Seeking Agricultural Produce Free of Pesticide Residues

Proceedings of an International Workshop held in Yogyakarta, Indonesia, 17–19 February 1998

Editors: **I.R. Kennedy, J.H. Skerritt, G.I. Johnson, and E. Highley**

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* A late paper for this earlier section

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Executive Summary

THIS workshop was supported by the Australian Centre for International Agricultural Research (ACIAR), the Australian Agency for International Development (AusAID), the Agency for Agricultural Research and Development (AARD) Indonesia and the Food and Agriculture Organization of the United Nations (FAO).

Some 70 participants from 12 countries reviewed options for monitoring and minimising the contamination of agricultural produce and the environment by residues of pesticides, while maintaining the benefits of pesticide use to agricultural production. The use of pesticides has been an important strategy in ensuring food security in many countries, but the contamination of produce and the environment is hampering agri-industry development and damaging human and environmental health. While recent research has provided new, cost-effective options for measuring and managing pesticide residues, in many countries the capacity to monitor contamination and to provide remediation is limited because of inadequacies in regulatory mechanisms, infrastructure support, laboratory facilities, or the availability of trained personnel.

The workshop arose as one outcome of the review in 1996 of a research project (PHT 93/09) funded by ACIAR in which simple test kits based on immunoassay methods were developed in collaborative research between CSIRO Australia and India's Central Food Technological Research Institute (CFTRI). While the project focused on analytical methods for residues in foods, it was obvious that the problem of contamination could not be treated in isolation from the environment in which food and fibre are produced. The simple test methods must be evaluated in the agricultural environment, where their results can help improve pesticide application strategies and develop remediation techniques.

The overall objective of the workshop was to contribute to the amelioration of non-target impacts of pesticides. Specific goals of participants were:

- to identify priorities in the development of appropriate test methods for environmental residue analysis aimed at abatement of pesticide residues in food through both improved application methods and remediation strategies;
- to disseminate information on country priorities and needs, and on research progress in test methods and remediation strategies; and
- to publish the results of the workshop as a monograph in the ACIAR Proceedings series, to facilitate dissemination of the work to developing country scientists and to policymakers.

Representatives from several Asian–Pacific countries reported on various aspects of the regional situation and exchanged information on recent developments aimed at ameliorating residue problems. From their reports and the ensuing discussions, it was clear that the significance of the problems regarding pesticides are widely recognised, and the need for integrated pest management (IPM) to reduce pesticide use well understood. Moreover, the reports published in these proceedings make it clear that much progress had already been made in understanding the means to overcome the problems. For example, simple tests for monitoring of pesticides in produce and the environment are available. They can be used to assess and deliver suitable yardsticks for measuring the ecotoxicological impacts of pesticides and, in some cases, to help remediation.

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Nevertheless, it was equally obvious from workshop discussions that further effort is needed to provide fully effective solutions.

Priorities for Action

As an outcome of the workshop, a set of priorities for future work was developed under the following four main headings:

1. Monitoring of pesticide residues in produce and the environment

- Obtain baseline data on the extent of the problem, to provide an accessible database for taking informed decisions regarding contamination.
- Review national policies, regulatory mechanisms, and infrastructure support, with a view to encouraging regional harmonisation of pesticide risk-reduction strategies and increased attention to pesticide residue monitoring and remediation of contaminated sites.
- Promote the use of simple test methods (TLC, spot tests, ELISA, bioassays) for monitoring, to assist decision-making regarding environmental protection.
- Ensure that the results of such monitoring provide effective feedback, leading to appropriate action and best management practice aimed at protecting human health and ensuring sustainable ecosystems.

2. Research

- Develop simple, affordable test methods for monitoring and research.
- Introduce measures to reduce the need for pesticides by increasing their efficacy, as part of integrated pest management (IPM).
- Develop the means to remediate pesticides by clean-up of soil and water, thus minimising contamination of produce and the environment.

3. Training and Extension

- Encourage national and international agencies to invest in the infrastructure and the technical facilities and people needed to mount effective national pesticide risk reduction strategies.
- Support training programs promoting the use of simple field tests, linked to quality assurance of the effectiveness of such training.
- Ensure chemical manufacturers, government, and industry agencies cooperate to develop better methods of pesticide application.
- Extend the applications of IPM so as to reduce the need for pesticides.

4. International standards, networking, and collaboration

- Harmonise procedures for registration and regulation in different countries (taking account of climatic differences and other factors affecting the fate of pesticides), including the establishment of maximum residue limits, with the aim of reducing costs and increasing trade.
- Coordinate the use of simple monitoring tests and standard protocols, ensuring quality assurance by linking their use to key analytical centres in each country.

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- Encourage international collaborative research and exchange of information to obtain solutions to pesticide residue problems.

Workshop participants recommended that these priorities for action be considered by both international and national agencies with responsibility for food security and human health, particularly the funding bodies charged with these responsibilities.

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Welcome and Opening Address

Dr Faisal Kasryno

Director General, Agency for Agricultural Research and Development, Indonesia

DR Sutatwo H. [Assistant Director General, FAO], Professor Sukanto [Chancellor, Gadjah Mada University], Dr G. Johnson [ACIAR representative], Distinguished Guests and Scientists, Ladies and Gentlemen:

First of all, I would like to warmly welcome you to Yogyakarta, Indonesia. Your participation in this workshop demonstrates your keen interest in exploring appropriate methods of environmental analysis through research and development aimed at abatement of pesticide residues in agricultural products, particularly pesticide residues in food. Allow me also to take this opportunity to express my deep appreciation to the organising committee for making this workshop successful, and to the Australian Centre for International Agricultural Research (ACIAR) for its sponsorship. I believe that this workshop will present a great opportunity to enhance awareness of environmental protection with respect to pesticide management.

Ladies and gentlemen, agriculture plays an important role for Indonesia's economy by providing enough food, increasing employment opportunities, producing raw material for industry, and by generating foreign exchange. During the First Long-term Development Plan (1969–1993), production and productivity of various agricultural commodities have increased significantly. Entering the Second Long-term Development Plan (1994–1998), the Ministry of Agriculture has responded to the various challenges of globalisation by launching an agribusiness approach as the basis of agricultural development. Nevertheless, food security is still considered as one of the most important issues in agricultural development.

Sustainable agriculture and highly efficient agribusiness systems is one of the scenarios in future agricultural development. Entering the era of globalisation and free trade, agricultural products should be highly competitive in term of their quality, including their permissible limits of pesticide residue.

It is said that the tropical climate is a paradise for insect pests and for diseases of food crops. Therefore, insects and diseases will remain serious limiting factors of development. Hence, crop protection will be an important focus.

Indonesia's crop protection policy is expressed in the implementation of integrated pest management (IPM). It is enforced and clearly stated in the Crop Cultivation Law No. 12 launched in 1992, and the Government Decree No. 6, 1995 on Crop Protection, Presidential Interaction No. 3, 1986, banned 57 insecticides for use on rice.

In the IPM system, pesticide use is virtually the final resort, when other control measures are not effective or inefficient. This indicates that an IPM system does not prohibit the use of pesticides. However, if in certain circumstances use is necessary, pesticides should be used wisely, adhering to legal requirements and ensuring correct and safe use.

We are all aware that pesticides are easily obtainable and are widely used for the benefits they bring to society. In addition to crop protection, pesticides also have veter-

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inary, fishery, storage, wood preservation, public hygiene, etc. uses. However, indiscriminate use of pesticides may likewise lead to adverse effects such as poisoning of humans and animals, induced pesticide resistance, bioaccumulation, residue problems, and destruction of the environment.

Considering the abovementioned matters, pesticides have to be properly managed so as to enable us to gain maximum benefits with minimum negative impact. In order to reduce or suppress the negative impact of pesticides which is likely to occur, the Indonesian Ministry of Agriculture, through the Pesticide Committee, has taken necessary steps such as:

- Tightening of registration requirements, particularly those relating to the safety of pesticides to people and the environment.
- Re-evaluation to all registered pesticides, making evaluations based on all data/information currently available, especially on aspects of safety to people and the environment.

Based on re-evaluation results, it is possible that the registration of certain pesticides will be withdrawn. A pesticide's registration can be revoked if it is proven to be highly dangerous to humans and the environment and there is any infringement of existing regulations.

At present, there are many violations to the existing regulations covering pesticide use, such as the distribution and use of adulterated, unregistered, and prohibited or restricted pesticides. I realise that some of our participants are from the pesticide industry and are involved in the development of pesticides, but are not directly involved in the use of pesticides. Nevertheless, I fully expect that you give attention to and actively participate in putting a stop to existing infringements, by distributing valuable information and providing guidance relating to pesticides. By these means we can hope that pesticide management (pesticide control) in particular can be more easily implemented.

One of the negative impacts of pesticide use that has been neglected so far is their residues in agricultural products. This will be very important in relation to agroindustry. The Ministry of Agriculture has issued a 'Guide book for pesticide residue analysis', and Ministerial Decree No. 985, 1997, in order to reduce or avoid the negative impact of pesticide use.

One of the objectives of this workshop is to assess appropriate test methods for environmental residue analysis aimed at abatement of pesticide residues in food through both improved application methods and remediation strategies. I am confident that the workshop will come up with useful solutions to these problems.

Finally, I wish you a successful exchange of views and hope that this workshop will end with important recommendations that provide the participating countries with the most favourable solutions.

With these all in mind I am wishing you all an enjoyable and pleasant stay in this part of the country. I now have the pleasure to officially open the workshop. Thank you.

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Achievements in Pesticide Application for Agricultural Use and in Residue Control in Indonesia

Kasumbogo Untung

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INDONESIA has adopted IPM as a basic policy for crop protection since the declaration of Presidential Decree No. 3, 1986. The main method in implementing IPM is the establishment of a large-scale IPM training program for farmers through an 'IPM Field School' system, which uses a participatory approach to teach farmers decision-making based on IPM principles.

Most IPM farmers have changed their attitude and behaviour in applying pesticides and managing their fields. Most IPM farmers have been able to limit the use of insecticides and fungicides very significantly, so they could save on production costs and increase their incomes. Reduction of pesticide application will reduce the risk of polluting the environment and of harming humans.

Pesticide residues in some agricultural commodities have been monitored by several institutions, especially the Directorate of Food Crops Protection. Extensive monitoring activities were conducted in 1988, 1989, 1990, 1991, and 1993. The results showed that most pesticide residue levels in several food crops at centres of agriculture were below WHO/FAO maximum residue limits. The latest records of pesticide residue monitoring, in 1996–97, showed that farmers were using less pesticides.

Pesticide has been known and used widely by farmers in Indonesia since the 1950s to protect their crop from pest attacks. Every year, thousands of tonnes of different kinds of pesticides are distributed, bought, and applied by farmers for controlling insect pests, diseases, and weeds. During the early years of the BIMAS (mass guidance) rice intensification program in the 1960s, pesticides were introduced and recommended by the government as the most reliable pest control method, and thereby to be used by BIMAS farmers as part of a technological package. The farmers were impressed with the results of pesticides application, and a practice at first confined largely to rice, became common practice in just about every crop. Up to now the most intensive uses of pesticides can be found in soybean and vegetable (high-altitude and low-altitude vegetables) cropping. Most of the farmers who apply pesticides expect a 100% kill of the entire range of insect pests, plant pathogenic organisms, and weeds.

This expectation led the government to subsidise the pesticides used in the BIMAS program by as much as 80% of their total cost. The government had to spent US$100–150 million annually for the pesticide subsidy. The total pesticide consumption for the BIMAS program increased dramatically every year, from less than 1000 tonnes in 1970, to 18 000 tonnes in 1987. Under the BIMAS program, times of pesticide application to the rice crop followed prescribed schedules, usually four times during the growing season, regardless of the population level of the pests. In the case of local pest outbreaks, the frequencies of sprays was increased. The kinds of pesticides which were popular with the farmers were broad-spectrum, effective, and 'fast- killing' pesticides. They killed not only the target pests but also beneficial and non target

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organisms including insect predators, parasitoids, wildlife, etc., and left residues in the environment.

Despite the increasing use of broad-spectrum pesticides, the pest problems were not solved, instead they were made worse by pest resurgence, secondary outbreaks, and pest resistance to pesticides. A new rice pest in Indonesia, namely the brown planthopper (BPH) had increased its populations and damage since the 1970s and reached the worst outbreaks in 1977 and 1979 where more than 600 000 ha of rice infested by the BPH was totally destroyed. Problems of BPH resurgence were followed by the development of BPH biotypes, resurgence, and resistance to pesticide. Problems with the heavy dependence on chemical control began to increase, and these problems were basically of an ecological and biological nature (Flint and van den Bosch 1981).

The next outbreak of BPH occurred in mid 1986 when it caused heavy damage to 75 000 ha of rice fields in Central Java. These consecutive outbreaks of BPH became a threat to the rice self-sufficiency that was achieved by the nation in 1984. This condition prompted the government to change its policy of pest control from a dependence on pesticides to a comprehensive IPM approach. The Presidential Decree No. 3, 1986 became a 'milestone' of the IPM implementation in Indonesia. This decree outlined the commitment of the government to maintain rice self-sufficiency through human resources development, environmental management, and protection of human health. In addition, the decree ordered the withdrawal of 57 previously registered formulations of broad-spectrum insecticides used on rice.

Indonesia has been successful in executing IPM principles in rice and other crops through a training program for farmers. The training program was started in 1989 and evaluation data showed that most IPM farmers could very significantly reduce the amount of pesticides they applied. This paper briefly discusses some achievements and methodologies of Indonesian farmers in reducing pesticides, and how these relate to pesticide residue management.

IPM Principles and Implementation in Indonesia

Integrated pest management (IPM) is an ecologically based control strategy that relies heavily on natural mortality factors such as natural enemies and weather, and seeks out control tactics that disrupt these factors as little as possible. IPM uses pesticides, but only after systematic monitoring of pest populations and natural control factors indicate a need for their use. IPM considers all available pest control action, and evaluates the potential interaction among various control tactics, cultural practices, weather, other pests, and the crop to be protected.

Presidential Decree No. 3, 1986 outlines some principles of pesticides usage in IPM in Indonesia as follows:

1. The type of insecticides used and the method of applications should take into account the protection of natural enemies of the BPH and other insect pests of rice.
2. The development of insect resistance to insecticides should be avoided through proper insecticide management procedures.
3. Insecticides should be used only if other control methods are not effective, and then in a judicious way.

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The decree demonstrated the consistent political will of the government that IPM should be the strategy of pest control. The decree ordered that the agricultural extension officers, farmer organisations, and individual farmers be trained to increase their skills for effective pest management. IPM is being challenged to develop human resources at the farmer level, i.e. the farmers themselves. They should be able to implement IPM in their own fields. The farmers should master IPM and become experts in it (Oka 1996).

IPM human resources development began in 1989 under the National IPM Program, which aimed to train different target groups i.e., farmers, field and extension workers, related government officials, community leaders, and the general public. Throughout the duration of the National IPM Project (1989–98) goals were set to train 2000 field pest observers, 6000 field extension workers, and 800 000 farmers. Initially IPM was aimed at rice fields only, but it was then extended to include soybeans and vegetables (cabbage, potato, tomato, shallot, and yardlong beans).

The IPM training methodology and system which have been developed by the National Program is an active, participatory, 'learning by doing' process, to enable farmers to make the right decisions in implementing IPM techniques under real field conditions. During a training period of one growing season, farmers learn and practice proper pest management decisions, based on the analysis of information which they gather through weekly monitoring. Four IPM principles which are implemented in the IPM field school are:

- grow healthy crops,
- conserve and use natural enemies,
- make weekly field observations, and
- develop farmers as IPM experts.

In groups of 25, farmers learn through experience how to monitor and make weekly ecosystem observations, practise ecosystem analysis, and make IPM decisions that will maintain the equilibrium between the pest population and its natural enemies.

IPM involves not only pest control activities, but also covers all aspects of crop management, including balanced and efficient fertilising, efficient use of water, crop rotation, soil conservation, and other agronomic measures. The overall objectives of IPM implementation are to bring higher crop productivity, increase farmers' incomes, maintain pest populations below an economic damage threshold, limit use of pesticides, and reduce the risks to human health and the environment.

Impact of IPM on Pesticide Use

Field surveys of 2013 farmers in 72 districts carried out in 1991, two years after the start of the program, indicated that average insecticide applications had fallen by about 56%. The result of a second impact study carried out in 1993, based upon 3335 farmers, indicated that this trend had been maintained. This enabled IPM-farmers to save about Rp. 100 000/ha as compared with those still using the insecticide-based system of pest control (Oka 1996).

The impact of IPM farmer training on pesticide use on vegetables is even more impressive. Compared with those still using insecticide-based pest control, cabbage farmers practicing IPM could reduce insecticide use by 80%, fungicides use by 95%, increase yields by 7.6%, and increase net return by $831.4/ha. The IPM technology

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implemented by farmers on potatoes reduced pesticide use by 89%, fungicide use by 81%, increased yield to 3.8 t/ha, and net return to $ 1710.64/ha. Similar results were gained from the implementation of IPM on tomato, shallot, and yardlong bean.

At the national level, recent statistics show that the average insecticide use by rice and soybean farmers has fallen, reflecting, at least in part, the success of the government's policy and regulations on IPM and pesticide management in changing the approach of farmers to pesticide use (Untung 1996). The reduction of insecticide applications to rice to about half their previous level and to about 2/3 their previous levels in highland vegetable crops has significantly reduced the risk of polluting the environment and endangering human health, and has helped to minimise pesticide residue levels in food and the environment.

Pesticide Residue Monitoring Program

The deep concern of society about environmental pollution, especially pesticide residues in food crops and products, has been growing in recent years. Efforts to detect pesticide residues in food crop products have been made by several institutions and scientists on an irregular basis. Because of many constraints and limitations, pesticide residue monitoring activities have been very limited in geographic spread, crop/commodity types, sampling frequencies and number of institutions involved. However, by using the limited and scattered data available we can get at least a glance at the pesticide residue picture in Indonesia.

The institution most active in collecting information on pesticide residues is the Pesticide Analysis Laboratory of the Directorate of Food Crops Protection (DFCP), Department Agriculture, in Pasar Minggu, Jakarta. DFCP has been monitoring pesticide residues in some food crops since 1980 (Siswomihardjo 1996). Monitoring activities were in the main production centres for food crops, especially vegetables in North Sumatra (Tanah Karo), West Java (Lembang), Central Java (Wonosobo), East Java (Batu), South Sulawesi (Enrekang), and Bali (Tabanan).

Extensive monitoring activities were conducted in 1986, 1988, 1990, 1991, and 1993, covering the following commodities: cabbage, tomato, potato, chilli-pepper, carrot, yardlong bean, celery, cauliflower, onion, shallot, grapes, apple, milled rice, and soybean.

According to DFCP's reports, most pesticide residue levels in those food crops sampled were below maximum residue limits based on WHO/FAO standards. Most crops contained organochlorines, especially DDT. Pesticide residues in some commodities were found to be above MRLs. These instances included: carbofuran in cabbage in 1986, potato in 1989, and tomato in 1989; chlorpyrifos-methyl in cabbage in 1989; fenvalerate in tomato in 1990; chlorpyrifos in cauliflower in 1991; and dithiocarbamate in potato and tomato in 1990. Table 1 gives maximum residue levels of pesticides detected in some food crops during monitoring by DFCP laboratories over the period 1986–1993.

DFCP's most recent pesticide residue monitoring activities were conducted in the 1996–97 fiscal year. During that year DFCP's Pesticides Laboratory monitored residues on cabbage, potato, tomato, yardlong bean, shallot, and chilli-pepper. Also sampled were some imported fruits, namely apple, pear, grapes, and orange. Due to budget limitations, the numbers of sampling locations and commodities were lower than those in previous years (1986–93).

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Table 1. Maximum levels (ppb) of residues of 12 pesticides detected in some vegetable crops in Indonesia during 1986–93

	Commodity						
Pesticide	Cabbage	Tomato	Potato	Chilli-pepper	Carrot	Yard-long bean	Grapes
Dithiocarbamates	1663	4913	570	160	145	–	90
Carbaryl	–	–	17	–	–	–	40
Diazinon	105	105	62	348	29	36	8.7
Chloropyrifos	2.7	–	13	46	54	15	–
MIPC	5.8	20	–	–	–	–	–
DDT	85	447	687	–	1634	7.7	–
Carbofuran	85	212	550	–	–	–	–
Fenitrothion	51	2.8	3.5	2.1	7	–	–
Cypermethrin	1261	234	30	–	–	13	–
Permethrin	10	15	–	–	–	–	–
Fenthoate	61	2.8	–	–	–	–	0.3
Cyhalothrin	1.1	39	1	–	–	0.2	–

The result of pesticide residue monitoring in vegetables and fruits in 1996–97 showed that residues were not detected in most samples and, where detected, were on average lower than in previous records. This trend is encouraging, and might indicate that growers of horticultural crops are using less pesticide and/or applying it most wisely.

Pesticide Residue Management

In August 1996, the government released the Joint Decision of the Minister of Agriculture and the Minister of Health on the Maximum Residue Limits of Pesticide in Agriculture Produce. This decision formally stipulated MRLs for more than 2000 pesticide/commodity combinations, applicable to domestic agricultural production and to imported commodities.

This regulation requires that all agricultural produce marketed and consumed must not contain pesticide residue levels higher than the MRLs listed in the annex of the Joint Decision. The list and values for MRLs are basically those from the latest MRLs promulgated by the Codex Alimentarius Commission, Joint FAO/WHO Food Standards Program.

Some action programs have been planned and implemented, as a follow-up to the government's decision on MRLs. The 'National Standard Method' for pesticide residue analysis on agricultural produce, based on international standards, has been completed. The standard was formally declared by the Chairman of the Pesticides Commission. We are in a process of evaluating and establishing a national network of laboratories for pesticide residue analysis.

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Opening Remarks

Soetatwo Hadiwigeno

Assistant Director-General and Regional Representative for Asia and the Pacific, Food and Agriculture Organization of the United Nations

MR Chairman, Distinguished Participants, Ladies and Gentlemen:

I am very happy to be with you all today, to deliver some brief opening remarks on this very important workshop. Recently, FAO has been very active in helping member countries in establishing food control systems in their respective countries, to comply with various international standards and in line with World Trade Organization (WTO) Sanitary and Phytosanitary (SPS) agreements. This workshop provides us with an opportunity to share with you our concern, as well as to inform you on FAO activities in the area of pesticide residues and food control in general.

The levels of pesticide residues we found in food products in the markets depended very much on three main group of factors:

- the types of pesticides being applied by the producers, namely the farmers;
- when, how much, and how pesticides are being applied to crops; and
- measures being taken and enforced to make sure that food products entering the market are in compliance with the established food safety standard, including the institutionalisation and the implementation of HACCP and GMP.

Accordingly, my contribution to this workshop will concentrate on those three issues.

Let me start with pesticide control and regulations. I hope you are well aware that FAO is very much concerned about the indiscriminate use of synthetic pesticides, particularly from the standpoints of environmental and health considerations. The problem with the use of pesticides, which during the last 15 years has become a focus of public interest in this FAO region, is the possible threat they pose to human health and the environment. In that context, an FAO conference in 1985 adopted the 'International Code of Conduct for the Distribution and Use of Pesticides' and recommended that all FAO member nations should promote the use of this code in the interests of safety and more efficient use of pesticides. FAO executed a regional project, from 1988–1993, for promoting awareness of responsibilities under the code of conduct on pesticide use in 27 developing countries of the FAO Asia–Pacific region. This project helped the participating countries to establish and strengthen: their pesticide registration scheme; technical capabilities of regulatory manpower; safe and efficient use of pesticides and implementation of 'prior informed consent' (PIC) for hazardous and restricted/banned pesticides.

However, our latest monitoring of the implementation status of the code shows that much remains to be done to enforce appropriate pesticide management in the developing countries of this region. Our observations also indicate that there is a large gap in setting out norms and standards to ensure safe pesticide use in most developing countries of this FAO region. There is a plan to survey the latest implementation situation of the PIC at country level through a 'Regional Meeting of the Designated National Authorities' of the PIC in August 1998 in Bangkok, Thailand. The 5th session of the

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intergovernmental negotiating committee for an international legally binding instrument for the application of the PIC for certain hazardous chemicals and pesticides in international trade will be held in March 1998 in Brussels, Belgium.

The second topic is the judicious use of pesticides and agro-chemicals. In this context, let me turn now to the subject of integrated pest management (IPM) which makes maximum use of all available non-pesticide means to manage the interaction between a crop and its pests, using synthetic pesticides only as last resort. FAO has been promoting IPM since 1967. It has been modestly successful in reducing the use of broad-spectrum pesticides, as well as in scaling down of government subsidies in pesticide consumption in several developing countries of this region. Therefore, FAO takes a great pride as a catalyst for IPM in this region. It is now almost 18 years since Asian rice farmers and other farmers of major crops and their village level technical advisers have been creating and operating IPM systems that work to minimise the pesticide exposure, through FAO-executed IPM regional projects and programs. It is, therefore, a matter of satisfaction to us that IPM is the Asia and Pacific region's distinctive and globally recognised contribution to sustainable agriculture in the 21st century. Even Agenda 21 as adopted at UNCED focused global interest on IPM as the practical response to concerns about pesticides in agriculture intensification.

In order to support this policy and to complement the technical activities further, the Agricultural Engineering Branch of the Agricultural Support Systems Division of FAO has over the last three years implemented a program on 'Safe and Efficient Application of Agro-chemicals and Bio-products'.

The objective of this program is to contribute to a reduction of the amount of pesticides used in agriculture, and the related hazards to human health and environment, by improving the quality of and working conditions associated with pesticide application techniques and equipment, and the skills and knowledge of farmers and operators using the equipment. This program is intended to complement the ongoing activities of the FAO Plant Protection Service in order to come closer to the common goal of using fewer synthetic inputs in agriculture so as to bring safer and more sustainable production.

The third topic concerns food control and food safety issues. Mr Chairman and distinguished participants you may be aware that FAO is committed to improving food security on a global basis, including the protection of both the environment and a healthy food supply. This commitment is contained in the preamble of the FAO Constitution which calls for 'raising levels of nutrition and standards of living of the people'. Thus, FAO has a very important role to play in improving nutrition and food security of both rural and urban populations, including ensuring the quality and safety of food and protection of the environment. This commitment was re-affirmed at the FAO World Food Summit held in Rome in late 1996.

The need for all countries of the FAO Asia–Pacific region to improve their national systems of food control, including control of pesticide residues, should be of high priority within the governments concerned. As you are aware, the requirements of the World Trade Organization (WTO) under its agreements on 'Sanitary and Phytosanitary (SPS) Measures' and 'Technical Barriers to Trade (TBT)' have had a very big impact on all countries to improve their control systems for food quality and safety, including ensuring that foods do not contain excessive levels of pesticides. Most countries have already started to strengthen their national food control programs, but much

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remains to be done, especially regarding ensuring good agricultural practices in the use of pesticides. FAO has been active in this region in providing technical guidance and assistance to the countries in these fields.

The joint FAO/IAEA (International Atomic Energy Agency) cooperative programs also are providing assistance to countries in the implementation of SPS and TBT agreements through training and quality assurance services with particular reference to chemical contaminants. All these development support programs of FAO/IAEA are being carried out at Seibersdorf Laboratory in Vienna, Austria, as a 'Training and Reference Centre on Food Quality and Pesticide Control'.

This workshop is one way to get a better understanding of the need for all countries in the region to take immediate action to use some of the newest technologies to ensure that foods are safe and nutritious, including freedom from excessive or non-permitted pesticide residues. As you know, risk management is only one part of the broad field of risk analysis which encompasses risk assessment, risk management, and risk communication—for this is the future methodology for controlling all food contaminants, including residues.

The work of the joint FAO/WHO Codex Alimentarius Commission, with a membership of 160 member countries, has received far-reaching acknowledgment by the WTO. The Codex standards, codes of practice, and guidelines are now the benchmark for trade that is used by the WTO for food safety matters, including for pesticide residues and certainly for enforcing maximum residue limits (MRLs). It is therefore imperative that, in the use of pesticides for food crops, there is a good understanding of good agricultural practices (GAPs), good food manufacturing practices (GMPs), and Codex. The Codex system has in fact introduced the whole spectrum of risk analysis into the overall application of standards, guidelines, and codes of practice.

Concluding Remarks

As we all know, the level of pesticide residues found in food crops depends very much on the types of pesticides being used, the amounts being used/applied, the crops on which they are being used, and the postharvest interval before marketing. Codex has taken all of these points into consideration in its work. FAO is using the Codex in providing technical guidance to countries in strengthening national infrastructures for food control, including ensuring good agricultural practices are used, and the overall control of pesticide residues. Such technical guidance includes legislation as well as laboratory activities related to testing procedures.

The WTO is, in fact, having a major impact on all countries of the world, especially on developing countries and certainly on all our countries in Asia. At the same time, the WTO is also having a large impact on the work of FAO. At least two of the WTO's agreements relate to food and in doing so have caused many countries to urgently review their food control procedures for domestic, exported, and imported food. The 'new' WTO agreements related to food involve the use of the work produced by the FAO/WHO Codex Alimentarius related to food quality and safety and require improved control systems as well as improved processing controls for industry. This has meant that the private sector and the public sector are indeed forced to work much closer together in their efforts to assure food quality and safety. This is most important,

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especially with the economic problems being experienced by all countries in the region. Establishing an operational and enforceable food control system it imperative to supplement and complement other measures including IPM, GAPs, and GMPs.

FAO is fully committed to assisting countries to ensure that their foods are safe and nutritious and that food security is met on both a rural and urban level. The control of the use of pesticides so as to ensure that foods are safe is essential and at the same time it is important to ensure that such use does not result in degradation of the environment. The use of scientific technologies such as risk analysis, IPM, GAPs, and Codex will certainly be a valuable asset in assuring our world's population that our food supply is safe.

Mr Chairman, finally, I would like to say once more that while the region as a whole has been developing the management of synthetic pesticide distribution and use for plant pest management, there are still some disquieting features in safe handling and judicious use of pesticides. Many of these are essentially continuing problems persisting in our view from infrastructure and policy deficiencies at the national plant protection organisation level.

However, I have no doubt that with the presence of distinguished experts, this workshop will provide guidelines to alleviate the problem.

I express my sincere thanks to ACIAR of Australia and ARRD of Indonesia for organising this very important workshop. I wish you all success.

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Defining the Scope of the Problem of Pesticide Residues

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Pesticides in Perspective: Balancing Their Benefits with the Need for Environmental Protection and Remediation of Their Residues

I.R. Kennedy*

Abstract

Worldwide, research data show that, without effective pest management, preharvest crop losses would average about 40%. Postharvest needs for pest control also must be met to prevent major losses of agricultural products. Nor is pest control solely an agricultural concern—it has many other rural, urban and industrial applications. Without effective pest control, the world's food and fibre production and environmental and human health would all be seriously threatened. As a result of publicity directed toward the use of chemical pesticides, it is commonly thought that all pest management programs depend entirely on chemical use. Nothing could be further from the truth. In fact, nonchemical techniques, such as sanitation, cultivation, crop rotation, resistant cultivars, and biological control (including the recent introduction of transgenic plants) are widely and increasingly used for pest control. Chemicals should be used primarily as a last line of defence.

Even so, many pests cannot be controlled adequately and there remains a continuing need for application of substantial quantities of chemical pesticides for the foreseeable future. It is essential to ensure that their benefits in allowing economic production of agricultural crops clearly outweigh possible disadvantages such as development of resistance and contamination of produce or of the environment of pesticide residues. In this paper, several case studies derived from experience in the Australian cotton industry are used to illustrate some of the key principles that must be considered to achieve environmental protection. It seems probable that public tolerance of pesticide residues in produce will lessen rather than increase as time goes by. Therefore, it must be assumed that protection of the environment in which agricultural produce is prepared and its remediation with a view to reducing these impacts will be essential. However, the best methods to achieve this protection are poorly known and applied at present. In managing pesticide use, there are three logical steps that can help to reduce the impact of residues:

- Minimise pesticide input;
- Contain the pesticide to the application site; and,
- Select for use pesticides with minimal environmental impact.

There also needs to be effective and inexpensive means of monitoring the possibility of impacts, either by analysis of produce or of the environment. In this paper, methods and practices consistent with these three steps will be discussed in the context of several case studies of particular pesticides.

WITHOUT pest control, food and fibre production and environmental health in all countries would be seriously threatened. Worldwide, research data show that, without effective pest management, preharvest crop losses could average 40%. O'Brien (Pimentel and Dinnette 1992) estimates that nearly one-third of the world's potential food supplies are lost to pre- and postharvest pests in any case (Walker 1983). Australia is no exception—if insects, diseases and weeds could not be controlled, production of many field and horti-

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cultural crops simply would not be economical. In most countries, the extra fertile land needed to reduce usage of pesticides would not be available and in other countries, significant environmental damage would result from land clearing. Nor is pest control solely an agricultural concern—it has many other rural, urban and industrial applications. The fact is, without pest control, food and fibre production and environmental and human health in Australia and worldwide would all be seriously threatened.

The value of chemicals was recognised early in the development of agriculture. Inorganic pesticides such as sulfur and the arsenical and mercurial compounds and botanical insecticides such as pyrethrums—although only moderately effective—nevertheless represented an important advance in pest control. But the development of DDT, 2,4-D and other synthetic organic pesticides around 1950 introduced a new era of pest management. For the first time, farmers could achieve excellent control of insects, weeds and plant diseases. It was even thought that, if applied in sufficient amounts, these chemicals would eliminate most pest species. This was not to be the case, because of the development of resistance, still one of the major constraints on cheap and effective control of pests such as insects and weeds. Furthermore, the recognition that organochlorine (OC) pesticides could bioaccumulate causing impacts on some species (Connell 1988) and very recently that they might even have oestrogen-like properties leading to effects on the fertility of humans (Colburn et al. 1997) has caused a strong reaction to their use.

Resistance and Rates of Pesticide Use

Pests, particularly insects, showed great capacity to develop resistance. Today, worldwide, more than 700 species of insects are resistant to insecticides; plant pathogens resistant to fungicides are widespread; weed resistance to herbicides is becoming common (see Charles et al. 1995). Widespread use of these first organic pesticides, particularly DDT and other organochlorine insecticides such as dieldrin and heptachlor led to another unforseen problem—they were persistent and bioaccumulated. This presented a potential residue problem in food, while from an environmental viewpoint, reproduction in fish and birds was affected.

It soon became clear that intensive use of these chemicals could no longer be tolerated and, in most developed countries, the persistent OCs have been phased out and their use banned. New chemicals, such as organophosphates, carbamates and pyrethroids were developed which were generally applied at lower rates and which did not bioaccumulate. But being less persistent, it often was necessary to apply them more frequently. Many pests are now becoming resistant to some of these pesticides and special strategies have been developed to slow resistance. For example, in Australian cotton production, the pyrethroid resistance strategy (Forrester 1990; Cox and Forrester 1992; Forrester et al. 1993) provided several years respite and allowed retention of some chemicals longer than otherwise possible. This strategy involved the use of endosulfan early in the season, followed by pyrethroids as the plant canopy filled, with finishing sprays a chemical in another class such as organophosphates (or chlorfluazuron, as discussed below).

As a result, it was obvious that chemical-based pest management programs could not be maintained and that, in future, acceptable levels of pest control would be achieved only through better combination of chemical and nonchemical management techniques. Experience with the persistent OC insecticides demonstrated the fallacy of assuming that pest control agents could be developed and introduced without considering potential side-effects. Establishment of international standards for pesticide residues in agricultural produce and environmental concern with pesticide use forced governments, including those in Australia, to establish agencies monitoring and regulating pesticide use. In most developed countries, research on pesticide behaviour and environmental impact quickly became an important component of developing pest management programs.

Benefit–cost Analysis for Pesticide Use

Analysing the relative benefits and costs involved in continued pesticide use would require the consideration of many factors. Amongst the benefits from pest control that need to be included in such analysis are:

- increased ease and degree of pest control. Apart from direct effects on production, effective pesticides obviously release food, fibre and timber producers to concentrate on other agronomic factors related to achieving efficient yields.
- greater production per unit area of land resulting from pest control. In fact, the increased level of

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food production per capita and relative food security that was happily achieved during the past 40 years, thus averting the spectre of world famine, needed very little change in land area. Increased yield has almost exactly matched population increase (Dyson 1996), as a result of improved crop genotypes, availability of fertilizers particularly nitrogen, and more effective pest control by both pesticides and better management. A very valuable consequence is that a lower proportion of the Earth's arable land is needed for food, fibre and timber production. Given that more marginal land of poorer fertility or accessibility (e.g. uplands) would otherwise have often been needed, the capacity to improve yield has been a very important factor in limiting land use.

- cheaper production per unit output usually results, even though many chemical pesticides are expensive and newer alternatives even more so.
- improved quality of produce (e.g. fruit and vegetables with less surface damage) is usually a by-product of pest control with pesticides. However, consumer preference for 'organic produce' clearly involves different standards of quality. There is no reason why both standards for the quality of produce cannot coexist.

Against these benefits, there are certainly risks and costs that attend the use of chemical pesticides. These include:

- contamination of food and fibre. In most countries, maximum residue levels (MRLs) have been set for agricultural produce, indicating permissible limits to contamination. Achieving these MRLs requires careful attention to application of pesticides and strict adherence to recommended withholding periods during which residue levels fall to acceptable levels as a result of dissipation (chemical and biological degradation, volatilisation, photodegradation, wash-off in rain, etc.) following the date of application. Thus, education programs and good farming practices are essential.
- direct human exposure of farmers, villagers, children. Particularly in public health programs for diseases such as malaria in highly populated regions, there is a risk to human health. In addition, the availability of pesticides also highly toxic to humans (e.g. organophosphates, carbamates) can lead to tragic consequences.
- environmental contamination and toxic effects on ecosystems. The risks to animal and plant species in ecosystems where pesticides may impact as a result of transport from the target sites on farms are real and environmental values must be considered. Data on the ecotoxicology of pesticides is now considered essential in registration processes for chemicals, measured by parameters such as the LD_{50} or LC_{50} indicating lowest doses or aquatic concentrations killing half a test population of the organisms concerned. In general, permissible levels of chemical contaminants are set using such parameters, with a safety factor included (often a ten-fold safety margin over the dose shown to exert measurable effects).
- the development of pest resistance is also a cost resulting from pesticide use. Increased insect or weed resistance leads to increased rates or numbers of pesticide applications, or to the substitution of new chemical pesticides. In the case of DDT, its banning from use has been a result of increased resistance by insects at least as much as recognition of environmental damage. While many of the replacements have been less damaging (e.g. the pyrethroids), the loss of an effective chemical may result in worse environmental impacts as a result of ignorance of such effects on ecosystems.

Little published material exists on the benefit-cost analyses of pesticide use (e.g. Brush and Clemes 1995; Cothern 1996). In order to improve risk assessment for pesticides, better methods are required. Several of the papers in these proceedings address the need for such methods and the development of 'yard-sticks' to illustrate the extent of impacts and to determine acceptable levels in the use of pesticides. In all likelihood, the standards set to safeguard human and environmental health are likely to become more stringent as time goes on, requiring consistent responses by scientists and others.

The Cotton Industry and Pesticides

Without effective management programs, losses caused by pests—particularly insects—would be so devastating that cotton production would not be economical. For entomologists attempting to cope with managing insecticide-resistant strains of insect pests while losing effective chemicals one after another because of environmental concerns, it has been a constant struggle. Nevertheless, effective management programs have been developed, more recently using a wider selection of less persistent insecticides from different chemical groups. But current cotton pest

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management programs still rely heavily on chemical use (Fitt 1994).

Unfortunately, while pest management research flourished, less emphasis has been placed on pesticide management research. This led to a number of problems, particularly the occurrence of pesticide residues in produce such as livestock and in environmental contamination of ecosystems (e.g. riverine systems). But in Australia, to their credit, cotton growers and irrigation water users have financially supported monitoring programs of the riverine system by the NSW Department of Water Resources (Land and Water Conservation) and Cotton Research and Development Corporation research on the fate of pesticides since about 1990. More recently, the scale of this research has been lifted including the development of the Cotton Cooperative Research Centre and a number of programs are now in progress to investigate the movement of chemicals off-farm, the behaviour of pesticides in the environment and accumulation and persistence in livestock and fodder. It is essential that this research should continue after the current problems are overcome. Just as chemicals vary in effectiveness against different pests, so also do they vary in their persistence and behaviour in the environment—a fact which always should be taken into consideration. Persistence and behaviour can only be established by appropriate research in pesticide management, preferably as part of the registration process for new chemicals.

Managing pesticide use

In managing pesticide use, there are three logical steps:

- Minimise pesticide input. This can be achieved by minimising the number and rate of applications. For example, monitoring pest pressure by direct counts can allow delay of spraying until the pressure has become tolerable according to some threshold. The time and meteorological conditions of spraying and the potency of chemicals can also affect the total amount of chemical that must be applied. The use of ground rigs and more directed band spraying, if possible, and the development of precision farming technology for direct herbicide application to weeds may also help limit the total amount of chemical needed. Application of the principles of integrated pest management (IPM), food-sprays to encourage the growth of beneficial predating insects that attack pests and the planting of plant buffer zones to grow beneficial insects can all contribute to reduced needs for chemical applications (see Kennedy et al. 1997b).
- Contain the pesticide to the application site. By minimising erosion, the transport of chemicals-associated eroded soil may be minimised. The use of flocculants in irrigation water (e.g. polymers such as polyacrylamide) may also contribute to reduced erosion and sediment transport. The recirculation of irrigation tail waters within an agricultural production system rather than release directly into a river system can also significantly reduce the transport of pesticide residues into the environment at large. Where it is possible, to maximise on-farm storage and the capacity to retain storm run-off will also contribute to containment of pesticides on farms.
- Select for use pesticides with minimal environmental impact. The physical and chemical properties of pesticides such as partitioning into soil organic matter, volatility, hydrolysis rates and effect of pH all contribute to their mobility in ecosystems (see Table 1 for a summary of environmental risk factors). The selection of pesticides with short 'half-lives' also helps minimise the risk of contamination of produce and the environment. Also, the existence of natural bioremediation by biodegradation (Van Zwieten and Kennedy 1995) is an important factor. For example, pyrethroids appear to present minimal environmental risk because they partition into the soil organic fraction yet are rapidly broken down, with a typical half-life of 1–2 weeks (Wang and Kennedy 1995; Wang et al. 1997). In principle, bioremediation using engineered microorganisms or plant-microbial systems (Van Zwieten et al. 1995; Feng and Kennedy 1997) may also be possible and other examples of bioremediation will be presented in the final section of this book.

All of the properties indicated in Table 1 for which quantitative measures may be determined are needed if a full assessment of the chemical's environmental risk is to be achieved. In many cases, such information is kept 'in-house' and may not be readily available to pesticide users and those advising them.

Reduction of pesticide use has been the goal of scientists concerned with insect control on cotton for many years, because it is important in terms of managing insecticide resistant pests. Through development of insect monitoring programs to determine pest pressure, and economic thresholds to indicate an maximum acceptable level of insect or weed damage

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Table 1. Properties of chemicals and environmental risk factors

Property	Parameter measured	Possible risk factor(s) assessed
Mobility	Volatility (Pa), Henry's constant	Damage to non-targets in air
Mobility	Solubility in water (g/L)	Leaching, run-off potential
Mobility	Binding soil/sediments (g/kg)	Run-off potential to rivers
Partitioning/hydrophobicity	Octanol-water K_{ow} , K_{oc}	Bioaccumulation in produce
Partitioning	Adsorption, deadsorption (K_D)	Release from run-off sediments
Partitioning	Binding K_D to media	Inability for containment
Persistence, dissipation	Half-life (days) in soil	Accumulation in environment
Persistence, dissipation	Half-life (days) in water	Transport in run-off
Persistence, dissipation	Half-life (days) on foliage	Transport in trash
Persistence, degradation	Ultraviolet degradation (per second))	Potential for accumulation
Persistence, degradation	Chemical hydrolysis (per second)	Potential for accumulation
Persistence, degradation	Effect of pH	Potential for accumulation
Persistence, degradation	Rate of biodegradation (per second)	Potential for accumulation

before a pesticide is applied and other techniques, substantial progress in reducing rates of pesticide application has been made. The pyrethroid resistance strategy discussed above also had the benefit of minimising application rates by delaying the appearance of resistance.

In order to illustrate some of the issues involved, two other case studies regarding reduced application rates and environmental impacts derived from recent Australian experience will be given. These studies demonstrate that simple, complete solutions are probably impossible but that the intelligent use of current knowledge and technology shows significant potential to reduce impacts. These cases relate to the development and use of transgenic cotton engineered to be resistant to insects and to the case of Helix (chlorfluazuron).

Transgenic cotton

The recent introduction of *Bt* (Ingard) cotton is expected to provide a major advance in reducing insecticide use. Gene technology designed by Monsanto to improve the expression of the crystal toxins of *Bacillus thuringiensis* in plants such as cotton has been applied by CSIRO to Australian cotton cultivars. As a result, transgenic cotton plants resistant to *Helicoverpa* (*Heliothis) armigera* and *H. punctigera* larvae have been prepared. Particularly in the case of *H. armigera,* these Australian pest species are particularly resistant to methods of pest control and this robustness extends to the Ingard biotechnology. Therefore, success in this Australian situation will be particularly welcome.

Small-scale field trials over several years have established the effectiveness of the toxin expressed in cotton foliage (Fitt 1996). In the 1996–97 season, many thousands of hectares of transgenic cotton were planted in NSW and Queensland in Australia, under licensing arrangements with Monsanto. The outcome was that the need for pesticide (kg/ha) in New South Wales (mainly of endosulfan) was reduced by 60%, with excellent yields with transgenic cotton + insecticide. In the 1997–98 season, about twice the area of transgenic cotton as in 1996 was planted. In a year with greater *Heliothis* pressure, the effectiveness of pest control has been less impressive, particularly in warmer areas of Queensland. However, significant control has still been achieved in NSW and it seems likely that an outcome in which chemical pesticide use is reduced by half for the time being is the best result possible. The management of Ingard cotton to reduce the likelihood of resistance developing will also require considerable resources. Provided the technology can be made available at reasonable cost and resistance is avoided, this transgenic cotton has a future and can help reduce the risk of contamination of produce and the environment. Maintaining transgenic plants requires a huge effort in manpower and expertise (e.g. the provision of refugia equivalent in area to the protected crop to maintain a large population of insects not subject to selection pressure for resistance). The cost of using Ingard cotton compared to using chemical pesticides will probably prove to be fairly cost neutral.

Despite this degree of success with transgenic cotton, it is obvious that chemical pesticides will still be needed!

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Helix (chlorfluazuron)

The case of Helix, a product marketed by ICI's Cropcare subsidiary in Australia for control of insects of cotton provides a salutary lesson in how a situation can develop where better management of registration and use could have avoided it. This product is manufactured in Japan, where it has been applied to horticultural crops.

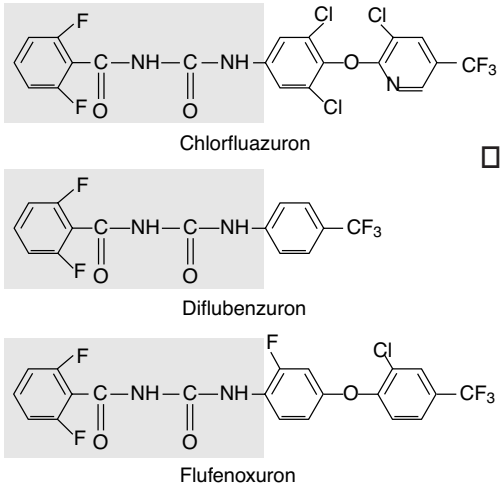

Figure 1. Three examples of benzoylphenylurea insecticides—chitin synthesis inhibitors

The active ingredient in Helix is chlorfluazuron, an insect-growth regulator, specifically affecting chitin synthesis, a key component of the insect exoskeleton. It therefore has the merit of being a selective insecticide only otherwise active on crustaceans or, potentially, some fungal species. It is characterised by a low vapour pressure, low solubility in water and firm binding to soil organic matter. It is effective at low doses and therefore exposure of the environment is reduced.

In Australia, Helix was regarded as a key candidate to be used in the introduction of transgenic cotton discussed below, as a single late season application. Its role would have been to prevent development of late season *Heliothis* pupae most likely to exhibit resistance to the *Bt* crystal toxin. The insecticide was not provided full registration, but licensed for limited use from one year to the next. Cotton farmers found it a very effective finishing spray and it was applied on a broad scale. In addition, during a 3-year drought period when stock-feed was in short supply, it was suggested that trash from cotton ginning might be used as emergency fodder.

Large quantities of trash, material difficult to dispose of in any case, was fed to cattle during the drought period in 1993–94. The trash was tested for residues of pesticides such as endosulfan, but no analyses for chlorfluazuron were performed. Chlorfluazuron was not included in the usual set of organochlorine standards and indeed, no straightforward method for its analysis was widely available. It seems likely that an attitude towards Helix that it was a benign product used at low rates, strengthened by its selective mode of action as well as almost complete ignorance of its chemical organohalogen nature, resulted in a lack of vigilance about this product, even though it was often the last pesticide sprayed on cotton foliage.

It is now known that chlorfluazuron is extremely persistent on cotton trash (Kennedy et al 1997a) and that the gin trash was almost certainly heavily contaminated. There is also anecdotal evidence that Helix drifted onto nearby pasture during aerial applications, further contributing to the problem. A vigilant analyst testing beef cattle for infringements of MRLs (as part of the national residue survey) discovered significant levels of chlorfluazuron in body fat, and large numbers of stock were subsequently quarantined from commercial use. Furthermore, chlorfluazuron was so strongly partitioned into the lipid phase (see Table 2) that its rate of elimination from body fat was negligible and quarantined stock had to be destroyed. Economic losses of $A100–200 million were claimed by beef producers in a legal class action in the Federal Court in Sydney and damages estimated to be of this order were awarded in 1997 on application to beef producers against the chemical supplier. The National Registration Authority was legally exempt from liability by statute and the state agencies involved were probably fortunate to be deemed not responsible, since they promoted the use of trash.

The Helix case is, in hindsight, an instructive one because all the information and warning signals needed to prevent this tragedy were available. It seems probable that at least one warning from a government chemist was overlooked and at no stage did any group of experts with the full range of expertise needed examine the situation. Those aware of the feeding of the gin trash had inadequate chemical expertise and those aware of the chemical nature of chlorfluazuron and the likelihood of its persistence were unaware that the trash was being fed. Furthermore, even limited animal trials by the chemical supplier would have indicated the strong likelihood of a

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problem developing. It is unfortunate that this insecticide with several desirable features, when used at low rates, should have been lost in this way. Used more carefully, with measures to prevent the feeding of trash and aerial drift, it is probable that Helix could have been in use in the Australian cotton industry for many more years.

Table 2. Octanol/water partition coefficients (logP) for benzoylphenylurea insecticides

Benzoylphenylurea	Log(K_{ow})
Diflubenzuron	3.9
Flufenoxuron	4.0
Teflubenzuron	4.3
Lufenuron	5.1
Chlorfluazuron	5.8

Source: The Pesticide Handbook; Eadsforth 1985

However, partly as a result of the experience with Helix, the National Registration Authority (NRA 1996) and Environment Australia now require much more stringent examination on their environmental fate in the registration of new chemicals, often insisting that field trials under Australian commercial conditions be performed.

Conclusion

Chemical pesticides will still be required to control some pests and risks to human health and the environment must be balanced against the benefits of efficient food, fibre and forest production Transgenic crops may even lead to increased applications of herbicides (Charles et al. 1995), despite denials by commercial interests. We need to ensure that harmful residues do not increase by proper selection of chemicals with shorter half lives as growers adopt reduced tillage techniques or as herbicide-tolerant crop varieties are introduced.

Even if total pesticide use is significantly reduced, the possibility that chemicals will continue to contaminate the environment will remain a concern. Attention will have to be directed toward developing ways to contain the residues to the greatest extent possible. And we have to ensure that where 'leakage' does occur—perhaps as a result of drift or run-off during heavy rainfall—the pesticides used will have little environmental impact.

It is important to remember that:

- complex problems have no simple solutions;
- best practice management is not in conflict with efficient farming options; and
- comprehensive solutions require coordinated efforts of chemical producers, registration bodies, industry organisations, scientists, regulators, and farmers/farm managers.

These solutions can be obtained, but their achievement will not be realised unless there is properly organised cooperation.

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Pesticide Management Policy in Indonesia

I. Daryanto*

Abstract

It has been realised that pesticides are generally hazardous substances that may have adverse effects on human health and the environment. On the other hand, their use may bring economic benefits in helping to develop, for example, the agricultural sector. It is essential therefore to manage the use of pesticides so as to maximise their benefits and minimise their negative effects.

Decree No. 7 of the Government of Indonesia, on the Control of Distribution, Storage, and Use of Pesticides, promulgated in 1973, is the basic instrument for pesticide management and legislation in the nation. This decree clearly states that pesticides for commercial distribution, sale, storage, and use in Indonesia must be registered and approved by the Minister of Agriculture. To assist its implementation, the Minister of Agriculture has promulgated some further decrees, including No. 280/1973 on Procedures of Pesticide Registration Application and Approval, No. 429/1973 on Packaging and Labelling Requirements of Pesticides, No. 944/1984 on Limitation of Pesticide Registration, and No. 536/1985 on the Control of Pesticides.

BY carefully considering the advantages and disadvantages of pesticide usage,it is essential that pesticides should be managed as judiciously as possible in order for us to obtain more benefits with less negative impact.

The use of pesticides is not forbidden in Indonesia, but the Government Law No. 12 of 1992 on crop cultivation System states that use of pesticides is virtually the last resort (if other applied control measures have been evaluated as inefficient and ineffective). For that reason, if in a certain circumstance pesticide use is necessary, it should be done wisely and taking account of three aspects—legal, correct, and safe.

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Definition of pesticides

According to the Government Decree No. 7 of 1973, the term 'pesticides' covers all chemicals, other substances, microorganisms. and viruses which are intended for:

- controlling pests and diseases on crops/parts of crops or agricultural products
- controlling weeds
- destroying and preventing unwanted growth of crops
- regulating/stimulating plant growth, excluding fertilizers
- controlling ectoparasites of domestic animals and livestock
- controlling and preventing aquatic pests
- controlling/preventing animals/microorganisms in the house, buildings (residential pests), and transportation means

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- controlling/preventing animals causing diseases to humans or animals by plant, soil, and water.

Regulatory basis

The Government Decree No. 7 of 1973 on the 'Control of Distribution, Storage and Use of Pesticides' is the basic instrument for pesticide management and legislation. It is clearly stated in this decree that pesticides for commercial distribution, sale, and use in Indonesia must be registered with and approved by the Minister of Agriculture.

To implement stipulations in this decree, some other decrees of the Minister of Agriculture have been instituted. Among these are Decrees No. 280/1973 on the Procedures of Registration Application and Pesticide Licensing, No. 429/1973 on the Packaging and Labelling Requirements of Pesticides, No. 944/1984 on the Limitation of Pesticide Registration, and No. 536/1985 on the Control of Pesticides.

Pesticides for use in crop management in particular have already been regulated in the Government Law No. 12 of 1992 on the Crop Cultivation System, and further spelled out in Government Decree No. 6 of 1995 on Crop Protection.

Registration and Approval

It is mentioned in the Government Decree No. 7 of 1973 that any pesticide has to be registered with the Minister of Agriculture and/or has to have permission from the Minister before its distribution, storage, and sale. Permission shall be given only to applicants whose pesticide meets registration requirements after technical and administrative data are completed and have been deemed acceptable. Technical data consist of safety to human and the environment and effectiveness to target pests.

Pesticide Committee

In the registration procedure, the Minister of Agriculture is assisted by the Pesticide Committee, a government institution under the direction of the Minister of Agriculture. The members of the committee include officials from different related institutions such as the Minister of Agriculture, the Ministry of Health, the State Ministry for Environment, the Ministry of Man Power, the Ministry of Forestry, the Ministry of Industry and Trade, and the universities.

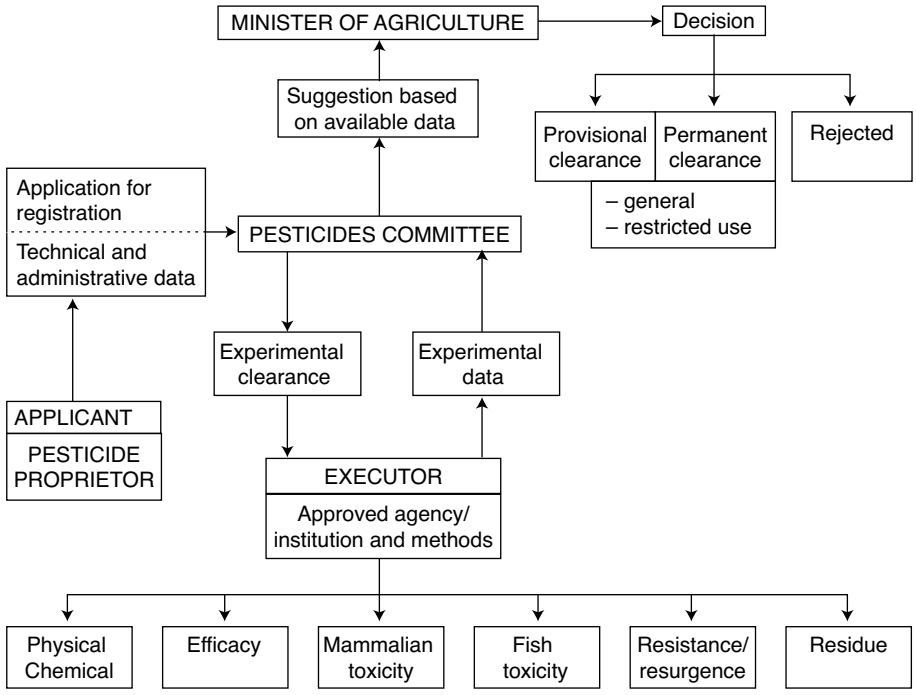

Figure 1. System for pesticide registration in Indonesia

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Registration procedures

The procedures for registration are shown in Figure 1. Figure 2 shows how the registration system controls the use of pesticides.

Registration criteria

In general, the criteria used in pesticide registration are as follows:

a. Physical and chemical properties of pesticides
b. The use to be registered
c. Mammalian toxicity
d. Environmental toxicity
e. Efficacy and phytotoxicity
f. Resistance and resurgence data, especially those to brown planthopper on rice
g. Residue data
h. Packaging
i. Labelling
j. Disposal procedures
k. Foreign registration
l. Biodata of applicant (registration holder, formulation proprietor, technical material of origin, etc.)

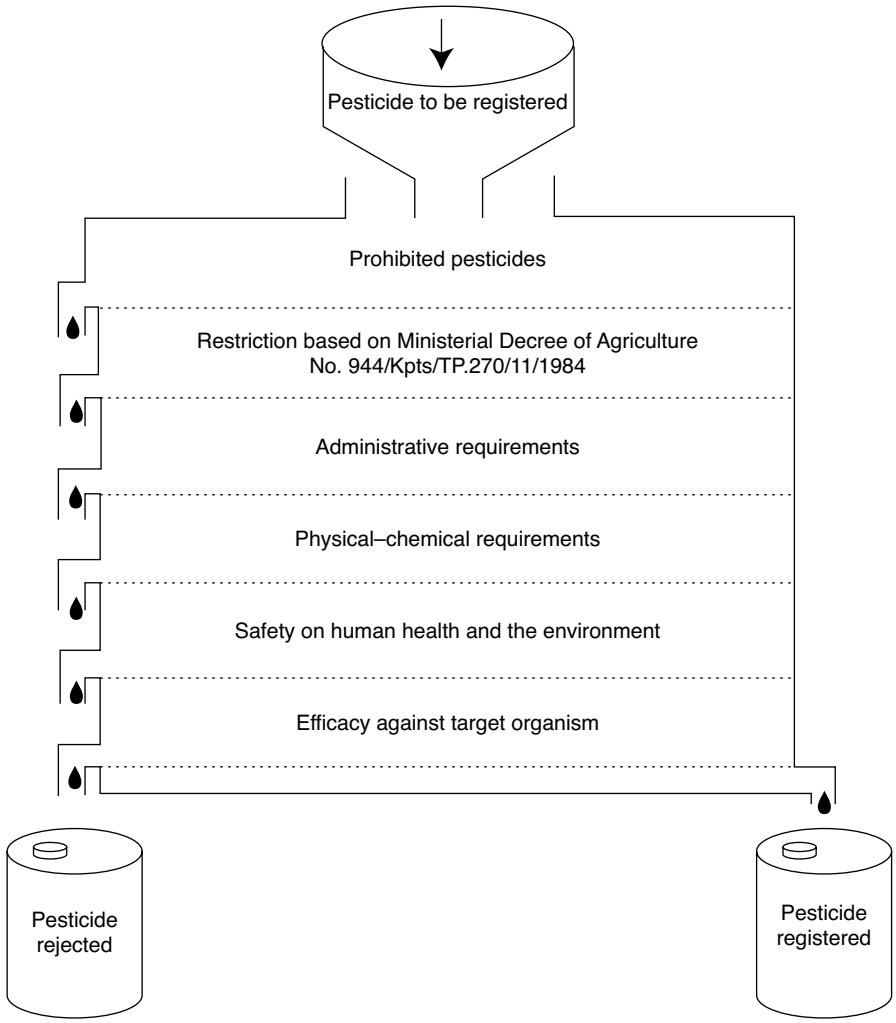

Figure 2. Diagram showing how the registration system controls the use of pesticides in Indonesia

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Permission

Based on the evaluation of data submitted and other available information, the approval or permission can be distinguished as:

Based on types

1. Trial permission or experimental clearance
2. Temporary permission or provisional clearance
3. Permanent permission or permanent clearance

Trial permission or experimental clearance shall be granted for pesticides to be used for experimental purposes in acceptance with conditions laid down by the Ministry of Agriculture for the period of 1 year.

Temporary permission or provisional clearance shall be granted for pesticides which may be put into restricted commercial use in accordance with conditions laid down by the Minister of Agriculture. Additional information is required in order to ensure the safe and effective use of these pesticides. The permission shall be granted for the period of l year.

Permanent permission or permanent clearance shall be granted for pesticides which may be put into commercial use for a period of 5 years. Nevertheless, the direction for use may be revised at any time within that period and, if necessary, the clearance may be withdrawn if the material is causing undesirable side effects.

Based on the properties

General

- it is addressed to the pesticides whose application does not require specific equipment, methods, skill, or other tools.

Restricted

- it is addressed to the pesticides whose application does require specific equipment, methods and skill.
- can only be used by a certain party who holds permission from the Minister of Agriculture/Pesticides Committee.
- it is prohibited to be freely distributed and stored by public.
- absolutely limited amount.
- the registration holder should provide an activity report in every 3 (three) months.
- orange label.

Registered pesticides

In accordance with the Decree of the Minister of Agriculture issued recently on registered pesticides and their permission, to date there have been 319 active ingredients of 770 formulations of pesticide registered with the Minister of Agriculture through the Pesticide Committee, with the following breakdown by scope of use:

- crop management 467 formulations
- environmental hygiene 230 formulations
- agricultural product stores 21 formulations
- forestry 43 formulations
- animal husbandry 7 formulations
- fisheries 2 formulations

Among the pesticides mentioned above, those which are most commonly and widely used are as follows:

1. glyphosate (herbicide)
2. mancozeb (fungicide)
3. 2,4-D dimethyl amine
4. propineb (fungicide)
5. maneb
6. paraquat dichloride (herbicide)
7. carbofuran (insecticide)
8. endosulfan (insecticide)—use in crop management now discontinued
9. BPMC (insecticide)
10. sulfosate (herbicide)

Control and Supervision

An important aspect in pesticide management is controlling and supervising distributed, sold, used, and stored pesticides in line with the proper rules.

Organisation

In order to coordinate efforts in pesticide control and supervision, a Pesticide Control Committee has been established at both provincial and district levels. Basically, the committee is a coordination or executive board which exists to assist the government in the following activities:

- To coordinate supervision from a diversity of institutions dealing with pesticide control
- To coordinate pesticide management conducted by related institutions.

The members of the committee have been officials from the Ministry of Agriculture, the Ministry of Health, the Ministry of Man Power, the Ministry of Industry and Trade, local government and other necessary parties depending on the problem being faced.

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Objective of supervision

Supervision needs to be addressed to the pesticide itself or the people who are responsible for distributing, storing, and using pesticides as ruled by Government Decree No. 7, 1973 and other existing regulations.

Pesticides

Subjects to be examined on pesticide supervision include name and type, quality, packaging, labelling, and information on whether or not they conform with the rules set forth by the Minister of Agriculture. Other than those, supervision activity is also directed to the quantity of pesticides stored, distributed, or used.

People handling pesticides

People who handle pesticides are the community which distributes, stores, and uses pesticides.

Pesticide Residue Control in Agricultural Commodities

Joint Decree on MRLs of pesticides

Two years ago, the Joint Decree of the Minister of Health and the Minister of Agriculture No. 881/Menkes/SKB/VIII/1996/(71 l/Kpts/TP.270/8/96) on the Maximum Residue Limits (MRLs) of Pesticides in Agricultural Products was promulgated, covering 218 active ingredients and 117 commodities of food crops, horticulture, plantation crops, animal husbandry/poultry products, and fish products which are either directly or indirectly consumed. The main points of this joint decree are:

a. MRLs of pesticides shall apply to agricultural products.
b. Agricultural products distributed in Indonesia (from Indonesia or from overseas) shall not contain pesticide residues exceeding the stipulated MRLs.
c. Imported agricultural products with excess pesticide residues shall be subject to refusal.
d. Pesticide residue analysis in agricultural products shall be carried out by laboratories appointed by the Minister of Health and the Minister of Agriculture.
e. Monitoring and control of the enforcement of this joint decree shall be conducted by the Minister of Health and the Minister of Agriculture.

This joint decree on MRLs of pesticides has the following aims:

a. To protect public health from the hazard of pesticide residues in agricultural products.
b. To control the importation of agricultural products containing hazardous pesticide residues.
c. To motivate farmers to be more rational in applying pesticides in compliance with the government program to implement integrated pest management.

As a follow-up to this joint decree, the Indonesian Pesticide Committee, Ministry of Agriculture is completing the following activities:

a. Preparing a standard method for pesticide residue analysis in agricultural products, which will be enacted in due course by the Chairman of the Pesticide Committee.
b. Making an inventory of, and evaluating 55 laboratories (within and outside the Minister of Agriculture) throughout Indonesia which are expected to be able to conduct pesticide residue analyses. Tentative evaluation shows that of these 55 laboratories, 20 in five major cities (Jakarta, Semarang, Surabaya, Medan, and Ujung Pandang) are capable of making the analyses.

Training for pesticide residue analysts

To increase the capability of analysts to handle and determine pesticide residues in agricultural products and to apply the standard method described above, training is considered essential. For that reason, training in pesticide residue analysis was held at the Directorate of Food Crop Protection from 11 November 1996. Eight analysts from the pesticide laboratories of Padang, Medan, Surabaya, and Ujung Pandang participated.

Workshop on monitoring of pesticide residues

A workshop on 'Monitoring on Pesticide Residues in Agricultural Products' was held at Cisarua, Bogor from 6–9 January 1997 and attended by 65 participants from various institutions. Participants unanimously agreed to nominate the Pesticide Laboratory of the Directorate of Food Crop Protection as the National Standard Test Laboratory.

Monitoring of pesticide residues

Monitoring of pesticide residues in food crops and horticulture is done by taking samples of fruits and

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vegetables in the centres of production in several major provinces throughout Indonesia and in a number of supermarkets in North Sumatra, South Sumatra, Lampung, Central Java, Bali, and DKI Jakarta. Commodities sampled include cabbages, onions, potatoes, chillies, tomatoes, grapes, oranges, apples, and pears.

Based on the results of analysis, it can be generally concluded that all of the commodities are still safe to be consumed as they contain pesticide residues which do not exceed the stipulated MRLs.

Conclusion

The registration and approval systems are the most important facet in pesticide management.

In pesticide registration in Indonesia, safety aspects of pesticides to human health and the environment are given the top priority.

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Appropriate Analytical Technologies for Monitoring Agrochemical Residues

J.H. Skerritt*

Abstract

Instrumental methods for agrochemical residue monitoring have become increasingly sensitive, in many cases along with an increase in the stringency of permitted maximum residue limits in food and environmental (soil and water) samples. Apart from the use of new sample extraction and clean-up methods such as microwave extraction, supercritical fluid extraction and solid-phase micro-extraction in sample injectors, the greater sensitivity of detection is often possible through use of secondary detection methods that are more specific for the target compounds, most notably mass spectrometry. While there are many technical advantages in these trends, a major disadvantage is the cost increase, both in terms of capital cost for the equipment and in per-sample analytical cost. Under these conditions, it can be difficult for developing countries to be able to monitor residues in enough samples at sufficient sensitivity.

Detailed multi-residue analysis of 'representative' samples may be appropriate for benchmark trade samples, but there is need for low-cost, high-throughput residue analytical methods that are not dependent on expensive equipment, high purity gases and uninterrupted electricity supplies. A range of alternative techniques, including thin-layer chromatography and colorimetric chemical methods based on detection using specific reagents has been developed. The colorimetric methods have been developed for organophosphates, organochlorines, pyrethroids and some fungicide residues and are usually quite simple but less sensitive than other methods. Methods based on solid-phase enrichment on disposable columns and detection of the target compound by fluorescence have also been valuable for mycotoxin analysis.

More sensitive and specific analysis is possible using enzymes and antibodies. Cholinesterase assays have been used for analysis of organophosphates and carbamate insecticides for over 40 years, but recently, more sensitive versions have been developed using an enzyme from insect head and the methods have been formatted into ELISA-like test kits. In the last 10–15 years, researchers have harnessed the specificity of antibodies and the simplicity and sensitivity of the ELISA format to develop a range of immunoassays for either individual pesticides or groups of pesticides. Although antibodies are biological molecules, they can be stabilised for long periods of time by drying, and so the technology is very suitable for use in developing countries. Immunoassays for most of the major agrochemicals have now been developed, and the major challenge is application to food and environmental matrices. There are also some logistic issues that need to be resolved to help dissemination of the technology. Some commercial kits may be too expensive for use in developing countries, although this can be offset by bulk purchases and use of cheap assay formats such as immunochromatography. Researchers in several developing countries are establishing the ability to develop the immunoassays in-country, and this is greatly facilitated through bilateral research and development projects, such as our ACIAR Project 9309, involving a partnership between researchers in India and Australia. The factors leading to the development of ACIAR Project 9309 are reviewed and an overview given of the progress of the project.

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A CENTRAL element in being able to adequately monitor the presence of agrochemical residues in foods intended for either domestic consumption or export, or in environmental samples, is the availability of analytical methods that are both reliable and affordable, and allow a sufficiently representative number of samples to be tested for the results to provide reassurance of the status of production as a whole. Instrumental methods, such as gas-liquid chromatography (GLC) or high-performance liquid chromatography (HPLC) are the standard reference methods used by most laboratories across the world and methods using this equipment form the basis of the vast majority of U.S. Environmental Protection Agency and AOAC international standard analytical methods. Over recent years, the methods have become increasingly sensitive. This increase in sensitivity has occurred, and indeed has been driven by, an increase in the stringency of maximum residue limits in food and environmental samples. One of the main criticisms of instrumental methods was that in attempting to measure trace quantities of chemical residues, litre volumes of organic solvents for sample extraction and clean-up had to be employed. This shortcoming has been overcome by the introduction of new sample extraction and clean-up methods, including microwave extraction, supercritical fluid extraction and solid-phase micro-extraction, although the cost of the equipment for some of these methods has limited their adoption in developing countries. Instrumental analysis of particular compounds has also become more specific due to availability of secondary, specific detection methods, especially mass spectrometry.

The major disadvantages with instrumental methods, especially the newer techniques are the very high capital and per-sample cost increases. Thus, it is difficult for developing countries to be able to monitor residues in enough samples at sufficient sensitivity. The lack of thorough monitoring systems can lead to health risks and hinder the development of export markets in agricultural produce, which could provide major cash income and an enhancement in standards of living in the country. For developed countries such as Australia, many of the same concerns apply. The high per-sample analytical costs can limit the number of samples actually tested. Twice during the last 12 years, Australian beef exports have suffered major threats because of the detection of chemical residues in carcasses after sale. Possibly, these crises could have been avoided if a higher proportion of samples had been tested.

It would be incorrect to assume that rapid quantitative screening methods will replace instrumental techniques, but they will provide valuable adjunct methods. For all countries, detailed multiresidue analysis of 'representative' samples are appropriate for benchmark trade samples. For example in some trade contracts with counties such as Japan, a limited number of these 'representative' samples must be screened for over 70 agrochemicals. This process requires use of several multiresidue analytical methods, employing GC-MS and HPLC, with the analysis cost of well over $1000. At the same time there is a need for low-cost, high throughput residue analytical methods that are also not dependent on expensive equipment, high purity gases and uninterrupted electricity supplies (an important factor for developing countries or for field use in developed countries). Some of these are discussed below.

Alternative Techniques for Simple, High-throughput Residue Analysis

Several approaches have been developed— before the advent of reliable GC methods in the 1960s techniques such as TLC and colorimetric chemical methods were the mainstay of pesticide analysis. The methods included thin-layer chromatography, colorimetric chemical methods (incorporating detection using specific reagents), bacterial growth assays, enzyme inhibition methods and immunoassays.

Thin-layer chromatography (TLC) is based upon the differential partition of a chemical between a solvent mobile phase and a stationary phase, typically a silica coating on a glass, aluminium or plastic solid support. A range of both nonspecific detection techniques (use of U.V. light, iodine spray, charring) and specific detection methods (group-specific chemical reagents, photosynthesis inhibition for herbicide detection, cholinesterase inhibition for organophosphate/carbamate pesticide detection) has been used in conjunction with TLC (Ambrus et al. 1981). One of the main advantages of TLC is that it requires minimal equipment, especially for qualitative detection of residues. While scanning and spot area measurement techniques can be used to quantify residues, this is less commonly done nowadays. Except for the cholinesterase or photosynthetic detection methods, the sensitivity of many TLC methods is only moderate. Thus for residue analysis, the method often does require extraction, concentration and some 'clean-up' of residues. Newer detection reagents and chroma-

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tography media (e.g. reversed-phase TLC) can add resolution and specificity to the analyses (Ambrus, these proceedings) .

A range of different colorimetric chemical detection methods using specific reagents have been developed, especially by Indian scientists (Seshaiah and Mowli 1987; Shivare and Gupta 1991; Pasha et al. 1996). Some of these techniques are reviewed by Pasha (these proceedings) and Tejada (these proceedings). These tests are done either in solution or the pesticide extract is spotted onto chemically-impregnated test paper. Methods include diazo- coupling for carbamates, radical ion formation for quarternary herbicides such as paraquat, use of chromogenes such as o-toluidine for chlorinated pesticides and copper (I) for dithiocarbamate fungicides. The methods are usually simple, although the toxicity of some of the reagents used for detection is of concern. In addition, the methods are usually of moderate sensitivity (detection of residues to ppm levels). They are also of only intermediate specificity. This can be an advantage in screening for pesticide contamination although, for example, the cross-reaction of chlorinated organophosphates with reagents used to detect organochlorine insecticides can pose difficulties in interpretation of results. A clever alternative (Karanth et al. 1982) is to 'tissue print' the food sample onto filter paper to evaluate the localisation of residues within fruit and vegetables, assisting in the determination of routes of contamination (e.g. soil uptake versus surface residues) and strategies for decontamination. Such information is obviously lost when food homogenates are sampled for analysis.

Bioassays can also be utilised for chemical residue analysis. The most widely utilised are commercial assays for analysis of antibiotics, but similar assays have been adapted for pesticides. These may utilise either organelles such as chloroplasts for analysis of photosynthetic inhibitory herbicides (Lawrence 1980; Tekel et al. 1994) or to utilise bacterial cells. Several groups have used particular strains of *Bacilllus thuringiensis,* as its growth is inhibited by dithiocarbamate fungicides, and the extent of growth can be assessed colorimetrically. The disadvantages of these assays is that they require culture of an organism and growth can also be affected nonspecifically by other sample components, such as heavy metals. The cholinesterase inhibition test, for detection of organophosphates and carbamates, is perhaps the most widely used enzyme inhibition screen for chemical residues. While the process can be monitored through a pH change, the most usual method is through the use of Ellman's reagent, dithionitrobenzoic acid (DTNB) (Ellman et al. 1961). Action of cholinesterase on acetylthiocholine (a thio-analogue for the natural substrate for the enzyme, acetylcholine) produces acetate plus thiocholine. This is the reaction which is inhibited by carbamate and organophosphates (thio-phosphates in the sample require conversion before analysis). The extent of conversion to thiocholine is measured spectrophotometrically by use of DTNB; a yellow colour forms with a maximum absorbance at 412 nm. Cholinesterase inhibition assays for detection of pesticide residues have been known for over 40 years (Archer and Zweig 1959), and have also been described in the papers by Tran Van An et al. (these proceedings) and Tejada (these proceedings). Recently, we have made key improvements to the method, such as using a unique high sensitivity enzyme and formatting of the assay into a stable test kit for small laboratory or field use, which can perform these assays with a high degree of precision (Figure 1).

Other enzymes, such as yeast aldehyde dehydrogenase have also been utilised for pesticide residue analysis (Wiegandrosinus et al. 1990; Marty et al. 1993), although the extent of their use is far lower than for cholinesterase. Enzyme assays for agrochemical residues have several advantages. A broad specificity screen can be developed for a group of compounds with a common mechanism. Much of the equipment for modern immunoassay, such as ELISA plates and readers could be utilised in the analysis. A key advantage is that nature has already provided the pesticide detection mechanism —the method does not require hapten synthesis and antibody preparation. However, there are also several disadvantages. Since most of the assays do not use an intermediate wash step, enzyme inhibition assays may be more subject to matrix inhibition than immunoassay. This limitation could in principle be overcome by immobilisation, but not all enzymes immobilise well. The sensitivity of analysis differs for different compounds (Figure 2), and is hard to manipulate, although different forms of cholinesterase for example, exhibit differing sensitivities for certain organophosphates. There are also a limited range of target enzymes, so the approach will only ever be suitable for some agrochemicals.

The final, and most widely used, form of simple test for pesticide residues involves competitive ELISA (enzyme-linked immunosorbent assays).

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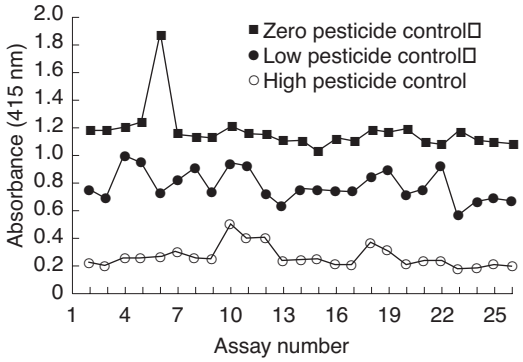

Figure 1. Precision of cholinesterase analyses: results from 25 sequential assays. Note that in assay number 6, stopping reagent was accidentally not added to the microwells corresponding to the zero pesticide controls.

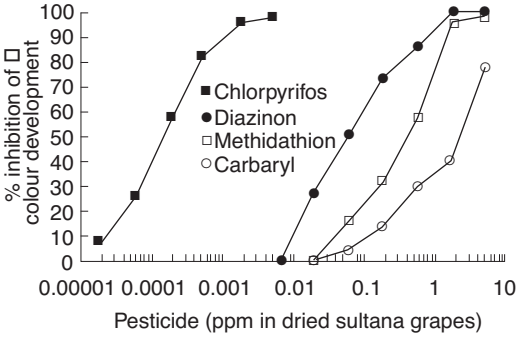

Figure 2. Cholinesterase inhibition by three organophosphate and one carbamate insecticide, spiked at different concentrations into dried sultana grapes.

There are two main assay formats. In competitive enzyme-immunoassay using immobilised antibody, a pesticide-protein conjugate is immobilised on the microwell or tube walls. After blocking of non-specific reagent binding, the sample (containing pesticide) and enzyme conjugate of an antibody to the pesticide are added and incubated. During the incubation period, there is competition between the pesticide in the test sample and the immobilised pesticide-protein complex for the limited number of antibody binding sites. Unbound pesticide and antibody-enzyme conjugate are removed by a washing step, and colour developer (enzyme substrate/chromogen) added. In direct competitive enzyme-immunoassays, the solid phase is instead coated with an antibody. The sample (containing pesticide) and an enzyme-pesticide conjugate are added to antibody-coated microwell. The subsequent steps are similar to the assay which uses immobilised antigen.

Immunoassays for Agrochemical Residue Analysis

Immunoassays are now established for most of the major agrochemicals, and although radioimmunoassays are sometimes used, the principal approach used is ELISA. While the assays are simple to use, this simplicity often belies the amount of research that has been needed to develop the original assay. Research required for each target pesticide includes the following.

Chemical synthesis of analogues of the pesticide and coupling to proteins and detection enzyme used in the assay

Small molecules such as pesticides do not elicit antibodies unless coupled to a macromolecule, such as a protein. Most pesticides do not have a functional group readily available for such coupling, and even in cases that do it is often not advisable to use the group. For example, a carboxylic acid herbicide, once coupled to a protein becomes a neutral amide, and the antibodies thus developed will often preferentially detect the amide in preference to the herbicide. It is preferable to undertake chemical synthesis of pesticide analogues which had an introduced functional group (e.g. -COOH, -NH_2, -SH) for coupling to the 'carrier' protein. A spacer arm (often $[CH_2]n$) is usually used to 'present' the pesticide hapten more efficiently to the immune system. Several different chemical approaches have been required for each pesticide for which we developed antibodies (Skerritt et al. these proceedings). Most of the analogues we made had not been synthesised before. In order to obtain assays of high sensitivity for 'free' pesticide, sometimes a different analogue must be coupled to the detection enzyme from that used in antibody production. Finally, often several haptens must be synthesised before appropriate assay sensitivity and sensitivity is achieved. The amount of work required can vary tremendously. For example, development of antibodies to trichlorobenzenes was simple as suitable chlorinated phenols and chlorinated phenoxyacetic acids are readily available. On the other hand, in the development of immunoassays for type II synthetic pyrethroids, we developed over a dozen different haptens.

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Production of antibodies to pesticide-protein conjugates

It is best to immunise at least two rabbits with each hapten, as animal-to-animal variation in antibody titre and affinity can be quite significant. We routinely couple each pesticide hapten to two quite different carrier proteins, for example, keyhole limpet haemocyanin and ovalbumin. This is essential if either the titre of pesticide-specific antibodies is to be determined or the test format to be developed involves indirect ELISA using immobilised antigen. Initial assay characterisation involves assessment of the sensitivity and specificity of the assay for free pesticide. In addition, assays to be used for analysis of food matrices require tolerance to the water-miscible organic solvents that are usually used to extract the target compounds.

Incorporation into a laboratory assay format

Antibody properties required for agrochemical immunoassays include appropriate sensitivity, specificity and dynamic response. The assay sensitivity for the target compound in food should match trading requirements or the Codex Maximum Residue Limits. Over-sensitivity is as undesirable as insufficient sensitivity, as positive responses may be of little practical consequence. Appropriate specificity refers to the cross-reaction of the assays with other pesticides and either pesticidally active or inactive metabolites, while a steep (or 'dynamic') concentration response curve is required for quantification of pesticide in the test sample to be precise. Ruggedness, which includes freedom from solvent inhibition, reagent stability, and reproducible performance are essential parameters if the assay is to be used routinely as an analytical tool. Finally, the assay performance must be assessed using relevant food matrices and research undertaken to remove sources of interference.

Incorporation into a kit format for industry users

The performance of the final kit with food and environmental matrices is usually reassessed, and the data obtained compared with instrumental analyses. Reagent stabilisation and stability trials are an important part of assay commercialisation (Figure 3).

Collaboration between Australia and India: ACIAR Project 9309

Strategies to reduce postharvest pesticide usage are of equal importance to Asian countries as they are in Australia. However, the higher cost of many of these strategies together with usual conditions of high humidity and temperature postharvest, mean that high levels of pesticide usage will continue for some years to come. For example, in India in 1991, 65000 t of insecticides were used, including 48000 t of organochlorines. Widespread use, high application rates, the persistent nature of the major compounds used (HCH and DDT) and the difficulty of policing appropriate application and withholding times each led to the regular finding of excessive and even unsafe residues in food commodities (Bull 1982; Ferrer and Cabral 1991; Kannan et al. 1992). The cost and logistic constraints to performing systematic surveys in a country the size of India are daunting. However, the monitoring of pesticide residues in food is an important aspect to minimising potential hazards to human health. When unacceptable residues are found, steps can be taken to identify the cause and prevent violations recurring. Failure to adequately screen and control residues in food (Lal et al. 1989), especially of compounds such as organochlorines (which many other countries have either banned or strictly limited use) is one factor that has limited the ability of India to effectively enter the export market for tropical fruits, vegetables, spices and other agricultural commodities as well as it otherwise could.

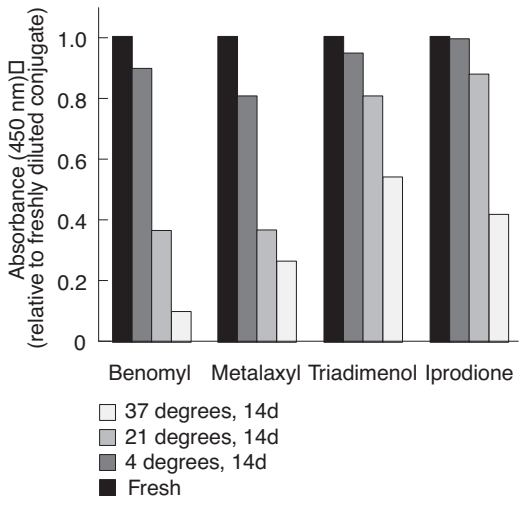

Figure 3. Long-term stability of kit components, such as horseradish peroxidase-fungicide enzyme conjugates can be predicted using accelerated stability trials at elevated temperatures.

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To monitor residues adequately, our collaborators at the Central Food Technological Research Institute in Mysore saw a critical need for simple, rapid and inexpensive methods for residue detection, and approached us to develop a joint ACIAR-sponsored research project. Their interest was despite the fact that they were equipped with the 'standard' analytical methods of GC, TLC and HPLC. The requirement for specialised equipment is somewhat lower than for 'instrumental' (GC or HPLC) methods of analysis, being limited to automatic micropipettes and a photometer, preferably one designed for microwell plates. Field-use tests are designed for either on- site monitoring or for use by poorly equipped laboratories or office situations, and require no fixed equipment, with a portable blender and colorimeter being sometimes used. Barriers to more effective pesticide testing in developing nations include the price of equipment, cost and the time taken for each analysis, problems with decentralisation of testing and coping with a statistically correct proportion of samples. Immunoassay and related methods can be considered an appropriate technology for countries such as India as they are simple (little training needed), have inexpensive running costs (few dollars/test) and do not require expensive equipment.

The project, which ran between 1993 (1994 in India) and mid 1998, aimed to develop, adapt and apply, in collaboration with laboratories of the Central Food Technological Research Institute of India, a range of simple-to-use test kits for detection and quantification of residues of agrochemicals in food (predominantly plant based foods, including fruit and vegetables, pulses, cereals and oilseeds, ground nuts and spices). Because of major residue problems, some extension of the methods to drinking water and milk was undertaken, although the emphasis was on major insecticides and fungicides used in Indian intensive and broad-acre agriculture. Key targets were major use compounds whose continued but more appropriate use is important for productivity of the agricultural industry and whose use is associated with risks of human exposure to unacceptable residues and damage to developing food export markets. While India has special concerns with residues of DDT and HCH (Nair et al. 1992), many of the insecticides and fungicides used in India are associated with residue concerns in food and the environment in other developing countries and in Australia and other developed countries. Indeed, these persistent organochlorines, especially DDT and its metabolites still contaminate grazing and cotton-farming soils, with very high concentrations found in cattle dip sites. Most pesticide residue concerns and monitoring needs for food products in Australia derive from our need to conform to residue specifications set by export markets for grain, fruit and meat. There is also pressure for more thorough environmental monitoring of pesticides. The ACIAR project benefited Australia directly through development of useful methods and establishment of a 'critical mass' for research and development in new methods for environmental monitoring in the cotton industry (Lee et al. 1997) and in horticulture (Skerritt et al., these proceedings).

The ACIAR project had two main phases. In the first phase (7/93 to 6/96), the focus was on development of test methods for pesticides (especially persistent organochlorines, toxic organophosphates and benzimidazole fungicides). This work was carried out in Australia. The second part involved the application of the methods to different food types, with a focus on major Indian export commodities (Karanth et al. these proceedings). In the second phase (7/96 to 6/98), training and equipment were provided for our Indian collaborators to develop assays and produce prototypes within India, ensuring on-going availability of reagents within India and the future independence of their research effort. By late 1997, three ELISA assays (DDT organochlorines, cyclodiene organochlorines and benzimidazole fungicides) had been developed in full within India. In addition, simple immunoaffinity chromatography methods were developed to clean up residues from 'difficult' sample matrices such as spices, tea and coffee, and immunoassays are being developed for botanical pesticides, such as the azadirachtin compounds from neem. In the immediate future, the new immunochemical tests will be integrated with other methods that are used in the CFTRI laboratory, including both GC as well as simple colorimetric tests (Pasha these proceedings), and the methods disseminated to food analytical laboratories and industry.

Although we experienced several logistic challenges in establishing the India–Australian collaboration, goodwill and hard work on both sides resolved potential problems. The initial delays in signing contracts by senior government officials in New Delhi was resolved by clearly emphasising the benefits of the work to both India and Australia. We made personal contacts with these senior officials and had government and industry champions for the project in India at the outset. Apportioning of intellectual prop-

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erty was addressed by application of a simple formula, namely that the results of research carried out in a particular country would be owned by the host country. Developing the skill base in India included training visits of senior and junior project staff in Australia, and, in the second phase of the project where development of immunoassays within India was required, we involved a second Indian laboratory (Osmania University), which had significant experience in the immunoassay analysis of small toxins. The implementation of the results was facilitated by working with food analysts in a food analytical facility, rather than an academic setting. Dissemination of the results of the project within India is being carried out through several approaches. These include on-site training by the Indian project scientists, in the new methods at other food analysis laboratories, conduct of residential workshops in Mysore and development of a training/ methods manual. Through distribution and trials of Indian-developed kits, the methods will be used within the Indian National 'Coordinated Research Program' on pesticides.

The outcomes of the project are varied, but all relate to the value of being able to economically and efficiently monitor for residues. A better understanding of chemical behaviour in sprayed crops or in treated produce will assist in Integrated Pest Management through more rational timing and use of sprays, thus avoiding the practice of 'spraying by the calendar'. Food for sale for the domestic market should be more readily able to be quarantined if found to be contaminated, and specified withholding times more readily enforced. The ability to demonstrate to potential export customers that an industry is able to quality control systematically and thoroughly for residues of concern, will be of value to both countries in developing or retaining export markets for agricultural produce. While not explicitly part of the current project, use of the tests with skin swabs could improve pesticide exposure assessment and assist in establishing appropriate re-entry periods into sprayed areas, both part of implementing improved occupational health and safety standards for agricultural workers. Indeed, one of the key advantages of immunoassay in biological monitoring is that only a small (finger prick) blood sample is required for the analysis.

Example: development of an immunoassay for monitoring DDT

One of the key targets for our collaboration with India was DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane). DDT has proven economical and versatile to use in both agricultural and public health applications, but bioaccumulation of DDT in the fat of higher animals and humans and higher in the food chain led to significant restrictions on its use. DDT is also converted to the even more stable non-insecticidal product, DDE (1,1-dichloro-2,2-bis (*p*-chlorophenyl)ethylene). Despite not being used by many countries for some years, the persistence of DDT and its metabolites in soil (including DDE and 1,1-dichloro-2,2-bis(*p*-chlorphenyl)ethane, DDD), means that residues remain of concern when considering land-use changes for potentially contaminated sites. In our research (Beasley et al. 1998), we developed a panel of antibodies to different DDT analogues and metabolites using a series of haptens, and their incorporation into ELISA assays. Some of these assays exhibited cross-reactions with other stable DDT metabolites making them useful as a screening tool for determining total DDT loads, while others were more specific for either DDT, DDE, DDA or dicofol. Five separate assays were optimised, predominantly detecting DDA (lower limit of detection, LLD = 0.1 ppb in buffer), DDE (LLD = 0.3 ppb), DDT (LLD = 2 ppb), DDT and DDE (LLD for DDT = 0.3 ppb) and dicofol (LLD = 0.5 ppb) (Figure 4). We also applied the assays to analysis of drinking and river water (Figure 5), soil and selected foods.

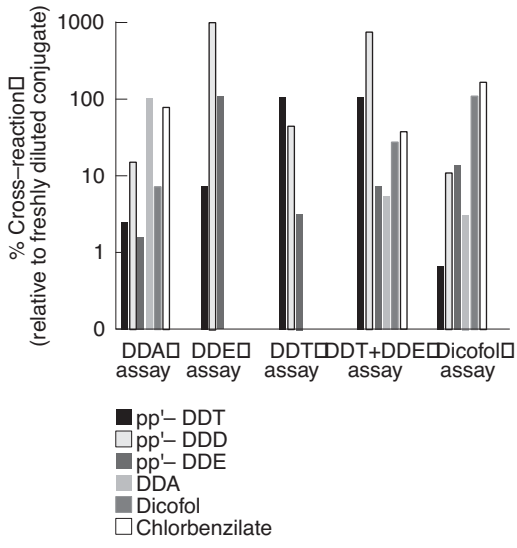

Figure 4. Specificities of different immunoassays for DDT and related organochlorines.

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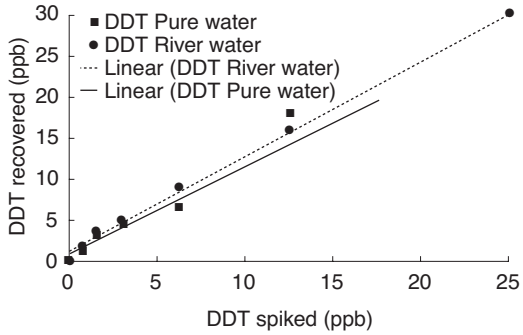

Figure 5. Recovery of DDT (spiked into river and laboratory water) using ELISA.

Conclusions

A range of rapid test methods have been developed for pesticide residue analysis, and these have important applications in both developing countries and in Australia. Methods using immunoassay can be developed for most groups of insecticides, fungicides and herbicides and can be made both simple and very sensitive. Assays can detect either individual compounds or groups, and can be applied to analysis of residues in complex food matrices. Clean-up is sometimes needed, but it can often be kept simple. Special methods are often needed for complex matrices (such as tea, coffee and spices), and within the ACIAR 9309 project immunoaffinity chromatography methods have been developed for organochlorine pesticide analysis in these complex sample matrices. It is however, important to validate the immunoassay or other rapid test against instrumental methods using residues extracted using the same solvent and procedure used for the rapid test, to ensure that any differences in the recoveries obtained using the two methods are not merely due to differences in residue extraction efficiency. This is best carried both with incurred and spiked residues for each matrix to be studied. Our ACIAR 9309 project also aimed to develop 3–4 key methods for residue extraction and clean-up from major food types (high or low in moisture, fat or sugar). This aim has largely been achieved.

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Agricultural Chemical Use and Residue Management in India

P.K. Seth, R.B. Raizada, and Rakesh Kumar*

Abstract

India's per hectare use of pesticides is very low compared with many other countries and the country is almost self sufficient in meeting its pesticide needs. Currently, approximately 80 000 tonnes of technical grade pesticides are applied each year (80% in agriculture and 20% in public health). Pesticide residues in food have been investigated in India over the last three decades. Varying amounts of DDT and BHC residues have been found in agricultural produce, milk, fats, meat, fodder, etc. Levels found have been mostly below stipulated MRL, but their presence remains of concern. Use of organophosphorus, carbamate, and the synthetic pyrethroid groups of pesticides has not led to any serious residue problems. The higher levels of residues sometimes observed have been the result of improper use or deliberate overuse. Cases of serious contamination of soil or water are rare. Monitoring of pesticide residues in human milk and tissues has shown a generally decreasing trend in residue levels.

Since 1970 there has been increasing awareness about the public health and environmental risk of pesticide use. BHC was phased out in 1997 and DDT may be used only for public health applications. The use of herbicides and fungicides has gradually increased. The use of environmentally benign and biopesticides is being promoted. To reduce the pesticide residue load, research seeking transgenic plants with resistance to pests has been implemented, in addition to integrated pest management (IPM) programs. There is a significant body of R&D seeking new, biologically based methods for pesticide-residue analysis and removal/decontamination. The pesticides to be used in the country are registered and adequate regulations for the manufacture, transport, storage, use, and importation of pesticides have been prescribed.

THE economy of India, like many other developing countries, depends heavily on agriculture. The green revolution achieved in India has been possible only because of the inputs to agriculture provided mainly by the energy sector, fertilizers and pesticides, and the effective land and water resource management.

Pesticides in general are chemicals used to kill or control unwanted pests. The unused pesticides and their degradation products and metabolites in various compartments of the environment may find their way into the human body through food chain, causing various health hazards. Besides agriculture, pesticides are important in public health for controlling the vector-borne diseases.

In India during last three decades there has been a rapid growth of the pesticide industry and India is self sufficient in pesticide production. Total insecticide production in 1994 was 74463 t (45% of it organochlorines) (Table 1). With the use of DDT restricted to public health purposes and a ban on use of HCH from April 1997, because of their long-term persistence in the environment, total insecticide production during 96–97 fell to 41 497 t (Anon. 1998) of which 80% found use in the agriculture sector.

The country has registered as many as 144 pesticides under *Insecticide Act 1968* and *Rules 1971*. The Act governs the manufacture, transport, and applica-

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tion of pesticides, in the interest of safety to human health and to protect the environment. A country such as India where the cultivated area extends to 175 million hectares to provide food to a very large population, and where there is a need to improve conditions of hygiene, the use of pesticide is indispensable. Every year India loses about 20–30% of its crops to pests and plant diseases.

Table 1. Production of technical grade pesticides in India

Pesticide	Production (t)	
	94–95	96–97
Insecticides	74463	41497
Fungicides	6099	8535
Herbicides	6184	8265
Rodenticides	543	250
Fumigants	1844	1300
Total	88890	59847

Source: Anon. (1998)

The pesticide usage pattern indicates that insecticides are the most used group, followed by herbicides, fungicides, and others. The pesticides are classified as organochlorines, organophosphorus, carbamates, thiocarbamates, synthetic pyrethroids, metal salts, and biopesticides. The per hectare consumption of pesticides in India is very low compared with Taiwan, Japan, Korea, the USA, and Thailand (Table 2). Compared with the rest of the world, percentage use of organochlorine pesticides in India is slightly higher, and the use of carbamate, synthetic pesticides, and biocides is low (Bami 1996).

Table 2. Consumption of pesticide per hectare of cultivated area

Country	Consumption (g/ha)
Taiwan	17000
Japan	12000
Korea	6559
Europe	3000
USA	2500
Thailand	1367
Argentina	960
Mexico	750
India	570
Africa	127

Health Hazard and Risk Assessment

The use of pesticides can cause adverse health effects to humans and animals. Global records of human poisoning cases and fatalities indicate that there are more deaths from intentional than unintentional poisoning (Table 3). Significant numbers of accidental poisonings from pesticides have been recorded around the world. Among the main causes of these occurrences were spillage during transport or storage, contaminated clothing, improper application, explosion, leakage, and consumption of contaminated food (Dikshith 1991). Experimental studies on animals have shown that pesticides may have mutagenic, carcinogenic, and/or teratogenic potential on long-term exposure. In addition, consumption of pesticide contaminated food may also damage the central and peripheral nervous system, liver, and kidney, or produce birth effects.

Table 3. Global record of pesticide poisoning cases and fatalities

Acute poisoning	No. of cases	% fatalities
Unintentional	1 million	2
Intentional	2–2.5 million	8

Source: World Health Organization 1986

Pesticide Residues

Pesticide residues have been identified in human blood, fat, and milk, with risk to human health. During the past two decades significant analytical data on pesticide residues in water (inland and marine), soil, sediment, food materials, vegetables, crop plants, milk and milk products, biota, human and animal tissues etc. have been generated.

Air

The atmosphere is a transport medium and a vast reservoir for pesticide residue. Pesticides can enter the atmosphere either intermittently or more continuously depending on the source, both in particulate and vapour form, from manufacturing sites, aerosol sprays, and/or from application sites. The concentration of HCH and DDT in different cities has been found to vary from not detectable (ND) to 21797 ng/m^3 for HCH and 0.08–528 ng/m^3 for DDT (Agarwal 1991; NIOH 1985; NIOHHEAL 1992; Singh 1993) Pesticide factories and storage areas have

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shown higher values of HCH and DDT as compared with other areas of the cities, but the maximum value recorded was less than the recommended permissible limits (0.5 mg/m^3 for HCH and DDT).

Water

The widespread use of pesticides makes it inevitable that some will reach water. The pesticide may persist in water depending on the type of chemical and water (surface, ground, and ocean). HCH (ND–12 ppb) and DDT (ND–0.433 ppb) have been observed in the drinking water of various cities (Jani et al. 1991; NEERI, 1992; Singh 1993; Thakkar et al. 1993; ITRC 1995).

Soil

Soil is considered as a reservoir for pesticide residues because of their direct application to plants and for soil treatment. The persistence of the pesticide residue in soil depends on the type of chemical and on the type of soil—its organic matter and clay content, mineral oil content, soil acidity, temperature, soil moisture, and crop pattern. Disappearance of pesticide residue in soil depends upon the soil microbes, volatilisation, leaching, and run-off.

The concentrations of insecticide residues found in soil are in the range 0.03–0.53 ppm for HCH and 0.03–15 ppm for DDT (Raizada 1996). Several studies in which DDT was monitored periodically at the one location revealed that residue levels were increasing with time, showing the accumulation and persistence of this compound in soil (Saxena et al. 1987).

Vegetables

The levels of HCH in different varieties of vegetables from various locations in the Lucknow district were found to range from a trace to 20 ppm and DDT from a trace to 5 ppm (Raizada 1996). Vegetable samples collected from the same area at two different periods (1991 and 1995) showed the presence of HCH in the range 0.0063–0.49 ppm in 1991 and 0.015–0.066 ppm in 1995, while DDT was in the range 0.0012–0.106 ppm in 1991 and 0.004–0.03 ppm in 1995 (Dikshith et al. 1992; Raizada 1996). In both studies residual levels were below the MRLs set by FAO/WHO (1986) and PFA (1954).

Edible oil and oil seeds

Because most pesticides are highly soluble in oil and fat, residue levels were studied in edible oils (mustard, coconut, sesame, safflower, and sunflower). HCH and DDT were found to be present in these oils at concentration in the range trace–22.97 ppm and trace–25.70 ppm, respectively (Agnihotri et al. 1974; Battu et al. 1980; Kalra et al. 1980; Jadhav 1986).

The survey studies on monitoring of pesticide in edible oil, revealed that the lowest residue levels of HCH and DDT were in coconut oil while other oils (sesame, groundnut, mustard, vegetable) had comparatively higher levels (Srivastava et al. 1983; Dikshith et al. 1989a). A few samples have however shown residues of DDT and HCH above the permissible limit for food grains prescribed by PFA (1954).

Cereals and pulses

Market surveys revealed that the contamination and range of pesticide residues (HCH and DDT) varies from place to place, depending upon the extent of use of pesticide during storage in godowns (Bindra et al. 1973; Jadhav 1986). In Bombay, field samples of rice and wheat showed DDT and HCH residues in the range 0.01–0.8 ppm, while in stored grains they varied from 1.0 to 13.9 ppm, representing a 15-fold increase. A decline in the residues of DDT and HCH has been observed in food grains (Table 4).

Samples of wheat collected from different locations in urban and rural areas of Uttar Pradesh and West Bengal have shown the presence of HCH and DDT residues in all samples. Residues of HCH in wheat from Uttar Pradesh were higher than in wheat from West Bengal, whereas reverse was true for rice. However, the values of these pesticides were below than the tolerance limits of WHO/FAO (1986) and PFA (1954) (Raizada 1995). Pulses from Uttar Pradesh, Hyderabad, and Mysore were found to be contaminated with appreciable levels of DDT (10–75 ppm) and HCH (7– 87 ppm) (Majumdar 1973; Lakshminarayana 1980).

Tea and coffee

The presence of HCH (0.09 ppm) and DDT (0.009 ppm) has been reported in tea leaves . The residue levels of DDT (0.0007 ppm) and HCH (0.020 ppm) in instant coffee were less than those found in filter coffee (DDT, 0.024 ppm; HCH, 0.23 ppm) (Table 5) (Huq 1995). Though no MRL values of HCH and DDT have been fixed for tea and coffee, the levels recorded are thought to be not high enough to have any adverse health effects.

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Table 4. DDT and HCH residues (ppm) in food grains

Place/year	Wheat		Rice		Reference
	DDT	HCH	DDT	HCH	
Bombay,1980	8.9	14	8.1	11	Noronha et al. (1980)
Ludhiana,1983	6.0	4.0	—	—	Bindra et al. (1973)
Delhi,1983	6.0	—	16	—	Verma (1983)
Parbhani,1986	1.8	3.92	1.32	5.3	Jadhav (1986)
Lucknow,1996	0.004	0.011	0.004	0.004	Mishra et al. (1998)

Table 5. Residues of total HCH and DDT in tea and coffee

Commodities	Residue levels (ppm)	
	Total HCH	Total DDT
Tea	0.0882 (trace–0.24)	0.0009 (trace–0.0025)
Coffee (instant)	0.020 (trace–0.037)	0.0007 (ND–0.002)
Coffee (filter)	0.2343 (trace–0.34)	0.0241 (trace–0.029)

ND = not detected.

Milk and milk products

A major program on surveillance of DDT metabolites and HCH isomers in cow's milk and its food products has been undertaken by several institutions under the sponsorship of ICAR (1979–84) and ICMR (1986–93). DDT residues were detected in about 82% of the 2205 samples of bovine milk collected from 12 States in the country (ICMR report 1993). The proportions of samples containing higher than MRL values of DDT were: more than 74% in Maharashtra, 70% in Gujarat, 57% in Andhra Pradesh, 56% in Himachal Pradesh and 51% in Punjab. Analysis of bovine milk samples from Bangalore city and of a popular brand of baby milk powder showed that 83.3% of bovine milk was contaminated with DDT and 100% with HCH, while no DDT was found in five brands of baby milk powder. All baby milk powder samples analysed showed the presence of HCH isomers predominated by beta isomers (Awasthi et al. 1995). Concentrations of gamma-HCH above the MRL were found in 10 of 23 samples of cow's milk collected from Panchayatha in the Trivandrum district of Kerala (Visalakshi et al. 1993). Kalra and Chawla (1983) have reported HCH (0.02–12) and DDT (trace–16 ppm) in a large number of butter samples collected from various parts of the country. Fifty samples of five brands of butter collected from Lucknow indicated HCH levels of 0.55–1.5 ppm and DDT levels of 2.0–10.80 ppm (Takroo et al. 1985).

Meat, eggs, and fish

Goat, chicken, buffalo, and sheep meat samples from some districts of Uttar Pradesh have shown the presence of HCH and DDT residues ranging from a trace to 5.1 ppm. Eggs showed low levels of HCH (0.01–1.01 ppm) and DDT (0.02–2.1 ppm) (Raizada 1996).

Fish samples collected from Madhya Pradesh, Rajasthan, Ludhiana, Calcutta, and Bombay contained HCH (0.01–8.5 ppm) and DDT (0.02–34 ppm) residues (Bhinge and Benerji 1981; Battu et al. 1984; Raizada et al. 1989b).

Fodder grass and cattle feed

Some common grasses used as cattle feed were found to be contaminated with DDT (0.2–0.45 ppm), HCH (0.39 ppm), and aldrin (0.16 ppm) (Kaphalia 1982; Dikshith et al. 1989).

Residue levels in humans

Levels of DDT and HCH in fat samples collected during autopsies were monitored in various studies

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undertaken over the period 1965 to 1992. They showed a decreasing, though not sequential, trend in residue levels. Dale et al. (1965) reported a maximum concentration of DDT (26 ppm) in autopsy fat sample collected from Delhi. Thereafter, the residue levels of DDT fell gradually to 21.8 and 22.3 ppm (Ramchandran et al, 1973, 1984), 4.7 ppm (Bhaskaran et al. 1979), 8.1 ppm (Saigal et al. 1985), and 3.9 ppm (Kashyap et al. 1993).

Some major metabolites of DDT (*p,p'*-DDE) and isomers of HCH (beta-HCH) in the general and exposed populations have also been analysed. The mean levels for total DDT and HCH were 213 ppb and 70 ppb in males, whereas females had 177 and 65 ppb. respectively (Kashyap et al. 1993). These values were lower than those reported earlier from various cities (Agarwal et al. 1976; Kaphalia and Seth 1983; Ramchandran et al. 1984). In an occupationally exposed population, concentrations of DDT and HCH residues were found to be approximately three-fold higher than the values reported from the general population (Gupta et al. 1984; Siddiqui et al. 1981a; Dua et al. 1996) without any overt significant adverse health effects.

The residues of organochlorine pesticides (*p,p'*-DDE, *p,p'*-DDT, aldrin, dieldrin and isomers of HCH) were frequently detected in placental and associated fluid samples from pregnant women. Newborn babies were found to show lower pesticide residue than their mothers, indicating that the placenta prevents transfer of some but not all of the DDT in the maternal blood (Saxena et al. 1980; Nair et al. 1996).

Breast milk was reported to contain pesticide residues at least twice as high as those in cow's milk. The DDT and HCH content in breast milk, collected from different cities, exhibited varied levels of HCH (0.1–0.47 ppm) and DDT (0.2 to 1.6 ppm) (Siddiqui et al. 1981b; Raizada et al. 1995; Nair et al. 1996; Kumar et al. 1996). The DDT and HCH residue levels found in human milk were more or less comparable to those encountered in developed countries (DDT, 0.01–4.1 ppm; HCH, ND–0.02 in Canada, the USA, Guatemala. and the U.K. (Smith 1991)).

Methods for Analysis of Pesticides

The analysis of pesticides is a painstaking procedure involving extraction, clean-up, and determination. In earlier days, the most commonly used methods were spectrophotometric and chromatographic. More recently, pesticide residue analysis in many laboratories throughout the country is done by gas–liquid chromatograph (GLC) and high performance liquid chromatography (HPLC). Over the past few years, a major program on ELISA-based kits for detection of some pesticides has been undertaken at CFTRI, Mysore, in collaboration with CSIRO Plant Industry (Karanth et al., these proceedings).

Reducing Risks of Pesticides

Decontamination/detoxification

Studies on decontamination/detoxification of pesticide-polluted soils/effluent through biodegradation in paddy fields, soil, and water using biotechnological approach have been undertaken (Raghu et al. 1966, 1989; Sethunathan et al. 1973, 1982, 1991; Qazi et al. 1993). Studies have shown that microorganisms can degrade endosulfan and malathion (Singh and Seth 1989; Singh et al. 1989; Awasthi et al. 1997).

Alternative methods

The products with insecticidal activity obtained from plants and bacteria, being less persistent in the environment and safer to humans and animals and other non-target species, are receiving priority today. The biocides based on *Bacillus* species are being developed and their toxicity has also been evaluated. The active ingredients of neem, e.g. azadirachtin, have been isolated and found effective against a number of pests. The toxicity evaluation of azadirachtin-based biopesticides has shown that these formulations are practically non-toxic to biological systems and do not persist in the environment. These products are rapidly replacing the synthetic pesticides in the country. A new neem-based product has been developed to kill the pests (Ayyanger 1992). Attempts are being made to develop pesticide-resistant genetically engineered plants (Tuli et al. 1989, 1994).

Management

Regulations on the use of pesticides, in the form of the *Insecticide Act 1968* and *Rules 1971* have been promulgated. The Central Insecticide Board (CIB) and the Registration Committee (RC) are two statutory bodies constituted by the Government of India under Ministry of Agriculture. The CIB advises the central government about the potential risk of pesticides, and safety measures necessary to prevent such risks. The RC carries out the registration of pesticides

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after proper scrutiny and specifies safety measures. Pesticides are registered under clauses 9(3), 9(3b), and 9(4) of article 9 of the *Insecticide Act*. The requirements for registration of pesticides include chemistry, bioefficacy and residues, toxicity to animals , data on health records of humans, and information on labelling and packaging.

The *Prevention of Food Adulteration Act 1954* under the Ministry of Health prescribes the maximum residue limits (MRLs),the legal limits for pesticides in food commodities. The MRLs are based on the maximum residues found following good agricultural practice, provided that these limits are toxicologically acceptable. The use of pesticides is regulated in such a manner that the intake of pesticide residues does not exceed the acceptable daily intake (ADI). ADIs are derived from the results of long-term feeding studies with laboratory animals. The government is concerned about the safety of humans and animals and about the contamination of the environment by pesticides. Several expert committees have been set up from time to time to look into restricting or banning the use of particular pesticides. As a result, HCH, endrin, aldrin, dieldrin, and heptachlor have been banned, and the use of DDT has been restricted in the country.

Integrated pest management (IPM) for crop protection is receiving great attention throughout the country and the accent is on the use of environmentally friendly products such as biocides and pesticides derived from plants.

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Agrochemical Use and Concerns in Pakistan

Umar K. Baloch and Muhammad Haseeb*

Abstract

Pakistan, an agricultural economy, is faced with severe problems of illiteracy, high population, and poverty, and consequently is struggling for food security like most of the Asian developing region. Agriculture contributes about one-third of the national income and employs half of the labour force. Pakistan is blessed with diverse agroecological zones, allowing all types of crops to be grown. However, pests consume about one-third of the yield of the various crops, mainly cotton, sugarcane, tobacco, wheat, rice, maize, pulses, and fruits and vegetables. The economic loss to crops in Pakistan is valued at 130 billion rupees, while invisible losses are probably twice this.

The environmental implications of the use of agrochemicals in the developing countries are generally ignored. The magnitude of the hazards to the environment and human health is multiplied due to lack of technical know-how in handling agrochemicals. The adverse effects of pesticides in particular are very high and long ranging. The toxicological impact is intensified because of illiteracy, poverty, inadequate implementation of pesticide control regulations, and a lack of research infrastructure.

To study the environmental impact of these chemicals on human health, wildlife, and non-target organisms, and to monitor residues of agrochemicals in the food chain, the Government of Pakistan (GOP), in collaboration with UNIDO through DANIDA assistance, has established an 'Eco-toxicology Institute' at Islamabad to serve the nation and other developing Asian countries. The UNIDO is seeking further support to strengthen the facility to extend co-operation to the central Asian states. The GOP, in view of the global concerns for seeking agricultural produce free of chemical residues, is looking to collaboration to meet the maximum residue limits for national food consumption and for international trade.

THE world food summit of November 1996 focused attention on matters relating to food security and took a critical look at high input agriculture, particularly chemical use, which results in serious health and environmental hazards. Food security is a complex issue governed by economic and socio-political factors. One of the major issues is the birth rate explosion in the Asian developing countries, which has doubled the population from 1.2 billion to 2.6 billion between 1950 and 1985. Today the figure is 3.1 billion out of a world total of 5.2 billion and is projected to climb to 3.3 billion by 2000. While overall economic growth in Asia has, until recently, been impressive (over 7%), although uneven due to high population growth, the poverty indices in these countries remain at 30-45%. Of the more than one billion poor people who live in slums and shanty towns and who earn less than a dollar a day per person, 900 million live in this region. Over 700 million people in the region still do not get enough food to lead a healthy and productive life (Gill 1994; Sato 1994).

Pakistan has a total area of 58 million ha, of which 30 million ha is designated cultivable area; 21 million ha (70%) of this are recorded as cultivated, with an annual cropping intensity of 100%. Irrigated agriculture is practiced on 16 million ha (76%), of which 11 million (69%), 4 million (25%), and 1 million ha

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(6%) are irrigated by canals, tube wells, and other sources, respectively. The quantity of irrigation water available is recorded as 144500 million m^3 at source. Some 91500 m^3 (63%) is surface water resources and 53000 million m^3 (37%) from groundwater. Fifty-eight per cent of the annual irrigation water supply is used in the kharif (autumn harvest) cropping season and 42% in the rabi (spring harvest) season. Irrigation water is reported to be single major constraint on full development of land and agricultural resources.

The economy of Pakistan has undergone considerable diversification over the years, yet the agriculture sector remains the mainstay of the economy, contributing about one-third of national income and employing over 50% of the labor force. It provides livelihood to over 70% of the rural population. It is also the largest source of foreign exchange earnings. Agriculture serves as the base sector for the country's major industries such as textiles and sugar, and provides raw materials for industrial development. It also consumes industrial products in the form of agricultural inputs (GOP 1995a). The major crops grown in Pakistan include wheat, rice, maize, cotton, sugarcane, several types of pulses and oilseeds, and fruits and vegetables (GOP 1995b).

Crop Losses

Insects, plant diseases, and weeds are a major constraint to higher yields of crops. Pakistan is blessed with diverse agroecological conditions capable of successfully supporting almost all types of crops. However, these conditions are also ideal in providing an environment conducive to a wide range of pests. Pakistan's crops are highly vulnerable to these enemies, resulting in considerable quantitative and qualitative losses of agricultural commodities.

To present statistically correct data on losses caused by the various pests is a very difficult proposition. Even in the most developed countries the magnitude of losses has not been adequately measured, as many variables are involved; so is the variation of damage caused by an organism or a group of organisms on a given crop and in a given area from year to year. It has been estimated that, on average, 40% of crop yield at preharvest and 10% at postharvest stage is lost or damaged by different pests and diseases. It is difficult to present statistically correct data on losses by pests. According to an agricultural inquiry committee in 1975, losses due to insect pests were estimated to cost 36 billion rupees per year (in October 1998 US$1 = ca 40 rupees). The Food and Agricultural Commission (GOP 1983) placed the losses at 10–15% of potential yield of crop and 50–100% in severe outbreaks. Most of the Pakistani references indicate aggregate pest losses over 50% but the invisible losses are twice those reported. On the basis of most generally agreed figure of 40% loss (insects 15%, weeds 10%, diseases 10%, and rodents 5%) the economic loss to crops in Pakistan value works out at 150 billion rupees. The major outbreaks of insect pests and crop diseases since creation of Pakistan are summarised below (GOP 1983; Baloch and Haseeb 1994; Baloch 1995).

- *1950s*. Many fields of rice were left unharvested and allowed to be grazed by cattle as a result of severe attack of rice borers resulting in production of grainless ears. In Hafizabad Tehsil alone, the intensity of rice borer attack reached 77%, thus resulting in a damage which amounted to 40 million rupees. A survey carried out 1956 revealed that the quantity of grain lost due to rodents in the Sialkot district in one year was enough to feed the population of Sialkot city (0.3 million) for 10 years.
- *1960s*. Pyrilla attack on sugarcane was so intense that it reduced the sugar recovery from 8–10% to 5% in North-West Frontier Province. Bollworms of cotton attained 'serious pest' status and rice borer problems also continued.
- *1970s*. Losses estimated in major crops were 1437 million rupees by insects, million rupees by diseases, and 1144 million rupees by weeds, giving a total of 3610 million rupees. The Agricultural Inquiry Committee reported that yields can be increased by 10–25% if proper plant protection measures were taken. The wheat crop was severely attacked by rusts in 1978 and the yield was reduced by 20% (authors' estimate).
- *1980s*. Gram blight diseases on chickpeas totally deprived farmers of a gram crop in 1982. The cotton crop faced a crisis situation because of insect pest (mainly cotton bollworms) outbreaks resulting in yield losses of over 40%. The export quantity and value of bananas was reduced to 5955 t and 29.1 million rupees during 1987–88, compared with 8621 t and 34.5 million rupees in 1984-85 because of a severe outbreak of banana bunchy top virus. The production of bananas was further reduced in the following year because of this epidemic, as many orchards in lower Sindh were completely wiped out and farmers replaced bananas with sugarcane. The banana disease epidemic rendered many farmers bankrupt.

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- *1990s*. The cotton yield fell from 12.822 million bales in 1991 to 9.054 million bales in 1992 and 7.8 million bales in 1993 due to heavy attack by cotton leaf curl virus transmitted by white fly. Heavy disease infestation was recorded in central areas of the Punjab Province. The area under cotton was almost the same in both the years. In 1991, about 14 000 ha of crop was heavily infested while in 1992 the infestation spread to about 118 000 ha and attacked the total cotton cropped area in Punjab during 1993.

Crop Spray Coverage

Despite the use of pesticides worth billions of rupees every year, their consumption in Pakistan is still far below that of the developed countries of the world. According to an estimate, only 30% of Pakistan's total crop area receives partial pesticide cover, while the remaining 70% of the farms completely lack any plant protection. However, there is a growing awareness among farmers about the use of pesticides to increase production. The major customer in the pesticide market is the cotton crop, which alone accounts for about 70% of the total pesticide consumption in Pakistan. According to report submitted to the Federal Pesticide Committee, 54% of the cotton crop was given plant protection coverage, consuming 1430 t during 1985, Some 60% of the crop was protected from pests, consuming 2010 t of pesticide during 1986, and during 1990, 65% of cotton crop was protected from insect pests, consuming 2236 t of pesticide. The report of the Agriculture Commission subgroup on pesticides estimated that demand for pesticides must increase by at least 10% to sustain growth of the country's agriculture sector (Baloch 1982, 1995).

Importation and Consumption of Pesticides

A major turn around in government policy was the withdrawal of subsidies, and transfer of pesticide sale and distribution to the private sector in February 1980. Since then the pesticide industry has developed rapidly. This trend is evident from the fact that pesticide usage jumped to 6865 t (active ingredient) worth about 6554 million rupees in 1992 as against 915 t costing only 213 million rupees in 1981. This was an about sevenfold increase in tonnage and over 31-fold increase in cost in 11 years (Baloch 1995).

The consumption of agricultural pesticides in Pakistan during 1980–93 also indicates a similar trend in terms of quantities (formulated and active ingredients) and value. The data on consumption of various categories of pesticides in terms of active ingredients show that insecticides are the major component in the crop protection sector in Pakistan. They account for more than 90% of the total pesticides used. In recent years, pyrethroids, mainly for pest control in cotton, have been the major insecticides used, followed by organophosphates. The organophosphates and synthetic pyrethroids form about 88% of the total insecticides used. The use of carbamates and organochlorines has fallen to about 4% of the total insecticides used. Herbicides, fungicides, acaricides, and rodenticides together account for less than 9% of the total pesticides used (Baloch 1995).

Local Formulation

Efforts at primary manufacture of pesticides in the country have been almost negligible. Manufacture of DDT and BHC was started at Kala Shah Kaku, Lahore and Nowsehra in the North-West Frontier Province, but both these factories have been closed down because of international bans and local pressure against the use of these pesticides. To increase local employment opportunities and to lower pesticide prices there is a need for pragmatic approach to the basic manufacture of some potential pesticides in the country; and complete local formulation to save foreign exchange spent on imports. It will be possible only through strengthening national policy in this direction.

An increasing amount of final pesticide products is being formulated locally. At present there are 19 formulation plants in the country, where 57% of total pesticides used are being formulated locally. Seven multinationals have established formulation plants and twelve are local entrepreneurs. The total annual installed capacity of formulation is 79360 t, on a one-shift basis: granules, dusts/wettable powders, and emulsifiable concentrates respectively account for 29391, 21 100, and 28869 t of formulation. Efforts are under way to increase formulation capacity to 100% in the near future. These efforts have been hampered by a number of factors, the most important being: a lack of facilities to assure standards and safe product quality; ineffective regulatory practices; and absence of basic technical information on. To ensure production of high quality formulations by local

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industry, incentives, strict standards, qualified personnel, and adequate quality control facilities must be provided (Baloch 1995).

Pesticide Legislation

The *Agricultural Pesticides Ordinance* was promulgated in 1971 and the *Agricultural Pesticides Rules* were framed in 1973. These rules are comprehensive and elaborate because they are based on guidelines first issued in 1962 by the Food and Agriculture Organization (FAO) of the United Nations, Rome and updated from time to time. These guidelines were prepared in consultation with international agencies and representatives of the developed and developing countries. However, the facilities for implementation of the rules are generally absent, except for the establishment of a Federal Pesticide Laboratory and a rudimentary cell in the Department of Plant Protection, Government of Pakistan at Karachi. The laboratory, which was established following the promulgation of the pesticide act and rules, has been capable of performing no more than physical and chemical analyses of the products submitted for registration and to meet the quality control requirements.

An Agricultural Pesticides Technical Advisory Committee (APTAC) has been established made up of representatives from the various universities, departments of agriculture of the provincial governments, and the federal Ministry of Food, Agriculture and Livestock including Pakistan Agricultural Research Council (PARC), Central Cotton Committee. The Department of Plant Protection, Ministry of Food, Agriculture and Livestock, Government of Pakistan serves as the secretariat of the Committee and the Director, Department of Plant Protection is the registrar. APTAC is assisted by a subcommittee comprised of scientists from the federal and provincial departments relevant to the use of pesticides.

The *Pesticides Ordinance 1971* was amended during 1991, 1994, and in 1997 to provide for importation of pesticides under generic names, and to strengthen provisions for punishment of defaulters.

Regular registration scheme

The scientific sub-committee reviews the results of physical/chemical analysis and bioefficacy tests performed by the various research organisations for the control of pests attacking various crops. It makes recommendations for accepting/rejecting applications for registration submitted by the pesticide companies for marketing of a pesticide brand in Pakistan for review by APTAC. The committee so far has recommended registration of 202 pesticide active ingredients under different trade names and issued certificates of registration to the various pesticide marketing companies.

Generic scheme registration

The issue of introduction of a generic scheme for agricultural pesticides was raised in 1988 under the previous People's Party regime with the intention of reducing prices of pesticides which are higher than in the international market and particularly in comparison to the Asia–Pacific Region. The matter was considered at several meetings of APTAC during 1989–91. Finally, the Pesticide Act and Rules were amended. It is generally believed that, in the absence of proper arrangements for pesticides monitoring, the scheme will be used to advantage by those who can exploit the situation. There are 139 products so far registered under the generic scheme, including 14 active ingredient insecticides, mainly organophosphates and pyrethroids

Deregistration of pesticides

The Pakistan Agricultural Research Council during the early 1980s initiated the case for deregistration of pesticides which were internationally banned or restricted for use in the developed countries. The issue was thoroughly considered in the various meetings of the Agricultural Pesticides Technical Advisory Committees with regard to their deleterious effects and economic implications. Ultimately, the importation of 21 pesticides, either in technical grade material or formulation, was banned through government gazette notification on 19 September 1994.

Pesticide Residues

Attempts have been made by the Pakistan Agricultural Research Council to monitor pesticide residues. The results show evidence of pesticide contamination in several samples of fruits and vegetables collected from the wholesale market of Karachi. In some cases, contamination exceeded the maximum residue limits proposed by FAO/WHO for particular pesticides. In another study, 70% of the cottonseed samples tested for residues were found to be contaminated by different pesticides. Similarly, the results of a project conducted by the Pakistan Council for Scientific and Industrial Research on cottonseed revealed the pres-

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ence of high residues in test samples. In addition, pesticide-monitoring studies on milk, feed, and cattle drinking water showed pesticide contamination (Parveen and Masood 1987, 1988; Masud and Hasan 1992; Jabar et al. 1993).

Impact Assessment

The environmental implications of the use of agrochemicals in the developing countries are generally ignored. The magnitude of the risks to environment and human health is multiplied because of lack of technical knowledge on how to handle agrochemicals. The adverse effects of pesticides, in particular, are very high and long ranging. The toxicological impact is intensified because of inadequate implementation of pesticide control regulations, illiteracy, and poverty, coupled with the lack of a research infrastructure.

Under present circumstances, it is impossible to confirm that all food supplies will be free of objectionable pesticide residues. To obviate risks of highly toxic/persistent pesticides and to internationalise the country's agriculture, adequate residue data for pesticides should be generated in Pakistan on an organised and systematic basis, and at a national scale. This information is essential to help fix tolerance/acceptable limits of various pesticides in consumer goods contaminated with these toxicants. Moreover, in order to protect and promote the quality of foodstuffs, it is now imperative to establish an infrastructure with adequate laboratory facilities.

To study the impact of these chemicals on human health, wildlife, non-target organisms, and the environment in general, residues of agrochemicals in the food chain are being monitored by the Government of Pakistan (GOP) in collaboration with United Nations Industrial Development Organization (UNIDO). With the assistance of the Danish International Development Agency (DANIDA) an 'Eco-toxicology Institute' has been established at Islamabad to serve the nation and other developing Asian countries. UNIDO is seeking further support to strengthen the facility and to extend cooperation to the central Asian states. The GOP, in view of the global concerns for seeking agricultural produce free of chemical residues, is looking to collaboration to meet the maximum residue limits for national food consumption and for international trade.

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Pesticide Use and Control in Malaysia

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Abstract

Since the introduction of synthetic organic pesticides in the mid-1940s there has been an increase in dependence on these chemicals in Malaysia. Estimates show that by the mid-1990s the annual end-user values of agricultural and public health pesticides are about RM300 million and RM190 million, respectively. Herbicides account for about 75% of the agricultural pesticides consumed, while insecticides, fungicides and rodenticides account for 16, 5, and 4%, respectively. The growth of the pesticides market in the country has averaged about 4–5% annually over the last 3 years.

The principal legislation for the control of pesticides in Malaysia is the *Pesticides Act 1974* and the rules and regulations implemented under it. There are, however, certain aspects of pesticides which are controlled under other legislation. The main thrust of the *Pesticides Act* is the control, through registration, of the manufacture, import, and sale of pesticides. Other aspects of control include the licensing of premises selling and storing pesticides for sale, labelling of pesticide products, control of importation of unregistered pesticides for research and educational purposes, control of advertising of pesticides, and control of the use of certain highly toxic pesticides.

It is envisaged that pesticides will continue to play a very important role in the agricultural sector in the country, particularly at this time when there is an increasing need to increase agricultural productivity due to the economic problems faced by the country. There is also an increasing awareness on the need to manage pesticides effectively so that the benefits derived from their use will not be negated by their adverse effects. There are various programs involving both legislative and non-legislative means in place in the country aimed at minimising risks posed by pesticides.

SINCE their introduction in the mid-1940s, synthetic organic pesticides have played a major role in modern intensive agriculture which has contributed to increased agricultural production. Pesticides have also played a very important role in the protection of public health in the last 50 years all over the world. While the benefits derived from the use of pesticides are many, excessive use and misuse of these chemicals have also brought about many undesirable side-effects. To address this problem, most countries, including Malaysia, have implemented relevant legislation and risk reduction programs to ensure that the use of these chemicals does not cause unacceptable adverse effects.

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Pesticide Use in Malaysia

In spite of the increasing importance of the manufacturing sector in the Malaysian economy in the last decade, the agricultural sector continues to play a very important role, contributing 12.7% of the gross domestic product (GDP) in 1996 with a total value of over RM16 million. The rapid economic development of the country has, among other things, resulted in an increasing problem of labour shortage in the agricultural sector. Agricultural inputs such as pesticides which can reduce this problem are thus of great importance.

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Oil palm, rubber, cocoa, paddy (rice), and fruits and vegetables are currently among the main crops grown in the country and will continue to be so for the foreseeable future. Although there is a gradual conversion of some rubber, cocoa, and marginal paddy land for industrial and urban development, the current economic downturn being experienced in the country, as with other countries in Asia, will result in greater emphasis on agricultural production and development. The country is expected to step-up efforts to increase production of certain food commodities so as to reduce the food import bill. It is therefore envisaged that inputs for agricultural production such as fertilizers and pesticides will play a more prominent role in the near future.

In the agricultural sector, oil palm remains the favoured crop, while rubber and cocoa have suffered a decline in production area due to the conversion of these lands to oil palm or commercial areas and because of labour shortages. The cultivation of fruits and vegetables is expected to increase so as to reduce the imports of such commodities. In 1996, oil palm area was estimated to be around 2 million ha, rubber 1.5 million ha, cocoa 45000 ha, paddy 504000 ha, fruits 262000 ha, and vegetables 32000 ha (DOA 1996) (Table 1).

Table 1. Areas (ha) planted to the main crops in Malaysia

Crop	Area (ha)
Oil palm	2021
Rubber	1586
Cocoa	45
Paddy	504
Fruit	262
Vegetables	32

The Malaysian Agricultural Chemicals Association (MACA) in its 1997 annual report (MACA 1997) noted that the annual end-user values of pesticides in Malaysia over the previous three years had been about RM300 million in the agricultural sector and RM190 million in the public health sector. Herbicides accounted for about 75% of the agricultural pesticides used, while insecticides, fungicides and rodenticides accounted for 16, 5, and 4%, respectively (Table 2). The amount of pesticides used in the new oil palm plantings, fruit orchards, and in the production of ornamentals and vegetables is expected to grow as output increases. The growth of the pesticides market in terms of end-user value in the country has averaged about 4–5% annually over the past three years.

Table 3. Estimates of expediture (RM million) at end-user level in the public health pesticide market in Malaysia

Household/public health	1995	1996	Growth (%)
Aerosols	80	85	6
Coils	85	86	1
Mats and others	20	21	5
Total	185	192	4

The household pesticides and public health markets increased at about 4% from 1995, to reach RM192 million in 1996 (Table 3). Aerosol products and coils made up more than 80% of the market.

Regulatory Control

Currently, there are several laws in operation in the country for the control of pesticides. The *Pesticides Act 1974* which is implemented by a Pesticides Board that has the Department of Agriculture (DOA) as its secretariat, is the main Act that controls pesticides. Other Acts/Ordinances that also control certain aspects of pesticides include the following:

Table 2. Estimates of the value (RM million) at end-user level of the crop protection chemical market in Malaysia

Chemical	1994	Growth (%)	1995	Growth (%)	1996	Growth (%)
Herbicides	210	5	220	5	227	3
Insecticides	41	5	43	5	47	9
Fungicides	14	8	15	7	16	6
Rodenticides	11	10	11	–	11	–
Total	276	5	289	5	301	4

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- the *Food Act 1983* and *The Food Regulations 1985* and (Amendment 1995*)* which control pesticide residues in food and are enforced by the Ministry of Health;
- the *Environmental Quality Act 1974* and (Amendment 1985 and 1996) which controls pesticide effluents from factories and is enforced by the Department of Environment;
- the *Occupational Safety and Health Act 1994* which controls worker safety, including aspects relating to pesticides and is enforced by the Department of Occupational Health and Safety; and
- the *Hydrogen Cyanide (Fumigation Act) 1953* (Revised 1981) which controls the fumigation of premises, including ships, and is enforced by Ministry of Health.

Under the *Pesticides Act*, various rules and regulations have been promulgated and implemented to ensure that the objective of the Act is achieved. The following are six major rules/regulations that are being implemented:

1. *Pesticides (Registration) Rules 1976*;
2. *Pesticides (Importation for Educational and Research Purposes) Rules 1981*;
3. *Pesticides (Labelling) Regulations 1984*;
4. *Pesticides (Licensing for Sale and Storage for Sale) Rules 1988*;
5. *Pesticides (Advertisement) Rules 1996*; and
6. *Pesticides (Highly Toxic Pesticides) Regulations 1996*.

Pesticides (Registration) Rules 1976

This set of rules is central to the control of pesticides in Malaysia and enables the Board to ensure that the pesticides imported, manufactured, and sold in the country are not only of acceptable quality but also not cause unacceptable, adverse effects on human health and the environment. Applicants are required to provide relevant data for evaluation by the Board before a decision is made on whether the application could be approved. These data must include a risk–benefit analysis. A pesticide is evaluated among other things on the following aspects:

- product chemistry/quality;
- toxicology including ecotoxicology;
- efficacy/use;
- residue chemistry/effects;
- environmental fate;
- packaging and labelling; and
- risk/benefits.

Pesticides (Importation for Educational and Research Purposes) Rules 1981

These rules allow unregistered pesticides to be imported into the country for research and education. Other than pesticide companies, research institutions and universities also import pesticides for research. This is in line with the country's policy of encouraging such bodies to carry out research and development. The results obtained from the research carried out on many pesticides have been used to support the registration of the products.

Pesticides (Labelling) Regulations 1984

These regulations prescribe the manner of labelling pesticide packages. This is to ensure that, among other things, users of pesticides are provided with appropriate information on the correct manner of use of the product. Label information covered under these regulations includes:

- trade name of product;
- active ingredient(s), concentrations, and formulation;
- direction for use and recommended dosages;
- toxicity class;
- preharvest interval;
- symptoms of poisoning;
- first aid and medical treatment;
- precautionary statements;
- guide to disposal of empty container(s); and
- date of manufacture.

Pesticides (Licensing for Sale and Storage for Sale) Rules 1988

These rules are enforced with the objective that premises which sell and/or store pesticides for sale conform to certain conditions for the safe and proper storage/display of registered pesticides. To date about 4000 premises have been licensed in Malaysia.

Pesticide (Advertisement) Regulations 1996

With the enforcement of these regulations, all advertisements on pesticides must have prior approval from the Pesticides Board before they may be published. The main objective of the regulations is to ensure that pesticides advertisements published are not misleading and provide useful information to prospective purchasers of pesticides.

Pesticides (Highly Toxic Pesticides) Regulations 1996

These regulations were formulated to control certain highly toxic pesticides which have been shown to

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have caused problems but are still considered to have a role to play. Under the regulations, greater accountability is placed on the employers and workers using or handling these highly toxic pesticides to minimise the adverse effects they pose.

Enforcement of the Pesticides Act 1974

Enforcement is a very important aspect in the control of pesticides in the country. The Board, in addition to the personnel required to implement the above regulations, has also established an enforcement unit which, among other things, is to ensure that the aforementioned regulations have been complied with by all concerned. Enforcement officers of the Board carry out regular inspections of premises selling and storing pesticides, and of firms manufacturing pesticides. These officers also carry out raids and seizures at premises which do not comply with the provisions of the Act, and they are also authorised to prosecute offenders in court. Appropriate legal and other action is taken against those found selling or manufacturing products which are found not to comply with the specifications of the registered products. Penalties as stipulated in the Act include fines and imprisonment.

Post-registration Monitoring

Other than data evaluation, greater emphasis is also placed on post-registration monitoring of the approved pesticides in order to determine the problematic ones and the extent of damage being done or likely to occur if their use continues. This involves collection and analysis of food samples for excessive pesticide residues, wider enforcement action, and the encouragement of farmers to self-regulate. The Board is also widening the scope of post-registration monitoring of pesticides through the establishment of a laboratory capable of analysing pesticides in the environment.

Control by Administrative Means or in Cooperation with Other Agencies

Control of aerial application of pesticides

A set of guidelines on aerial application of pesticides was drawn up and implemented in collaboration with the Department of Civil Aviation (DCA). Under this arrangement, the DCA has made it a condition in the Aerial Application Certificate that written clearance from the Board must be obtained before the start of each spray operation. The Board, for its part, will ensure that the operation is needed before an approval is given, and will allow only certain pesticides to be used for such aerial applications. The applicants have therefore to justify to the Board that the aerial application is the only practicable method of control.

Participation in other international activities

At the international level, the Pesticides Board is represented in other interagency committees, directly or indirectly, to discuss global issues concerning pesticides such as the Prior Informed Consent (PIC) procedure, Persistent Organic Pollutants (POPs), and the FAO International Code of Conduct on the Use and Distribution of Pesticides.

With increasing awareness on the need to protect the health of the local population as well as facilitate interregional trade among ASEAN countries, efforts have recently been made to try to harmonise pesticide maximum residue limits, particularly for commodities commonly traded among the member countries. An expert group has been set up to look into this matter.

Pesticide Risk Reduction Activities

The sound management of pesticides is imperative for the sustainable development and well-being of the country, and various pesticide risk reduction programs are currently being undertaken by the DOA, government agencies, research institutions, and the industry. The strategies used in Malaysia include the use of both legislative and non-legislative approaches.

Legislative approaches

There is no denying that legislative control will continue to play a major role in ensuring that pesticides are used with minimal deleterious side-effects. To ensure this, the Board has taken various initiatives towards the sound management of pesticides in the country. These include:

- the review of registration status of pesticides to meet present-day standards;
- placing greater emphasis on the evaluation of ecotoxicological and environmental fate data for registration;
- restricting the availability and use of certain pesticides;

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- checking on impurities present in certain pesticides;
- taking enforcement action against registrants whose products do not comply with the required specifications; and
- reviewing existing legislation with the view to implementing amendments that will increase its effectiveness.

Non-legislative and other approaches

Non-legislative activities have also played an important role in minimising the possible adverse effects of pesticides in the country. These include:

- educational programs on the safe handling of pesticides;
- research into biopesticides by research institutions;
- implementation and promotion of integrated pest management (IPM), such as the use of barn owls to control rats in paddy fields;
- research into alternative pest control methods;
- introduction of better pesticide application technology; and
- promotion of alternative farming methods, including organic farming and hydroponics for the production of vegetables.

Conclusion

Pesticide usage will continue to feature prominently in agricultural, social, and economic development, but it is becoming increasingly clear that their use must be compatible with the need to protect human health and the environment. Sensitivity towards their use is increasing as more and more information regarding their possible deleterious effects becomes available. Successful management and use of pesticides in future will depend very much on how well the risks associated with their use can be managed.

The Pesticides Board will continue to keep abreast with current developments and take appropriate actions for the sound management of pesticides in the country. Regulatory changes and refinement of administrative procedures for the registration of pesticides will encourage the use of less hazardous products. The aim is to work with all parties to make a smooth transition to less chemically intensive agriculture.

The public also has a responsibility to ensure that pesticides are used only for the crops and purposes intended, that they are applied as recommended on the label, and that the measures recommended to protect their health and safety while handling pesticides are strictly observed.

The industry can also play an important role in the management of pesticides in the country. It could foster development of cleaner and safer products and technologies. In this respect it might be appropriate to consider encouraging research into formulations of pesticides which are more environmentally acceptable. Such pesticides could include ready-to-use formulations that do not require prior measuring and mixing, which could expose users and their surroundings to contamination. Pesticides of low concentration but high efficacy would require lower dosage and, ultimately, would also reduce contamination of the surrounding environment. Industry could also carry out a greater amount of self-regulation by, for example, incorporating better quality control systems in its production, and withdrawing outdated products from the market.

Finally, it is imperative that all the agencies involved in the control and management of pesticides in the country perform their duties on the basis of a common national policy on pesticide safety. This requires close cooperation between all bodies concerned.

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Pesticide Residues in Food and the Environment in Thailand

Monthip S. Tabucanon and Ruchaya Boonyatumanond*

Abstract

Like most other developing countries, Thailand has benefited from the availability of a growing spectrum of pest control chemicals (some 2000 formulations are sold in Thailand) but has produced its share of pesticide poisonings and wider environmental contamination.

A number of trends can be discerned in Thailand's use of pesticides. Localised problems have emerged, and the mismanagement of pesticides, particularly in the agricultural sector, underlines the need for improved controls and better education of pesticide users on the hazards involved in using these materials. The total amount of pesticides used is clearly growing. If there is a drive to improve the efficiency of Thai agriculture in future years, because the total area of land available for farming is constantly shrinking, then the use of crop protection chemicals is likely to grow. The most buoyant demand has been for the relatively safe pyrethroid group of insecticides, although to date the total quantities involved are much smaller than for the organochlorine, organophosphate, or carbamate compounds.

Three major issues related to pesticides have emerged. First, there is the question of what can be done to ensure that Thailand's growing consumption of pesticides does not buy increased agricultural productivity at the expense of human health and environmental quality. Second, there is the question of whether enough is known about the country's growing number of major pesticide plants. Third, we need to determine what can be done to ensure that all pesticide residues are identified and properly managed.

This paper describes the residues of pesticides found in food and the environment in Thailand, and discusses the challenge for Thailand to manage the use of pesticides.

OF all the chemical products in extensive use in Thailand, pesticides probably have the greatest potential for harming both people and their environment. Total consumption of pesticides in Thailand grew from 12 000 t in 1981 to over 20 000 t in 1994 (Fig. 1).

The pesticides used come: (1) as formulations from other producing countries; (2) as imports of active ingredients which are locally formulated into final products, and (3) from local manufacturing plants.

Pesticide imports have fallen from the 50% share of the market they enjoyed in 1980, partly because their cost has encouraged increased local formulation of final products from imported active ingredients, and partly because Thailand has begun to build up its own pesticides manufacturing base. While insecticides accounted for the major proportion of pesticide imports during the 1970s, growing volumes of herbicides and fungicides have been used in Thailand through the 1990s.

Herbicides, in particular, have been enjoying a rapidly growing market, and as a result insecticides have not regained the dominant market share which they held in the 1970s.

The total amount of pesticides used is clearly growing. If there is a drive to improve the efficiency of Thai agriculture in future years, because the total area of land available for conversion to farming is

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constantly shrinking then use of crop protection chemicals is likely to grow further.

Herbicides are gaining growing acceptance among farmers. Although herbicides are toxic chemicals, they tend to be very much less so than most insecticides and are also perhaps less likely to cause long-term environmental problems. But like all agricultural chemicals, their formulation, distribution, and use may lead to problems and should be closely monitored. The human health and ecological problems alleged to have been associated with 2,4,5-T underline the need for careful quality control in the production of such materials.

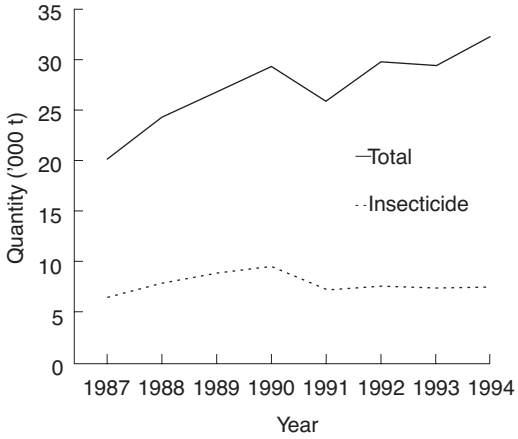

Figure 1. Quantities of insecticides and all pesticides imported into Thailand during 1987–94.

Legal and Institutional Framework

An array of regulations and agencies is involved in dealing with pesticides in Thailand. A summary of some of the important responsibilities and linkages is provided in Figure 2. Summaries follow of some of the key Acts impinging on pesticide use.

Poisonous Substances Act

The *Poisonous Substances Act*, as amended in 1973 and 1992, controls the import, export, manufacture, marketing, storage, transport, and use of poisonous substances. Under the Act, these substances are broadly described as 'active ingredients' whose properties may endanger human and/or animal health, crops, or property. Controlled substances which are used in agriculture, industry, and public health come under the joint control of the Ministry of Industry (MOI) and the Ministry of Public Health (MPH).

The respective ministries are empowered to include a chemical on the list of poisonous substances. Once a chemical has been listed, it is subject to the provisions of the Act. Permission must be obtained from the respective ministries before the import, export, or manufacture of listed chemicals. The ministries can issue ministerial regulations governing the storage, transportation, manufacture, use, labelling, and disposal of poisonous substance and their containers.

Factories Act

The *Factories Act* empowers the MOI to control the establishment and operation of factories. The MOI can issue regulations imposing limits on discharges of air pollutants, effluents, or waters from factories, and setting standards for the working environment.

National Environmental Quality Act

The *National Environmental Quality Act*, amended in 1978 and 1992, authorises the National Environment Board (NEB) to perform functions that are mostly concerned with policy development and coordination with other government agencies on matters relating to environmental quality.

Regulation of the Office of the Prime Minister on Accident Prevention

The *Regulation of the Office of the Prime Minister on Accident Prevention* created the National Safety Council of Ministers which acts on matters related to accident prevention, proposes new laws or regulations to the Council of Ministers, and promotes public awareness and public relations.

Other Acts

The *Public Health Act* covers the handling of refuse and solid wastes, nuisances, and food sales.

Currently, only substances that have been specifically named in ministerial regulations under the Act are subject to control. Because of commercial secrecy, the amount of locally formulated pesticides had to be established using the known figures for imports of active ingredients (Table 1) and conversion factors recommended by the Agricultural Division of the Economic and Social Commission for Asia and the Pacific (ESCAP). Public education and

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awareness programs, together with measures designed to ensure public participation in major planning decisions, are essential in order to pursue a vigorous program for the good management of pesticides in Thailand.

Table 1. Quantities (t) of active ingredients of different types of pesticides imported into Thailand during 1985–94.

Year	Insecticide	Fungicide	Herbicide	Others	Total
1985	5146	2646	4830	210	12 832
1986	5799	2512	4262	204	12 777
1987	5881	4530	3967	247	14 625
1988	7050	4362	5596	205	17 213
1989	6937	4724	6747	317	18 725
1990	7176	2800	8272	346	18 594
1991	5560	2087	7071	311	15 029
1992	6098	3513	8450	418	18 479
1993	5305	3988	9056	476	18 825
1994	5252	4885	9554	640	20 331

Overview of Pesticide Residues in Food and the Environment in Thailand

Organochlorine pesticides in green mussels (*Perna viridis*) in the Gulf of Thailand

Monitoring of trace toxic substance in the aquatic environment using mussels or other bivalves as biological indicators is a well-established procedure (Butler 1973; Phillips 1980; O'Connor 1992).

Materials and methods

Green mussel (*Perna viridis*) samples were collected from 11 stations along the coastal area of the Gulf of Thailand (Fig. 3) during 1993–95. The samples were cleaned and frozen at –20°C until analysis. The samples were analysed for 23 organochlorine pesticides—aldrin, dieldrin, endrin, hexachloro-cyclohexane (HCHs; α, β, γ, and δ isomers), heptachlor, heptachlor-epoxide, chlordane, hexachlorobenzene (HCB), and DDT (o,p'- and p,p' isomers). Homogenised green mussel samples were

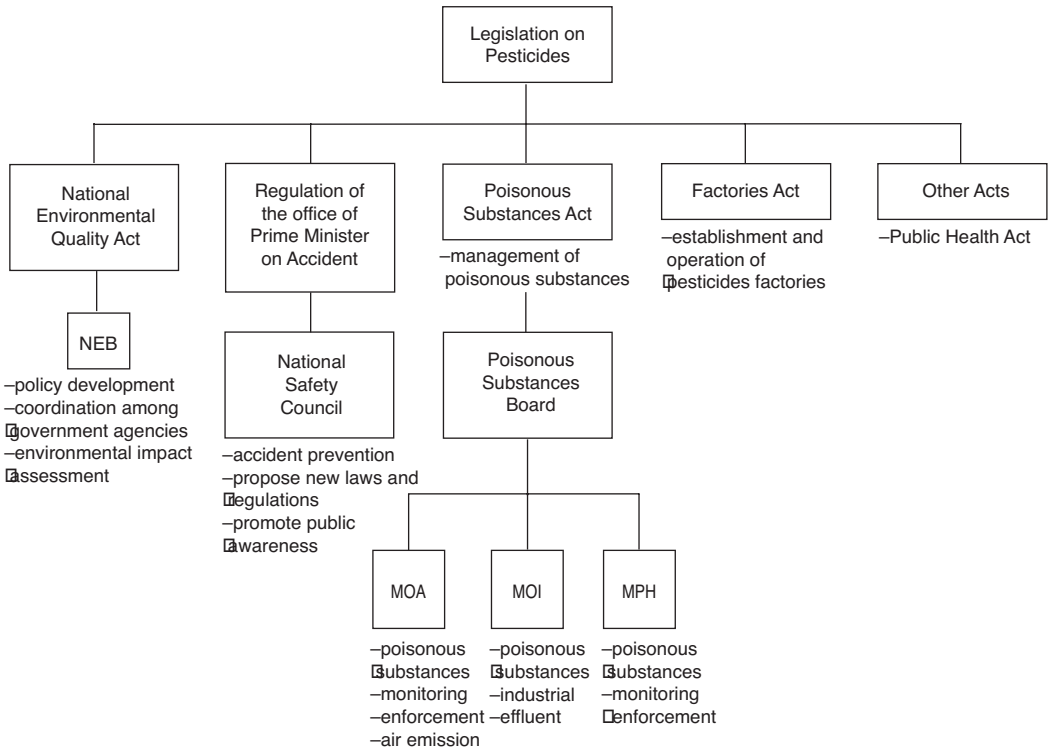

Figure 2. Legislation regulating pesticide use in Thailand

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extracted with hexane and acetone, followed by Florisil dry column and Florisil chromatography column clean-up (Ruchaya Boonyatumanon et al. 1996). The final extract was analysed by gas chromatography. For the methods used, the detection limits and recovery rates of organochlorines in spiked samples were in the range 0.6–8.2 ppb (ng/g wet weight) and 70–90%, respectively.

Results and discussion

The concentrations of organochlorine pesticides in green mussels from the Gulf of Thailand in 1993–95 were analysed on a wet weight basis. The results are shown in Tables 2 and 3.

Figure 3. Sites where mussel samples were collected

A low concentration of α-HCH (0.86 ng/g) was detected at Chonburi station in 1993, but none was detected in 1994 and 1995. β-HCH was found at the range 3.2–26 ng/g at Trad, Surachatani, and Petchaburi in 1993 and 1994, with the highest concentration at Petchaburi station. γ-HCH was found at 3.9 ng/g at Pattani station in 1995. δ-HCH was found in the range 1.0–6.0 ng/g at Ranong, Chonburi, and Surachatani stations in 1993.Endosulfan is used as an insecticide against a range of insects on a variety of crops. Endosulfan I was found at 5.7 ng/g at Trad station. Endosulfan II was found at 5.1 ng/g at Petchaburi station. Considering DDT and its isomers (*o,p'* and *p,p'* of DDT, DDE, DDD) only *p,p'*-DDE was found, at 24 ng/g at Pattani station.

Residues of heptachlor, heptachlor epoxide, aldrin, endrin, dieldrin, and chlordane were not detected. The trend in frequency of organochlorine pesticide residues was *p,p'*-DDE > β-HCH > α-HCH > δ-HCH. In a previous study during 1989–91, residues of organochlorine pesticide such as aldrin, dieldrin, DDTs, α-HCH, β-HCH, and heptachlor were often found and at concentrations higher than those in the 1993–95 data. The trend in residues from 1989–95 is shown in Table 4.

p,p'-DDE was the most frequently found organochlorine pesticide residue in green mussels during 1993–95 (Fig. 4). The major source of *p,p'*-DDE is degradation of *p,p'*-DDT under oxidising conditions.

Organochlorine pesticides in the Chao Phraya River

To determine the distribution and concentrations of residues of organochlorine insecticides in the Chao Phraya River, water samples for analysis were collected at 21 stations on the river during 1993–94 and 22 stations during 1988–91 (Fig. 5, Table 5). Samples were collected in both the wet and the dry seasons.

Materials and methods

The water samples were analysed for hexachlorobenzene (HCB), hexachlorocyclohexane (HCHs; α, β, γ, and δ isomers), aldrin, heptachlor, heptachlor epoxide, dieldrin, endrin, and DDTs (o,p'- and p,p'-isomers of DDT, DDE, and DDD). Sampling was carried out according to the procedure outlined in standard methods (ONEB 1987).

The water samples were analysed with liquid–liquid extraction by using a homogeniser and Florisil chromatography clean-up. Organochlorine compounds were quantified using a Hewlett Packard model 5890 gas chromatograph equipped with a 63Ni electron capture detector.

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Table 2. Concentrations (ng/g) of organochlorine pesticide residues found in green mussel samples from Prachuabkirikhan, Surachatani, Petchaburi, Trad, and Ranong on the Gulf of Thailand during 1993–95

Organochlorine pesticide	Prachuabkirikhan			Surachatani			Petchaburi			Chonburi			Trad			Ranong			MDL[a] (ppb)
	1993	1994	1995	1993	1994	1995	1993	1994	1995	1993	1994	1995	1993	1994	1995	1993	1994	1995	
1. α-HCH	—[b]	–	–	–	–	–	–	–	–	0.86	–	–	–	–	–	0.80	–	–	0.8
2. BHC	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0
3. β-HCH	–	–	–	–	22	–	–	26	–	–	4.7	–	3.2	–	–	–	–	–	1.5
4. γ-HCH	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.82	–	2.4
5. δ-HCH	–	–	–	–	–	–	6.0	–	–	2.2	–	–	–	–	–	1.5	–	–	1.4
6. heptachlor	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.6
7. aldrin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.4
8. heptachlor epoxide	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.5
9. oxychlordane	NA[c]	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	2.8
10. trans-chlordane	NA	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	2.4
11. o,p'-DDE	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.7
12. endosulfan I	NA	–	–	NA	–	–	NA	–	–	NA	–	–	NA	5.7	–	NA	–	–	5.5
13. cis-chlordane	NA	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	2.9
14. dieldrin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2.8
15. p,p'-DDE	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3.8
16. o,p'-DDD	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9.3
17. endrin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4.2
18. endosulfan II	NA	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	2.0
19. p,p'-DDD	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3.8
20. o,p'-DDT	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7.8
21. endosulfan sulfate	NA	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	3.2
22. p,p'-DDT	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6.0
23. methoxychlor	NA	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	NA	–	–	8.2
Wet weight (g)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
% fat content	0.86	1.2	1.4	0.88	0.81	1.0	2.0	1.7	2.0	1.1	1.3	1.5	1.6	1.0	1.1	1.5	0.83	0.76	
% moisture content	87	85	82	89	86	87	86	86	83	85	86	84	82	87	84	85	87	85	

[a] MDL = minimum detection limit; [b] – = not detected; [c] NA = not available.

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Table 3. Concentrations (ng/g) of organochlorine pesticide residues found in green mussel samples from Chumporn, Samutsongkarm, Nakornsri Thammarach, Pattani, and Trung on the Gulf of Thailand during 1993–95

Organochlorine pesticide	Chumporn			Samutsongkarm			Nakornsri-Thammarach			Pattani			Trung			MDL[a] (ppb)
	1993	1994	1995	1993	1994	1995	1993	1994	1995	1993	1994	1995	1993	1994	1995	
1. α-HCH	–[b]	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	0.8
2. BHC	–	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	1.0
3. β-HCH	–	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	1.5
4. γ-HCH	–	–	–	–	–	–	–	NA	–	–	3.9	–	NA	–	–	2.4
5. δ-HCH	–	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	1.4
6. heptachlor	–	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	0.6
7. aldrin	–	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	1.4
8. heptachlor epoxide	–	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	2.5
9. oxychlordane	NA[c]	–	–	NA	–	–	NA	NA	–	NA	–	–	NA	–	–	2.8
10. trans-chlordane	NA	–	–	NA	NA	–	NA	NA	–	NA	–	–	NA	–	–	2.4
11. o,p'-DDE	–	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	2.7
12. endosulfan I	NA	–	–	NA	–	–	NA	NA	–	NA	–	–	NA	–	–	5.5
13. cis-chlordane	NA	–	–	NA	–	–	NA	NA	–	NA	–	–	NA	–	–	2.9
14. dieldrin	–	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	2.8
15. p,p'-DDE	–	–	–	–	–	–	–	NA	–	–	24	–	NA	–	–	3.8
16. o,p'-DDD	–	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	9.3
17. endrin	–	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	4.2
18. endosulfan II	NA	–	–	NA	–	–	–	NA	–	NA	–	–	NA	–	–	2.0
19. p,p'-DDD	–	–	–	–	–	–	–	NA	–	NA	–	–	NA	–	–	3.8
20. o,p'-DDT	–	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	7.8
21. endosulfan sulfate	NA	–	–	NA	–	–	–	NA	–	NA	–	–	NA	–	–	3.2
22. p,p'-DDT	–	–	–	–	–	–	–	NA	–	–	–	–	NA	–	–	6.0
23. methoxychlor	NA	–	–	NA	–	–	–	NA	–	NA	–	–	NA	–	–	8.2
Wet weight (g)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
% fat content	2.4	1.0	1.4	1.7	2.2	1.0	0.97	NA	1.2	1.3	1.2	0.79	NA	1.4	1.2	
% moisture content	85	89	86	79	82	1.0	89	NA	87	83	85	89	NA	85	82	

[a] MDL = minimum detection limit; [b] ND = not detected; [c] NA = not available.

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Recoveries from spiked samples were in the range 75–95% . The detection limit in river water samples was 1.0 ng/L for all organochlorine pesticides.

Table 4. The trend of organochlorine pesticide residues during 1989–95

Year	Trend in occurrence of organochlorine pesticides	Trend in occurrence of organochlorine pesticides—DDT isomers
1989	DDT > aldrin > dieldrin >HCB > heptachlor > HCHs	p,p'-DDE > p,p'-DDT > p,p'-DDD > o,p'-DDD > o,p'-DDT > o,p'-DDE
1990	DDT > dieldrin > aldrin > HCHs	p,p'-DDE > o,p'-DDE > p,p'-DDT > p,p'-DDD > o,p'-DDT > o,p'-DDD
1991	DDT > aldrin > dieldrin > heptachlor	p,p'-DDT > p,p'-DDE > p,p'-DDD > o,p'-DDD > o,p'-DDE > o,p'-DDT
1993–95	β-HCH > α-HCH > δ-HCH > endosulfan II	p,p'-DDE > o,p'-DDE > o,p'-DDT

Sources: 1989 data, Cherdchan Siriwong et al. (1991); 1990 data, Monthip Tabucanon et al. (1990); 1991 data, Suthep Ruangwises et al. (1994).

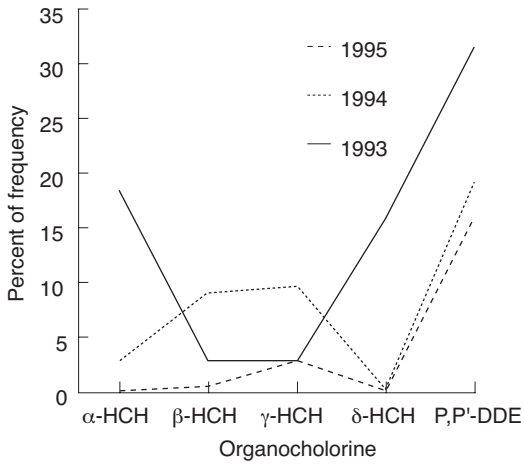

Figure 4. (above) Percentage frequency of occurrence of the organochlorine pesticides most commonly found in green mussels from the Gulf of Thailand in 1993–95.

Table 5. List of water sample locations

Location[a]	Condition
River , station 20	paddy field
Canal (near station 4); S-1(lower stream), S-2 (upper stream)	industrial area
River, station 3	urban area
River, station 1	municipal sewage

[a] See also Figure 5.

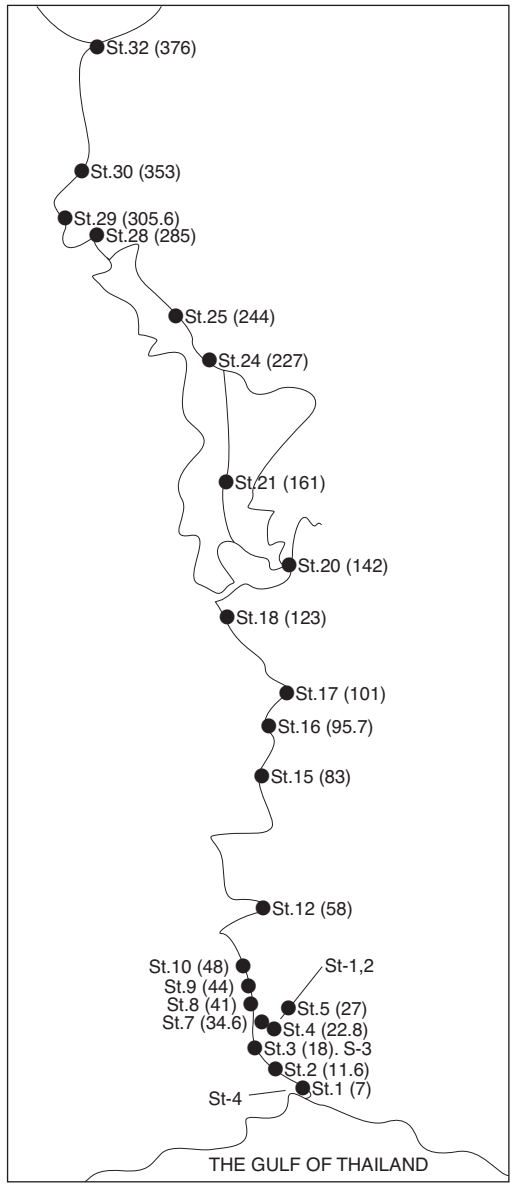

Figure 5. (right) Stations on the lower Chao Phya River where water samples were collected for organochlorine pesticide residue analysis.

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Table 6. Concentrations (ng/L) of organochlorine pesticides in the lower Chao Phraya River, Thailand, 1988–93

Chemical	Frequency[a]	Range	Median	Frequency	Range	Median	Frequency	Range	Median
	Nov. 1988 (n = 10)			May 1989 (n = 10)			May 1990 (n = 17)		
α-HCH	100	0.11–9.0	0.68	90	0.07–0.70	0.18	29	0.21–0.56	NA[b]
β-HCH	60	0.20–19	0.22	100	0.23–1.5	0.64	41	0.31–2.6	NA
γ-HCH	100	0.13–4.3	0.31	60	0.08–0.36	0.14	12	0.67–0.71	NA
Aldrin	90	0.57–5.5	13	100	4.7–22	13	100	1.3–8.1	3.2
Dieldrin	80	2.7–17	5.6	80	6.7–24	8.0	100	2.8–7.4	4.4
pp'-DDE	100	0.20–18	0.38	100	0.30–1.5	0.73	53	0.27–0.94	0.29
pp'-DDD	100	0.23–18	0.66	60	0.12–0.52	0.12	59	0.24–1.7	0.25
pp'-DDT	70	0.31–29	0.41	70	0.21–1.1	0.26	59	0.30–6.7	0.51
	Oct. 1990 (n = 9)			Jan. 1991 (n = 12)			Apr. 1991 (n = 12)		
α-HCH	22	0.09	NA	75	0.12–0.61	0.37	92	0.22–0.3	NA
β-HCH	0	NA	NA	17	0.49	NA	0	NA	NA
γ-HCH	0	NA	NA	0	NA	NA	0	NA	NA
Aldrin	44	0.09–0.51	NA	92	0.87–2.4	1.6	67	0.79–4.3	1.4
Dieldrin	89	0.2–0.82	0.42	92	0.44–1.7	1.1	92	0.17–.054	0.38
pp'-DDE	11	0.09	NA	ND[b]	NA	NA	8	0.27	NA
pp'-DDD	0	NA	NA	ND	NA	NA	8	0.47	NA
pp'-DDT	22	0.15–0.16	NA	ND	NA	NA	25	0.30–0.82	NA
	Feb. 1992 (n = 8)			Nov . 1992 (n = 10)			May 1993 (n = 4)		
α-HCH	ND	NA	NA	10	0–1.0	NA	ND	NA	NA
β-HCH	ND	NA	NA	ND	NA	NA	ND	NA	NA
γ-HCH	ND	NA	NA	30	3.6–5.0	4.3	ND	NA	NA
Aldrin	ND	NA	NA	20	2.0–7.7	4.9	ND	NA	NA
Dieldrin	ND	NA	NA	ND	NA	NA	ND	NA	NA
pp'-DDE	ND	NA	NA	50	1.0–6.5	4.25	ND	NA	NA
pp'-DDD	ND	NA	NA	ND	NA	NA	ND	NA	NA
pp'-DDT	ND	NA	NA	ND	NA	NA	ND	NA	NA

[a] Frequency of occurrence in %
[b] NA = not available; ND = non detected.

Results and discussion

The concentrations of organochlorine compounds in water samples over the 6-year period are given in Tables 6 and 7 and are plotted against distance from the river mouth in Figure 6. The number of upstream stations sampled increased to provide more data on the background concentration of organochlorine pesticides. Relatively higher residue levels of aldrin were observed upstream; e.g. at stations 22, 25, and 30. These results seem to reflect the fact that the upstream basin of the Chao Phraya River is mainly an agricultural area. In some areas (stations 7 and 10) the concentration of DDTs is higher than 20 ng/L, because of the use of DDT in a malaria eradication program until 1994.

As expected, the trend in persistent organochlorine pesticides was lower in 1993–95, not only in the range of residues detected but also in the concentrations found in green mussel and river water samples.

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Table 7. Concentrations (ng/L) of organochlorine pesticides in the lower Chao Phraya River connected at Prathumthani Province

Chemicals	Frequency[a]	Range	Median	Frequency	Range	Median	Frequency	Range	Median
	Feb. 1993 (n = 4)			Nov. 1993 (n = 4)			Apr. 1994 (n = 4)		
α-HCH	ND[b]	NA[b]	NA	ND	NA	NA	ND	NA	NA
β-HCH	ND	NA	NA	ND	NA	NA	ND	NA	NA
γ-HCH	ND	NA	NA	ND	NA	NA	ND	NA	NA
Aldrin	ND	NA	NA	ND	NA	NA	25	1.3	NA
Dieldrin	ND	NA	NA	ND	NA	NA	25	1.0	NA
pp' DDE	ND	NA	NA	ND	NA	NA	ND	NA	NA
pp'-DDD	ND	NA	NA	ND	NA	NA	ND	NA	NA
pp'-DDT	ND	NA	NA	ND	NA	NA	ND	NA	NA
	Jul. 1994 (n = 4)			Feb . 1995 (n = 3)			Jul. 1995 (n = 3)		
α-HCH	ND	NA	NA	ND	NA	NA	ND	NA	NA
β-HCH	ND	NA	NA	ND	NA	NA	ND	NA	NA
γ-HCH	ND	NA	NA	ND	NA	NA	ND	NA	NA
Aldrin	ND	NA	NA	ND	NA	NA	ND	NA	NA
Dieldrin	ND	NA	NA	ND	NA	NA	ND	NA	NA
pp'-DDE	ND	NA	NA	ND	NA	NA	ND	NA	NA
pp'-DDD	ND	NA	NA	ND	NA	NA	ND	NA	NA
pp'-DDT	ND	NA	NA	ND	NA	NA	ND	NA	NA
Endosulfan I	NA	NA	NA	NA	NA	NA	ND	NA	NA
Endosulfan II	NA	NA	NA	ND	NA	NA	ND	NA	NA
Endosulfan sulfate	NA	NA	NA	ND	NA	NA	100	20.0–26.0	23.0

[a] Frequency of occurrence in %
[b] NA = not available; ND = non detected.

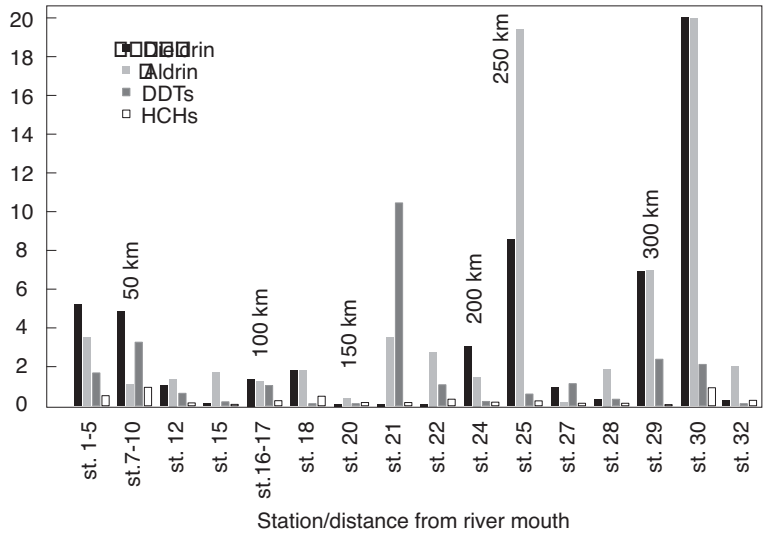

Figure 6. Spatial changes in median residue levels of total HCHs, total DDTs, aldrin, and dieldrin at various sampling points on the Chao Phraya River, Thailand

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Table 8. Organochlorine pesticide banned or restricted by the Ministry of Agriculture and Cooperatives, Thailand

Pesticide	Year banned or restricted
HCHs	1980
endrin	1981
DDTs	1983
aldrin	1983
endrin	1983
toxaphene	1983
heptachlor	1988

Monitoring of Organochlorine Pesticides in the Mekong River Basin

This monitoring program is a regional collaboration between Thailand, Laos, Cambodia, and Vietnam—the 'Water Quality Monitoring Network in the Lower Mekong Basin Project'. The main aim is to monitor the trends in pesticide residues in the water and fish in order to evaluate water quality criteria for an agreement between the countries bordering the Mekong on water quality standards and as a basis for water pollution control legislation.

Water and fish samples were collected twice a year in the wet and dry seasons. From analyses of 100 fish samples and 20 water samples it was determined that concentrations of organochlorines were below accepted maximum residue limits (MRLs).

The Royal Thai Government has banned many organochlorine pesticide compounds from use in agriculture (Table 8). DDT continued to be used, mainly for mosquito control, until 1994. The Department of Communicable Disease Control, Ministry of Public Health has used synthetic pyrethroids instead of DDT since 1995. The concentrations of residues in aquatic animals were lower than the MRLs set by the Ministry of Public Health of Thailand (e.g. DDTs, 5 mg/kg in food; aldrin, 0.1 mg/kg).

Conclusions

A number of trends can be discerned in Thailand's use of pesticides. Localised problems have emerged and some mismanagement of pesticides, particularly in the agricultural sector, underlines the need for improved controls, more vigorous enforcement of legislation, and better education on the hazards involved in using these materials. Although the level of pesticide is not yet particularly pronounced, concern has been expressed about the levels of pesticide residues found in food and the environment in Thailand.

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Pesticide Residues in Food and the Environment in China

Shu-Gui Dai, Yong-Sheng Chen, Zhi-Chao Zhang, He Xu, and Xiao-Qin Liu*

Abstract

The current status of agrochemical remediation and analysis in China is briefly described in this paper. Research and application studies on remediation have made progress in a number of areas, including use of microorganisms to degrade pesticides, promotion of pesticide degradation by rare earth elements, and the development of more readily degradable fertilizers to replace inorganic fertilizers. As regards analysis, besides routine analytical work, a number of new research directions have been implemented recently. These include: solid-phase extraction and supercritical fluid extraction techniques used in the pre-treatment of environmental samples for pesticide residue analysis; multi-chromatographic methods and multi-residue analysis; simultaneous analysis of the mother pesticide and its degradation products; immunoassays and immunoaffinity chromatographic methods of pesticide residue analysis; investigation of pesticide residues and their metabolism by radioactive isotope tracer method; and the enantio-selective analysis of chiral pesticides.

AGRICULTURAL development is very important to help accomplish modernisation in China. Though subject to many natural disasters, the production of Chinese agriculture has grown steadily for many years, aided by government policy and inputs such as pesticides and other agricultural chemicals. Since pesticides are toxic chemicals, their increased use has also brought increased risks to human health and the environment.

General Situation of Production and Use of Pesticides in China

The development of the Chinese pesticide industry began in 1950, but most of the materials and equipment currently in use have been introduced over the past 10 years. Except for a number of established pesticides which were introduced from foreign countries, many categories of pesticides have been developed and made in China. About 150 different pesticides had been produced in China up till 1990. Production was about 226 kt/year, making China the third largest producer in the world. Insecticides account for 78.7% of production, fungicides 11.1%, and herbicides 9.3%. Persistent organochlorine pesticides have been banned in China since 1983. Since then, production of organophosphorus, esters of amino-formate, and pyrethroids etc. (low residue insecticides) has expanded, as well as the development and manufacture a many high efficiency insecticides and herbicides. At present, the main focus of the Chinese pesticide industry is to study and develop the so-called super-high efficiency pesticides which are highly selectivity, are used in small amounts, and are friendlier to the environment, and will thus help conserve the agricultural environment while achieving good pest control.

According to statistical data of the Chinese Ministry of Agriculture, plant diseases and insect pests affect, on average, almost 200 million ha per year. The area to which pesticides are applied is now about 150 million ha, preventing grain losses of about

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20–30 Mt and cotton losses of about 0.6–0.5 Mt. Statistics on the use of the main categories of insecticides, fungicides, and herbicides in China are shown in Tables 1, 2, and 3 (Hua and Shan 1996), respectively. China covers a vast area, and economic development between regions is uneven, leading to obvious difference in levels of pesticide use.

Pesticide Residues in Food and the Environment

Pesticide residues in food

In China, the main staple foodstuffs for most of the people are grain, vegetables, tea, and fruit.

During the 1960s–1970s, a great quantity of very persistent organochlorine pesticides such as HCH and DDT were used. This category of pesticide not only is persistent in the soil but also easily accumulates in living tissue, so their residues continue to contaminate in crops produced over a broad area long after they were banned in China in 1983. Country-wide sampling of grain in 1983, detected HCH residues in over 90% of grain. Over 7% of wheat, paddy, and maize samples had residues above allowable levels. The average residue level was 0.11 mg/kg. However, DDT contamination of grain was not as serious: 0.2% of samples exceeded allowable limits, and the average residue level was 0.02 mg/kg. In 1988–1989, HCH and DDT residues in grain had fallen by more than one order of magnitude compared with those at the beginning of 1980s. For instance, HCH residues in wheat had fallen from 0.093–10.8 mg/kg to 0.003–0.14 mg/kg.

Organophosphorus and organonitrogen pesticides were substituted for organochlorines, bringing their own residue problems. For example, random sampling for methamidophos in paddy produced by a particular county which used a high quantity of pesticide, revealed that its concentration reached 6.6–470 μg/kg, 33% over the acceptable limit. In addition, residues of the herbicide nitrofen in rice and chlorotoluron in wheat reached nearly 100% over the allowable limit.

Pesticide pollution of vegetables is very serious, but is associated with the pesticide category and variety, and with the specific surface area of the type of vegetable. For example, the initial residual concentrations of four kinds of pyrethroid-type pesticides contaminating various vegetables are shown in Tables 4 and 5 (Zhang et al. 1990). It can be seen from Tables 4 and 5 that the initial residue level is highest in leafy vegetables, lower in legumes, and lower still in eggplant and cauliflower. These clear differences were mainly caused by variation in the specific surface areas of the edible portions of the different vegetable types.

In some city vegetable markets, pesticide residue problems remain critical. For example, in some cities of Shandong Province, residues of dimethoate in radish, trichlorfon in Chinese cabbage, and parathion-methyl in celery were 4.1, 2.5, and 8.4 times over-standard, respectively. Furthermore, according to investigations by an agricultural institute in 1992–1993, the maximum detected value of pyrethroid pesticide residue in vegetables produced in the suburbs of Shanghai was 8.53 × 10 mg/kg, equal to 8.53 times the acceptable limit, and the detection rate of methamidophos in vegetables in the suburbs of Guangzhou city was more than 70%. However, the overall assessment concerning pesticide residues in vegetables of Suzhou city was not as bad; no residues were found in tomatoes or lotus root. The pesticide categories most often found to exceed standard limits were dimethoate in Chinese cabbage, methamidophos and dimethoate in greens, and dimethoate in spinach, but the rates of over-standard occurrences were all below 10%.

Most Chinese people like to drink tea and it is also an important export commodity, so great attention is paid to its sanitary condition . Residues of HCH and DDT were investigated in 108 representative tea gardens located in Fujian Province. The results indicated that HCH is distributed throughout the tea bushes and soil, but most of it has remained in the soil and has already degraded to lower concentration. DDT, on the other hand, has concentrated within the trees, leading to an obvious difference in distribution between the tea tree and the soil (Sun et al. 1997). It is therefore concluded that HCH contamination is the result of persistent residues of a pesticide used a relatively long time ago, or so-called 'old pollution', whereas DDT contamination was caused mainly by the acaricide dicofol used in the later 1980s, or so-called 'new pollution'. Levels of residues of HCH and DDT in tea garden soil and tea leaves are shown in Table 6. HCH was found in all 108 samples taken: 98.2% of samples had residues < 9 μg/kg; 1.8% were in the range 10–100 μg/kg; and there were no over-standard (> 200 μg/kg) samples. The average residue level was 2.8 μg/kg. DDT was also found in all samples: 47.7% of samples had residues <9 μg/kg; 45.9%

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Table 1. Use of the main categories of insecticide in China (t, active ingredient)

Year	Inorganic		Organochlorine		Organophosphorus								Amino-formate esters	Pyreth-riod	Other		Total
	NaF	$PbAsO_4$	HCH	DDT	Pt[a]	Ptm	Tc	Dv	Dm	Om	Mmd	Is	–	–	Am	Cd	
1950	117	96	–	–	–	–	–	–	–	–	–	–	–	–	–	–	213
1960	1568	–	68 310	8 100	473	–	1 916	–	–	–	–	–	–	–	–	–	80 362
1970	–	–	171 672	18 992	4357	3869	7 507	4 257	6 790	–	–	–	–	–	–	–	217 444
1980	–	–	241 613	16 428	6407	9400	11 135	29 563	13 578	935	2 975	–	1978	–	270	2803	337 085
1990	–	–	–	–	6300	7500	12 000	26 000	16 000	9040	35 000	2847	4303	1400	18 000	7000	145 390

[a] Pt = parathion; Ptm = parathion methyl; Tc = trichlorfon; Dv = dichlorvos; Dm = dimethoate; Om = omethoate; Mmd = methamidophos; Is = iscarbophos; Am = amitraz; Cd = chlordimeform

Table 2. Use of the main categories of fungicide in China (t, active ingredient)

Year	$CuSO_4$	Carbendazim	Triadimefon	EBP	Iprobenfos	Zineb	Tricyclazole	Fenaminosulf	Jinggangmycin	Total
1950	152	–	–	–	–	–	–	–	–	152
1960	9707	–	–	40	–	–	–	–	–	9747
1970	5400	–	–	–	–	677	–	–	–	6077
1980	15120	1188	–	1273	640	395	–	120	90	18826
1990	6704	7497	1322	1194	1052	800	728	850	1500	21647

were in the range 10–19 μg/kg, and 6.4% were over-standard (i.e. >200 μg/kg). The average residue level was 183 μg/kg.

With regard to pesticide residue contamination in fruit, levels of seven kinds of pesticides contaminating oranges (ripe or unripe) are listed in Table 7 (Yu et al. 1997).

Pesticide residues in the environment

Pesticide residue pollution has spread throughout the environment to affect the atmosphere, water bodies, the soil and ecosystems in general.

It is well known that pesticide can be transported long distances via the atmosphere: organochlorine pesticides have been detected on the snow-covered peaks of 4250 m mountains on the Qinghai–Xizang Plateau. However, atmospheric concentrations are usually of the order of 10^{-12} g/kg or below. There are two main types of hazards arising from pesticides in the atmosphere: one is caused by unreasonable use resulting in poisoning of person who sprayed the pesticide and affecting sensitive organisms living in the surroundings, such as the Chinese silkworm; the other is endangering the health of workers in pesticide factories when not enough attention is paid to the safety of the working environment.

Survey data concerning pollution of the aquatic environment by pesticides are quite rare. Investigation of some sections of the Yellow river, detected no organophosphorus pesticides. Of the organochlorine pesticides, DDT was not detected, but HCH was present, mostly as the a-isomer, in all samples, at lev-

Table 3. Use of the main categories of herbicide in China (t, active ingredient)

Year	Nitrofen	Buta-chlor	2,4-DB	Chloro-toluron	MCPA	Gly-phosate	Atrazine	Pro-metryn	Triflur-alin	Dibromo-chloro-propane	Total
1950	–	–	–	–	–	–	–	–	–	–	
1960	–	–	–	–	–	–	–	–	–	–	
1970	259	–	808	–	–	–	–	–	–	–	1067
1980	1132	–	682	20	50	–	100	110	300	540	2934
1990	2625	2467	2429	1260	1399	902	690	365	300	–	12437

Table 4. Initial residual concentration of pesticide in leafy and legume vegetables

No.	Vegetable	Pesticide	Quantity used (g/ha)	Initial residual concentration[a] (ppb)	Estimated concentration 10 days later (ppb)	Specific surface area (cm^2/g)
1	greens	sanmarton	36	4313	269	41.8
2	greens	sanmarton	36	4599	436	41.8
3	packchoi	sanmarton	18	1837	171	15.9–18.0
4	packchoi	phenothrin	24	1906	138	15.9–18.0
5	packchoi	cypermethrin	24	1695	125	15.9–18.0
6	packchoi	sanmarton	24	1862	159	15.9–18.0
7	packchoi	deltamethrin	24	1935	144	15.9~18.0
8	chicken feather vegetable	sanmarton	48	5608	240	
9	cowpea	sanmarton	36	420	26	6.9
10	cowpea	sanmarton	48	831	201	5.8–6.9
11	sword bean	sanmarton	48	975	42	5.3

[a] Analysed value of sample collected about one hour after pesticide spraying.

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els ranging from 0.04 to 2 μg/L. As to the groundwater, over-standard residues have been found in areas with higher groundwater levels and sandy soils.

Pesticide pollution in soil is more common and serious (Gao 1992). Organomercury and organoarsenic pesticides were banned in 1972 in China, but their residues were still present in 1982 because their half-lives in soil are 10–30 years. On the bases of the detection data of Hg, as in paddy in 1982, the Hg content was 0.01–0.05 mg/kg, and arsenic content less than 1 mg/kg (as As_2O_3).

After the prohibition of organochlorines in 1983, their levels in soil have gradually fallen. The following are typical data: the maximum residues of HCH and DDT in soil of Fujian Province were 0.896 mg/kg and 1.040 mg/kg, respectively, but the highest contaminations of HCH in the soil of Beijing city and Henan province were 1.007 mg/kg and 1.498 mg/kg, respectively. Organophosphorus, amino-formate ester, and pyrethroid etc. pesticides took the place of the organochlorines, but they have not caused wide-scale soil pollution yet, because they readily degrade in soil. Nevertheless, in some localities where large quantities of pesticides have been used, serious soil pollution has resulted. Residues in soil of the herbicide nitrofen, for example, are in the range 0.16×10^{-6}–5.98×10^{-6} μg/kg, average 1.21×10^{-6} μg/kg, in certain counties. Also, chlorotoluron residues in soil

Table 5. Initial residual concentration of pesticide in various vegetables

No.	Vegetable	Pesticide	Quantity used (g/ha)	Initial residual concentration[a] (ppb)	Estimated concentration 10 days later (ppb)	Specific surface area (cm^2/g)
12	cucumber	sanmarton	36	320	0.4	1.3
13	cucumber	sanmarton	36	88	3.9	1.3
14	cucumber	sanmarton	36	119	13.6	1.5
15	tomato	sanmarton	36	354	1.94	1.1
16	tomato	sanmarton	36	116	11.7	0.86
17	tomato	sanmarton	36	72	2.18	0.86
18	tomato	sanmarton	48	79	3.7	0.89~0.92
19	tomato	sanmarton	48	135	7.0	0.89~0.92
20	sweet hot pepper	sanmarton	48	237	7.2	2.3~2.9
21	eggplant	sanmarton	48	75	3.1	2.1~2.7
22	cauliflower	sanmarton	36	151	20.5	<1
23	cauliflower	phenothrin	36	106	2.2	<1
24	cauliflower	cypermethrin	36	91	10.2	<1
25	cauliflower	sanmarton	36	145	7.8	<1
26	cauliflower	deltamethrin	36	155	13.3	<1

[a] Analysed value of sample collected about one hour after pesticide spraying.

Table 6. Pesticide residue condition of HCH and DDT in tea garden soil and tea leaves

Soil	Depth (cm)	Number of samples	HCH			DDT		
			Detection rate (%)	Range (μg/kg)	Average (μg/kg)	Detection rate (%)	Range (μg/kg)	Average (μg/kg)
Tea garden soil	0–15	108	88	0–81	3.7	70	0–386	8.3
	16–30	108	87	0–62	3.5	44	0–23	1.4
Background soil		19	79	0–2	1.4	28	0–8	0.5
Tea leaves		108	100	0.4–15	2.8	100	0.1–5162	183

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averaging 0.28×10^{-6} μg/kg, and up to 0.45×10^{-6} μg/kg have been measured. The average concentration of methamidophos residues in soil was 0.14×10^{-6} μg/kg, the highest reaching 0.64×10^{-6} μg/kg.

The successive use of pesticides has led to the enhancement of resistance to the action of the pesticide among pests, and it has become more serious day by day. Before 1985, the bollworm was very sensitive to pyrethrin. By 1986, however, the resistance of bollworm to deltamethrin and pydrin has increased by 25–36-fold and 58-fold, respectively. Furthermore, bollworm resistance to deltamethrin has already reached over 100 times, resulting in an increase in the amounts of spray used and the number of times spraying is done.

Table 7. Pesticide residue in unripe and ripe orange (mg/kg)

Pesticide	Unripe orange	Ripe orange 1[a]	Ripe orange 2[b]
Trichlorfon	0.051	ND	ND
Dichlorvos	0.32	0.024	1.20
Methamidophos	ND	ND	ND
Dimethoate	0.63	0.34	0.29
Omethoate	0.017	ND	ND
Parathion-methyl	ND	ND	ND
Isocarbophos	ND	ND	ND

[a] No pesticide was used on green fruit during September
[b] Dichlorvos and mixture of lime with sulfur were used in September
ND Denotes not detected

Factors Affecting Pesticide Pollution

The extent of environmental pollution caused by pesticides depends upon their intrinsic toxicities and residual levels in the environment. However, it can also depend on interactions between residues and physicochemical conditions in the environment.

Toxicity of pesticides

Toxicity of pesticides is an important index for determining if a pesticide will jeopardise the environment, livestock, and human health. Nowadays, the pesticides in common use in China are methamidophos, monocrotophos, parathion, parathion-methyl, phorate, omethoate, isocarbophos, carbofuran etc., all of which are high toxicity compounds. They are 10–100 times more toxic than the organochlorines, although the quantity used can be reduced one order from that for organochlorines. On the other hand, impurities in pesticides and degraded intermediates, as well as the end-product itself, may increase the toxicity of a pesticide; for example, the herbicide 2,4,5-T was banned because it contains micro-amounts of dioxin. Some pesticides have higher chronic toxicities in terms of carcinogenicity, mutagenicity, or teratogenic characteristics, while their acute toxicities might be low, so it may be some time after their introduction into the environment before their side-effects are seen. This can be exemplified by nitrofen which has been used about 20 years in China and was originally considered to be a low toxicity herbicide. Recently, however, it has been banned internationally because of its extreme potential toxicity. So it will likely also be withdrawn from use in China (Hua and Shan 1996). In addition, the degree of harm to organisms living in the environment is related to the exposure dose and time with pesticide.

Factors influencing pesticide residue levels in the environment

Physicochemical properties of pesticides have a fundamental influence on the residue levels in the environment. Important properties and indexes in the context of the environment include: water solubility (S_w); octanol/water partition coefficient (K_{ow}); adsorption constant of soil organic carbon (K_{oc}); biological concentration factor (K_b or BCF); and degradation half life ($DT_{1/2}$) (Mo et al. 1994). In general, S_w is negatively correlated with K_{ow}, K_{oc}, and K_b, i.e., K_{ow}, K_{oc} and K_b fall with increasing S_w; however, K_{ow} is positively correlated with K_{oc} and K_b, i.e, K_{oc} and K_b rise with increasing K_{ow}. In other words, pesticides with large S_w are easily transported in soil and readily enter waterbodies or groundwater via leaching from fields, thereby causing pollution or acute harm to biota. Conversely, pesticides with small S_w, large K_{ow} are readily accumulated into organisms, leading to chronic effects. The stability of a pesticide in the environment depends upon $DT_{1/2}$. Three degrees of residual life are generally recognised: pesticides with $DT_{1/2} < 3$ months are classed as easily degradable pesticides with $DT_{1/2} = 3$–12 months are middle residual compounds; and pesticides with $DT_{1/2} > 12$ months are the so-called persistent pesticides. Some pesticide can be isomerised in soils and crops. The circumstances of a pesticide's use also affects residual levels. Misuse is one important factor causing pesticide pollution, especially in economically developed regions where there is a tendency to use the higher toxicity compounds.

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Table 8. Safety factors and coefficients calculated for various pesticides

Pesticide	Residual quantity after 7 days (mg/kg)	Maximum quantity entered human body via tea drinking (μg/day)	ADI (mg/kg/day)	Safety factor[a]	Safety coefficient 1/safety factor
Permethrin 10EC	1.75	0.49	0.05	1/6000	6000
Cymbush 10EC	1.52	0.13	0.05	1/22000	22000
Bestox 5EC	0.92	0.08	0.05	1/37000	37000
Decis 2.5EC	0.32	0.03	0.01	1/21000	21000
Sumisidin 20EC	4.48	0.65	0.007	1/640	640
Karate 2.5EC	0.52	0.05	0.02	1/24000	24000
Biphenthrin 10EC	2.34	0.79	0.02	1/1500	1500
quinalphos	5.00	2.39	0.00015	1/4	4
dimethoate	1.47	2.48 (omethoate)	0.0005 (omethoate)	1/12	12

[a] Safety factor = [maximum quantity entered/human body weight (60 kg)] ÷ ADI

Climate, hydrological, and geological conditions such as temperature, pH, rainfall, light radiation, and irrigation all affect volatilisation, transport, degradation of pesticides.

Safety assessments of pesticide must be based upon comprehensive information. Recently, some Chinese researchers reported the results of an assessment and study on the safety of pyrethroid applied in tea gardens (Wang et al., 1997). In this paper, the degradation dynamics of seven kinds of pyrethroids in tea plant were studied by simulated field tests and GC method. The loss of pyrethroids during tea processing and the residues extracted from tea in infusion were also detected. According to the experimental data obtained, the residues that could potentially enter humans were calculated and the safe application of pyrethroids in tea gardens was assessed with reference to the human toxicity of the pesticides. The safety factors for the various pyrethroids studied are shown in Table 8.

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Sources of the Problems—Industry Case Studies of Chemical Use and Residue Dissipation

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Pesticide Use and Residues in Stored Grains in the Philippines

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Abstract

A review of current practices in storage and pest management of grains is presented. Chemical control remains to be a major weapon in combating pest infestation, a persistent source of loss in grain storage. The most commonly used pesticides are described and the implications of pesticide usage in food and feed commodities are discussed. There is a dearth of information available on the extent of pesticide residues contaminating treated grains. Recommendations are outlined to address this serious concern.

PHILIPPINE rice production has climbed steadily during the past five years, growing by an average of over 4% annually. In 1997, the country produced 11.4 Mt of rice and 4.5 Mt of maize. In spite of this, however, the Philippines remains in a deficit position, failing to meet growing local demand. Thus, 655000 t of rice and 145659 t of maize were also imported last year.

Unfortunately, the shortfall in production is made worse by the enormous postharvest losses. Insect infestation remains a major source of loss in storage. Based on a nationwide survey by Maranan et al. (1996), storage loss in rice which can be attributed mainly to pests is in the range 0.35–5.2% for a storage period of up to 151 days. On the other hand, losses in maize range from 2.8 to 3.3% over 17 days of storage. Other grains such as mungbean and soybean also suffer from the ravages of pests, which cause similar if not greater levels of loss. This situation necessitates the use of pesticides and attendant concerns about their effect on health and the environment.

The Grain Trade and Storage Practices

Farmers usually dispose of their entire grain production immediately after harvest. There are one to two cropping seasons a year in rainfed areas but irrigated farms manage two or more sales annually. The grain is sold to local merchants, agents of rice and maize millers, central warehouses, or the NFA (National Food Authority), a government agency that provides a ready market for the farmers' produce and maintains security reserve stocks for the country. It absorbs about 1–3% of the rice produced and less than 1% of maize and owns the biggest national network of warehouses and mills. A significant portion of its maize inventory is imported.

The main bulk of the farmers' produce is procured by the private merchants. Grain traders and processors are situated in major trading centres in the urban and rural areas.

Storage practices in the country have changed little over the last decade. About 80–90% of the harvested produce is stored in rural areas, with the rest in urban warehouse facilities (Picar 1986). There are three levels of storage namely: farm, commercial/cooperative, and government level.

Farm-level storage

Farmers usually hold their produce in jute or synthetic bags, or in bulk, using wooden bins and bamboo cribs, baskets made of straw, and clay jars kept inside, under, or beside the farmhouse. Sometimes, the grains are also stored in bulk inside a small hut

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raised above the ground and made of indigenous materials such as bamboo or tree bark. Typically, the storage capacities are small (3–100 bags of 50 kg each). Almost all of the produce is sold within a week after harvest; the remaining few bags, which are for household consumption, are kept for about 3 months, or until the next harvest. The level of hygiene and sanitation in these structures is low, minimal inspection is carried out, and sound storage management principles are absent. Most of these traditional structures are usually not rodent-proof or gastight, but they are well-ventilated (Caliboso and Acda 1992).

Grains intended for seed also form the bulk of grains stored by the farmers. Legume seeds are stored for 4–9 months (Tiongson et al. 1997) in sacks or in large metal cans or in covered plastic containers. Farmers need to resort to long storage to make sure they have seed for the next cropping season. The absence of commercial seed producers, particularly for peanuts, mungbeans, and soybeans, is a serious problem confronting farmers. Peanut growers find it difficult to maintain the viability of seed during long storage. Peanut farmers are therefore obliged to plant a second crop during the rainy season primarily for seed production. Legumes for human consumption are held for 1–3 months days. Most farmers store about 1–4 bags of unshelled peanuts for seed and 1–10 bags of mungbeans for seed.

Commercial/cooperative-level storage

Rural traders and processors store bagged commodities in poorly-ventilated warehouses made of concrete or galvanised-iron sheets with wooden or iron frames. A concrete drying pavement is usually built next to the building. Ricemills seldom stock more than a few weeks supply of milled rice; commonly, paddy and maize are stored for from a few days to 3 months (Gonzales 1986; Paz et al. 1991). However, due to the shift in government policy wherein the private sector must equally share the mandatory provision of a 90-day buffer stock, storage periods have gradually lengthened in private mills. Capacities of storage structures in local traders and farmer-cooperatives range from 200 to 24000 bags of 50 kg each, while wholesalers and processors have huge capacities of 100000 bags or more. Tiongson et al. (1997) reported that while the turnover of legumes is generally rapid, there are instances when these crops are stored for longer. For mungbean and soybean, the storage period noted was 1–2 months, while peanuts were kept for 1–8 months.

Most of the yellow maize produced and imported goes into the warehouses and silos of feed manufacturers, while white maize is bagged and stored in warehouses. It is milled to corngrits for human consumption and also processed into other food products such as baby food, snack items (corn chips, etc.), corn oil, and cornstarch. Yellow maize comprises 50% of livestock feed. The feed millers stock several months supply.

Wheat is also imported, and is stored in concrete silos by flour mills for a few months before processing. Flour is frequently unloaded immediately after milling.

Government-level storage

Storage structures owned and maintained by the government have steel frames, concrete floors and corrugated iron walls and roofs. Because of its mandate, the NFA holds grains in storage for extended periods. This averages from 3 months to 1 year but may extend to 3 years. Prolonged storage exposes the grain to an extremely high risk of damage due to pest attack. Warehouse capacity is generally huge ranging from 100 000 to 200 000 bags, of 50 kg each.

Pest Control Practices

Grains stored on-farm, although exposed to insect infestation, seldom receive any kind of protective treatment. This can be attributed to the rapid disposal of the stocks and also an inadequate understanding and appreciation of the pest problem. Some farmers employ indigenous materials for insect control, such as ash, wood shavings, and plants that have a repellent effect on insects. In legumes, other natural methods include mechanical agitation, stepping on the pods, and repeated washing and drying. A few farmers soak their sacks in insecticides (Tiongson et al. 1997). Unavailability of the appropriate pesticides and the cost involved in pesticide application are major constraints to pesticide use at farm level. When farmers apply pesticides to grain, it is usually for the protection of seed material. Some farmers admix carbaryl or malathion with mungbean seeds to control bruchids, using an improvised inclined sieve. Others use whatever insecticides are left after the crop is harvested. Often these are insecticides that are not registered for use in stored grains. Although directly treated grains are intended to be sold as seed materials, whatever quantity is left after marketing is also

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sold later for food. This leaves the consumer open to the twin risk of exposure to highly toxic chemicals and excessive residues, and emphasises the need to educate the farmers on the selection of appropriate insecticides.

To equip farmers with advanced technologies, the government through the *Gintong Ani* ('Golden Harvest') Program implemented by the Department of Agriculture and with the postharvest component undertaken by the Bureau of Postharvest Research and Extension (BPRE), distributed Volcani cubes to 150 farmer cooperatives throughout the country. The program is designed to intensify rice and maize production and covers activities from growing to marketing. The cube makes use of the hermetic storage principle and is made of tailored PVC, 0.8 mm thick. The technology resulted from a collaboration between BPRE and the Agricultural Research Organization (ARO) of Israel with funding support from the United States Agency for International Development (USAID)-Cooperative Development Research (CDR) Project . It is also planned to introduce the sealed plastic technology for outdoor storage developed jointly by the BPRE and CSIRO (Commonwealth Scientific and Industrial Research Organisation) of Australia with funding assistance from ACIAR.

Rice millers generally do not pay much attention to pest abatement owing to the fast turn-over of their rice stocks. On the other hand, some wholesalers who handle huge inventories, particularly of maize, employ a pest control technician (in-house) or secure the services of a commercial pest exterminator. Warehouse spraying and fumigation is resorted to in case of infestations.

Food processors, feed millers, and flour millers who have to maintain high quality food and feeds for considerable periods also apply pest control treatments by contracting commercial pest control applicators or by employing in-house pest control technicians to fumigate or apply residual sprays. Aside from yellow maize, warehouses of feed millers contain a variety of feed ingredients such as bran, fishmeal, and soybean meal which are highly susceptible to pest damage. In addition, these warehouses are seldom emptied, leaving pockets of residual infestation in hard-to-reach places. Warehouse fumigation is frequently resorted to under this situation.

The warehouse is made airtight by covering all holes and gaps with polyethylene plastic strips pasted to the surface using packaging or masking tapes. The windows and doors are closed and then sealed using the same material. Obviously, this manner of sealing does not conform with the minimum requirement for gastightness; gas leakage is likely to be high, exposing people and animals within the immediate vicinity to great danger, not to mention the likelihood of a fumigation failure. Because of its ease of application, phosphine is the most commonly used fumigant. Methyl bromide, on the other hand, is only applied when it is so specified, as in fumigation of grain consignments in ships and barges. In food and feed mills, the entire warehouse and silo are kept under fumigation for 72–96 hours, after which they are ventilated for another 24–48 hours. About 1.5–2 g/m^3 of phosphine in aluminium or magnesium phosphide formulation is applied. The walls, floors, and the outer layers of the bag stacks are sprayed with a layer of 1% malathion or pirimiphos-methyl, or 0.4% cyfluthrin applied at 1 L/20 m^2. For stack fumigation, the same dosage of phosphine gas is applied. Stacks are covered with 0.2 mm thick plastic sheets, held to the floor by masking or packaging tape, or using sand snakes and/or chains. Fogging of the warehouse is also practiced, using 2% pirimiphos-methyl or 0.5% deltamethrin. Furthermore, rodent baiting is done using coumatetralyl, brodifacoum, or zinc phosphide.

The pest control system in government warehouses has remained basically the same, comprising of stack fumigation, residual spraying of warehouse fabric and bag stacks, and fogging. Similarly, the insecticides, dosages, and rates of application in current usage have not changed over the past decade (see Caliboso et al. 1986, Semple 1986). Malathion, permethrin, fenithrothion, pirimiphos-methyl, and dichlorvos are applied for fabric treatment of walls and floors of the warehouse, while malathion and dichlorvos are used for fogging. NFA maintains its own pest control services unit which carry out regular monitoring and inspection of stocks and periodic application of pesticides. Recently, however, the Authority has adopted the controlled atmosphere storage technique for extended storage of milled rice developed jointly by CSIRO and BPRE under the auspices of ACIAR. Whole bag stacks are covered with tailored plastic envelopes glued together using chemical adhesives. Phosphine is released inside the stack at a rate of 1 g/m^3. This is part of a strategy for maximising warehouse space by the long-term storage of milled rice rather than paddy and addressing temporal distribution requirements of the government.

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NFA is a heavy user of pesticides. Based on its pesticide procurement of 1996, it consumed around 353 kg of active ingredient (a.i.) of insecticides and rodenticides and 180 kg a.i. of phosphine fumigants. The agency spent over US$200 000 on pesticides in 1996 alone.

Pesticide Residues

Monitoring of pesticide residues in raw grains is vested mainly in the Bureau of Plant Industry (BPI). Unfortunately, due to lack of facilities, equipment, and trained personnel, and the high cost involved in analysis, BPI cannot enforce regulatory standards. At present, the Bureau's facilities have been strengthened to monitor pesticide contamination in fruits and vegetables alone. For this reason, most of the information available in the literature is for perishables. Data on the extent of pesticide residues remaining in treated grains are restricted to the results of a few laboratory studies on degradation of insecticides applied directly to grains (Varca et al. 1988; Gragasin et al. 1993, 1994). Perhaps the most useful insight into residual contamination in grain commodities arising from simulations of grain industry pesticide application practices can be gained from the investigations of Sayaboc et al. (1992) and Tejada et al. (1992). Results of these studies indicate that residue levels in treated materials remain below the MRLs established by FAO. It is worth mentioning that residues in all imports of rice, wheat, and maize are checked by NFA and so far have never been found to exceed the safe limit.

Summary and Recommendation

In general, storage and pest control practices of the grains industry do not lead to serious pesticide contamination of the grain commodities. An exception, however, is in seed grain stocks which are often admixed with insecticides—these commodities may eventually find their way on the dinner table. Another concern is the direct grain treatment practiced by some feed millers, food processors, and flour millers. Information is not available on residues in maize and wheat from these processors. Regulatory agencies must be trained on residue analysis and must have access to relatively cheap and rapid methods of residue analysis to be able to check and monitor food safety. Twenty-three years ago, three major areas of concern were already identified by Magallona (1975) in the monitoring of pesticide residues: (1) lack of trained personnel; (2) lack or inadequacy of equipment; and (3) high cost of equipment, supplies and reagents. These remain valid today.

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Integrated Monitoring and Dissipation Studies for Development of Best Practice Management of Chemicals Used in Cotton Farming

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Abstract

In recent years the Australian cotton industry has been the focus of significant research on pesticide management with a view to reducing its impact on the riverine systems and reducing the extent of contamination of agricultural produce such as beef. In this research, pesticides such as endosulfan, pyrethroids and benzoylphenylureas such as chlorfluazuron have been examined for aerial transport in drift, erosion of cotton fields in surface run-off and their rate of dissipation. The outcome sought from this research is the development of best practices for the cotton industry that will minimise the impact of the 30–40 different insecticides and herbicides used in production.

From an integrated program of on-field monitoring of the fate of endosulfan as a case study, the following conclusions can be made:

- Apart from about 5% aerial drift at application, the dissipation of α- and β-endosulfan (half-lives 4–30 days) from cotton fields occurs mainly by volatilisation in the first 2–3 weeks after application, particularly of the α-isomer (70%); β-endosulfan was more strongly sorbed to plant and soil materials,
- Endosulfan residues on cotton plants, including the metabolic product endosulfan sulfate, is quickly metabolised, with a composite half-life of 3–4 days. In two weeks only 2–3 % of the amount applied in one spraying remains in the foliage. This contrasted strongly with the comparative persistence of chlorfluazuron, with 70% remaining after 47 days,
- Unfortunately, some persistence of endosulfan in the field occurs because of the formation of endosulfan sulfate—a toxic oxidation product—with a 'half-life' in soil of about 110 days. However, by the start of the new growing season only about 2% of the endosulfan applied (2.25 kg/ha) remained on field as endosulfan sulfate, so there is little or no long-term accumulation,
- Run-off waters contain 1–2% of the total endosulfan applied to cotton fields in the season, with major storms accounting for up to half of this amount,

Based on this data set, field testing of new chemicals and farming best practices that help limit the future impact of pesticides on produce and the environment can be recommended.

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IN the development of farming practices to minimise the extent of contamination of produce and the environment with chemical pesticides, knowledge of the environmental fate of particular chemicals following application is essential. In this paper, the results of integrated monitoring studies for several pesticides in Australian cotton production systems, focusing on endosulfan will be described, illustrating possible outcomes and their role in decision making. This study was performed as part of a major research program ('Minimising the impact of pesticides on the riverine environment using the cotton industry as a model'), funded by the Land and Water Resources Research and Development Corporation (LWRRDC), the Cotton Research and Development Corporation (CRDC) and the Murray–Darling Basin Commission (MDBC).

The monitoring of river systems used to provide irrigation water for cotton growing has shown significant contamination of river water by endosulfan residues (Fig. 1). The maximum levels of contamination shown in the Figure 1 are related to the cotton growing season in summer, following several aerial applications of endosulfan on crops in the late November–January period in response to insect pressure.

The physicochemical characteristics of a particular chemical and its susceptibility to biodegradation will determine whether it is transported to other sites and its degree of persistence in soil and water. Persistence and dissipation are commonly measured with reference to the half-life the time necessary for half the amount of chemical applied to disappear from a site. Although simple in principle, the field half-life possibly results from several processes acting simultaneously and may be difficult to estimate with accuracy. The same chemical applied under different weather or soil conditions, for instance, can have quite different half-lives. For this reason, half-life figures are normally referred to particular environments, or given as a range and they should be used as an indicative guide only.

From time course data it is possible to calculate half-lives (Fig. 2) according to the following mathematical model:

$$dC/dt = kC$$

where C is concentration, t time and k a constant.

For a first-order reaction, the rate constant for this equation is $k = \ln(Co/Ct)/t$ and the half-life ($t_{1/2}$) is calculated as

$$t_{1/2} = \ln(0.5)/k = -0.693/k$$

The field half-life of a particular chemical is strongly determined by its physicochemical proper-

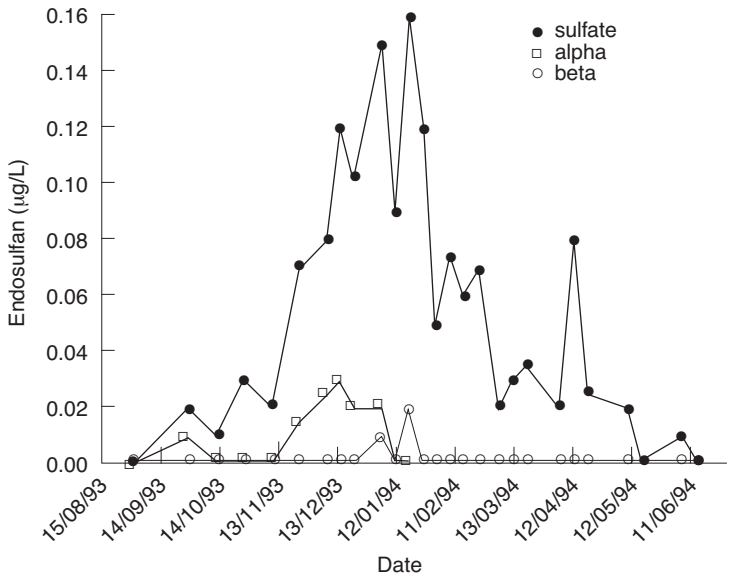

Figure 1. Endosulfan residues in river water; replotted from data collected by the Department of Land and Water Conservation, NSW, Australia obtained in the North-West Rivers Water Quality Program (Cooper 1996).

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ties and can vary according to the medium: air, water, soil and organic matter. For example, endosulfan is stable at neutral pH but undergoes chemical breakdown in water (hydrolysis) under alkaline conditions (Cotham and Bidleman 1989; Singh et al. 1991; Peterson and Batley 1993; Southan and Kennedy 1995), forming the less toxic product endosulfan diol.

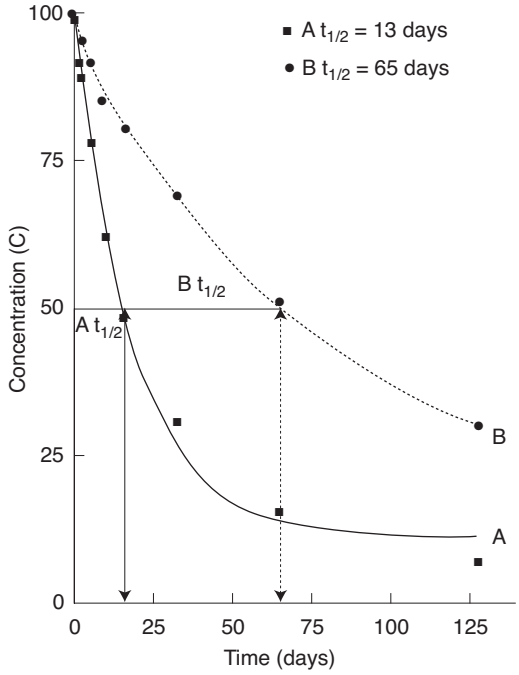

Figure 2. Decay curves for chemicals in the environment. To compute the half-life, logarithmic plots are prepared and the half-life estimated from the resultant linear plot.

Experimental Plan

Full details of the experimental methods used in this study will be published elsewhere (Kennedy et al. 1997a, preliminary report). However, for the illustrative purposes of this paper, the following information on methods is supplied.

Description of field sites

Farms at Narrabri and Warren in the Namoi and Macquarie river valleys of New South Wales were chosen for the three consecutive years of this study. In the first year (1993–94 season), field 21 (82 ha) at Auscott, Narrabri, was selected for study. In the second year, the 1994–95 season, because of severe drought in the Namoi valley, the study was continued in the Macquarie valley on the Auscott, Warren farm on field 4 (57 ha) near Nevertire. Comprehensive studies of irrigation run-off were also carried out for the Warren field site, including hydrological measurement of flows off the field. Soil pH values were measured in the range 7.5–8.2, with only minor variations over the field. A similar range of values was found in the Narrabri soils.

Sampling design

In the first year, a stratified design of 90 regular blocks was chosen for field 21 at Auscott, Narrabri, for convenience of both sampling of soil and plant material, and to allow methodological analysis of sampling intensity and spatial-temporal variability in soil residues. To investigate the effect of furrow aspect on spatial distribution of pesticide, separate sets of samples were taken from the top, edge and bottom of the furrows. Following intensive baseline soil samples of the strata on field 21, sampling schedules were determined by the date of three endosulfan sprayings—December 13, 23 and January 4—and physical conditions on-field, as a result of irrigation or rainfall. In addition, two flumes were set for sampling of water during irrigation events.

Similarly, at the Warren field site, another stratified design of 18 blocks on field 4 was chosen for soil and foliage sampling, so as to statistically detect the spatial and temporal variability of residues in the field. Sediment samples from the tail drain were also taken throughout the season. Based on the experience of the previous year, sampling was more intensive immediately after the endosulfan sprayings. At some aerial sprayings, sampling was coordinated with integrated studies on volatilisation and aerial drift, in collaboration with NSW Agriculture (V. Edge and N. Ahmad) and the University of Queensland (N. Woods and I. Craig). To estimate the amount of pesticide falling on both soil and canopy immediately after spraying, a set of filter paper strips on wooden slats and another set on aluminium plates 1 m above the canopy were placed in the field (Fig. 3). Plant cover of soil by the foliage of cotton plants was calculated using a shadow technique to measure horizontal cover near noon, also measuring the height and width of plants on 1 m long sections of 10 furrows.

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Figure 3. Plates and slats used to verify chemical application and deposition rates on cotton fields. Filter papers were extracted with acetone, and endosulfan estimated by GLC and ELISA analyses.

Data collection

Soil sampling was done in accordance with the standard sampling protocols (Kennedy et al. 1995). This involved combining 6–10 cores (5 × 4.5 cm) from the top layer, which were crushed and thoroughly mixed on a glass tray, the resulting sample representing a single stratum of the field. In the first year, 20 cores were used for each composite soil sample but statistical analysis showed little improvement in the variance of averaged data from more than 6–10 soil cores in composite samples. A simple statistical test of the results showed that no significant differences in the mean values and their variance could be obtained by using half that number of cores (Fig. 4). Subsequently, in the second year each soil sample was a composite of only six cores taken on a logarithmic basis along a transect. However, no difference was observed between separate soil samples taken at close or wider separation within a block on a particular field. Thus, in the final year of the study, composite soil sample for analysis was made up of ten cores chosen at random throughout the field.

Foliage sampling also experienced some variations through the program. In the first year, leaves from a number of plants within the same stratum were collected in glass jars, with residue analysis conducted on a surface area basis ($\mu g/cm^2$). In the second year, foliage was sampled on each occasion as nine whole plants, thus allowing the calculation of a pesticide load per plant and a chemical balance for the whole field. As the season proceeded and the size of cotton plants increased, plants were separated for analysis into outer and inner leaves, stems and bolls. A similar procedure was followed in the third year, by taking whole plants within a 1 m section along a furrow from random points on the field.

To estimate initial deposition of endosulfan on soil, paper strips (placed on field during sprayings 2 and 3 at field 4) were removed from the wooden slats and plates within one hour of spraying. Each paper strip was transferred to a glass jar for storage in the deep-freeze and acetone was added to extract the pesticide for further analysis by immunoassay and gas chromatography. Volatilisation of endosulfan was measured by the application of carbon filters placed above the crop through which air was pumped at a measured rate (Ahmad and Edge 1996).

Water run-off samples were taken in 1 L amber glass bottles closed with teflon seals during irrigation or storm events. Information on the total run-off via the return drain exiting from fields through a 'drop-box' was obtained using stage height indicators recording on data loggers, and also by equipment installed by the NSW Department of Land and Water

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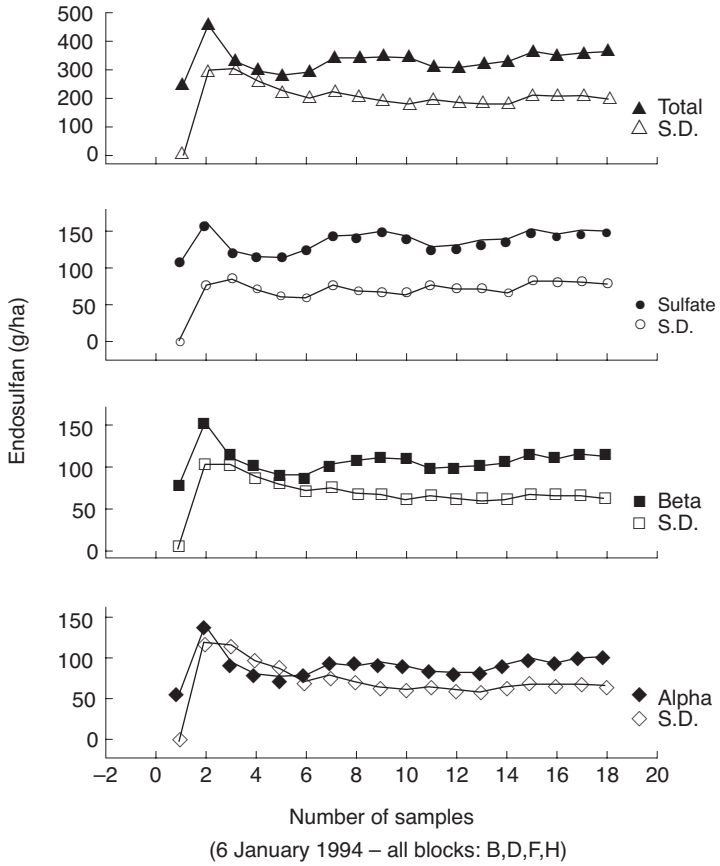

Figure 4. Mean values (filled symbols) and standard deviations (hollow symbols) of analyses for α-endosulfan, β-endosulfan and endosulfan sulfate for repeated soil cores (6 January 1994—all blocks: B, D, F, H). Five cm cores were analysed separately and the results calculated for 1 to *n* samples selected at random.

Conservation (Tuite 1996). In the second year of the study, three storms produced sufficient discharge to take run-off samples, one being of very large volume of less than 1 in 25 years probability.

Analytical methods and quality assurance

As part of the research program, a validation of immunoassays (ELISAs) for soil and water samples using CSIRO immunoassay kits for endosulfan (Lee et al. 1995a,b, 1997) has been of particular importance, since it has allowed the analysis of a much larger number of field samples than possible by GLC. The main advantages of the technique are its speed and the relatively low cost, each analysis being less than 10% of the cost by solvent extraction and gas chromatography. The compromise is a loss of specificity (all cyclodienes yield positives) and some loss of accuracy, with a total detection limit of 0.2 ppb in soil and a practical range of 0.2–50 ppb in water, without the possibility to distinguish between the three toxic forms of endosulfan.

A separate project on quality assurance has shown that the three main analytical centres involved in this research program produced gas–liquid chromatography (GLC) data following solvent extraction of water and soil samples within acceptable limits of quality assurance (Kennedy 1995). Analytical work by three different laboratories (the Biological and Chemical Research Institute at Rydalmere, the NSW Department of Water Resources at Arncliffe and Department of Natural Resources at Indooroopilly in Queensland) proved that confidence in the accuracy

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of the analytical results obtained during this program was justified.

Analytical methods and quality assurance

Agreement between results obtained by gas chromatography and immunoassay for soil and run-off water (see Fig. 5) was excellent ($r^2 = 0.9$), but at least 10 g of well-mixed soil was required for reliable analyses using methanol extraction. However, immunoassay sometimes gave greater values for residues in run-off samples than analysis by GLC. This may indicate real differences at the time of analysis, since immunoassays are conducted soon after being collected, before inevitable losses occur by volatilisation or hydrolysis of the α- and β-isomers during transportation (processes with half-lives of only 1–2 days at ambient temperature of 25°C and pH 8.5).

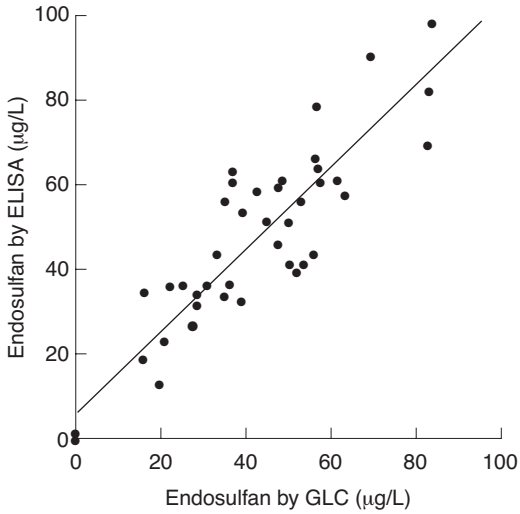

Figure 5. Comparison of endosulfan analyses in run-off water by solvent extraction and gas-liquid chromatography (GLC) or by immunoassay (ELISA) (Lee et al. 1997). Analyses shown are for the sum of both isomers and endosulfan sulfate.

Agreement between the two methods is much better when the more stable endosulfan sulfate is the main residue in run-off several weeks after spraying. It was also found preferable to freeze water samples for immunoassay, or to add acetate buffer (pH 5.5), if analysis could not be conducted immediately.

Data was studied using relevant statistical procedures. They helped improve the precision in calculating pesticide loads in run-off using the Cotton Farm Simulation Model developed by John Tuite (1996). Also, extensive validation using the GLEAMS modelling program was performed in collaboration with Robin Connolly (Queensland Department Natural Resources, Toowoomba) on predictions made of risk factors concerning impacts on the riverine environment.

Results and Discussion

Dissipation of pesticides in cotton production systems using endosulfan as a model

Although pesticides may be transported away from the site of application, as indicated earlier, degradation takes place in situ, by chemical, biological or photodegradative processes. The time course for dissipation of endosulfan in cotton fields at the Warren field site is shown in Figure 6 (foliage) and Figure 7 (soil). These profiles indicate multiple applications and the subsequent transport processes such as volatilisation, run-off in surface water from soil as well as degradation on leaves and in soil in situ. However, most of the dissipation observed in the first few days has been shown in this study to be by the physical process of volatilisation rather than degradation.

The patterns for foliage and soil are similar, although soil can be seen to stabilise β-endosulfan and endosulfan sulfate so that these compounds persist there much longer. Unlike previous organochlorines such as DDT, endosulfan dissipates rapidly, with the formation of endosulfan sulfate as a major degradation product. Unfortunately, endosulfan sulfate is also toxic to fish and some other biota.

Dissipation patterns

Baseline data from soil taken from the field sites showed a low endosulfan concentration at the start of the study: the concentration observed before spraying on field 21 was less than 0.08 mg/kg (equivalent to 60 g/ha), occurring mostly in the sulfate form (0.06 mg/kg, ppm). Three sprayings with endosulfan raised the maximum total residue concentration recovered in soil to 0.7 mg/kg (or 530 g/ha). In the first month following the final application, this value fell rapidly, mainly because of the disappearance of the α-isomer by volatilisation. A more stable period followed for about two months with complete disappearance of α-endosulfan, some retention of the β-isomer in soil

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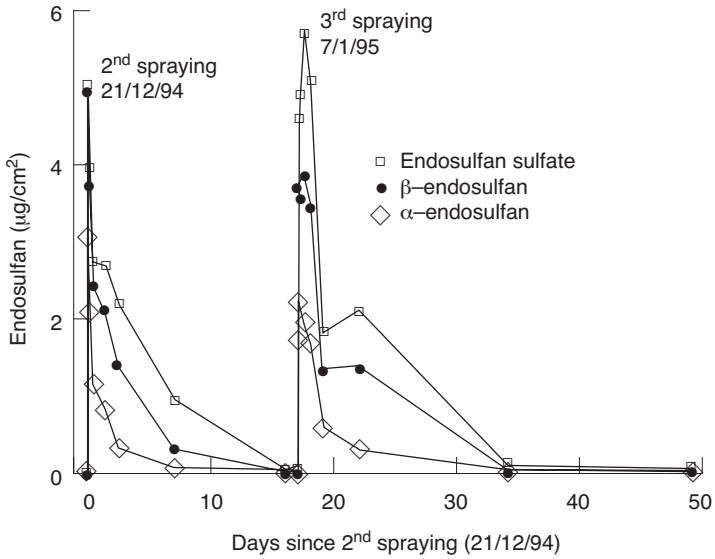

Figure 6. Rapid decline of endosulfan residues in cotton foliage at Warren, NSW, Australia in 1994–95. Even endosulfan sulfate, a metabolic product of endosulfan, particularly the α-isomer, is degraded rapidly.

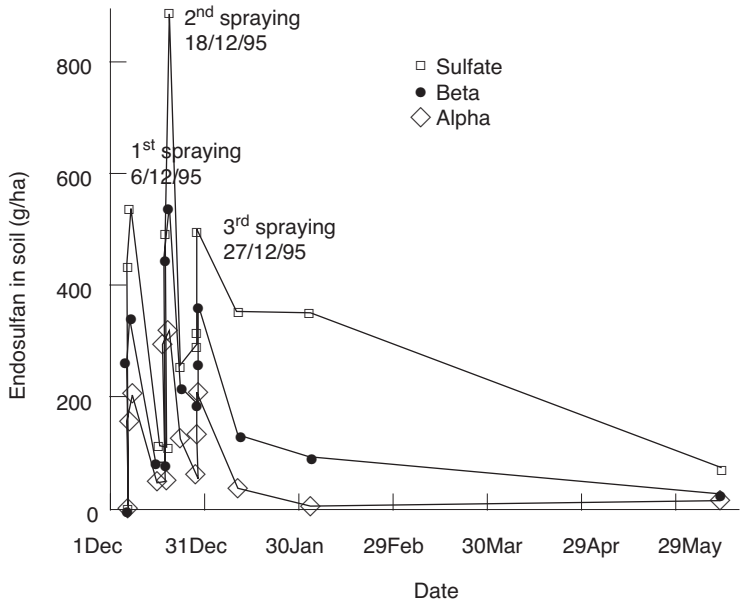

Figure 7. Dissipation of endosulfan from soil on a cotton field at Warren, NSW, Australia in 1995–96. Endosulfan sulfate, probably mainly formed by fungi in soil, is the most persistent residue.

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Table 1. Estimated half-lives (days) for total endosulfan in soil of cotton growing areas

Year	Field	Phase	α-endosulfan	β-endosulfan	E. sulfate (days)	Total endosulfan
1993–94	21	1	5.5	6.8	–	4
		2	10.5	103	120	180
1994–95	4	1	1.8	4.1	–	2
		2	65	86	152	105
1995–96	20	1	5.7	–	–	6
		2	35.7	40.2	63	129
1995–96	7	1	3.7	4	–	5.2
		2	7.8	119	105	137
Average		1	4.2±1.8	4.9±1.6	–	4.3±1.7
		2	29.7±26.6	87±34	110±36.9	137.7±31.3

and an increasing amount of the sulfate form. Eight months later, residues had fallen steadily to reach the same concentration as in the year before. This result indicates there was no build up of endosulfan residues in these alkaline grey-cracking soils (vertisols). from one season to the next (Fig. 6). This lack of accumulation is consistent with another preliminary study (Kimber et al. 1995a,b) conducted on several farms in the Namoi Valley, showing that cotton farming systems have the capacity to dissipate endosulfan residues, even where there is continuous growth of cotton for up to five applications annually.

A low baseline concentration of pesticide residues, below the detection limit of 0.05 mg/kg for endosulfan sulfate, was also obtained in the 1994–95 season on field 4. A maximum peak of residue in soil was reached at 0.86 mg/kg (440 g/ha) on the day of the second spraying, when field plant cover was 25%. Another spraying 16 days later showed a lower peak of 0.68 mg/kg (345 g/ha) when the plant cover was 50%. More intensive sampling after the three spraying events revealed detailed information on the rapid disappearance of α-endosulfan and the slower disappearance of β-isomer.

Thus, it is apparent that there is a complex pattern of dissipation of endosulfan in soil: in the first stage, the two parent isomers (α- and β-endosulfan) dissipate quickly but at different rates coincident with a build up of endosulfan sulfate, the latter reaching a maximum (0.2 mg/kg or 100 g/ha) about two weeks after the final spraying.

The faster dissipation of endosulfan in the first phase could be explained as a loss of the parent isomers by volatilisation, the extent of which is dependent upon environmental conditions such as temperature, wind and soil moisture (Southan and Kennedy 1995). This was confirmed by direct measurements using air from above the crop filtered through carbon filters. In the second phase the slower rate of dissipation seems to reflect the degradation in soil of the more stable product endosulfan sulfate as well as dissipation of remaining β-endosulfan.

Persistence of endosulfan in soil: half-life

Apparent half-lives for each of these two phases have been estimated from a large number of data points (Table 1), with crude mean values of about 4 days and about 140 days for total endosulfan residues in each of the two phases. However, these values are approximate only and more careful analysis of this data will be described elsewhere (Southan and Kennedy 1995). The half-life in soil also varied from field to field as the environmental conditions (time of day, temperature, wind speed, etc.) varied for each spraying. In general, the decline in α-endosulfan concentration in soil was much faster than that for β-endosulfan, whilst endosulfan sulfate was formed quickly as α-endosulfan disappeared and afterwards degraded relatively slowly. On one field, the peak quantity of endosulfan sulfate represented about half the α-endosulfan plus β-endosulfan deposited on soil

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Table 2. Transport of endosulfan residues in run-off water (Field 4, 1994–95)

Event	Date	Days since last spraying	Discharge (ML[a]/ha)	Endosulfan in water (mg/L)	Endosulfan removed (g/ha)	% endosulfan washed off
Irrigation 1	8/12/94	8	0.97	7.65	7.42	4.3
Irrigation 2	28/12/94	7	0.84	6.98	5.86	2.9
Storm 1	5/1/95	15	0.037	6.11	0.23	0.1
Irrigation 3	13/1/95	6	0.69	6.96	4.8	2.7
Storm 2	19/1/95	12	2.35	8.85	20.8	9.9
Storm 3	28/1/95	21	0.022	6.09	0.13	0.07
Irrigation 4	6/2/95	30	1.03	1.62	1.67	0.9
Irrigation 5	20/2/95	44	0.73	0.75	0.55	0.3
Irrigation 6	4/3/95	56	0.75	0.15	0.11	0.08
Total			7.42		41.57	1.9

[a] ML = megalitres.

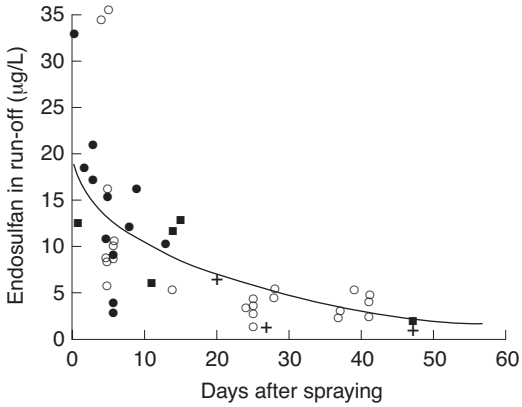

Figure 8. Endosulfan residues in run-off from 19 cotton fields at Warren, NSW, Australia in 1995–96. The decline in residue concentration in irrigation run-off in several return drains is well correlated with the declining concentration in soil (see Fig. 7).

or one-fifth the total endosulfan applied in the three aerial sprayings.

Difficulties in estimating half-lives under field conditions can arise from several causes, the main being the frequency of sampling immediately after the spraying.

Spatial uniformity

Statistical analysis (ANOVA) of the data for the stratified design by rows and columns on fields 21 and 4 indicates that there was no significant difference in concentrations of endosulfan residues between strata. There was no evidence of significant redistribution of the pesticide downfield (i.e. in irrigation run-off water). Thus, aerial application provides an even spread of pesticide on the field, and the rate of conversion to endosulfan sulfate also seems to occur evenly across the field, at least during the first weeks when the concentrations are highest.

Movement through soil layers

At field 21, a special set of four cores from the field, tail-drain slope, tail-drain scow, and return

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drain was taken for analysis of endosulfan residues in soil layers. As observed in a previous CRDC-funded study (Kimber et al. 1995a,b), most of the endosulfan was present in the top surface layer, its concentration declining with depth and being negligible beyond 8–10 cm. All samples showed similar residue concentrations, but residues from return drain were significantly lower. Given the low solubility of endosulfan and its high affinity for organic matter, leaching seems improbable, particularly in these vertisols; rather, any presence of endosulfan below surface layers may be explained by soil and contaminated dust falling into the wide and deep cracks often appearing in this type of soils.

Endosulfan in run-off water

There was significant pesticide contamination of all irrigated run-off water after the first spray application. The residues found were generally in the range 1–30 μg/L in run-off at the drop-box, depending on the number of days from the previous aerial application. Values declined to about 2 μg/L one month after spraying, corresponding with the decline in on-field soil residue concentration (see Fig. 8 and Table 2).

In Table 6, the calculated amounts of endosulfan residues for different levels of measured run-off are shown. This data indicates that 2–4% of the residues on-field are typically washed off the field in an irrigation event, with up to 10% leaving the field through the drop-box during a major storm. Normally, residues in irrigation run-off are recirculated in the farming system, and do not enter riverine systems except in major storms.

Conclusions

From data such as these, a chemical balance for pesticides like endosulfan can be prepared. As a result of these field analyses, the following conclusions were made:

Develop strategies to minimise the pesticide load on cotton fields.

In general, retaining pesticide residues at the site of application in soil and cotton plants or trash where they may dissipate in situ is desirable and reduces vulnerability to further run-off. Strategies that should be considered to minimise loads include:

- minimise the number of pesticide applications. This may be achieved by careful attention to monitoring of pest pressure, and by choices related to time of spraying (e.g. volatility of endosulfan is greater in hotter conditions, reducing effectiveness and requiring more frequent spraying),
- use band spraying where possible, minimising the contamination of soil by focusing the application of insecticides on plants where efficacy is greater and breakdown faster,
- apply the principles of integrated pest management and encouragement of beneficial predators, to reduce insect pest pressure (e.g. food-sprays, plant buffer zones to grow beneficials) and so reduce the need for pesticide application.

For endosulfan, frequent sprayings lead to further building-up residues in the soil, since the intervals normally used (10–15 days) are not sufficiently long to allow for a substantial degradation in soil. But with late spray applications with 60% or more of plant cover, the residues added to soil were shown to be smaller in magnitude. Reducing total pesticide load on soil can be achieved if the actual spraying schedules are changed: given the half-lives and degradation times of endosulfan it is evident that the main potential for contamination is given by the early aerial sprayings with 85–95% of the field surface exposed. In these conditions very little endosulfan goes onto the plants where it could dissipate more rapidly. While the requirements for pest control may limit flexibility, this principle could be kept in mind where alternative strategies for pest control exist. The introduction of transgenic Ingard cotton, shown in the 1996–97 season to reduce the need for early season spraying with endosulfan, will allow this benefit to be gained. The availability of transgenic Ingard cotton now provides an opportunity to reduce endosulfan application, particularly early when soil exposure is greatest, and in sensitive areas near stock routes, wetlands and rivers.

Minimise run-off and sediments in run-off and the possibility of contaminated return-drain water joining the rivers.

Obviously, from the concentrations of endosulfan residues found in run-off from cotton fields in this study, irrigation tail water should never be made available to livestock, even just before defoliation. Operation of the Farm Simulation Model (Tuite 1996) using data from the NSW Fieldsite at Warren suggests that the strategy of quantifying flows and transport of pesticide residues on individual farms can help provide very useful results regarding the

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benefits of reducing run-off, particularly by increasing on-farm storage, thus allowing the quarantining of more pesticide residues on-farm.

The relevant strategies are:

- recirculate all tail waters on farms and provide the maximum capacity for their retention in ponds or depressions during storms. Sediment traps where the velocity of water flow is significantly reduced provide an opportunity to minimise transport of pesticide residues to rivers.
- schedule irrigations by careful monitoring of soil moisture conditions. Irrigations just before storms provide conditions for more run-off than usual, so attention should be paid to weather forecasts in irrigation scheduling where possible.
- reduce erosion by using flat furrow gradients particularly near and in tail drains. Sediment load is directly related to velocity of flow. Cultivation of soil increases erodibility in storms and should be used sparingly or replaced by minimum tillage herbicide treatments that should also be carefully monitored for environmental impacts.
- reduce erosion by retaining stubble and other vegetation on cotton fields. Such techniques (as shown in this study in 1994–95) prevent sealing of the soil surface by reducing the impact of rain water, improving infiltration into soil and reducing run-off during irrigations and in storms (Silburn 1996).
- develop farm environmental plans, including amelioration practices such as strategic location of fallow fields to retain and receive storm waters, particularly near outlet points on farms, directing storm run-off away from sensitive wetlands and streams as part of a storm water management plan. Such farm environmental plans, possibly prepared by a specialist consultant, could include application of models such as the Simulation Model or other models, tailored to provide a user-friendly output to form a reliable basis for recommendations and providing an analysis that incorporates the specific features of the particular farm and the neighbouring locality.
- conduct more research to further define the capacity to limit pesticide transport and to remediate soil and water.

A knowledge of likely pesticide loadings could help make prudent decisions regarding controlling run-off and recycling of water in the farm system. For instance, up to 60 g of endosulfan was removed from field 20 during the peak of the flow (1.5 ML/hour) after the two first sprayings, but in the irrigation following the last spraying the amounts of endosulfan residues removed were four times lower despite showing a similar flow.

New strategies to reduce pesticide loading in run-off, such as the use of flocculants in irrigation water, are still subject to investigation; it may be premature to recommend their application until their capacity to retain residues on-field has been evaluated, and any environmental impacts assessed. However, there is no doubt of the ability of small amounts of polyacrylamide to substantially reduce sediment loads in run-off.

Ponding of water as such does not encourage endosulfan dissipation, as the half-lives of this pesticide in the pond experiment were found to be similar to those in soil, and there was a 50% deposition as sulfate on the bottom floor (S.W.L. Kimber and I.R. Kennedy, unpublished data). However, hydrolysis in ponds could be fostered by alkaline treatment, since endosulfan is quickly degraded under high pH (Southan and Kennedy 1995), or by bioremediation with mixed microbial cultures or enzyme preparations, possibly including cyanobacteria (Kennedy et al. 1997b). In part, the technology required for such approaches has been developed during this study such as the validation of an endosulfan immunoassay for field analysis, allowing the time for quarantine of run-off waters in storage. However, these new solutions demand the development of new technology, which is also recommended as part of an on-going process of reducing the environmental impact from pesticides used in cotton farming. Any such new procedures can readily be incorporated into the farm environmental plan.

Acknowledgments

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Residue Monitoring in Wool, and the Environmental Impacts of Pesticide Residues

P.W. Robinson, H.L. Boul, N.I. Joyce, and T.C. Reid*

Abstract

Pesticide (ectoparasiticide) residue analysis has become a vital tool for improving farmer practices and demonstrating the overall cleanliness of New Zealand's wool and wool production. Demonstration of this cleanliness is imperative for protecting market access. This paper reviews residue issues and solutions throughout the wool production and processing chain, and the development of analytical techniques for residue testing.

Results are presented for on-farm soil contaminations as a result of sheep dipping and the disposal of excess dip wash. Contamination with historical compounds such as arsenic, dieldrin, and carbophenothion is evident at some sites. Low-level soil residues are apparent with most modern dips but significant contamination can result following use of some water-soluble compounds. Soil degradation, however, results in little or no long-term contamination from these compounds. Chemical methods for the treatment of excess dip wash before land disposal have potential to reduce soil contamination, but filtration, using a wool-based filter, may offer a cheaper and more robust long-term solution.

Ectoparasiticide residues are removed from wool during wool scouring. Removal of these compounds can result in highly toxic effluents. Treatment of these waste waters is essential to minimise environmental impacts. High rate anaerobic destabilisation allows for the removal of pesticides from wool processing effluents. The resultant sludge may be composted or pelletised before land spreading or landfill disposal.

International environmental legislation restricts the discharge of pesticides in textile processing effluents. Compliance with this legislation offers the possibility of some wool importing countries to use ectoparasiticide residues as a technical barrier to trade. Taken to their ultimate extent, these legislative restrictions could result in a ban on certain organophosphate or pyrethroid-based compounds. The implications of this to the New Zealand farmer are discussed.

NEW Zealand has an agricultural based economy, heavily reliant on exports of primary produce. Wool production contributes significantly to the gross domestic product, but because it is a commodity that is subject to the vagaries of supply and demand and fluctuating currencies, this contribution is inconsistent. Recently, Wools of New Zealand has introduced a global marketing strategy based on a country of origin brand, the Fernmark™, in order to differentiate New Zealand wool from that supplied by other countries. The Fernmark™ strategy relies heavily on the 'clean green' imagery associated with New Zealand. Wools of New Zealand and the Wool Research Organisation of New Zealand (WRONZ) have embarked on a significant program to understand and manage pesticide (ectoparasiticide) use on New Zealand sheep farms to ensure that this image is soundly based.

Ectoparasiticide use is essential in sheep farming to protect stock from the ravages of the sheep blowfly and lice. Blowflies lay their eggs on live sheep, and the hatched maggots burrow into the skin, causing large lesions. Heavy infestations may be fatal. In contrast, lice are obligate parasites of sheep. Problems occur

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when lice numbers rapidly multiply. High numbers of lice affect the pelt and may cause wool to become cotted (entangled) and discoloured due to the animal rubbing itself to ease discomfort. An increased incidence of mycotic dermatitis may also occur.

Despite the need for ectoparasiticides, their potential affect both on the environment and trade means that their use is subject to intense scrutiny. Compounds must be used in a sustainable manner and, as with the production of other agricultural products, transparency within the farm-to-fabric (pasture-to-plate) pipeline is essential. The dipping of sheep may result in the contamination of soil and may produce significant quantities of excess dip wash for disposal. In addition, residues remaining on the wool may be transferred to processing liquors when the wool is scoured (washed) or dyed. The transfer of ectoparasiticide residues to processing effluents may render these toxic to receiving waters. This toxicity may be overcome by suitable treatment technologies. However, wool residues have potential to become a technical barrier to trade under the terms of the General Agreement on Trade and Tariffs.

Analysis of Ectoparasiticides on Wool and Wool Products

Sheep dipping may be carried out in a plunge pool or shower (where the whole animal is soaked to the skin) or by hand-jetting, which applies less chemical to selected parts of the sheep. Pour-on treatments for lice may be applied as a narrow strip along the spine or as a spot between the shoulders.

Most currently used ectoparasiticides (Table 1) are lipophilic and thus strongly associate with the wool grease component of the sheep fleece. Cyromazine, a hydrophilic compound, is an exception to this. The variable chemistry of the compounds used, large variations within the wool, and the behaviour of lipophilic compounds in a lipophilic matrix, make the analysis of pesticide residues on wool both time consuming and costly.

Table 1. Ectoparasiticides currently registered for use in New Zealand, and some of their physicochemical attributes.

Chemical	Target pests	Octanol:water partitioning coefficient (log P)	Half-life in field soils (days)	References—physicochemical attributes
Organophosphates				
Chlorfenvinfos	Fly and lice	3.8–4.2	10–22	Tomlin 1994; Rouchard et al. 1988 .
Chlorpyrifos	Fly and lice	4.7–5.3	4.8–27	Tomlin 1994; Frank et al. 1991; Racke 1993.
Coumaphos	Fly and lice	4.1		Tomlin 1994.
Diazinon	Fly and lice	3.1–3.8	14–45	Tomlin 1994; Frank et al. 1991; Racke 1993; Ferrando et al. 1992
Dichlofenthion[a]	Fly and lice			
Propetamfos	Fly and lice	3.8		Tomlin 1994.
Pyrethroids				
Cyhalothrin	Lice	6.8	28–84	Tomlin 1994.
Cypermethrin	Fly and lice	6.6	ca 100	Tomlin 1994; Agnihotri et al. 1986, 1989; Takahashi et al. 1985.
Deltamethrin	Lice	4.6–5.4	14–72	Tomlin, 1994; Muir et al. 1985; Mestres and Mestres 1992.
Others				
Cyromazine	Fly	–0.06	50–107	Tomlin 1994; Mumma and Bogus 1981.
Diflubenzuron	Fly and lice	3.89	10	Tomlin 1994; Hornsby et al. 1996
Ivermectin	Fly and lice		28	Hornsby et al. 1996

[a] This material was withdrawn from use in New Zealand in 1997.

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Sampling of wool for analysis

It has been estimated that there is a 36–42% variation in pesticide residues levels between sheep and a similar level of variability on a single animal, even when compounds are applied using a shower or plunge dip (Rammell et al. 1988). It might be expected that this variability would be greater where compounds are applied using a jetting system or as a pour-on. For most survey work, where farm lots or composite farm samples are required, standardised core sampling in accordance with International Wool Textile Organisation (IWTO) Core Test Regulations 1996 is used. Under these regulations core samples of wool are taken from each bale within a lot, the number of cores being dictated by the size of the lot. Where individual animals are to be tested, a hoop sample is shaved from the middle of the animal, blended, and then analysed.

All samples received are blended in an air blender. A 50 g subsample is then selected from the bulk sample. This subsample is cut into 5–8 mm lengths, using steel cutting blades which can be cleaned between samples. After cutting, the samples are further air blended before being stored in clean glass containers. Opened sample containers are placed in a controlled temperature and humidity room (20°C, 65% r.h.) for 24 hours before weighing and extraction.

Extraction of wool samples for analysis

In traditional solvent-extraction methods for wool residue analysis, wool is Soxhlet extracted with dichloromethane (DCM) or ethyl-acetate/hexane. Soxhlet extraction is relatively slow, with extractions taking from 4–18 hours (Greene 1993; Greene and Wimbush 1995); but multiple extractions can be performed simultaneously. Unfortunately, it is our experience that these extractions do not fully recover all compounds, and also provide extracts that are hard to clean up before GC analysis. This latter observation is believed to result from the high amounts of internal wool lipids these techniques extract. The extraction method of choice at WRONZ is supercritical fluid extraction (SFE) for all compounds except cyromazine, for which methanol extraction is used. Table 2 compares the merits of Soxhlet extraction and SFE. WRONZ uses a Dionex SFE 703 which is capable of taking 8 extraction cells simultaneously. Extractions can be performed in 30 minutes but extra time is required to clean and set up the apparatus for the next extraction. Although SFE provides cleaner extracts than solvent extraction, subsequent clean-up is still required.

Clean-up of extracts

Sweep co-distillation has found favour in the past for the clean-up of samples containing high levels of wool grease (Peaker et al. 1991a,b). However, Jones (1992) suggested that this method had a number of deficiencies, including: pesticide recoveries are not quantitative, particularly for some pyrethroids (although our experience suggests that this may be a function of extraction rather than clean-up); and the analyte solutions from the method contain small amounts of volatile wool grease components and non-volatile woolgrease esters that produce significant background noise, peak broadening, and drifts in retention times.

Jones (1992) and Greene (1993) suggested gel permeation chromatography (GPC, or size exclusion chromatography) as the method of choice for analysis

Table 2. Relative merits of Soxhlet extraction versus supercritical fluid extraction (SFE) for the analysis of wool residues

Factor	Soxhlet	SFE
Cost	Low cost with many labs having access to equipment	High capital cost—may be offset by lower operator charges
Time	Relatively slow (4–18 hours), but fast turnaround	Fast (30 mins), but more time is required for set-up
Recoveries	Generally good, although lower for some pyrethroids	Excellent, although thermally labile compounds such as diazinon may cause problems
Reproducibility	Good	Fair, can be improved using trapping techniques
Environmental	High volumes of solvents require disposal	No solvents to dispose of

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of samples containing woolgrease (i.e. extracts of wool and wool scouring waste waters). Jones (1992) claimed recoveries ranging from about 70 to 110% for all pesticides likely to be found in woolgrease (but did not analyse for cyromazine). Greene (1993) attempted to repeat this work on both greasy wool and wool scouring effluents. Results suggested somewhat lower (and more variable) recoveries for some of the later-eluting compounds, such as cypermethrin and deltamethrin. Greene also indicated that contamination of the GC, especially the electron capture detector (ECD), may be a significant problem. Both Greene and Jones processed limited samples, and the broad applicability of their results is therefore hard to ascertain.

Work carried out on this method at WRONZ suggested that, because of the problems identified by Greene, it was not robust enough for routine analysis of a large number of samples (Robinson and Joyce, unpublished data). Significant amounts of woolgrease components carry over into the analyte solution, resulting in GC column contamination, peak broadening, a drift in retention times, and loss of sensitivity. Such results are not surprising when it is considered that woolgrease is a complex mixture of compounds with molecular weights ranging from about 200 to 1000, and that the pesticides of interest have molecular weights in the range 280–510. Separation by size, or molecular weight, is unlikely to be very efficient.

Building on earlier work by Gillespie and Walters (1986, 1989), WRONZ developed a clean-up process based on reverse-phase high performance liquid chromatography (HPLC). Sample preparation time was similar to GPC but cleaner samples were produced, reducing GC down time. This method has now been used successfully for four years, processing many hundreds of samples. The current system at WRONZ uses standard 4.6 mm diameter HPLC columns, and while these restrict sample volumes they can be operated much more rapidly than the preparative columns used by Jones (1992) and Greene (1993). Experience to date indicates that GC column and precolumn life may be twice that achieved using GPC clean-up. In addition, it is likely, but not quantified, that less contamination of the detector occurs, further reducing down time.

Final analysis of samples

All ectoparasiticides listed in Table 1, with the possible exception of ivermectin, may be analysed by GC. However, for convenience both cyromazine and diflubenzuron are analysed by HPLC. Cyromazine and its degradation product, melamine, appear to interfere with GC columns and or detectors when large numbers of samples are analysed (Robinson 1995), while diflubenzuron requires derivatisation before GC analysis. All pesticide GC analyses at WRONZ are performed on Shimadzu QP5050A or QP5000 GC/MS instruments. The use of a mass spectrometric detector provides greater confidence in results and eliminates the risk of false positives. To allow simultaneous analysis of all the organophosphates and pyrethroids listed in Table 1 (plus a large number of organochlorine compounds), injections are performed using a cold on-column injector. This injector prevents degradation of thermally labile compounds, such as diazinon, occurring in the injection port.

Contamination of Soils On-farm

Saturation dipping techniques, such as plunge and shower dips, generally require large volumes of surplus dip to be disposed of at the end of the day. Plunge dips may possess sumps of 1000–10 000 L capacity, whereas shower dips typically have a sump volume of 500– 1000 L. In addition to the disposal of this liquor, the ground adjoining the dip site may become contaminated with pesticides by splashing and fleece drainage.

Compounds no longer used

Soil contamination in localised areas around sheep dipping sites was analysed on a group of 15 farms on New Zealand's south island. Ten farms were sampled in each of two years, with five farms common to both samplings. Soil samples were taken from the splash zone adjoining the dip site, in the drainage area (pasture close to the exit of the dip), and in the area where dip wash was disposed of by land spreading after dipping. Samples were taken using standard cores from both 0–7.5 cm and 7.5–15.0 cm depths at several localities in each of these areas and pooled. Samples were also taken on three separate occasions: immediately before any dipping for a season; within two days of dipping; and two months after dipping.

Soil samples were initially analysed for contamination by dipping compounds such as arsenic and organochlorines which are no longer used. Arsenic levels were significantly higher in areas next to dipping sites than in control areas on the same farm (Table 3:

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$p < 0.05$, Student t-test). Arsenic concentrations were also higher on farms operating permanent shower type dips than on farms using mobile showers (Table 3: $p < 0.05$, Student t-test); this result despite similar periods of operation in some cases. Two sites where dipping had occurred for less than 7 years showed no difference between background and dip site levels of arsenic.

Levels of DDT and its metabolites were similar to typical levels for farms in the region, and did not significantly differ between dip sites and their respective controls. Levels up to 2.6 mg/kg were recorded for total DDT residues (including DDE), but values were generally less than 0.8 mg/kg. This is close to the estimated mean DDT levels of 0.44 mg/kg for farms in the Canterbury region (Roberts et al. 1996). Lindane residues were below the limit of detection of the method in all soil samples, although traces were occasionally observed. Dieldrin was associated with many dipping sites and very high levels were recorded in soil samples taken from two farms. Levels up to 45 mg/kg were found in the splash zone of a dip and in areas where surplus dip had been disposed of. These residues are consistent with the use of dieldrin as an ectoparasiticide until about 1970. In contrast, DDT and its metabolites tended to occur in control samples rather than in samples specifically associated with dip sites. Again, this is consistent with the intensive field use of DDT for grass grub control rather than as a sheep ectoparasiticide. Aldrin was consistently associated with the dip site on one property sampled in both years with levels ranging from 2–10 mg/kg. The origin of these residues is unclear, as it seems that aldrin was never registered as an ectoparasiticide.

Bromophos-ethyl and carbophenothion residues were detected on some farms. Both these products were withdrawn from availability several years ago, and detection of significant residues was therefore somewhat surprising. Bromophos-ethyl residues were generally low with the highest recorded value being 0.8 mg/kg. In contrast, carbophenothion residues were detected at levels up to 23 mg/kg on one farm.

While this study revealed some low-level contamination of dip site soils by dip formulations used in the past, a similar study conducted in the Waikato region (North Island, New Zealand) found greater contamination. Levels of arsenic up to 1630 mg/kg were recorded, although the volcanic origin of the soils might have contributed to these high levels. Levels for dieldrin were comparable with the results presented here (McBride 1994). The North Island study, however, found measurable lindane (up to 4.2 mg/kg on one site).

Currently registered compounds

Five farms were selected for their use of the compound, cyromazine (Vetrazin™), an insect growth regulator. High levels of cyromazine were detected in and around dipping sites, in drainage areas, and disposal sites (Table 4). Samples taken within one or two days of dipping indicated high levels of contamination (up to 85 mg/kg) in the top 7.5 cm of the soil. Generally lower residues were recorded in the lower 7.5–15 cm profile, but samples taken from the dis-

Table 3. Soil arsenic levels associated with sheep dipping sites in Canterbury, New Zealand

Farm location	Arsenic levels (mg/kg)		Dip type	Period of use
	Control	Drainage		(years)
Christchurch	6.10	16.20	Permanent	50
Peel Forest	3.84	5.86	Portable	>15
Claremont	2.41	14.60	Permanent	15-20
Darfield	2.99	6.20	Permanent	20
Aylesbury	3.03	31.80	Permanent	>20
Amberley	0.54	2.29	Portable	10–15
Oxford	3.39	3.97	Portable	3
Swannanoa	1.99	2.05	Portable	>10
Amberley	1.13	0.86	Portable	<7
Rotherham	2.73	6.00	Portable	>15

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posal site, where the ground was generally saturated, also sometimes had high levels of cyromazine (up to 36 mg/kg) in the 7.5–15 cm zone.

Table 4. Mean cyromazine contamination of soil (mg/kg) in and around dipping sites at three different sampling periods

Sample site	Sample depth (cm)	Concentration of cyromazine and time of sampling		
		Pre-dip	Post-dip	Two months post-dip
Control	7.5		0.0	
Control	15.0		0.0	
Dip site	7.5	<0.1	24.0	6.1
Dip site	15.0	<0.1	15.5	4.7
Disposal	7.5	<0.1	55.9	28.7
Disposal	15.0	<0.1	11.2	11.8
Drainage	7.5	<0.1	12.5	3.6
Drainage	15.0	<0.1	3.6	2.0

Post-dipping contamination varied from farm to farm, especially in areas next to the dipping site. Levels of cyromazine were generally lower in samples taken 2 months after dipping, reflecting losses through degradation. Loss of cyromazine, between samples taken within 1–2 days of dipping and 2 months later, varied from 20–98%, with values typically around 70%. Losses from the 7.5–15.0 cm zone were lower than in the top 7.5 cm of the soil profile. In some cases, increases in concentration were apparent in the latter zone which may reflect some movement of cyromazine through the soil column, coupled with slower degradation (Table 5).

Overall loss rates for individual farms were relatively consistent between the three sampling localities but, as indicated, there was variation between farms. There was no correlation between the loss of cyromazine from individual sites (and movement down through the soil column) and the cation exchange capacity or organic matter content of the soils. The soils had very low moisture contents. Most sheep dip sites were situated in the open, with little or no vegetation cover. This, coupled with low rainfall over the months of this study, would be expected to result in low microbial activity and low cyromazine degradation.

The reported degradation rate for cyromazine in soils is highly dependent on soil moisture and microbial biomass and, as such, the DT50 has been

Table 5. Loss of cyromazine from soils in and around sheep dips

Farm location and sample site	Total concentration (mg/kg) of cyromazine in 0–15 cm profile and (in brackets) percent present in 7.5–15 cm profile.		Overall percent reduction
	Post-dip	2 months post-dip	
Swannanoa			
Dip	2.6 (0.0)	1.4 (8.0)	46.2
Disposal	53.7 (6.1)	33.8 (24.4)	37.1
Drainage	10.0 (4.0)	8.0 (46.3)	20.0
Amberley			
Dip	63.2 (32.3)	6.4 (40.6)	89.9
Disposal	97.4 (12.1)	31.5 (27.0)	67.7
Drainage	20.7 (13.0)	4.5 (33.3)	78.3
Oxford			
Dip	82.4 (44.7)	1.2 (58.3)	98.5
Disposal	80.8 (19.3)	48.4 (40.1)	40.1
Drainage	26.5 (18.6)	1.8 (27.8)	93.2
Amberley			
Dip	48.0 (41.9)	26.8 (37.3)	44.2
Disposal	49.2 (38.4)	19.0 (36.8)	60.4
Drainage	37.8 (26.2)	8.0 (32.5)	78.8

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reported as being between 3 and 142 days (Mumma and Bogus 1981); although values between 50 and 107 days seem more typical. Microbial degradation is the primary loss mechanism, although leaching can be significant in sandy soils. Cyromazine does not hydrolyse or photolyse (Burkhard 1979a, b). There was an interval of 65–76 days between immediate post-dip and 2-month post-dip samples, with average degradation ranging from 34 to 78%. These figures equate to half-lives of approximately 40 to 100 days, which is consistent with observations from other parts of the world.

Soil samples were collected from a further nine farms using either diazinon or chlorfenvinphos. In general, soil contamination with these compounds was consistently lower than the contamination recorded with cyromazine. However, in one 0–7.5 cm sample, at a dip disposal site, chlorfenvinphos at a concentration greater than 530 mg/kg was recorded. It is possible that this level was associated with a spill of concentrate. Table 6 summarises the mean levels of both diazinon and chlorfenvinphos found in the soils of the farms sampled (the 530 mg/kg found at one farm has been excluded from the calculation of these means).

As with the farms sampled for cyromazine, contamination was noticeable immediately after dipping but levels were significantly reduced 2 months after dipping. The low and variable nature of the data preclude the calculation of half-lives for these compounds, but they are clearly short. Some carryover from previous dipping was apparent, as significant concentrations were detected in some 'pre-dip' samples.

We believe that the significant difference between soil contamination associated with cyromazine and that associated with diazinon or chlorfenvinphos can be explained as follows. Cyromazine is a water-soluble compound which essentially does not strip (actively partition onto the wool from the dip). In contrast, both diazinon and chlorfenvinphos are lipophilic and are prone to stripping. Stripping of the active compound by woolgrease, results in a gradual fall in its concentration in the dip wash (Charleston 1985). Label requirements for replenishment, reinforcement, and replacement should ensure that the dip wash is always maintained above a specified concentration. It is possible that some farmers do not stick rigidly to label requirements, resulting in significant drop-offs in active concentrations. This drop-off has been recorded in the field (Table 7) and may be responsible for the lower soil contamination observed with lipophilic products and, more importantly for the farmer, the ultimate failure of pest protection.

Disposal of Excess Dip Wash

Dip disposal is the most serious risk to the on-farm environment. Most farmers can dispose of dip on land well removed from surface waters, but a WRONZ survey in 1994 revealed that approximately 30% of the 300 or so farmers who answered the survey questionnaire indicated that their dipping yards and/or disposal sites were within 100 m of permanent surface water (P.W. Robinson, unpublished data, 1994). While there has been no direct evidence for surface-water contamination as a result of sheep dip-

Table 6. Typical diazinon and chlorfenvinphos contamination of soil in and around dipping sites after three different sampling periods

Sample site	Sample depth (cm)	Concentration (mg/kg) of diazinon and time of sampling			Concentration of chlorfenvinphos and time of sampling		
		Pre-dip	Post-dip	Two months post-dip	Pre-dip	Post-dip	Two months post-dip
Control	7.5		0.0			0.0	
Control	15.0		<0.1			0.0	
Dip site	7.5	<0.1	0.4	<0.1	0.0	2.0	0.2
Dip site	15.0	<0.1	0.1	<0.1	0.2	0.1	<0.1
Disposal	7.5	0.1	4.5	<0.1	1.1	9.0	0.4
Disposal	15.0	<0.1	0.7	<0.1	0.3	2.0	0.2
Drainage	7.5	<0.1	0.2	<0.1	0.0	<0.1	<0.1
Drainage	15.0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

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ping in New Zealand, there is circumstantial evidence to suggest that it might be a problem (Scott et al., 1994). In addition, there is recent circumstantial evidence in the U.K. that contamination of waterways might be a significant problem (Anon. 1997).

Table 7. Measured and calculated concentrations of dip wash active ingredient on nine farms using shower or plunge dips.

Calculated dip concentration (mg/L active ingredient)	Measured dip concentration (mg/L active ingredient)	Percent (%) of calculated dip concentration present in final dip wash
440	101.2	23
400	104.5	26
400	88.0	22
400	20.5	5
325	63.3	19
230	94.0	41
205	21.2	10
186	3.1	2
120	57.6	48

Laboratory experiments indicated that pesticide residues in spent dip solutions can be destroyed by oxidation using pool chlorine or Fenton's reagent. The use of chlorine was proven on dirty liquors, such as spent dip solutions. However, there was doubt about the efficacy of Fenton's reagent in treating these liquors. Chlorine appeared suitable for the treatment of all flystrike control compounds except chlorfenvinphos and cypermethrin. Fenton's reagent appeared effective against all organophosphates (including chlorfenvinphos) and cypermethrin, but was ineffective against cyromazine. Experiments have not been conducted on either diflubenzuron or ivermectin. A summary of the treatment methods is given in Table 8.

Field trials were conducted on nine farms that were using a variety of dipping compounds. Chlorine tended to provide good treatment of spent dip solutions, with removal efficiencies being always greater than 55% and, in most cases, greater than 80%. This was significantly lower than the 80–100% removal efficiencies observed in the laboratory using cleaner solutions. Fenton's reagent gave variable results ranging from no effect though to 69% removal. Again, results were somewhat poorer than laboratory experiments had given. In addition to the poor performance of the treatments in the field, farmers indicated that they found handling the chemicals unnerving. Further work has concentrated on non-chemical removal methods, the most encouraging of which so far is the use of wool-based filters.

Table 8. Chemical treatments for the destruction of surplus dip solutions

Active ingredient of dip wash	Chlorine treatment	Fenton's reagent
Chlorpyrifos, coumafos, dichlofenthion,	20 kg pool chlorine per 1000 litres of dip wash	1/2 cup of ferrous sulfate, stir well, then add 10 litres of peroxide per 1000 litres of dip wash
Chlorfenvinfos	NA[a]	as above
Diazinon, propetamfos	10 kg pool chlorine per 1000 litres of dip wash	as above
Cypermethrin	NA	as above
Cyromazine	20 kg pool chlorine per 1000 litres of dip wash	NA

[a] NA = treatment not applicable

Laboratory experiments have been conducted using small filters made from glass columns filled with 10 g knops (small balls of wool) of wool. Wool knops have been used both clean and with added wool grease up to 500% w/w. Wool knops were packed into 25 mm diameter glass columns and a solution containing propetamfos, diazinon, chlorfenvinfos, and coumafos applied. Samples of the filtered liquor were taken at intervals for analysis. In the first experiment, 2500 mL of clean liquor was passed through the column, and samples taken after 300, 500, 1000, 1500 and 2500 mL. The initial mean removal for all four compounds was 92.7%, and the final mean removal 88.8%; giving a cumulative removal of more than 200 mg of pesticides for every gram of wool. In a second experiment, both clean wool knops and wool knops containing 470% w/w of woolgrease were packed into two separate columns. Excess dip wash was collected from a local farm and fortified with the four compounds of interest, then 2000 mL of liquor was passed through the clean wool filter and 3500 mL through the greasy wool filter (Table 9). The greasy wool filter initially removed more pesticides than the clean wool filter (89% ver-

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sus 52.0%) but its efficiency fell as more liquor was processed. Further experiments are planned to investigate whether cumulative removal may be achieved by recycling liquors through a filter several times and also to determine the ultimate loading capacity of such filters.

Table 9. Percentage removal of pesticides from excess dip wash using wool-based filters.

Volume of liquor (mL) processed	Wool only filter	Wool + woolgrease filter
250	52	89
500	54	74
1000	56	70
2000	56	65
3500	–	60

Toxicity of Pesticide Residues in Processing Effluents

Wool scouring removes on average 95% of all ectoparasiticides from the shorn wool, with most of this removal occurring in the first three scouring bowls. Water consumption by this point is typically 1.5–2.0 L/kg of greasy wool scoured. Little removal occurs in the subsequent rinse bowls, where detergent concentrations are low and the water is usually cold. The scouring (or flow down) liquor from the first three bowls is passed through a series of grease recovery centrifuges where typically 30% of the woolgrease is recovered. Approximately 30% of the lipophilic pesticides are removed at this point also. The remaining effluent is then discharged through a variety of treatment systems to the environment. Treatment of wool scouring waste waters in New Zealand varies from complete evaporation and incineration, at one plant, to untreated municipal ocean discharges at three others.

The ectoparasiticides used on sheep are highly toxic in aquatic environments. Table 10 indicates the approximate maximum levels than can be sustained in fresh waters, based either on the application of a 100-fold safety factor being applied to the 48-hour EC50 for *Daphnia* spp. (for organophosphates (OPs), pyrethroids, and diflubenzuron) (Hill 1989; Xiu et al. 1989; Fischer and Hall 1992; van Wijngaarden et al. 1993; Tomlin 1994) or chronic toxicity bioassay results (for cyromazine; Robinson and Scott 1995). Table 10 also indicates proposed U.K. environmental quality standards (EQS) which are to be set for pyrethroids, organophosphates, and diflubenzuron in order to protect all aquatic life. If the ectoparasiticides are present in fresh water at levels below these 'no effect levels' then there should be no harm to any aquatic life.

Due to the levels of ectoparasiticides, effluent produced from scouring greasy wool is also highly toxic. Both raw and treated wool scouring effluents were collected from a number of wool scours throughout New Zealand and subjected to both chemical analysis and toxicity bioassays. The toxicity of effluents was determined by diluting with clean water and assessing the dilution required to reduce toxicity to a point where only 50% of the test organisms were killed in 48 hours (48-hour EC50, Table 11). The no observable effect dilutions for these effluents are probably 10 –100 times greater than the 48-hour EC50 concentrations.

The 'toxic units' for each effluent constituent were calculated based on the measured concentration of the contaminant and its known toxicity to the test species (*Daphnia magna*) (Amato et al. 1992) (Equation 1).

$$\text{Toxic unit} = (\text{concentration of the contaminant}) \div (\text{published } EC_{50} \text{ for } \textit{D. magna}) \quad (1)$$

Toxic units can be used for both pesticides and non-pesticides, provided the units of measurement are equivalent. Correlation coefficients were then determined for standardised variates of each contaminant. A standardised variate is calculated according to Equation 2:

$$\text{Standardised variate} = (\text{value } n^{\text{source}} - \text{mean}^{\text{source}}) \div \text{standard deviation}^{\text{source}} \quad (2)$$

In all cases it was shown that the concentration of organophosphates or total pesticides (organophosphates plus pyrethroids) present in the effluent was highly correlated with its overall toxicity (Table 12). These results indicated a simple additive action for the pesticides present with no apparent synergistic or antagonistic effects evident despite the high concentrations of grease and suspended solids which might be expected to reduce bioavailability.

Rinse waters were shown to be slightly toxic after settling. Some pesticides were detected at low levels (μg/L range), with organophosphates proving to be the principal toxins. Dilutions of 20–150 times

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Table 10. Toxicity and approximate no effect level for ectoparasiticides in fresh waters

Compound	48-hour EC_{50} *Daphnia* spp. (μg/L)	Proposed U.K. EQS or no effect level	Toxicity reference
Chlorfenvinphos	0.3	0.01	Tomlin 1994
Chlorpyriphos	0.8		van Wijngaarden et al. 1993; Tomlin 1994
Coumaphos	1.0	0.01	Tomlin 1994
Cyhalothrin	0.1	0.001	Hill 1989; Tomlin 1994
Cypermethrin	0.15	0.001	Tomlin 1994
Cyromazine	93 000	5.0[a]	Tomlin 1994; Robinson and Scott 1995
Deltamethrin	0.04	0.001	Xiu et al. 1989
Diazinon	0.8	0.01	J Reeve, pers. comm., MAF, NZ, 1993
Diflubenzuron	7.1	0.03	Fischer and Hall 1992; Tomlin 1994
Ivermectin	0.025	0.0002[b]	Bloom and Matheson 1993
Propetamphos	14.5	0.01	Tomlin, 1994

[a] Based on chronic toxicity to freshwater chironomids (Robinson and Scott 1995)
[b] Based on a 0.01× *Daphnia* 48 hour EC_{50}

should be adequate to reduce the toxicity of these effluents to no observable effect levels. Flow down liquors were highly toxic, with no observable effect dilutions being of the order of 100 000 to 1 000 000 times. However, treatment in an anaerobic pond reduced the toxicity by 50–60% and treatment in a Bioloop™ system (see below) reduced the toxicity by approximately 90%.

Table 11. Toxicity (48-hour EC_{50}) of woolscouring waste waters to freshwater aquatic life

Effluent	48-hour EC_{50} dilution	Effluent	48-hour EC_{50} dilution
Untreated flowdown	10 800	Bioloop™ [a] treated flowdown	550
Flowdown, ex. anaerobic pond	6 300	Settled rinse water	5.7

[a] See text for description.

Treatment of wool scouring waste waters

WRONZ has developed a high-rate anaerobic digester to handle wool scour effluents. Known as Bioloop™, this system destabilises the wool-grease/water emulsion with a hydraulic retention time of 2–4 days. Effluent from the Bioloop™ plant is then separated in a settling tank, with the settled sludge being further thickened using a decanter centrifuge. Pesticides in the effluent to be treated by the Bioloop™ plant partition into the sludge phase. Even for the water-soluble cyromazine, partitioning between the sludge and the clarified liquor strongly favours the sludge (mean proportion in the sludge = 86.1 ± 3.7%). Thickened sludge may then be composted or mixed with waste fibre before land spreading or landfill disposal.

Table 12. Correlation coefficient of the standardised variate for organophosphate (OP) concentration and woolscouring waste water toxicity

Effluent	Correlation coefficient for OP concentration
Untreated flowdown	0.72[a]
Bioloop™ [b] treated flowdown	0.70
Flowdown, ex anaerobic pond	0.70
Settled rinse water	0.71

[a] Correlation coefficient for total pesticides.
[b] See text for description.

Composting of wool scour sludges has been carried out in forced-air aerated stacks, with temperature of the compost controlled by a computer linked to the aeration fans. Composting is a biological process which converts complex wastes into a rich organic substance which can be applied to land with a net benefit in terms of soil quality. Obviously, with the demonstrated high affinity of ectoparasiticides for the

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Table 13. Percentage degradation of pesticides in material before and after four composting and curing trials (solvent extractable 'grease' fractions)

Sample name and date	Propetamphos	Diazinon	Dichlofenthion	Chlorpyriphos	Chlorfenvinphos	Cypermethrin
HR1 composted	38	55	14	9	57	–24
HR2 composted	34	58	24	18	61	
HR2 cured 21/11	70	100	84	68	89	
HR2 cured 3/2	95	100	98	95	98	
HR3 composted	80	84	58	67	89	54
HR4 composted	65	62	10	45	85	24

Note: Bromophos-ethyl, carbophenothion, cyhalothrin, coumaphos and deltamethrin were also analysed for but not detected.

sludge phase of anaerobic digesters (and thus Bioloop™ and pond sludges) it was important to investigate the degradation of these compounds during composting.

Table 14. Approximate half-lives for diazinon and chlorfenvinphos on crossbred and merino sheep at different times of the year

	Half-life (days)	
Time of year and type of sheep	Chlorfenvinphos	Diazinon
Summer, crossbred	25	12
Winter, crossbred	39	32
Summer, merino	56	47

Sludges from a Bioloop™ pilot plant were composted in the presence of wool waste and sawdust. Pesticides were quantified in solvent extractable 'grease' fractions of material before and after composting, as well as after curing. The results presented here (Table 13) are from a preliminary trial. It can be seen that the degree of degradation of each pesticide after composting varied between samples. This variability was associated with variations in the operating conditions of the individual composts and the degree of biological activity. After 8 weeks curing (sample HR2 cured 3/2; Table 13) virtually all the pesticides had been lost from the compost. At this stage it is not certain whether this loss was the result of degradation or leaching, as curing piles were stored outside and leachate was not collected or analysed. However, given the low water solubility and high K_{ow} values for these pesticides, and the high affinity they have for organic matter, it is unlikely that leaching would result in significant loss of pesticides from the curing piles.

Marine toxicity

Most wool scours, indeed most sewage discharges in New Zealand, ultimately discharge into the marine environment. Unfortunately, the toxicity of pesticides to marine organisms is largely unknown. We have found, however, that the toxicity of many organic compounds, including pesticides, can be predicted based on their toxicity to the freshwater flea (*Daphnia* spp.). The toxicity of organics to a typical marine organism (a mysid shrimp) has been shown (Fig. 1; Le Blanc 1984; P.W. Robinson, unpublished data) to be approximately twice that of the standard freshwater test species (*D. magna*). This would suggest that the EQS values indicated in Table 10 need to be halved to adequately protect the marine environment.

Many of New Zealand's municipal ocean outfalls are of essentially untreated effluent. In some cases wool scour effluent can represent a significant proportion of this effluent. The New Zealand Coastal Policy Statement of 1994 specifies an allowable mixing zone equivalent to a 200-fold dilution for ocean outfalls. In the worst case, one New Zealand scour contributes 8.1% of the average dry weather flow (ADWF) of a municipal sewage outfall and the effluent receives no treatment other than on-site grease recovery. These figures, in addition to within-scour water consumption, can be used to calculate maximum acceptable greasy wool limits and hence potential compliance with allowable discharge amounts. Indications are that many New Zealand wool scours will have to invest in effluent treatment technology specifically to address the problems posed by the discharge of ectoparasiticide residues.

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Ectoparasiticide Residues and Overseas Legislation

Ectoparasiticide residues have the potential to be used as technical barriers to trade following the Uruguay Round of the GATT talks. Ectoparasiticide residues are both a market acceptance (consumer acceptance) and a market access issue. Consumers do not like the thought of the wool products they wear, or walk on, containing pesticide residues. It does not matter that the levels found in final products are well below accepted no-risk levels, and that the residues are, in all probability, not bioavailable. The European consumer is not aware of the need to treat animals on farms to prevent them suffering from ectoparasites. Two large consumer/industry groups in Europe have imposed very strict limits on pesticide residues in consumer goods. Most of these limits relate to organochlorines, which are still used in some developing countries but are largely absent from New Zealand's wool. The German Association for Environmentally Friendly Carpets, however, has also indicated that neither diazinon nor dichlofenthion should be present at 'detectable concentrations' in the final product. This is generally not a difficult condition for New Zealand producers to meet, but the Fernmark™ specifies only 60% New Zealand wool content in a final product. The potential therefore exists for some Fernmark™ products to contain significant residues, especially of diazinon, and particularly if poorly scoured wool from another source is used in the blend. In cases where this occurs it is the Fernmark™ reputation that may be damaged rather the component containing residues, a problem that may require stricter controls to be imposed over what wool can be blended into Fernmark™ branded products.

Ectoparasiticide residues on wool result in toxic scouring effluents and have potentially adverse effects on receiving waters. Both the U.K. and Europe have recognised this and enacted legislation that prescribes limits for ectoparasiticides on greasy or scoured wool to be processed. The U.K. legislation is based on an EQS approach using the values outlined in Table 10; although the specific figure for cypermethrin may be 10 times lower than the figure indicated here for pyrethroids. By making allowances for removal during effluent treatment, and dilutions within the environment and the sewage system, the U.K. has indicated possible maximum acceptable greasy wool residues levels. These are:

- 10 mg/kg for organophosphates
- 1.0 mg/kg for pyrethroids, and
- 3.1 mg/kg for diflubenzuron.

No limits have yet been set for cyromazine.

WRONZ, in association with Wools of New Zealand, surveys the national greasy wool clip annually. Based on these surveys, we have an informed estimate of mean ectoparasiticide residue levels, and

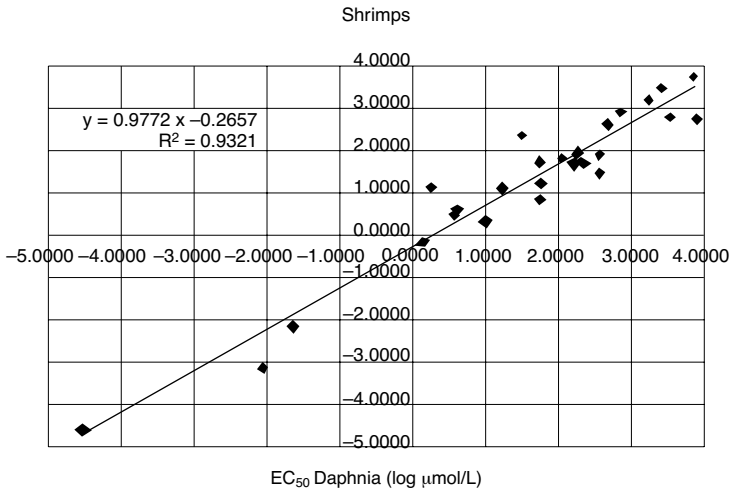

Figure 1. The toxicity of organics to a typical marine organism (a mysid shrimp) Le Blanc 1984; P.W. Robinson, unpublished data).

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these data can be used to calculate compliance with the above limits, assuming this wool is exported greasy. In general, crossbred wool has no trouble complying with the limits for organophosphates and diflubenzuron, but a proportion fails to comply with the limit for pyrethroids. It is believed the biggest problem with pyrethroid compliance is both late season (long wool) applications for lice, and the use of some high strength cypermethrin formulations. Cypermethrin appears to have a very long half-life on wool which may restrict its use in the future; especially if the U.K. persists in lowering the acceptance criterion for this compound.

Of much greater concern than residues on wool from crossbred sheep, are residues associated with the halfbred and merino sectors of the clip. Compliance with either the organophosphate or pyrethroid limit is low. Late season treatments, in particular autumn dipping followed by shearing before lambing in early spring, are the main problems but the lower degradation rate for ectoparasiticides on fine wool in comparison to coarse-woolled animals is also a factor (Table 14). Based on a review of the data available it appears as though the average half-lives for diazinon, chlorfenvinphos, and propetamphos on merinos, applied using either a plunge dip or jetting system, are 1.85 times longer than corresponding half-lives on Romney sheep (Rammell and Bentley 1989; Burman 1995; Burman and Edwards 1995; Burman and Strong 1995).

While the U.K. has set limits based on no observable effect levels in the freshwater environment, the Europeans have set discharge limits for different types of textile mills ostensibly to protect the marine environment. The European legislation has been formed as a result of several conventions collectively known as OSPARCOM (the Oslo, Paris conventions on the protection of the marine environment). OSPARCOM regulations set limits for chlorine and phosphorus-containing compounds, but they are based on a measure of total chlorine or phosphorus and not the toxicity of individual compounds. This may result in overly conservative limits for compounds having both chlorine and phosphorus moieties, and potentially ineffective control of highly toxic compounds, such as deltamethrin, that contain neither.

OSPARCOM limits are primarily concerned with late-stage processing, such as dyeing and finishing, rather than raw wool scouring. At present it appears that 90% of New Zealand's scoured wool may comply with these limits. Currently, non-compliance is associated with the presence of either chlorfenvinphos or cypermethrin, owing to the persistence of these compounds. In fact, only 80% of scoured wool complies if chlorfenvinphos has been used in any one of the farm lots making up a commercial scouring package.

Conclusions

The dipping of sheep for the control of fly-strike and lice may result in both direct and indirect environmental contamination. Soil contamination may occur near dipping sites but this appears to be partially mitigated by the stripping of the active compounds from the dip wash. The disposal of surplus dip wash from both shower and plunge dips is the most obvious form of environmental contamination. Disposal on to well-grassed flat land, well away from any surface waters, appears to be the easiest way of controlling this form of contamination but methods for treating excess dip wash are being evaluated. Wool-based filter systems offer a possible solution and one that may be applied to other liquors containing pesticides, such as residual sprays and the like.

Ectoparasiticides remaining on wool at shearing result in the contamination of wool scouring effluents. These effluents are toxic and, unless adequately treated, may have adverse effects on both the freshwater and marine environments. Anaerobic destabilisation followed by sludge composting offers a feasible and sustainable mechanism for dealing with these wastes.

Both the U.K. and Europe have recognised the toxicity of ectoparasiticides and have imposed limits on residues on either greasy or scoured wool. While the criteria for scoured wool residues can generally be met, compliance with greasy wool residues may prove more difficult to achieve; especially for the finer wool in our clip. International pressure may see some compounds forced from the market. The British Labour Government has already signalled that it would like to see a ban on organophosphates and more recently it has been suggested that pyrethroids should also be withdrawn because of their high aquatic toxicity and suspected interference with the human endocrine system (Anon 1997). Removal of either, or both, of these classes of compounds would severely restrict ectoparasite control options for farmers and would significantly increase the cost of that control.

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Contamination of Animal Products by Pesticides and Antibiotics

T.B. Murdiati, Indraningsih, and Sjamsul Bahri*

Abstract

Chemicals such as pesticides and antibiotics have been widely used in agriculture to improve product quality and output. However, over-use of those chemicals has caused contamination of animal products either directly or indirectly. While livestock is intentionally given antibiotics for disease treatment or for growth promotion, the exposure of pesticide to livestock usually comes from contaminated animal feed. Pesticide or antibiotic residues above the maximum residue limits are reported from a number of places in Indonesia. For food of animal origin, excessive antibiotic residues are reported more often than pesticide contamination.

NOWADAYS, the demand for high quality food has become as important as the food supply itself. Food quality includes not only highly acceptable organoleptic characteristics and nutritive value, but also the safety of the food. Food safety has been an important criterion of food quality of agricultural products, which includes freedom from chemical residues and contaminants such as antibiotics and pesticides. Pesticides, antibiotics, and other chemicals have been used intensively in agriculture to maximise production. In animal husbandry, antibiotics are used intentionally for disease treatment or growth promotion, while the exposure of animals to pesticides is mostly unintentional through contaminated feed (Sosromarsono 1977; Prescott and Baggot 1988).

In a developing country such as Indonesia, the effort to reduce the use of pesticides and antibiotics through biological control and integrated pest management has not yet had a significant impact. The amount of pesticides and antibiotics used in agriculture increases every year. The number of veterinary medicines registered in Indonesia increases every year. In 1991 there were 1281 registered veterinary medicines, while in 1993 this had increased to 1378. Similarly, the amount of antibiotics used increases every year: it was 27 592 kg in 1992, which increased to 29 525 kg in 1993 (Wiryosuhanto 1994). The number of pesticides registered and approved is also increasing every year (DEPTAN-RI 1997).

In the past few years there has been increasing concern over the extensive use of antibiotics in animal husbandry and their addition to animal feeds. Improper used of antibiotics in food-producing animals has resulted in the presence of antibiotic residues in animal products. Public concern on the hazards of pesticide contamination in food appeared earlier than concern about the hazards of antibiotic residues, probably because pesticides themselves are classified as toxic compounds, while antibiotics are not.

Human exposure to chemicals through food usually occurs at low doses; acute illness rarely occurs. However, continuous low-level exposure to chemical-contaminated food has been reported to cause cancer, allergies, hypersensitivity, and toxic effects. The most frequently reported allergic reactions was to β lactam antibiotic. It was reported that levels of penicillin as low as 5–10 IU are sufficient to produce an allergic reaction in sensitised humans (Paige et al.

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1997). Other antibiotics such as tetracyclines, aminoglycoside, and sulfonamide in a few cases may also cause allergic reactions in sensitised individuals. Prolonged used of antibiotics at low doses has led to antibiotic-resistant bacteria (Smith 1977; Bensink and Botham 1983). In 1968, the Swann Committee established by the British Government made a number of important recommendations on the use of antibiotics in animal husbandry and veterinary medicine. The recommendations were adopted and implemented by many other countries (Brander 1977; Bell 1986).

The degree of toxicity of pesticides to humans and other organisms differs considerably between each type of pesticide: a small amount of one pesticide might cause severe illness or even death, while larger quantities of another pesticide might have little or no effect, even if ingested. A pesticide might also have selective toxicity, causing death of one type of organism, but having little or no effect on other types of organisms (Hassall 1987). Some pesticides degrade quickly on the crop and in the soil, whereas others may persist in one form or another for longer periods. These persistent pesticides, which are mostly lipophilic, accumulate in animal fat and in particular tissues such as liver and kidneys, and the pesticide levels become higher than those in crops. Continuous consumption of either animal fat, liver, or kidney might caused serious health problems (Oudejans 1991).

Antibiotics in Food-producing Animals

In food-producing animals, antibiotics have therapeutic and non-therapeutic uses. Therapeutically, they are used for disease treatment and non-therapeutically as growth promoters. Ideally, the choice of antibiotic for disease treatment should be based on the identification and known sensitivity of the causal organism. However, in practice the treatment often does not wait for laboratory examination, as the aim is to return the acutely ill animals to optimal production as rapidly as possible. The infected animal might also spread the disease to other animals in the flock or herd. And, to achieve an effective therapeutic effect, antibiotics should be administered at a therapeutic dose rate (Bell 1986). Legally, antibiotics for disease treatment are available only on prescription, and only those antibiotics registered and approved are available in the market. However, antibiotics are easily obtainable without any prescription.

As growth promoters, antibiotics are usually added to feed at low concentrations, below therapeutic dose rate. Antibiotics added to the feed of the growing animal improve the growth rate and feed efficiency. The growth response of animals to antibiotics is a complex interaction of nutritional, physiological, and disease factors. It varies with animal species, age, the feed environment, and other factors. The growth-promoting properties of antibiotics have led to improper use of antibiotics. Farmers have been using any antibiotics available in the market, with no consideration for the hazards of those antibiotics to the animals or to the consumers of animal products.

When antibiotics are administered to food-producing animals, the appropriate withholding period should be observed before the animal products are consumed. Withholding time is the time taken for the concentration of a drug to fall below the acceptable level after the last dose has been administered. The withholding time for drugs given to farm animals affects the presence of residues of the particular drug in the animal products. Thus, it is expected that no residue will be detected or the residue will be below the recommended maximum residue limit (MRL) if the animal products are consumed after the withholding period. The withholding time is influenced by a number of factors, such as the dose rate, the dosage form of the drug, the route of the administration, the physical and chemical properties of the drug and the patho-physiological status of the animal (Baggot 1977; Debackere 1990). Table 1 lists the recommended use and withholding periods for antibiotics approved for use in food-producing animals in Indonesia.

Antibiotics added to animal feed have increased the exposure of animals to the antibiotics, as animals would be exposed to the antibiotics for their life time. Treatment with antibiotics should be terminated a few days before the animals are killed. In animals producing egg and milk, the products should not be consumed while the animals were on medication. In practice, for economic reasons, farmers have been selling all products, even those from animals which are under medication. Reports from Australia, the USA, and Indonesia indicated that most likely the appearance of antibiotic residues in animal products is the result of failure to observe withholding times (Herrick 1993; Spence 1993; Kusumaningsih 1996).

Like those antibiotics for medication, only antibiotics registered and approved as feed additive are allowed to be marketed for that purpose. Table 2 lists

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Table 1. Recommended usage and withholding period for antibiotics registered for food-producing animals in Indonesia

Antibiotic	Form	Animal species	Withholding period (days)
Ampicillin	injectable	poultry	5
	injectable	cattle	6
Amprolium	oral	cattle	1
Dihydrostreptomycin	injectable	cattle	30
	injectable	pigs	30
Streptomycin	oral	poultry	4
	oral	cattle	2
Furazolidone	oral	poultry	5
	oral	pigs	5
Nitrofurazon	oral	poultry	5
	oral	pigs	5
Carbadox	oral	pigs	70
Chlortetracycline	injectable	poultry	15
Oxytetracycline	injectable	poultry	15
Tetracycline	injectable	poultry	15
	oral	cattle	5
Penicillin G	injectable	cattle	5
	injectable	poultry	5
Penicillin + streptomycin	injectable	cattle	30
	injectable	pigs	30
Erythromycin	injectable	pigs	7
	injectable	cattle	14
Sulfonamides	oral	cattle	7–15
Monensin	oral	poultry	3
Tylosine	oral	pigs	2

compounds approved as feed additives, classified as either non antibiotic and antibiotics (Anon. 1994). Attempts to minimise production costs, have led farmers to use the cheapest antibiotics available on the market. For example, chloramphenicol is used despite the fact that it is banned for use in food-producing animals. Exposure to even small quantities of chloramphenicol may result in fatal aplastic anaemia in such animals. Nevertheless, chloramphenicol remains the drug of choice for treating certain diseases, especially typhoid, where the advantages appear to outweigh the potential risk (Bell 1986).

Pesticides in Food-producing Animals

Farm animals are usually exposed to pesticides unintentionally through contaminated animal feed, or a contaminated environment in which the animals were raised. Pesticide contamination of animals products due to direct application of pesticides to the animals for disease treatment is very rare. Ticks, flies, and scabies are the common animal disease usually treated with pesticides. However, in Indonesia, the use of pesticides to control livestock diseases is not common (Sosromarsono et al. 1977).

In Indonesia, as in most other countries, pesticides are more extensively used in crop production than in other areas of agriculture. The intensification program for crop production has brought an increased use of pesticides. Furthermore, efforts to reduce production cost in crop farming have caused more intensive use of pesticides. As a result crop products have been contaminated and some of these products then used as animal feed ingredients. The use of pesticides in stored products is another possible sources of crop contamination with pesticides. A report from the Diagnostic Laboratory of Balitvet supports this argument (Table 3). Feed factories or individual farms sent samples for pesticides analysis only if contamination was suspected (Indraningsih and Yuningsih 1995).

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When pesticides are deliberately added to stored crops, the chance of pesticide residues being present is higher than the use of pesticides during the growth of the crops. In addition to the type of application, the type of pesticides applied would also affect the presence of residues in the crop products. A great many types of pesticides are available, each type having different physical and chemical properties. Some pesticides are quite persistent, while others degrade rapidly. Farmers lacking education and knowledge, especially in developing countries, invariably choose the cheapest available pesticide rather than the safest ones. About half of the poisoning cases and nearly three quarters of the human deaths due to pesticides are estimated to occur in developing countries, although they use only 20% of the total world pesticides (Oudejans 1991).

Table 2. Compounds approved for use as feed additives for food-producing animals in Indonesia.

Non antibiotics	Antibiotics
Aklomide	Zinc bacitracin
Amprolium	Virginiamycin
Buthinorate	Flavomycin
Clopidol	Hygromycin
Dequinate	Monensin
Ethopabate	Salinomycin
Levamisole	Spiramycin
Piperazine	Kitasamycin
Tetramisole	Tylosin
Robenidin	Lasalocid
Roxarsone	Avilamycin
Sulfachlorpyridazine	Avoparcin
Sulfadimethoxine	Envamycin
Sulfanitrane	Colistine
Sulfaquinoxaline	Lincomycin
Buquinolate	Maduramycin
Nitrofurasone	Narasin
Furasolidone	Natacyn
Phenothiazine	
Halquinol	
Pirantel tartarte	
Olaquindox	
Aluminum silicate	
Nitrovin	

Antibiotic and Pesticide Contamination

Animal products contaminated with antibiotics and pesticides have been reported not just from Indonesia, but also from developed countries (Spence 1993; Nakazawa 1995; Gibbons et al. 1996). This suggests that there is global concern on the health impact of chemical residues in food. Consumers are concerned more than ever before about the quality and safety of food products, including food of animal origin. Food safety concerns not only consumers, but also veterinarians, producers, processing facilities, and regulatory officials throughout the world (Riviere 1991; Stenholm and Waggoner 1992; Angulo 1996).

A study was conducted by the Food Safety and Inspection Service of the United States Department of Agriculture to describe patterns of chemical residue violations in beef in 12 states of the USA during 1991, 1992, and 1993. The total number of violations fell during the 3 years studied. However, the residues in beef from slaughtered dairy cows increased. In 1991, there were 3249 residues found in 2734 carcasses, in the second year 3132 violative residues were found in 2813 carcasses and, in 1993, there were 2317 violative residues were found in 2051 carcasses. Neomycin was the most frequently identified chemical residue, followed by tetracycline, gentamycin, oxytetracycline, and penicillin (Gibbons et al. 1996).

Balitvet (1990) found residues of oxytetracycline in 65 of 93 chicken meat samples, and 35 of the samples were contamined above the recommended MRL. Chicken meat was collected from slaughterhouses in West Java and the Jakarta area (Table 4). Analyses were carried out only for tetracycline and sulfonamide residues. Pesticide contamination of chicken meat was also reported from the same study. However, although contamination by pesticides was detected, there was no sample with a pesticide level above the MRL. Field studies of farms in West Java found that different types of antibiotics were heavily used on poultry farms (Murdiati and Bahri 1991). Tetracycline residues and sulfonamides were also reported in chicken meat sold in the market in Bali and Kalimantan (Dewi et al 1996; Anon.1996).

While antibiotic tetracyclines have been widely used in poultry farming, β lactams are the antibiotics most widely used in dairy farming in Indonesia. Penicillin residues were detected in fresh milk collected from farmers and milk men in West Java, and in pasteurised milk from the supermarkets. Residues of penicillin were detected in 43% of 31 pasteurised

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milk samples, while 24% of 416 and 35% of 128 samples residues were detected in fresh milk collected from farmers and milk men, respectively (Sudarwanto 1990). Antibiotic residues were detected in 27 of 120 milk samples collected from dairy farms in Jakarta, 5 samples were positive for penicillin, 6 for tetracyclines, 9 for aminoglycosides, and 7 for macrolides (Lastari and Murad 1995).

Organochlorine pesticide residues were reported in domestic bird eggs obtained from Bogor markets and also from wild bird eggs taken from nest sites on Seribu Island (Indraningsih et al. 1988). The domestic bird eggs included eggs of local chickens, eggs of improved chickens, duck eggs, and quail eggs. The improved chicken eggs had lower levels of pesticides residues than eggs from other species, suggesting that free-ranging birds had access to pesticide contamination, as improved chickens were raised in intensive confined farming. Although the residue levels were below the MRL, the observations indicated that environmental pesticide contamination has occurred in Indonesia. Later reports on the pesticide residues in bird eggs found both organochlorine and organophosphate in more than 50% of 90 samples, with a diazinon level above the MRL in 1 of 30 duck eggs, and 5 of 30 either local chicken eggs or improved chicken eggs (Balitvet 1995).

Conclusions

Agricultural industry, including livestock production depends on drugs and other chemicals to control disease and to improve animal performance. In some cases, this dependence has caused violative residues

Table 3. Pesticides residues in animal feeds and feed ingredients received by the Diagnostic Laboratory Balitvet in 1984–1995

Pesticides	Animal feed (n = 31)		Feed ingredients (n = 15)	
	Number of positive samples	Range of concentration (μg/g)	Number of positive samples	Range of concentration (μg/g)
Diazinon	11	0.018–0.47	3	0.015–0.12
Malathion	4	0.105–0.99	–	–
Fention	–	–	2	0.69–0.85
Endosulfan	6	0.100–2.50	2	0.66–1.00
Lindane	-	–	2	0.05–0.56
Heptachlor	-	–	2	2.30–4.55
Metoxychlor	1	5.00	–	–

Table 4. Antibiotics and pesticide residues detected in chicken meat collected from slaughterhouses in West Java and Jakarta

Sample	n	Residues	Positive samples		Samples above MRL	
			n	%	n	%
Chicken meat	93	Oxytetracycline	65	70	35	37
		Chlortetracycline	28	30	15	16
		Tetracycline	–		–	
Chicken meat	61	Lindane	38	62	–	
		Aldrin	18	29	–	
		Endosulfan	15	25	–	
		DDT	20	33	–	
		Dursban	6	10	–	
		Diazinon	3	5	–	
		Heptachlor	37	61	–	

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in animal products, especially if the chemicals were used improperly.

Antibiotic residues were detected more often than pesticide residues, since animals are deliberately exposed to antibiotics, while the exposure to pesticides is usually unintentional through a contaminated environment.

Public concerns are continuously growing on the pesticides and antibiotics contamination of animal products, and other aspects of food safety of animal origin.

Consideration of food safety and quality must start at the farm and receive attention at all stages in the food chain of animal products. All segments of the food industry, the regulatory officers, and the scientific communities have attempted to reduce the potential problem of food safety.

The hazard analysis critical control point (HACCP) approach has been a promising concept for controlling contamination of food.

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Approaches to Risk Assessment of Chemicals in the Environment

C.J. Van Leeuwen*

The methodologies to assess the risks of chemicals in the European Union (EU) are generic and may include local and regional assessments. For realistic site-specific analyses of risks of contaminants in water, soil, and air, more refined models are needed. Models must be as simple as possible and as complex as needed. The bottleneck for risk assessment is the lack of adequate data on the production, use, disposal, fate, and effects of chemicals. Even for well-known pollutants, most of the environmental quality criteria for water are based on a few acute toxicity data for fish, daphnids, and algae. The situation is even worse for aquatic sediments and soil, despite the enormous costs involved in clean-up operations. The scientific justification of quality standards and the financial consequences these may have are unbalanced. In some areas in the Netherlands, pesticide concentrations in rain are acutely toxic to aquatic life. Drinking water quality standards are exceeded in some cases. It illustrates the need to further explore the fluxes of chemicals in the air and their ecological consequences in, for example, shallow surface waters and top soils. For the monitoring of mixtures of chemicals in surface waters integrated approaches are needed.

AFTER Japan and the USA, the European Union (E.U.) is the largest producer of chemicals in the world. Historically, there are differences in the national policies on chemicals in the E.U. member countries. In order to improve the internal market harmonisation of the various national approaches is taking place. This has resulted in a myriad of E.U. directives and regulations dealing with the safe production, use, import, storage, transport, and disposal of chemicals in order to protect humans and the environment. Human health (workers, consumers, indirect exposure of people through the environment) and ecosystem health have now received equal attention.

During the past decade there have been many developments in the area of risk assessment, e.g. within the Organisation of Economic Co-operation and Development (OECD) and the E.U.. In the E.U. there are several legislative instruments regarding new chemicals (the seventh amendment to Directive 67/548/EEC), existing chemicals (E.C. Council Regulation 793/93), plant protection products (Directive 91/414/EEC), and biocides (in preparation). Risk assessment in the E.U. involves three parties, i.e. the European Commission (policy co-ordination), the Member States (responsible for risk assessments and enforcement), and the producers and importers (submission of data). This three-party process requires an open, transparent process of communication and co-ordination. Academia plays a role in risk-assessment-related research. A short description of these regulatory frameworks is provided by Vermeire and Van Der Zandt (1995) and Van Leeuwen et al. (1996a).

A harmonised approach is needed for the assessment of new chemicals and the approximately 100 000 existing chemicals, including pesticides and biocides. An important instrument for the notification and evaluation of new and existing chemicals is the technical guidance document (TGD). The TGD contains methodologies for the toxicological and ecotoxicological risk assessment of new and existing chemicals (Commission of the European Communities 1996). This guidance takes the form of detailed

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descriptions in terms of data needs, release estimation, fate parameters, exposure models, and effect models. This guidance is seen as the state of the art. Deviation from this guidance remains possible, provided that clear further information or explanation is submitted. The TGD has been developed in close cooperation between the European Commission, the chemical industry, and the E.U. member states (Commission of the European Communities 1996). It should be noted that the TGD and all other risk assessment procedures, contain numerous science-policy decisions (Milloy et al. 1994).

A recent development is a European Union System for the Evaluation of Substances (EUSES 1996), based on the TGD. EUSES is a simple, straightforward, and transparent set of risk-assessment methodologies, recognising: (a) the limited data sets usually available; (b) the accuracy and variability of these data; and (c) the uncertainties in many of the assumptions. A new version of EUSES will be developed during 1988. It will include risk assessment methodologies for plant protection products and biocides. A standardised approach for ecological risk assessment (ERA) and human health risk assessment has three advantages:

1. Risk assessment is accompanied by complete transparency of methods, assumptions, and uncertainties. Risk assessments become predictable and their mutual acceptance will increase, leading to an increased co-operation and 'sharing the burden'.
2. Conditions are created for regular methodological improvements and adaptations on the basis of both scientific and legislative developments.
3. Time and money can be spent more effectively on 'priority chemicals', chemicals for which in-depth ERA and expert judgment are required.

In this paper, only four aspects of chemical risk assessment will be discussed, i.e. (a) the generic nature of risk assessment methodologies described in the TGD and its role in aquatic ecosystem management, (b) the data gap problem in deriving environmental quality criteria for water, aquatic sediments and soil, (c) the importance of dry and wet deposition from air and the impact on water quality, and (d) approaches to the determination of mixture toxicity.

The Generic Nature of Risk Assessment

The TGD (Commission of the European Communities 1996) is comprised of the following elements: a standardised minimum data set (the so-called base-set) which contains (short-term) toxicity and ecotoxicity data, basic physicochemical data, use information, and import/production data. A standardised realistic worst-case emission scenario is used by applying emission scenarios for 'use categories' based on use-specific emission patterns and emission factors. PECs are determined for local and regional circumstances using multimedia exposure models. These models operate by simplifying environmental media— air, water, soil, sediment, and biota—into homogeneous compartments or boxes and then tracking movement, degradation, and accumulation of chemicals from box to box. The TGD also contains modules for indirect exposure of humans through the environment (via drinking water, fish, plants, milk, and meat). Geographic, hydrological, and climatic variability are excluded in these exposure models as 'standardised environments' are used for the exposure calculations. For instance, it is assumed that 70% of the waste water passes through sewage treatment plants. For the regional PEC a Mackay Level-III model is used.

Procedures for assessment of chemical effects are also provided in the TGD. Extrapolation factors and methodologies are clearly described. Estimation methodologies, i.e. quantitative structure–activity relationships (QSARs) and a critical assessment of their limitations are given for physicochemical parameters, (bio)degradation, sorption, and (eco)toxicity data. Bioconcentration in fish and earthworms is used to estimate biomagnification. Two routes are considered: water –fish–fish-eating mammal or fish-eating bird, and soil–earthworm–worm-eating bird or mammal. For most existing chemicals, lack of data is the rule not the exception. Often, basic physicochemical properties, monitoring data, and toxicological information are not available (Van Leeuwen 1992, Van Leeuwen et al. 1996a). Because of the lack of data, assessment of effects in soil and sediments will often be based on the principle of equilibrium-partitioning between water and sediments and soil, respectively (Di Toro et al. 1991).

The shortcomings of this generic approach for ecological risk assessment (ERA) are obvious. Many ecological aspects are neglected. Numerous assumptions and arbitrary decisions are made to arrive at a risk characterisation for humans and ecosystems. These include assumptions concerning equilibrium partitioning, instantaneous mixing in the compartments of the exposure model, simplistic representations of aquatic and terrestrial ecosystems,

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simplifications of exposure, and the use of only a few biomagnification routes. Probably the greatest uncertainties are related to actual emissions of industrial chemicals during the various stages in their life cycle, with little known about the actual use pattern of industrial chemicals. The use of product registers can only partly overcome this problem. For agricultural pesticides this lack of knowledge is less problematic, as crop-specific dosages are known, leading to better prediction of the actual emissions of pesticides

The enormous variations in climatic (e.g. temperature and precipitation), hydrological, and geographic (e.g. soil type) conditions, and the variability in actual measures and techniques to reduce emissions or treatment of waste (water) are captured by assuming 'average' values. This implies that the ERA methods developed in the E.U. are focused on the assessment of 'generic risks of chemicals in the E.U.'. They are not suitable to assess site-specific or actual risks (Van Leeuwen et al. 1996a). For these questions—which are entirely different—more sophisticated models are needed. However, more sophisticated models will greatly increase the data requirements and thus the costs for authorities and industry. Even then, complete reduction of uncertainties is impossible. The quotation to be used here is the one from Aristotle: 'It is the mark of an instructed mind to rest easy with the degree of precision which the nature of the subject permits and not to seek an exactness where only an approximation of the truth is possible.'

Deriving Environmental Quality Criteria

According to the OECD (1989) assessment of the effects of chemicals follows a tiered system in which preliminary, refined, and comprehensive stages can be distinguished. For the derivation of predicted no effect levels (PNECs) for chemicals on the basis of limited ecotoxicological data, assessment factors are used. These assessment factors are modifications of assessment factors which were originally developed by the United States Environment Protection Agency (EPA). Limited ecotoxicological data include estimates on the basis of QSARs, short-term toxicity data on fish, daphnids, and algae, and a few (less than 4) long-term toxicity data (Table 1). Refined effects assessment is applicable in case more than four no observed effect concentrations (NOECs) are available. In this case, statistical extrapolation methodologies may be used, e.g. the one developed by Aldenberg and Slob (1993). Comprehensive risk assessment is applicable in cases where reliable field tests are available. This information is rarely available (Van Leeuwen et al., 1994). In order to derive PNECs for aquatic sediments and soil it is preferable to use sediment and soil-dwelling organisms. Since this ecotoxicological information is rarely available, the PNECs are estimated on the basis of the equilibrium-partitioning (EP) between water and sediment or soil. Van Leeuwen (1995) gives a detailed description of the extrapolation techniques and the equilibrium-partitioning.

Table 1. Assessment factors to derive a PNEC (Commission of the European Communities 1996)

Available information	Assessment factor[a]
At least one short-term L(E)C_{50} from each of three trophic levels of the base-set (fish, *Daphnia* and algae)	1000
One long-term NOEC (either fish or *Daphnia*)	100
Two long-term NOECs from species representing two trophic levels (fish and/or *Daphnia* and/or algae)	50
Long-term NOECs from at least three species (normally fish, *Daphnia* and algae) representing three trophic levels	10
Field data or model ecosystems	x[b]

[a] Detailed explanation is given in the Technical Guidance Document (Commission of the European Communities 1996).
[b] Reviewed on a case-by-case basis.

In order to illustrate the current attributes of environmental quality criteria, an inventory was made of ecotoxicological data available for well-known chemicals, i.e., 17 metals, 10 polycyclic aromatic hydrocarbons, 45 volatile chemicals, and 78 pesticides. The data were obtained from the project 'integrated environmental quality' in the Netherlands. Five data categories were distinguished (the extrapolation methodologies are given in parentheses):

a) fewer than four short-term ecotoxicity data (EPA);
b) more than three NOECs (A&S);
c) no data or not determined (ND);
d) EP using fewer than four short-term aquatic data (EP/EPA);
e) EP using more than three aquatic NOECs (EP/A&S).

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The results of this inventory are presented graphically in Figure 1. It can be concluded that, even for well-known chemicals, most of the PNECs are based on short-term ecotoxicity data. Note too that about 15% of the chronic toxicity of the chemicals is estimated on the basis of QSARs. In fact, most of the environmental quality criteria are based on PNECs using preliminary effects assessments. For soil and sediment, many standards are based on very few toxicological tests. In order to overcome this problem the concept of equilibrium partitioning is applied and this is probably the best we can at the moment, but it is certainly not scientifically justified for many chemicals. Moreover, many species in soil are exposed in a totally different manner. It is clear that some investment in refined effects assessment would lead to more realistic standards. When we compare this with the enormous costs of soil and sediment clean-up (this is approximately one-third of the Netherland's national environment budget) it can be concluded that the scientific justification of quality standards and the financial consequences this may have, are unbalanced.

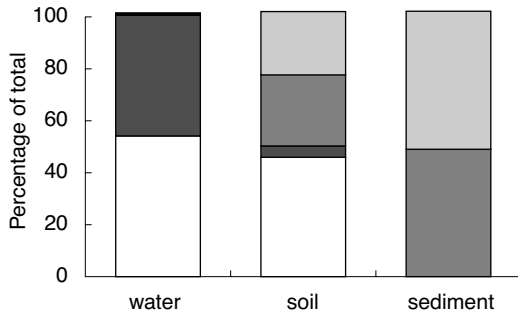

Figure 1. Data availability for the derivation of PNECs for 150 chemicals: Aldenberg and Slob (1993); EP/EPA; EP/Aldenberg and Slob; EPA; no data. See text for details.

Deposition and Water Quality

In 1994, the Province of South-Holland published a report on the occurrence of pesticides in rain (Provincie Zuid-Holland, 1994). These monitoring data were compared with environmental quality criteria for drinking water and ecotoxicological data published in the literature. From these data it appeared that the concentrations in rain were high. The concentrations of dichlorvos, parathion, and endosulfan exceeded the lowest LC_{50}-values obtained from short-term laboratory studies (Table 2). For dichlorvos, the mean and maximum concentrations exceeded the LC_{50} by factors of 3 and 14, respectively. At some places in the Netherlands rain is acutely toxic to aquatic life and adverse effects at the ecosystem level cannot be excluded. Concentrations of dichlorvos and parathion in rain exceed the drinking water standards of 0.1 μg/L, whereas for endosulfan, the highest concentrations measured equalled the drinking water standards. Very often, pesticide concentrations in rain are higher than their concentrations in surface water.

Table 2. Quotients of the mean and maximum concentrations measured in rain and the lowest LC_{50}-value reported in the literature for three pesticides (De Poorte en Van Leeuwen 1997).

Chemical	Quotient of mean concentration in rain and the lowest LC_{50}	Quotient of maximum concentration in rain and the lowest LC_{50}
Dichlorvos	3.4	14.3
Parathion	0.8	8.3
Endosulfan	0.2	1.5

Similar observations were made in other places in the Netherlands (Heemraadschap Fleverwaard 1993). They can be explained on the basis of the use and emission patterns of the pesticides and their environmental distribution. On the basis of the inventory of emissions of pesticides to water, soil, and air, it appeared that the emissions to air account for approximately 96% of total emissions (Table 3).

Table 3. Emissions of pesticides in 1995 expressed as active ingredient (Commissie van Deskundigen 1996)

Compartment	Emission (tonnes active ingredient/year)	Percentage
Soil	41	1.3
Groundwater	26	0.8
Surface water	46	1.5
Air	3090	96.4
Total	3203	100

Surface waters, which receive 1.5% of all emissions, are the most frequently monitored environmental compartment. Direct emissions to surface water still cause some problems. The soil compartment, including groundwater, receives 2.3% of all

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emissions. These compartments are monitored less frequently. Concentrations of pesticides in air and rain are not monitored on a routine basis. The ecological effects of dry and wet deposition of pesticides on shallow surface waters or topsoils, are hardly known. The same is true for industrial chemicals.

The occurrence of pesticides at acutely ecotoxic levels in rain suggests that biodiversity may be adversely affected, even in nature reserves. It shows the need to restrict the use of mobile, persistent, and ecotoxic pesticides. In the Netherlands, it is concluded that only 5% of the pesticides cause about 95% of the environmental problems. This offers a good opportunity for further sanitation and environmental improvement.

This example shows the relatively high importance of diffuse pollution and transboundary transport of chemicals. The importance of these transport routes has received attention, for example at the last ministerial conference on the North Sea. Nevertheless, the magnitude of the fluxes of many chemicals and their ecological effects still need to be explored in greater detail.

Approaches to the Toxicity of Mixtures of Chemicals

Much of the information available on the ecotoxicity of substances relates to single chemicals tested under laboratory conditions. Yet, it is unusual to find an aquatic ecosystem which is polluted by just a single toxicant. Mixture toxicity has received considerable attention by the European Inland Fisheries Advisory Commission (1987). Several reviews have been published, e.g. Calamari and Vighi (1991 and Hermens (1989). Based on the results of mixture toxicity studies carried out with aquatic organisms it can be concluded that the concept of additivity of the effects must be taken into account in the derivation of water quality criteria. Recently an attempt was made to summarise the options to deal with the problem of mixture toxicity (Van Leeuwen et al. 1996b). These options are summarised in Table 4.

One of the approaches presented in Table 4, i.e. group parameters, may be less familiar. The so-called group, or surrogate, parameter approach is an analytical procedure that is able to quantify the total

Table 4. Components of an integrated approach to water quality-based toxics control (modified after USEPA 1991)

Control approach	Capabilities	Limitations
Chemical-specific methods	Human/ecological protection	Do not consider all toxics present
	Generally accepted approach	Bioavailability not measured
	Straightforward treatability less	Neglect interactions of mixtures
	Expensive in case of only few chemicals	Complete testing is very expensive
	Allows predictions of impacts	Biological impairment is not measured
	Fate understood	Complete fate and toxicological data are available for very few chemicals
Group parameters	Aggregate groups of chemicals	Does not consider all toxics present
	Unknown toxicity partly covered	Biological impairment is not measured
	Relatively cheap	No direct human/ecological health
		Bioavailability partly measured
Toxicity tests	Aggregate and accurate toxicity	No direct human/ecological health
	Unknown toxicants addressed	Incomplete toxicology (few species may be tested)
	Bioavailability measured	Incomplete knowledge of the causative toxicants
	Prevents impacts	No direct treatment
		Persistency not taken into account
Bioassessments	Measure actual effects historical	Impact has already occurred
	Trend analysis total effects of all sources	Cause of impact not identified
	No differentiation of source	No direct human health protection

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amount of compounds from a single toxicological class that are present in a sample, or alternatively, the total amount of biological activity associated with that particular class. In practice, most group parameters that have been described to date are severely limited in either their relevance (cf. AOX, EOX, TOX measurements), both chemically and toxicologically, or in the number of groups or effects that can be monitored. One promising approach has been described recently for the quantification of the total narcosis-related toxicity of a mixture (Verhaar et al. 1995; Van Loon and Hermens 1995). Other groups that could in principle be monitored in a similar way are: reactive compounds, which could be quantified by assessing the amount of modified substrate in an assay that uses an appropriate substrate for a certain type of reactivity; and receptor-specific compounds.

It can be concluded that toxicity tests are cost-effective tools to detect, control, or predict biological effects of (mixtures of) chemicals. The application of short-term bioassays should be the first step in a tiered testing system. The next step should be the identification of the causative chemicals, using analytical chemical tools. The application of group parameters with a clear toxicological background may reduce costs and enhance our understanding of toxicological effects.

Conclusions

1) The risk management process is greatly hindered by the lack of knowledge of actual concentrations and effects of chemicals. Generic risk assessments are suitable to assess the risk of chemicals, but are often not suited to assess site-specific risks.
2) Data on environmental quality criteria are not available for most chemicals and the scientific quality of what environmental quality data are available on well-known chemicals is relatively poor.
3) For scientific and practical purposes there is a need to increase our knowledge of the impact of transport of chemicals through the air and their ecological effects. For instance, 95% of pesticides in the Netherlands are emitted to the air. Pesticide concentrations in rain are relatively high, but few data on the chemical monitoring of these pesticides exist . For some pesticides the measured concentrations were acutely toxic to aquatic life.. The magnitude of the fluxes of many chemicals in the air compartment and their ecological effects still need to be explored in greater detail.
4) Integrated approaches to water quality monitoring are needed to deal with the problem of mixture toxicity.

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Residues and Dissipation of Selected Pesticides in Paddy Rice Agroecosystems in Malaysia

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Abstract

A broad-scale survey was conducted on pesticide residues in surface and groundwater in rice agroecosystems of the Muda rice-growing areas, the 'rice-bowl' of the country, and the agricultural plain of Kelantan in Malaysia. Dissipation of lindane, 2,4-D, and paraquat was assessed through laboratory studies.

Pesticide residues were evident in the surface/groundwaters of the Kelantan Plain and MADA agroecosystem. Samples taken from piezometers and wells generally showed similar results. The cyclodiene insecticide endosulfan, in the form of the β-isomer, and its oxidation product endosulfan sulfate, was found extensively. The organochlorine residues detected were lindane, aldrin, dieldrin, and DDT. Their residues were generally low.

In many samples from the Kelantan Plain 2,4-D was found but, because it is not persistent, is not expected to cause a problem. The occurrence of 2,4-D, paraquat, and molinate residues in the surface waters of the Muda rice ecosystem was seasonal and more widespread during the period when the herbicides were applied. Residues of two commonly used pesticides, thiobencarb and carbofuran, did not pose a problem to the area's water resources.

RICE is a staple food crop in several Asian countries, including Malaysia. Pests continue to pose a threat to its production in spite of efforts to produce resistant varieties. Pesticides remain an essential tool for protecting rice crops in Malaysia, as a component of integrated pest management. Lindane, the only organochlorine insecticide allowed for agricultural use, offers effective control of the main stem borer species, *Tryporyza incertulas* and *Chilo polychrysus* (Lim and Heong 1977). The popularity of 2,4-D for weed control in rice is reflected in the $M4 000 000 spent on purchases of it each year (Cheah and Ooi 1988). Other commonly used pesticides include carbofuran, endosulfan, BPMC, MIPC, paraquat, thiobencarb, molinate, propanil, pretilachlor, glyphosate, metsulfuron, quinclorac, and fenoprop-ethyl (Ho et al. 1991). These chemicals are frequently discharged into the rice ecosystem.

The paddy land under the jurisdiction of the Muda Agricultural Development Authority (MADA) in Kedah State, and the agricultural plain of Kelantan, are two of the most important rice-cultivation areas in Malaysia. Water is an important resource to be conserved, and water has been recycled in the MADA agroecosystem which has an established network of irrigation and drainage channels. In the latter ecosystem, groundwater is an important source (70%) of potable water for rice farmers

Materials and Methods

Sampling sites

In the MADA agroecosystem, 12 sites were identified for the study, with surface water samples collected from 5 sampling points at each of these. The

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sampling points included farmers' fields, canals, drains, and rivers, and were selected to include partially and totally recycled water. Water was sampled monthly from November 1992 until July 1993. There were about 60 samples taken in each collection.

In the Kelantan Plain, 30 sites were selected for the study. The choice of sampling sites for groundwater was based on location, site elevation, cropping system, source of irrigation, and type of farm. A piezometer was installed at each site to collect water seeping from the surrounding paddy area.

Pesticides examined

The pesticides examined were: the organochlorine pesticides aldrin, dieldrin, lindane, and DDT and its metabolites DDE and DDD; endosulfan alpha and beta isomers and their oxidation product endosulfan sulfate; and carbofuran. The herbicides investigated were paraquat, 2,4-D, molinate, and thiobencarb.

Analytical procedure

Organochlorines

Organochlorine pesticide residues in water were analysed by liquid–liquid extraction and gas chromatography method as described in APHA (1989). The extracts were examined on a gas chromatograph equipped with an electron-capture detector (Model 8500, Perkin-Elmer Corporation, Connecticut, USA). The capillary column used was 25 m long and packed with 3% phenyl methyl silicone gum, 0.32 mm × 0.25 mm film thickness. Injector and oven temperatures were maintained at 190˚C and 200˚C, respectively. Oven temperature was programmed at a rate of 2˚C per minute from 120˚C to 250˚C. Under these conditions, the minimum detection levels were 0.002 ng/mL for lindane, aldrin, dieldrin, and endosulfan I (α-isomer) and 0.004 ng/mL for endosulfan II (β-isomer), endosulfan sulphate, and DDT and its metabolites DDD and DDE. To confirm the results, the extracts of randomly selected samples were rechromatographed on another column of different polarity.

2,4-D and paraquat

2,4-D and paraquat residues were determined using a Strategic Diagnostics immunoassay test kit. Standard solutions of 0.5, 10, and 100 ng/mL and a negative control were supplied with the kit. Three replicates per sample were conducted. Optical density values for each well reaction on the microtitre plate were obtained using an enzyme-linked immuno sorbent assay (ELISA) plate reader at 450 nm wavelength. A calibration graph was prepared with readings from the standard solutions, and was used to compute sample concentrations. The minimum detection limits for 2,4-D and paraquat were 0.5 ng/mL and 0.03 ng/mL, respectively.

Cabofuran, molinate, and thiobencarb

Carbofuran was determined using solid-phase extraction (SPE) and liquid chromatography in the method of Beauchamp et al. (1989). Molinate and thiobencarb were also analysed through the use of C18 and C8 SPE cartridges, respectively, and liquid chromatography with UV detection. The extracts were examined on a high performance liquid chromatograph (Waters Modular System with 501 HPLC Pump, Lambda-Max Model 481 LC spectrophotometer and Model 745 data module). The detection limits were 2 ng/mL for carbofuran and 0.1 ng/mL for molinate and thiobencarb.

Results and Discussion

MADA agroecosystem

The cyclodiene endosulfan, in the form of β-isomer and endosulfan sulfate, was found everywhere in the water of the MADA agroecosystem. Residues of the insecticide were detected in most of the water samples at levels ranging from <0.005 ng/mL to 25.5 ng/mL (Table 1). It is interesting to note that although the α-isomer is relatively more stable than the β-isomer (Guerin and Kennedy 1992), the results generally showed a higher number of samples containing the β-isomer. Residue levels were also generally higher. The observation may be attributed to the loss of the α-isomer through volatilisation. The ubiquity of endosulfan sulfate reinforces the conclusion that the oxidation product of endosulfan is very stable and considerably more persistent than the parent isomers (Miles and Moy 1979; Guerin and Kennedy 1992). The half-lives of endosulfan sulfate in aqueous media have been reported to be in the range 140–184 days.

The occurrence of other organochlorine insecticides such as lindane, DDT, aldrin, and dieldrin was sporadic. Except for lindane, use of organochlorine insecticides has been restricted or banned in Malaysia. Residue levels in most of the samples were below 0.1 ng/mL (Table 1). Lindane residues ranging from <0.002 ng/mL to 0.23 ng/mL found in the study were in the same order as those reported in India (Sujatha et al. 1993; Iwata et al. 1994).

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Table 1. Pesticide residues in water resources in Muda agroecosystem

Time of sampling	No. analysed	Pesticides detected	No. detected	Concentration range (ng/mL)
November 92	26	Lindane	11	<0.002–0.15
	26	Aldrin	1	<0.002–0.01
	26	Dieldrin	16	<0.002–0.12
	26	Endosulfan	25	<0.002–0.37
	26	DDT	14	<0.004–0.08
	55	2,4-D	9	<0.5–0.70
	54	Paraquat	6	<0.03–0.10
	55	Carbofuran	9	<2.0–2.2
December 92	42	Lindane	7	<0.002–0.15
	42	Aldrin	6	<0.002–0.06
	42	Dieldrin	13	<0.002–0.10
	42	Endosulfan	33	<0.002–25.5
	42	DDT	13	<0.004–0.30
January 93	51	Lindane	22	<0.002–0.09
	51	Aldrin	26	<0.002–0.16
	51	Dieldrin	7	<0.002–0.04
	51	Endosulfan	49	<0.002–0.32
	51	DDT	4	<0.004–0.09
	30	Carbofuran	0	<2.0
February 93	33	Thiobencarb	0	<0.1
	34	Molinate	28	<0.1
April 93	20	Lindane	5	<0.002–0.23
	20	Aldrin	3	<0.002–0.15
	20	Dieldrin	3	<0.002–0.04
	20	Endosulfan	13	<0.002–0.04
	20	DDT	2	<0.004–0.05
	38	2,4-D	30	<0.5–2.80
	6	Thiobencarb	0	<0.1
	39	Paraquat	33	<0.03–0.09
	6	Molinate	0	<0.1
May 93	6	Lindane	2	<0.002–0.04
	6	Aldrin	3	<0.002–0.06
	6	Dieldrin	3	<0.002–0.06
	6	Endosulfan	5	<0.002–0.54
	6	DDT	2	<0.004–0.05
	57	2,4-D	6	<0.5–4.5
	52	Paraquat	5	<0.03–0.097
	9	Thiobencarb	0	<0.1
	9	Molinate	1	<0.1–0.2

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Table 1. (Cont'd) Pesticide residues in water resources in Muda agroecosystem

Time of sampling	No. analysed	Pesticides detected	No. detected	Concentration range (ng/mL)
June 93	27	Lindane	17	<0.002–0.14
	27	Aldrin	11	<0.002–0.04
	27	Dieldrin	11	<0.002–0.09
	27	Endosulfan	23	<0.002–2.3
	27	DDT	15	<0.004–0.03
	54	2,4-D	13	<0.5–120
	52	Paraquat	5	<0.03–0.19
	22	Thiobencarb	2	<0.1–0.9
	23	Molinate	6	<0.1–17.0
July 93	7	Lindane	7	<0.002–0.05
	7	Aldrin	6	<0.002–0.01
	7	Dieldrin	0	<0.002
	7	Endosulfan	7	<0.002–0.04
	7	DDT	7	<0.004–0.02

Lower lindane residues were generally observed in the river and estuarine waters of Thailand (non-detectable–0.018 ng/mL), Vietnam (0.00036–0.012 ng/mL), and Indonesia (0.00084–0.0037 ng/mL) (Iwata et al. 1994). The results of laboratory studies (Mathur and Saha 1975; Scheunert et al. 1987; Cheah et al. 1998), suggest that lindane is very persistent in soils, but several field studies have indicated rapid dissipation of lindane. In these cases the loss was attributed to rapid volatilisation from soils, rather than biodegradation (Samuel et al. 1988; Tanabe et al. 1991; Waliszewski 1993).

The occurrence of 2,4-D, paraquat, and molinate residues was seasonal and more widespread during the period when the herbicides were applied. Many water samples were found to contain 2,4-D residues exceeding the European Union drinking water standard of 0.1 ng/mL. These residues were not expected to persist in the environment in view of the short half-lives of these materials, which were reported to range from 3.4 days in an aerobic muck to 9.3 days under anaerobic conditions (Table 3) (Cheah et al. 1998). None of the samples in which paraquat was detected contained residues exceeding 0.1 ng/mL. Its unique property of strong adsorption to soil colloidal particles (Damanakis et al. 1970) may account for this observation. Residues of molinate were higher—up to 12.7 ng/mL. Thiobencarb and carbofuran residues did not pose a problem in the waters of this ecosystem. Thiobencarb was detected in only two samples, at levels close to the limit of detection. Carbofuran also was found in only two samples, with residue levels close to the limit of detection of 2 ng/mL.

The source of aldrin contamination was difficult to establish. Consistently low residue levels suggested that these residues may have originated from another locality, as long-range transport of aldrin has been demonstrated (Butarelli et al. 1991). In general, dieldrin was found more frequently than aldrin and, as with aldrin, it was difficult to locate the source of contamination. Contamination arising from its illegal use in the ecosystem, and from non-point sources as discussed previously, was possible.

Though the frequency of detection of DDT was low, the residue levels detected were higher than those observed in river and estuarine waters of several countries in Asia and Oceania. Lower DDT residue levels of 0.00023–0.00250 ng/mL, 0.00029–0.025 ng/mL, 0.00019–0.00027 ng/mL, 0.0000065–0.000016 ng/mL, 0.0000095–0.00019 ng/mL, 0.0000013–0.0011 ng/mL were found in water samples from Thailand, Vietnam. Indonesia, Japan, Taiwan, and Australia, respectively (Iwata et al. 1994).

Kelantan Agricultural Plain

Endosulfan was also found as a ubiquitous contaminant in the rice ecosystem of the Kelantan agricul-

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tural plain (Table 2). Other organochlorine insecticides such as lindane, aldrin, dieldrin, and DDT were also present. There was little difference in the type of pesticides found, the number of samples in which they were detected, and their levels between groundwater from piezometers and drinking water. The presence of organochlorine residues in surface water and groundwater has been reported in Malaysia previously (Tan 1992; Cheah et al. 1994). The occurrence of organochlorine pesticides arising from normal agricultural use has also been reported in the USA (Ritter 1990).

Table 2. Pesticide residues in water resources of rice agrosystem in the Kelantan Plain. The figures in parenthesis indicate the total number of samples analysed and the number of samples containing detectable residues.

Time of sampling	Pesticides detected	Concentration range (ng/mL)				
		Piezometer	Well	Drain	Irrigation canal	River
July 92	Endosulfan	<0.002–1.54 (45)(37)	<0.002–0.6 (36)(28)	<0.002–0.52 (35)(32)	<0.002–0.029 (5)(4)	<0.002–0.15 (3)(3)
	Lindane	<0.002–0.34 (45)(21)	<0.002–0.31 (36)(16)	<0.002–0.26 (35)(16)	<0.002–0.066 (5)(1)	<0.002–0.15 (3)(2)
	Aldrin	<0.002–0.17 (45)(19)	<0.002–0.17 (36)(18)	<0.002–0.31 (35)(18)	<0.002–0.06 (5)(2)	<0.002–0.076 (3)(1)
	Dieldrin	<0.002–0.046 (45)(18)	<0.002–0.097 (36)(17)	<0.002–0.03 (35)(13)	<0.002–0.005 (5)(1)	<0.002 –0.027 (3)(2)
	DDT	<0.004–0.241 (45)(6)	<0.004–0.061 (36)(9)	<0.004–0.66 (35)(4)	<0.004 (5)(0)	<0.004 (3)(0)
	2,4-D	<0.5–0.95 (23)(10)	<0.5–44.0 (13)(5)	<0.5–0.85 (14)(11)	<0.5–0.56 (2)(2)	<0.5 –0.70 (3)(3)
	Paraquat	<0.03–0.08 (11)(11)	<0.03–0.13 (7)(7)	<0.03–0.13 (8)(8)	<0.03–0.03 (1)(1)	<0.03–0.10 (1)(1)
September 92	Endosulfan	<0.002–0.14 (33)(21)	<0.002–0.13 (29)(21)	<0.004–0.15 (22)(17)	<0.002–0.02 (4)(2)	<0.002–0.022 (3)(2)
	Lindane	<0.002–0.13 (33)(25)	<0.002–0.10 (29)(18)	<0.002–0.26 (22)(14)	<0.002–0.066 (4)(2)	<0.002 (3)(0)
	Aldrin	<0.002–0.09 (33)(15)	<0.002–0.17 (29)(7)	<0.002–0.11 (22)(6)	<0.004 (4)(0)	<0.002 (3)(0)
	Dieldrin	<0.002–0.13 (33)(26)	<0.002–0.072 (29)(21)	<0.002–0.09 (22)(11)	<0.002–0.013 (4)(20)	<0.002 (3)(0)
	DDT	<0.004–0.26 (33)(5)	<0.004–0.14 (29)(3)	<0.004–0.2 (22)(2)	<0.004–0.17 (4)(3)	<0.004 (3)(0)
February 93	Endosulfan	<0.002–0.15 (61)(47)	<0.002–0.48 (55)(28)	<0.002–1.52 (35)(34)	<0.002–0.087 (9)(6)	<0.002–0.14 (6)(5)
	Lindane	<0.002–0.08 (61)(3)	<0.002–0.007 (55)(3)	<0.002–0.026 (35)(3)	<0.002–0.008 (9)(1)	<0.00 –0.018 (6)(1)
	Aldrin	<0.002–0.033 (61)(15)	<0.002–0.10 (55)(10)	<0.002–0.015 (35)(4)	<0.002–0.024 (9)(1)	<0.002–0.017 (6)(1)
	Dieldrin	<0.002–0.13 (61)(26)	<0.002–0.022 (55)(8)	<0.002–0.11 (35)(14)	<0.002 (9)(0)	<0.002–0.012 (6)(2)
	DDT	<0.004–0.18 (61)(9)	<0.004–0.11 (55)(3)	<0.004–0.18 (35)(8)	<0.004–0.046 (9)(3)	<0.004–0.034 (6)(3)

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Table 3. First-order rate constants (k), half-lives ($t_{1/2}$) with 95% confidence limits and determination coefficients (r^2) in an aerobic and anaerobic rice-field soil

Soil	Pesticide	r^2	k (/day)	$t_{1/2}$ (days)	$t_{1/2}$ 95% c.l.[a]
Aerobic muck	2,4-D	0.89	0.21	3.4	2.8 –4.2
	Lindane	0.97	0.0019	369.3	343.0–400.0
	Paraquat	0.96	0.0014	499.2	405.1–650.9
Anaerobic muck	2,4-D	0.73	0.075	9.3	7.2–13.0
	Lindane	0.93	0.0012	609.1	548.9–683.6
	Paraquat	0.76	0.00024	2614	2289–3652

[a] c.l. = confidence limits
Source: Cheah et al. 1998

The detection of 2,4-D in the groundwater was not surprising as it has been shown to be moderately mobile in Malaysian agricultural soils (Cheah et al. 1997). Residue levels were, nevertheless, found to be low, ranging from <0.5 to 44 ng/mL (Table 2), for the entire sampling period.

Paraquat residues were found in the surface water (drainage, irrigation, and river) and their presence was not unexpected. The detection of the herbicide in the groundwater, however, was rather surprising considering its strong and irreversible adsorption to soils (Knight and Tomlinson 1967; Damanakis et al. 1970; Cheah et al. 1997). The presence of paraquat residues may be attributable to preferential flow or by-pass through the soil profile. Attempts were made to confirm the residues by ion-exchange and UV determination, but this was not successful because of the extremely low concentrations. Although long half-lives of 499 days and 2614 days in aerobic and anaerobic muck, respectively, were reported in agricultural soils (Cheah et al. 1998), paraquat is not expected to have any adverse effect on the ecosystem because of its rapid inactivation through soil colloidal particles.

Several studies have shown that there is little or no accumulation of pesticide residues in paddy crops (Iman and Rejesus 1977; Cheah and Lum 1993), but the impact of the residues on the biodiversity of the ecosystems needs to be examined in detail.

Acknowledgments

The authors thank Ms Rosmawati Selamat, Ms Jamiah Jaafar, and Mr Lim Teck Poi for technical assistance.

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Pesticide Use and Dissipation in Paddy Rice in Thailand

Nuansri Tayaputch*

Abstract

Pesticide use in Thailand has been increasing in the last 10 years in parallel with the increased production of agricultural commodities. The major types of pesticides used are organophosphates, carbamates, and pyrethroids, and various types of herbicides and fungicides. From surveys in 1991–92 on pesticides used in paddy fields, it was found that at least 25 different chemicals were used, some of which left residues in field soil and water. Further extensive surveys were made in 1996 on pesticide residues in milled rice and husked rice from 45 growing areas and one organic-rice-growing area. They revealed that trace amounts of organochlorine insecticides, mostly long banned from agricultural use, could still be found in most of the samples, but not at concentrations to cause concern. Some organophosphosphate and carbamate insecticides, such as monocrotophos, malathion, carbofuran, MIPC, and carbaryl were also found at low levels in some of the samples, but none in organic rice.

AGRICULTURAL production in Thailand has long depended heavily on pesticides. The evidence relating to pesticide use since pesticides were first imported into Thailand shows a continuing trend of increasing use over time. After years of intensive use of pesticides, problems concerning pesticide residues in food and the environment, resistance to pesticides, and pesticide hazards to humans and non-target organisms have been reported. However, the full impact has not yet been quantified.

Use of Pesticides in Agriculture

Pesticides were needed in agriculture because roughly one-third of the production of food, feed, and fibre was being lost to insect pests and plant and animal diseases. The reasons for extensive use of pesticides in Thailand are the wide variation in climate and crops, and the great multitude of pests and diseases. There are many types of pesticides now in use in Thailand: insecticides, herbicides, fungicides, and other minor categories including plant growth regulators, nematicides, and rodenticides. Among these, more herbicides are used than any other type of chemical. In the past, insecticides were the major class of pesticides used by farmers, but now the increasing cost of labour has made herbicide dominant over insecticides. The major insecticides currently in use are organophosphosphate, carbamate, and pyrethroid compounds. Almost all organochlorines used actively in the past have now been replaced by newer compounds. Dithiocarbamates and quaternary ammonium compounds are the major compounds used as fungicides and herbicide in Thailand.

Extent of Pesticide Usage

Over 60% of the overall pesticide usage in agriculture Thailand, in terms of amounts applied per unit area, is on rice, cotton, and fruits and vegetables. The remaining 40% is used to control pests in field crops, oil crops, rubber, ornamentals, etc. The trend of increasing pesticide use is reflected in the increasing

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quantities of active ingredients being imported (Table 1). Most of the pesticides used in the country are imported in the form of finished products or technical grades which are subsequently formulated into various products.

Pesticide Usage on Major Crops in Thailand

Rice is Thailand's most important crop. Over 50% of the total cultivated area is planted with rice, while cassava, maize, and mungbean are the major field crops grown in northeastern Thailand. The areas planted to other economic crops such as cotton, sugarcane, peanut, and soybean have fluctuated widely in recent years due to variations in world markets and local demand. Fruits and vegetables are increasing in importance at present, because of their high domestic and export values.

Table 2 gives the numbers of pesticides used on major crops as reported by many sources. A crop with serious pest problems and the greatest use of insecticides is cotton. Because of the heavy use of insecticides on cotton, cultivation of this crop in Thailand was sometimes reduced to accord with government policy for pesticide risk reduction.

Pesticide Dissipation in Paddy Rice

A study of the impact of pesticide residues on the rice paddy environment was carried out in Thailand during 1991–1992. Samples of paddy soil and water, and surface and run-off water, were collected from the experimental sites over two consecutive rice-growing seasons and analysed for all chemicals reported as being used. They were 25 different pesticides commonly used in rice-paddy field: 12 insecticides, 7 herbicides and 6 fungicides (Table 3).

From the overall analysis, it was found that there were two types of residues. One type consisted of residues that could be detected throughout the survey period at trace amounts. The other type was of residues that could be detected only shortly after their application and then disappeared.

Table 2. Numbers of pesticides used on major crops in Thailand in 1987

Crop or crop class	Insecticides	Fungicides	Herbicides
Fruits	35	30	4
Vegetables	30	26	small
Legume	29	13	5
Cotton	28	small	small
Rice	18	16	13

Source: Tayaputch (1990).

Table 3. Pesticides used in rice-paddy fields in Thailand during 1991–1992

Insecticides	Fungicides	Herbicides
monocrotophos, cartap, MIPC, methamidophos, isoprocarb, EPN, methyl parathion, carbofuran, endosulfan, triazophos, BPMC, cypermethrin	propineb, tricyclazole, benomyl, iprobenphos, carbendazim, isoprothiolane, oxadiazon	2, 4-D, bensulfuron, butachlor, metsulfuron, benthiocarb

Source: Tayaputch (1996).

The residues that could be detected throughout the whole period at low levels were endosulfan and oxadiazon, both of which were found in paddy soil and paddy water at different levels. However, at the end

Table 1. Quantities (t) of the main groups of pesticides imported into Thailand during 1992–1996

Type of pesticide	1992	1993	1994	1995	1996
Insecticides	6 098	5 305	5 252	6 573	6 608
Fungicides	3 513	3 988	4 885	4 828	4 446
Herbicides	8 450	9 056	9 554	11 934	14 041
Other[a]	418	476	640	727	446
Total	18 479	18 825	20 331	24 062	25 541

[a] Includes rodenticides, nematicides, and plant-growth regulators

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of the growing period or at harvest time, residues of these compounds were not detected.

Many other residues of organophosphophosphate and carbamate insecticides, and herbicides and fungicides such as monocrotophos, methyl parathion, triazophos, EPN, 2,4-D, butachlor, pretilachlor, bensulfuron, and carbendazim were found shortly after application and then disappeared from paddy soil, paddy water, and run-off water. Generally, organophosphates and carbamates degraded rapidly, especially in water unless they are carried in suspension and adsorbed onto particulate matter. Under flooded rice culture, the rapid disappearance of pesticides has been observed and documented (Chambers and Levi 1991).

Pesticide Residues in Rice

Due to the growing public concern over the hazardous effects of pesticide residues in food, the Agricultural Toxic Substances Division of the Department of Agriculture, Thailand has been conducting regular field monitoring on agricultural commodities to determine pesticide residues which remain after prolonged usage. An extensive survey on rice paddy was made in 1996 by collecting 171 samples of milled rice from 45 producing areas, and 46 samples of milled rice and husked rice from a single organic-rice-producing area. The samples were analysed for pesticide residues, yielding the results shown in Tables 4 and 5.

Persistent pesticides such as organochlorines remain on crops and in the environment for long periods. The persistence varies with climatic conditions and types of agroecosystems. Traces of organochlorine insecticide residues, mostly banned from agricultural usage for many years were found in a relatively large number of samples, as shown in Table 4 and 5. However, the levels of accumulation of most of the residues ranged from traces to quite low levels—lower than our ERLs which set the limit for most of the organochlorines at 0.1 mg/kg. Organophosphorous and carbamates insecticides such as diazinon, monocrotophos, malathion, carbofuran, MIPC, and carbaryl were also detected in some samples, also at low levels. No residues of these compounds were found in organic rice.

Conclusions

In general, the environmental impact of organophosphorous and carbamate pesticides is small,

Table 4. Pesticide residues in milled rice from 45 producing areas in Thailand in 1996

No. of samples analysed	Positive samples	Pesticides found	Amount in ppm (mg/kg)
171	91	BHC, aldrin, dieldrin, heptachlor & heptachlor epoxide, endrin, DDT & metabolites	0.003–0.02
	8	Diazinon, malathion, monocrotophos	0.001–0.01
	12	Carbofuran, MIPC carbaryl	0.003–0.02

Table 5. Pesticide residues in milled and husked rice from one organic rice field in Thailand in 1996

No. of samples analysed	Positive samples	Pesticides found	Amount in ppm (mg/kg)
Husked rice			
23	1	Heptachlor & heptachlor epoxide	0.002
	9	Aldrin & dieldrin	0.001–0.004
	2	Endosulfan	0.003–0.007
	2	DDT & metabolites	0.001–0.004
Milled rice			
23	4	Heptachlor & heptachlor epoxide	0.001–0.003
	12	Aldrin & dieldrin	0.001–0.008
	8	DDT & metabolites	0.001–0.006

Source: Sukmark (1997).

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because of dissipation by processes such as volatilisation, leaching, and run-off. The disappearance of these compounds may also occur aided by biotic and abiotic degradation. Organochlorine insecticides which persist in the environment long after their use was stopped, appeared in trace amounts in agricultural commodities. The concern over persistence of organochlorine compounds resulted in their use in agriculture being prohibited. However, the stability of these compounds and their widespread distribution in the environment mean that it will take a long time for their concentrations to diminish.

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Dissipation of Pesticides in Rice Paddy in the Philippines

L.M. Varca and A.W. Tejada*

Abstract

The dissipation of pesticides in rice paddy and rice paddy-fish culture was investigated.

Carbosulfan was rapidly converted to carbofuran in all components of the rice paddy ecosystem, except on rice leaves where it remained up to 7 days as parent compound. The major metabolite, carbofuran, remained up to 30 days in soil. Hydrolysis was the major route of degradation in water, with a higher rate at higher pH and temperature. The concentration in water was biomagnified up to 100 times in fish (*Tilapia* sp.), with the highest residues in gut tissues, then in fillets, and least in cranial tissue. Uptake by aquatic plants was observed up to 20 days and declined thereafter.

Similarly, BPMC (2-sec-butyl phenyl N-methyl-carbamate) declined rapidly at higher pH with half-lives of 2.4 hours, 5.6 hours, 7.6 hours and 8.4 hours at pH 10, 6, 7, 6, and 4, respectively. The rate of loss was higher in flooded than in unflooded soil, and followed a pseudo first-order kinetics with half-lives of 1.5 and 2.2 days, respectively.

In a chlorpyrifos-treated rice paddy fields, residues in soil and water remained longer in higher pesticide use farms than in lower pesticide use farm. Residues were also found in larger animals collected within the vicinity of the treated rice fields but the levels did not exceed the tolerance limit set by FAO/WHO.

Monocrotophos, methyl parathion, and cypermethrin in rice–fish culture had no apparent effect on fish survival if recommended practice was followed. Laboratory studies showed, however, that these pesticides were very toxic to fish. A 10-day interval between field treatment and harvest of fish may be considered tolerable for consumption.

Even the persistent DDT, when exposed in the open field, degraded rapidly, with a half-life of 31 days. In soil, DDT and its metabolite, DDE, dissipate faster than in temperate countries with half-lives of 235 and 161 days, respectively.

IN developing countries like the Philippines there is a growing concern on the effects of pesticides in the environment. One segment of the environment which is being threatened by pesticide pollution is the rice paddy ecosystem. About 60% of the total cultivated land area in the Philippines is devoted to rice production, and the drainage water from it finds its way into lakes, rivers, and the sea. This substantially increases the opportunity for widespread contamination of the terrestrial and aquatic environments. The problem is heightened when rice fish culture, which is being promoted throughout Asia, is practiced. The paddy-reared fish are exposed to pesticides, and thus may bioaccumulate residues above safe limits. The movement of these pesticides along the food chain may also result in the build-up of residues in organisms higher in the food chain, posing hazards to consumers or to the environment in general.

There are already some reports on the fate of pesticides in the paddy environment in the Philippines (Argente et al. 1977; Catahan 1977; Seiber et al. 1978;

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Lucero 1980; Varca 1981; Bajet and Magallona 1982; Barril and Tejada 1982; Tejada and Magallona 1985).

This paper reports studies on the movement, distribution, and loss of carbosulfan/carbofuran, BPMC (2-sec-butyl phenyl N-methyl-carbamate), chlorpyrifos, and DDT in the rice paddy ecosystem.

Field Experiment

An existing rice paddy and fish pond (7 × 4 m) next to each other at the National Crop Protection Center, Los Baños, Philippines were used in the study.

Rates of uptake, distribution, and loss of carbosulfan were determined in different components of the rice field and pond ecosystem over a 30-day period.

Field sampling

Rice paddy ecosystem

Samples of paddy water, soil, snails, and rice plants were taken at 3 hours, and at 1, 2, 3, 5, 7, 15, and 30 days after spray application (90 days after transplanting). At harvest time, rice plant samples were divided into stems, leaves, and grains and these were analysed separately.

Fish pond

Before paddy water was run into the pond, samples of water, fish *(Tilapia nilotica)*, 'kangkong' plants *(Ipomoea aquatica),* and snails *(Pila luzonica)* were taken to measure the background level of pesticide.

After draining the water from the rice paddy into the fish pond, samples of fish, kangkong, and pond soil and water were collected and analysed at various at 3 hours and at 1, 2, 3, 7, 15, and 30 days after the last application of the insecticides. The fish were divided into head, intestines, and fillets for analysis.

Fate of Carbosulfan

Rice

Carbosulfan residue was detected only on rice leaves, where it remained for up to 7 days after the last application of the insecticide. As carbosulfan concentration fell, carbofuran concentration increased. The maximum conversion of carbofuran was reached after one day, falling thereafter (Fig. 1). The rate of loss followed pseudo first-order kinetics with a half-life($t_{1/2}$) of 1.5 days. Carbofuran residue was concentrated on the leaves, followed by the stems, and was least on the grains (Fig. 2).

The effect of rainfall was very great. After 8 mm of rain, carbosulfan and carbofuran residues on rice leaves fell by 75–80%, and by 95% after 50 mm of rain (Table 1).

Table 1. Effect of rainfall on residues of carbosulfan and carbofuran on the leaves of rice plants.

Days	Rainfall (mm)	Residues on rice leaves			
		Carbo-sulfan	% reduction	Carbo-furan	% reduction
0	0	3.5		0.3	
1	0	2.2	37		
2	8.4	0.8	63	2.5	52
3	0	0.2	75	1.0	80
4	50		95	0.2	90
5	1.4	0.1		0.08	
6	0.6				
7	5.4	ND		0.01	

Sunlight enhanced degradation of the insecticide. Photodecomposition studies in the laboratory confirmed more rapid degradation in the field, giving a half-lives for carbosulfan and carbofuran of 1.4 hours and 3.2 days, respectively (Tejada 1983).

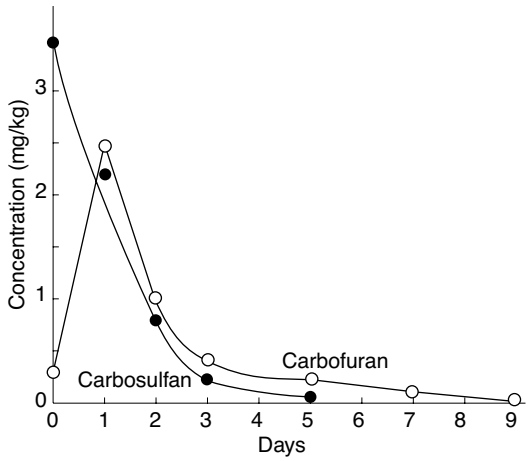

Figure 1. Residues of carbosulfan and carbofuran in rice plants 0–7 days after spray application

After conversion of carbosulfan to carbofuran, the latter was oxidised at the 3-position of the ring, giving rise to 3-hydroxy phenol. Also detected by thin layer chromatography and gas chromatography 3-

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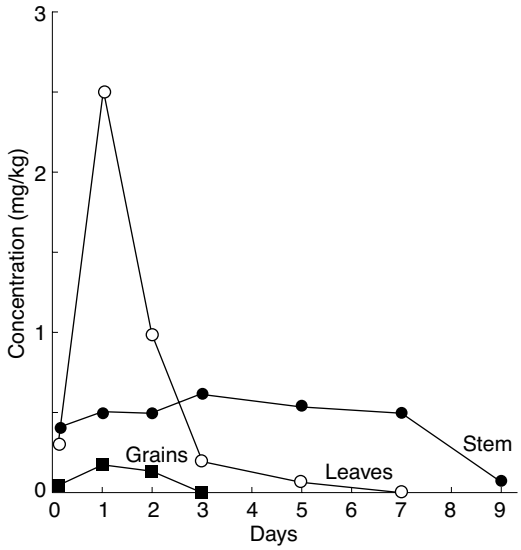

Figure 2. Distribution of carbofuran in different parts of rice plants

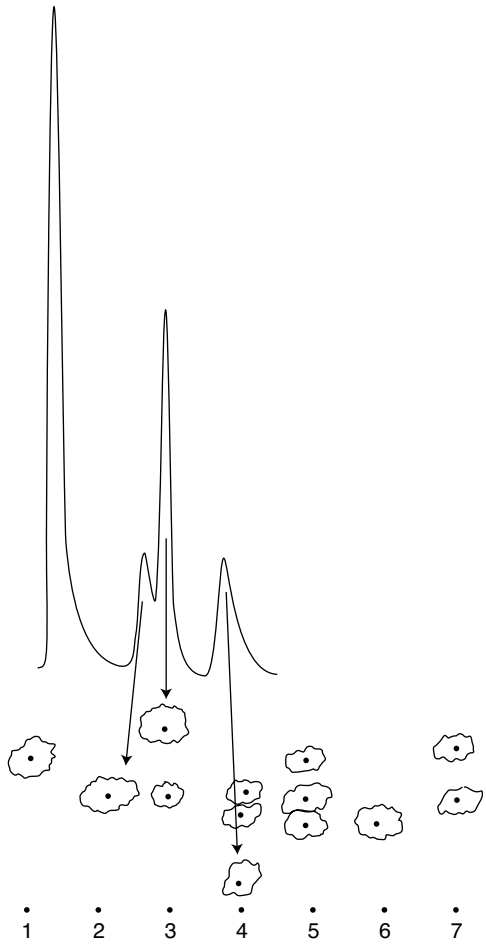

Figure 3. Thin-layer and gas chromatograms of carbosulfan and its metabolites: 1. carbofuran phenol standard; 2. carbofuran standard; 3. carbosulfan standard; 4. 3OH carbofuran; 5. extract of rice leaves harvested 2 days after spraying; 6. 3 keto carbofuran; 7. extract of rice leaves harvested 3 hours after spraying.

ketophenol and 7-phenol (Fig. 3). From these data, the possible metabolic pathway is shown in Figure 4. The same metabolites were reported by Umetsu et al. (1979) in maize and cotton plants and by Callejas (1982) in tomatoes. After 7 days they reported other metabolites such as *n*-hydroxyl methyl carbofuran.

Water

Carbosulfan residues were not detected in paddy water one day after application of the insecticide but the major metabolite, carbofuran, was detected up to 15 days. Repeated application did not result in the accumulation or build-up of carbofuran residue in paddy water (Fig. 5). This may be attributed to its relatively high solubility in water (700 mg/L). Carbosulfan/carbofuran is 60 000 times more soluble in water than is DDT (solubility is 0.0034 mg/L). Compounds such as DDT which are relatively insoluble in water have high partition coefficients (DDT = 1.55×10^6) and thus have greater potential for bioaccumulation.

As the level of carbosulfan in paddy water decreased, the level of carbofuran increased. Again the maximum conversion of carbosulfan to carbofuran occurred one day after spray application with the residue level declining thereafter. The rate of loss was pseudo first order with a $t_{1/2}$ of 5.8 days. Hydrolysis is the major degradation route of carbosulfan and carbofuran residues in water. The rate of hydrolysis was more rapid at higher pH and temperature (Fig. 6). The maximum pH occurred during the hottest part of the day between 1400–1600 hours.

This is the time when algal photosynthesis shifts the CO_2 equilibrium away from HCO_3 to CO_3 (Obcemea et al. 1971; Nikkelsen et al. 1977) thus increasing alka-

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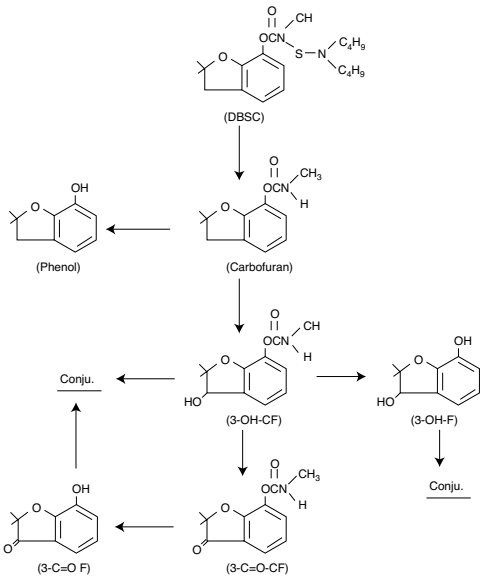

Figure 4. Metabolic pathway of dibutylaminosulfenyl carbofuran (after Umetsu et al. 1979).

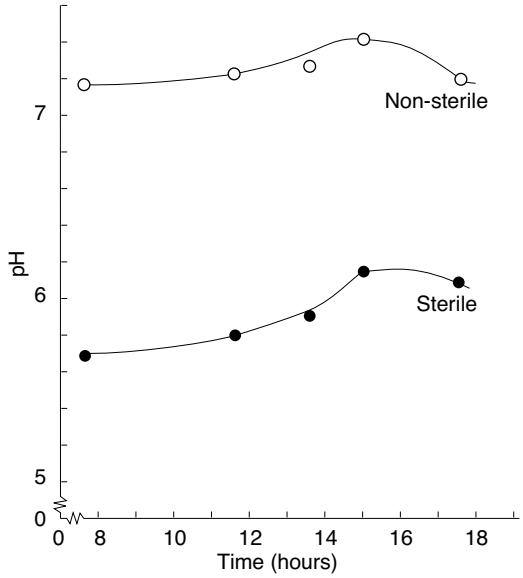

Figure 6. Fluctuation in pH at different times of the day under sterile and non-sterile conditions.

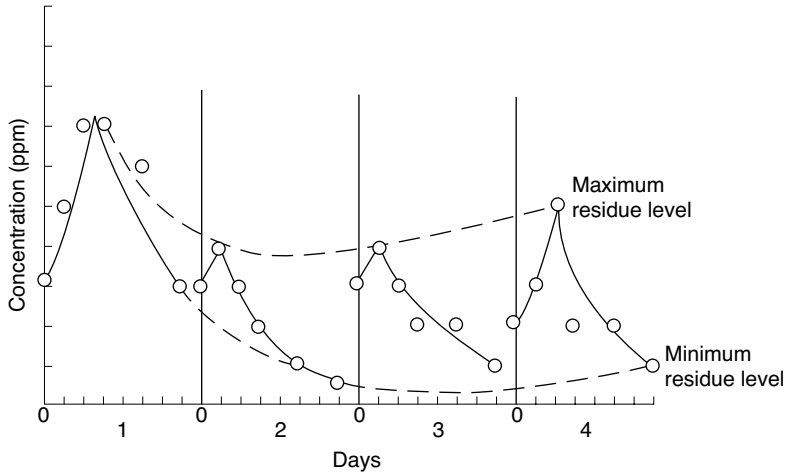

Figure 5. Levels of carbofuran in water folowing periodic application

linity. On the other hand, at low pH, carbosulfan is very stable, e.g. at pH 2.5–3.8 in the rat stomach (Umetsu and Fukuto 1982). This could be the reason for its lower mammalian toxicity.

Soil

Carbosulfan residues were not detected in soil. This could be expected because upon reaching the soil, the parent compound has already been converted to carbofuran in paddy water. Carbofuran remained the major metabolite in soil for 30 days (Fig. 7). The $t_{1/2}$ is 4 days. Siddaramapa and Watanabe (1979) reported that degradation of carbofuran in flooded soil was slow, because of strong adsorption. This has been considered the determining factor in the fate of insecticide in the soil. Adsorption is highly correlated with the organic matter and clay content of the soil (Stevenson 1975; Edwards 1966). Adsorption also reduces the biological activity and chemical degradation in soil (Ogawa et al. 1976), hence the residue is retained for longer.

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Snails *(Pila luzonica)*

Carbosulfan residues were not detected in snails even at 3 hours after application. The major metabolite detected was carbofuran. About 50% of the residues were obtained on the surface of the shell, suggesting that it might be a good protective covering against pesticide. The maximum uptake was obtained one day after spray application with no detectable residues after 7 days in the soft tissues of snails (Fig. 8).

Fish pond

Most of the pesticides used in rice paddy are highly toxic to fish (Table 2).

After inflow of the paddy water into the fish pond 3 hours after insecticide application to the rice paddy, all components were contaminated with the insecticide.

Table 2. Toxicity of some insecticides to Nile tilapia *(Oreochromis niloticus)*.

Insecticide	LC_{5O} (mg/L) at 48 hours	Rank[a]
Azinphos ethyl	1.0×10^{-6}	C
Endosulfan	6.9×10^{-4}	C
Cyfluthrin	1.6×10^{-2}	C
Chlorpyrifos	3.0×10^{-2}	C
Fenvalerate	3.0×10^{-2}	C
Cypermethrin	3.1×10^{-2}	C
Triazophos	3.5×10^{-2}	C
Etofenprox	9.1×10^{-2}	C
Thiodicarb	0.12	C
Carbosulfan	0.17	C
Alpha cypermethrin + BPMC[b]	0.22	C
Monocrotophos + fenvalerate	0.25	C
BPMC + chlorpyrifos	0.28	C
Fenitrothion	0.49	C
BPMC	0.64	C
Malathion	1.48	B
Metamidophos	2.96	B
Methyl parathion	3.50	B
Carbaryl	3.52	B
Monocrotophos	13.8	A

[a] Rankings are adapted from Nishiuchi (1974):
A = LC_{50} > 1 0 ppm: low toxicity
B = LC_{50} 0.5–10 ppm: moderately toxic
C = LC_{50} < 0.5 ppm: extremely toxic

[b] BPMC = 2-sec-butyl phenyl N-methyl-carbamate

Since carbosulfuran was already converted to carbofuran even before inflow of the paddy water, the major residue detected was carbofuran. The residue in water was detected up to 15 days. The mechanism of loss was pseudo first-order with a $t_{1/2}$ of 2.9 days.

As a consequence of contamination of water, uptake of residues by aquatic plants was significant. Maximum uptake was reached on the 15th day, when it levelled off up to 20 days, and declined thereafter (Fig. 9). Residues could still be detected at up to 30 days, but usually at low levels. Absorption through the roots is the normal pathway for systemic insecticides such carbofuran.

Residues were detected in Nile tilapia fish. The major metabolite detected in fish was carbofuran. The residue was highest in gut tissue, followed by fillet, and least in head tissue (Fig. 10). Similar results were reported by Argente et al. (1977).

Residue levels of carbofuran in fish were up to 100 times those in the pond and 10 times those in snails. Nevertheless, the bioconcentration factor is much lower than that in chlorinated insecticides, which can reach 10000–100000 times with DDT. Other insecticides examined (Table 3) have also bioaccumulated in fish.

Table 3. Bioaccumulation of ^{14}C labelled pesticides in fish

Pesticide	Fish (ppb)	Water (ppb)	Bioconcentration factor
Methyl parathion	8.3	1.000	8.3
Carbofuran			
Fillet	10.5	0.090	117
Intestines	17.0	0.090	189
Carbosulfan	1.0	0.004	239
Endosulfan I	0.9	0.011	82
Endosulfan II	1.5	0.007	214
Chlorpyrifos			
Fillet	1.8	0.027	65
Intestines	4.1	0.027	152

Fate in lactating goats

The residues detected on rice plants were simulated by spiking rice fodder fed to goats with ^{14}C-carbofuran (ring labelled) at rates of 0.5 and 1 mg/kg/day. Feeding was carried out for 7 days. Excretions in the faeces, urine, and milk were monitored. Accumula-

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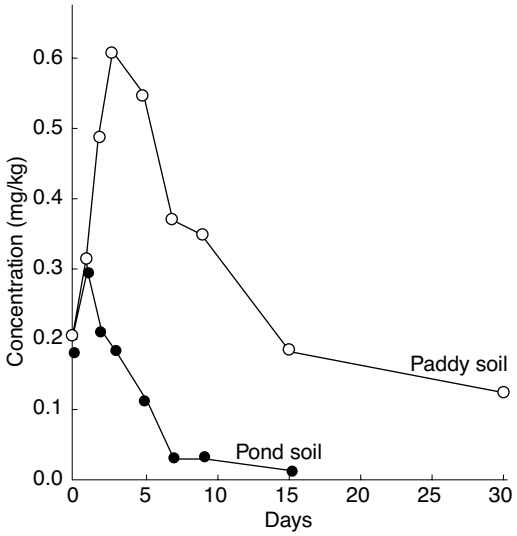

Figure 7. Adsorption and decline of carbofuran in soils from 0–30 days after spraying

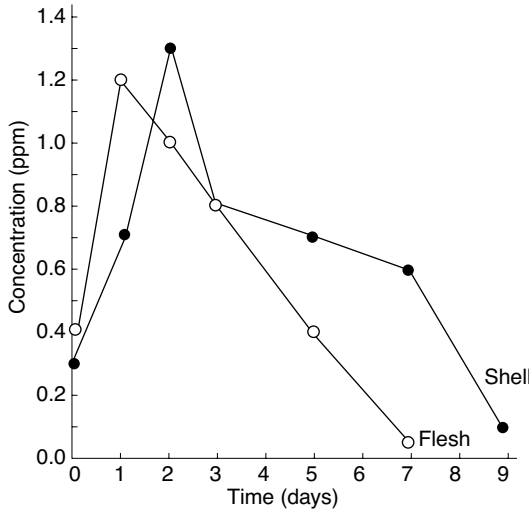

Figure 8. Carbofuran residues in snails (*Pila luzonica*), 0–9 days after spraying

tion and distribution in various organs and tissues of the goats were determined. The total milk and urine samples were taken twice daily, and the faeces once a day. The goats were sacrificed after 7 days.

Carbon-14-carbofuran equivalents were detected in various organs and tissues of the goats in decreasing order of concentration: omental fat > liver > kidney > subcutaneous fat > muscle > heart > brain (Table 4). Seventy-seven percent of the ^{14}C-carbofuran applied were excreted in the urine, 3% in the faeces, and 0.05% in the milk.

Table 4. Radioactivity levels in different tissues of lactating goat (ppm carbofuran equivalent)[a]

Tissue	Group I[b]	Group II[c]
Blood	0.08	0.07
Liver	0.55	0.69
Omental fat	0.84	1.42
Subcutaneous fat	0.23	0.41
Kidney	0.25	0.43
Brain	0.27	0.34
Heart	0.17	0.33
Muscle (biceps femoris)	0.19	0.34
Muscle (longissimus dorsi)	0.02	0.36

[a] Average values from the two goats in each group.
[b] Animal dosed with 0.5 mg/kg/day.
[c] Animal dosed with 1.0 mg/kg/day.

Fate of BPMC

In water

Insecticide application at the recommended rate of 0.75 kg of active ingredient per ha would theoretically give a 2.5 mg/kg residue level at the prevailing water level in the field. However, 1.3 mg/kg was present in the paddy water 3 hours after application. This means that about 50% of the applied material does not reach the target rice plants.

There was a rapid loss of BPMC in water (Fig. 10). The loss followed a pseudo first-order kinetics with $t_{1/2}$ = 13.9 hours. The probable causes of loss are volatilisation, weather factors, hydrolysis and partitioning into the soil component. The role of sunlight in the degradation of pesticides cannot be discounted. Haque and Freed (1974) found that most of the organic pesticides decompose in the presence of sunlight or ultraviolet radiation.

In soil

The rate of loss of BPMC from paddy mud was much less rapid than that from water over the first 2 days after application.

Adsorption is correlated with organic matter content and soil structure (Edwards 1966). Paddy soil, because of its high clay content, has greater adsortion for BPMC. The residue is thus retained longer in paddy mud than in paddy water.

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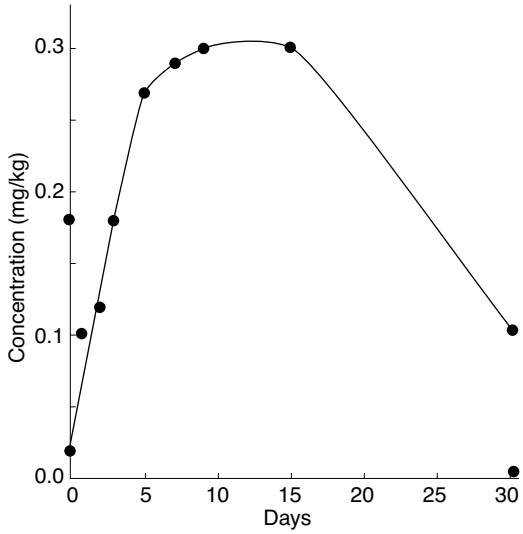

Figure 9. Uptake by, and decline of carbofuran in 'kangkong' (*Ipomoea* sp.) plants

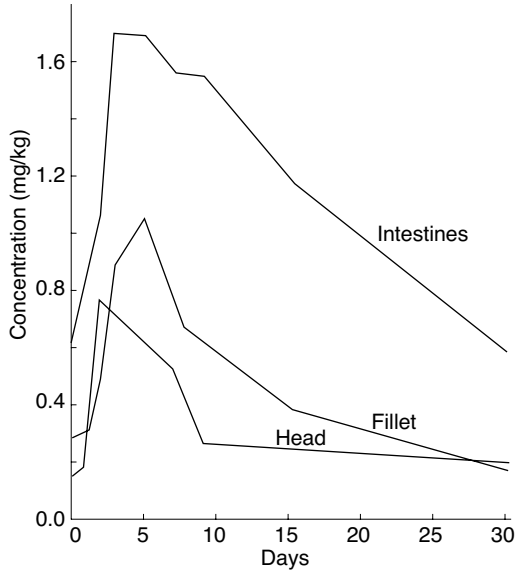

Figure 10. Distribution of carbofuran residues in fish, 0–30 days after spraying

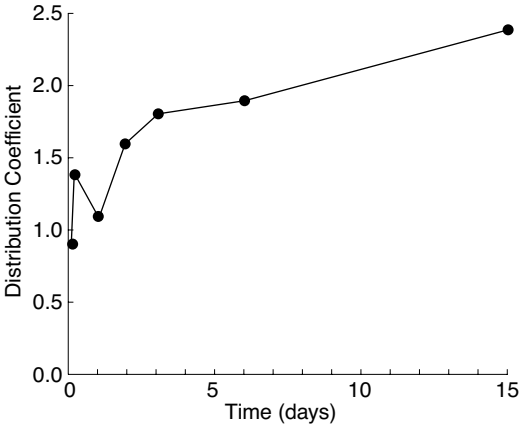

Figure 11. Distribution coefficient of BPMC in soil and water between 0 and 15 days after spraying

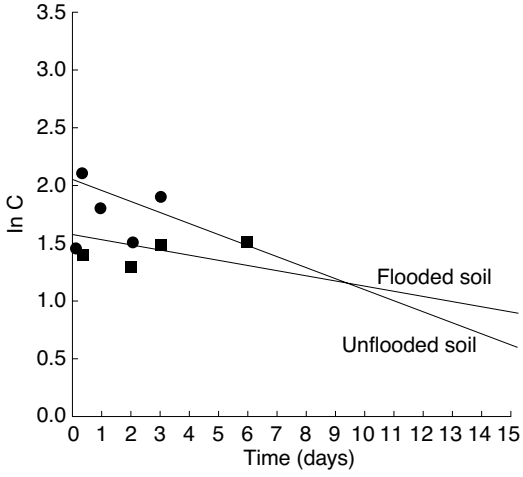

Figure 12. Decline curves for BPMC in flooded and unflooded soil

Solubility in water is another factor determining the extent to which pesticides will partition in this substrate. BPMC is insoluble in water. The distribution coefficient (K_d) is obtained using the equation of Hamaker and Goring (1976):

K_d = quantity absorbed in soil (μmoles/kg) ÷ quantity in solution (μmoles/L)

The distribution coefficient increased with time showing that the soil was the ultimate sink for the insecticide (Fig. 11).

The total amount of insecticide from the soil and water portions showed unaccounted increases in residues with time. It might be inferred that these are the result of soil bound residues or actual losses from degradation.

The degradation rate differed between flooded and un-flooded soils (Fig. 12), BPMC degrading more rap-

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idly in flooded soils. Half-life values were 1.5 days and 2.1 days in flooded and unflooded soils, respectively. This more rapid decomposition under flooded conditions might be attributable to the participation of anaerobic microorganisms in flooded soils (Venkateswarlu et al. 1977).

In flooded soil, the water molecules complete for sites in soil particles so that desorption exceeds adsorption, thus resulting in more rapid degradation of the insecticide (Edwards 1966).

Distribution in rice plants

Residues were highest in rice leaves, followed by those in rice stems and were lowest in rice grains. The high level on the leaves is only to be expected because application was by spraying which was directed towards the thick foliage covering the plants. After a day, almost 90% of the residue was removed from the leaves, declining rapidly to 1.5 mg/kg after 3 days.

Ogawa et al. (1976) found that BPMC was taken up by the roots of rice plants and translocated rapidly to the upper portions of the plants. The decline on the stem was slow compared with that on the leaves because of the protective covering of the foliage shielding the stems from agencies of degradation.

On rice grains, residues were higher on rough rice than on milled rice. The low level in milled rice can be attributed to loss during milling.

Degradation Studies of Chlorpyrifos in a Farmer's Field

Chlorpyrifos was degraded rapidly in paddy water taken from the field of a farmer who was a high user of pesticides, with residues detectable only up to 3 days (Table 5). This supports the findings of Zulkifli et al. (1983) and Smith et al. (1966). Coincidentally, this is the time when chlorpyrifos residues were highest on rice stalks. This could be the period during which the rice plants absorbed the chemical in the water by capillary action. The residues then started to decline, with a half-life of 187 days.

Chlorpyrifos residues on rice leaves were detectable at up to the 15th day after application. Analysis of soil from a high-pesticide-using farmer showed that the levels were higher than in rice plants, i.e. 1.25 mg/kg in soil and 0.09 mg/kg on leaves at day zero. This could be due to rainfall after application of the insecticide, which washed out the residues from the leaves and deposited them in the soil. The soil retained the residue longer than any component of the rice paddy due to adsorption and bound-residue formation. In addition, the soil is the ultimate sink of pesticide deposition.

Samples of rice leaves contained the highest levels of residues among the components analysed (Table 5). The size of the plant per volume of spray solution will affect the distribution of pesticide in the plant. Rice grains taken at harvest time did not contain any pesticide residue either in dehusked or rough rice. The usual practice of harvesting rice 40 days after the last application may be considered from the consumer's standpoint. However, stored rice treated with pesticide in warehouses contained residues for a long period (Tejada 1995).

Dissipation of DDT

Natural water from a river was collected and treated with ^{14}C-DDT. Samples were analysed daily for total radioactivity for up to 6 months. Sediments were centrifuged from water and analysed separately.

Table 5. Residues of chlorpyrifos from a high pesticide user farm (wet season, 1989)

Days after application	Water	Residues (mg/kg)		
		Rice stalks	Rice leaves	Soil
0	–	0.003 ± 0.001	0.09 ± 0.01	0.25 ± 0.04
1	0.001 ± 0.004	0.006 ± 0.0001	0.12 ± 0.005	0.23 ± 0.01
2	0.001 ± 0.007	1.009 ± 0.0003	0.10 ± 0.02	0.21 ± 0.02
3	0.001 ± 0.005	0.010 ± 0.005	0.07 ± 0.03	0.11 ± 0.07
5	<0.001	0.002 ± 0.0002	0.01 ± 0.003	0.06 ± 0.01
7	<0.001	<0.001	–	0.08 ± 0.01
15	<0.001	<0.001	0.003 ± 0.005	0.05 ± 0.02

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The decline of DDT was rapid in water, with a reduction of 36% in one day, then more gradually to 22% after 14 days. Only 1.69% of the applied ^{14}C-DDT remained after 6 months. The rapid decline maybe attributed to volatilisation, codistillation, and adsorption to suspended particles (Bowman et al. 1959; Acree et al. 1963).

Under field conditions, ^{14}C-DDE dissipated rapidly, with a half-life of 151 days, comparable with DDT, which has a half-life 105 days. DDD was detected a year after treatment of the soil.

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Dissipation of Organochlorines into Malaysian River Systems: a Survey

Guan H. Tan*

Abstract

A systematic study of organochlorine chemical pollution in Peninsular Malaysian waterways has been undertaken in anticipation of the enactment of the Malaysian Water Quality Standards pertaining to river water pollution from industrial, agricultural, and domestic sources. This study is also helping to verify the baseline levels for the implementation of water quality standards for various beneficial uses of water.

Malaysia is a country in which large areas of land have been used for agriculture. Rubber and oil palm are two major plantation crops which occupy 3.8 million hectares, about 70% of the total agricultural land area. In addition to this, rice-growing is also an important agricultural activity, contributing nearly 75% of the country's annual rice requirements. The use of pesticides in the agricultural sector in Malaysia has been increasing over the years, with use of some of the organochlorine pesticides such as lindane (γ-HCH), endosulfan, and dieldrin still being permitted. The distribution and fate of these organochlorine pesticides in the aquatic environment as a result of agricultural development has been studied by monitoring their residue levels in water and sediment samples from selected rivers in Peninsular Malaysia. The monitoring program was carried out in two major rivers flowing through rice-growing areas on the west coast of Peninsular Malaysia. A survey of organochlorine pesticides residue levels was also undertaken for water samples from 25 major rivers in Peninsular Malaysia.

The survey has shown that levels of organochlorine pesticides are still tolerable in terms of water quality for domestic use but that some have exceeded the critical levels to maintain aquatic life. Endosulfan, dieldrin, lindane, and DDT are some typical organochlorines found in the river water and sediment samples from Peninsular Malaysia.

SINCE the introduction of DDT as a pesticide in the late 1930s and the subsequent development of other organochlorine pesticides, the residues of these compounds have been found in many parts of the world (George and Fear 1966; Keith 1966; Pionke et al. 1968; Hattula et al. 1978).

Because these compounds are lipophilic and have low chemical and biological degradation rates, they tend to accumulate in biological tissues and increase in concentrations at higher levels in the food chain (Moore and Walker 1964; Kennedy et al. 1970; McEwen and Stephenson 1979). Their persistence and accumulation of organochlorine pesticides in aquatic organisms have led to their use being largely discontinued in the agricultural sector.

Large areas of Malaysia are devoted to agriculture. Rubber and oil palm are two major plantation crops which occupy 3.8 million hectares, some 70% of the country's total agricultural land area. Rice-growing is another important agricultural activity. The use of pesticides in the agricultural sector in Malaysia has been increasing over the years. The application of some of the organochlorine pesticides, such as lindane (γ-HCH), endosulfan, and dieldrin, is still permitted (Pesticides Board 1991).

The study reported here was carried out to measure the extent of environmental contamination by organochlorine pesticides of river waters in Peninsular

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Malaysia. Water samples were collected from different stations along 25 major rivers. Sediment samples were also collected from two rivers on the west coast of the peninsula.

Experimental

Two methods for extracting the sediment samples were compared: a soxhlet extractor consisting of a 500 mL round-bottom flask, a temperature-controlled heating mantle, a 150 mL capacity extractor, and a Liebig water-cooled condenser; and ultrasonic extraction with a Branson Ultrasonicator Model 3200.

The method of Tan (1992) was used for liquid–liquid extraction on the water samples.

Sampling procedure

Sediment

Sediment samples were collected from sampling stations along the Sungai Selangor and Sungai Kelang. Water samples were taken at stations where high organochlorine pesticide residue levels had previously been found (Tan 1992).

The samples were extracted from the river bed using a home-made excavator (a diagonally cut metal can fixed to a 1.5 m pole) and stored in wide-mouthed amber bottles. Sediment samples were taken from under shallow water near the river bank. Only surface sediment samples (approximately 5–10 cm deep) were collected. Duplicate samples (ca 1 kg each) were taken at each station. Concentrated sulfuric acid (2 mL) was added to each sample and stirred thoroughly to eliminate all biological activity. Sediment samples were then taken to the laboratory for analysis.

The sediment was spread out uniformly in a large, shallow, aluminium foil-lined tray and allowed to dry at room temperature (27–29°C) in a contaminant-free area for four days. The samples were stirred and mixed occasionally during drying to homogenise them. This process was continued until samples appeared dry and were free-flowing.

Water

Duplicate 2 L water samples were collected and stored in amber bottles. Samples were acidified to pH 2 with sulfuric acid to eliminate biological activity in the water. The samples were kept at ambient temperatures (approximately 27–29°C) and transported to the laboratory for subsequent extraction and analysis. Before solvent extraction the water samples were filtered to remove any solid material.

Quantitative GC analysis

The separation and quantification of the pesticide residues were performed with the Shimadzu GC-17A gas chromatograph fitted with an electron capture detector (ECD) using BPX5 capillary column (0.25 mm i.d. × 25 m).

A VG-Micromass Prospec GC-MS was used to confirm the identity of the pesticides.

Soxhlet extraction

Dried sediment (100 g) was placed in an extraction thimble. It was then wetted with 20 mL water. Hexane and acetone (1:1 by volume; 250 mL) and some pre-cleaned boiling chips were added to the round-bottom flask. The sample was extracted for 10 hours. The hexane/acetone extract was filtered through hexane-washed sodium sulfate, then concentrated in a rotary vacuum evaporator to about 10 mL before florisil column chromatography clean-up.

Ultrasonic extraction

Dried sediment (100 g) was placed into a 250-mL conical flask. Two hundred mL of hexane/acetone (1:1 by volume) was then added and the sample subjected to ultrasonication for 4 hours. During this procedure, the conical flask was covered with aluminium foil to minimise contamination and evaporation. The extract was then filtered through a phase-separator filter paper containing sodium sulfate to ensure quantitative transfer. It was then evaporated to dryness and immediately rinsed with 10 mL petroleum ether. The concentrated extract was then quantitatively transferred to the florisil column for clean-up.

Recovery experiments

The efficiency of the soxhlet and ultrasonic extraction technique for the pesticide residues in the sediments was determined by spiking the dried sample (100 g) with the organochlorine pesticide standards (5.0 μg/mL; 50 μL). This is equivalent to a spiking level of 2500 ng/kg for each pesticide.

The spiked sediment samples were then wetted with water (20 mL) and immediately subjected to the entire analytical procedure described above. This was repeated on a further four spiked samples to obtain a set of data for the efficiency of each extraction method.

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Results and Discussion

The recoveries of the soxhlet extraction of 12 organochlorine pesticides spiked into the sediment samples are shown in Table 1. Recoveries are in the range 91–103%.

Table 2 shows the recoveries using unltrasonic extraction of six organochlorine pesticides spiked into the sediment samples. The recoveries using ultrasonic extraction are in the range 45–105%.

Table 1. Recovery of organochlorine pesticide standards added to sediment samples (n = 5) using soxhlet extraction with 1:1 hexane–acetone

Organochlorine pesticides	Mean recovery (±S.D.) %
α-HCH	95 (±6)
β-HCH	93 (±8)
γ-HCH	97 (±7)
p,p'-DDE	94 (±5)
p,p'-DDT	96 (±9)
Heptachlor	95 (±8)
Heptachlor epoxide	96 (±7)
Endosulfan I	98 (±6)
Endosulfan II	96 (±8)
Endrin	103 (±7)
Dieldrin	94 (±9)
Aldrin	91 (±8)

When these are compared with the results of an earlier study on river water samples (Tan 1992), the recovery rates of these organochlorine pesticides using these two techniques are very similar to those for the water samples, which range from 85 to 103%. However, when the relative standard deviations for the recovery of the pesticides from the sediments were compared to those obtained for the water samples, it was found that the soxhlet and ultrasonic extraction recovery showed a wider range in standard deviations, from 5 to 10%. For the water samples the relative standard deviations for the recovery of the pesticides using solvent extraction with hexane ranged from 3.8 to 5.2% (Tan 1992). This may be attributable to the incomplete homogeneity of the sediment samples before the soxhlet and ultrasonic extraction.

Table 3 shows the results of the analysis of organochlorine pesticide residue levels in sediments from the rivers using the soxhlet extraction technique.

γ-HCH, heptachlor, aldrin, endosulfan and DDT were found in the sediments from these rivers. The relatively higher levels of endosulfan and γ-HCH in the sediments could be a result of their current use for controlling insect pests in rice fields.

Table 4 shows the results of the organochlorine pesticide residue levels in water samples from selected rivers in Peninsular Malaysia using the solvent extraction technique.

The residue levels of organochlorine pesticides found in the sediments are generally about ten times the concentrations in the river water. Since these compounds degrade very slowly and tend to accumulate in the sediments, they may subsequently leached out into the surrounding aquatic system.

Table 2. Percentage recovery (% ± S.D.) of organochlorine pesticide standards in sediment samples by ultrasonic extraction

Organochlorine pesticide	Station numbers					
	601	603	607	624	Sg Sel 1	Sg Sel 2
γ-HCH	98 ± 6	85 ± 7	75 ± 8	65 ± 6	65 ± 8	70 ± 6
Heptachlor[a]	70 ± 7	80 ± 5	84 ± 8	98 ± 5	99 ± 7	104 ± 5
Aldrin	73 ± 6	95 ± 6	75 ± 6	78 ± 7	105 ± 6	99 ± 6
Endosulfan[b]	65 ± 5	91 ± 7	73 ± 6	65 ± 7	70 ± 6	45 ± 5
Dieldrin	55 ± 7	75 ± 6	65 ± 9	58 ± 6	75 ± 6	55 ± 6
DDT	70 ± 5	91 ± 6	98 ± 6	95 ± 7	86 ± 5	70 ± 5

[a] Heptachlor = heptachlor + heptachlor epoxide
[b] Endosulfan = endosulfan I

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Table 3. Organochlorine pesticide levels (ng/kg) in Sg. Kelang and Sg. Selangor sediments

Station nos.		Organochlorine pesticide					
		γ-HCH	Heptachlor	Aldrin	Endosulfan	Dieldrin	DDT
Sg. Kelang	601	98	598	4036	nd	nd	1973
	603	1798	575	450	892	185	1795
	607	102	276	nd	758	839	1098
	624	3331	190	nd	nd	458	1506
Sg. Sel.	No. 1	4030	1275	462	960	1045	8764
	No. 2	312	258	324	277	373	923
	No. 3	157	134	316	122	2518	371

Table 4. Average levels (ng/L) of organochlorine pesticides in Peninsular Malaysian rivers (Sg. = sunga = river)

River	t-HCH	Endosulfan	Heptachlor	t-DDT	Aldrin+Dieldrin
Standards A[a]	2000	10000	50	100	20
Standards B[b]	130	ND	60	4	8
Sg. Perlis	30	159	11	33	nd
Sg. Kedah	50	24	16	42	11
Sg. Merbok	32	111	15	35	15
Sg. Muda	60	145	16	69	nd
Sg. Prai	65	108	26	48	15
Sg. Juru	54	47	18	52	nd
Sg. Perak	9	nd	5	25	2
Sg. Bernam	320	62	119	193	40
Sg. Selangor	280	313	103	113	48
Sg. Klang	1	nd	nd	10	4
Sg. Linggi	3	nd	nd	8	1
Sg. Melaka	4	33	16	74	3
Sg. Muar	nd	9	22	122	3
Sg. B. Pahat	nd	10	10	103	2
Sg. Pontian	8	12	13	44	9
Sg. Tebrau	1	1	29	59	14
Sg. Johor	nd	13	11	65	34
Sg. Mersing	4	47	10	88	3
Sg. Pahang	1	nd	4	10	nd
Sg. Kemaman	6	nd	4	nd	nd
Sg. Kerteh	2	nd	2	nd	nd
Sg. Dungun	nd	nd	nd	nd	nd
Sg. Terengganu	1	nd	1	nd	nd
Sg. Besut	nd	nd	nd	nd	nd
Sg. Kelantan	nd	nd	1	nd	nd

nd : not detected (i.e. below detection limit)
[a] Recommended Interim Standards of Malaysian ambient water quality for domestic use.
[b] Recommended Interim Standards of Malaysian ambient water quality for aquatic life.

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Conclusion

Because the sediment samples obtained from the river bed are less prone to variations caused by climatic changes than are surface waters, they may be an alternative sample matrix for monitoring the environmental fate of those organochlorine pesticides which are still being used in agriculture.

The efficiency of the recovery of these pesticides from the river sediment samples using the ultrasonic extraction is not significantly different from that of soxhlet extraction.

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Dissipation of Organochlorines in Northern Indian Soils

Hari C. Agarwal and Dileep K. Singh*

Abstract

Different components of the Indian environment, including soil, are contaminated by moderate to high levels of organochlorines such as DDT and HCH. Measured average DDT concentrations in human whole blood were in the range 0.12–0.68 mg/L in Delhi, and DDT was detected in all but 2 of 50 samples of soil and earthworms. The atmosphere, rainwater, and the Jamuna River ecosystem in Delhi contain appreciable residues of DDT and HCH. Significant concentrations of DDT, HCH and, to a lesser extent aldrin, dieldrin, chlordane, heptachlor, and other compounds have been detected in human and bovine milk and in butter, meat, and vegetables in different parts of the country. In view of the widespread contamination of the environment with organochlorines it is essential to study how these insecticides dissipate, to gather information which may be useful in the management of their residues, especially in soil.

The half-lives of DDT and HCH in soil under field conditions, measured using ^{14}C-labelled compounds, were 234–317 days, 319–343 days, and 124–147 days, respectively. At an altitude of 1211 m above sea level, DDT had a half-life of 250 days, and DDE a half-life of 271 days. Of the total residues, about 8% DDT and 13% HCH were bound to soil in one year, and later partly released over 2–3 years. A method was developed to chemically release the soil-bound residues without damaging them. Volatilisation and, to a lesser extent, mineralisation were the main mechanisms of loss of DDT, DDE, HCH and metabolites from the soil and were higher at higher temperatures. Solar radiation, especially UV radiation, and flooding of soil enhanced volatilisation of DDT and HCH.

THE widespread and often indiscriminate use of insecticides has resulted in pesticide failure, pest resurgence, development of resistance in insects against the insecticides, contamination of the environment with the toxic residues of persistent insecticides, and harm to non-target organisms. The residues of organochlorine insecticides, especially DDT and HCH are extremely common in the environment, even in areas where these pesticides have not been used (Dhaliwal and Singh 1993). Studies around Delhi, northern India have shown the presence of DDT and its metabolites in all samples of human blood examined, at concentrations in the range 0.117–0.683 mg/L (Agarwal et al. 1976). DDT was detected in all but 2 of 50 samples of soil and earthworms in Delhi (Yadav et al. 1981). The atmosphere, rainwater, and the Jamuna river ecosystem in Delhi all contain appreciable residues of DDT and HCH (Agarwal et al. 1986, 1987; Kaushik et al. 1987). Significant concentrations of DDT, HCH and, to a lesser extent aldrin, heptachlor, and other organchlorines have been detected in human and bovine milk, and in butter, meat, and vegetables in different parts of the country (Nair et al. 1992; Dhaliwal and Singh 1993).

Once these insecticides are released into the environment they may be redistributed in soil, air, water, and other abiotic and biotic components. The soil is a major sink for all of them. The persistence of an insecticide in the soil depends primarily on the rate of its dissipation from the soil. Most of the studies reported so far on the persistence of pesticides are from temperate countries where climatic conditions are quite different to those in the tropics and subtropics. A half

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life of up to about 10 years has been reported for DDT in temperate conditions (Edwards 1973). However, under tropical and subtropical conditions where temperature and humidity are much higher and ultraviolet radiation may be more intense, insecticides may dissipate much more rapidly. A significant proportion of the insecticide residues reaching the soil may bind with soil organic matter by peptide–lipid interaction and by hydrophobic bonding (Haque 1975). The formation of bound residues may be considered as a significant mechanism of the pesticide retention in the soil. It is, however, possible that after a certain period under suitable conditions this bound form may be released, at least in part, into the environment and may adversely affect living organisms.

DDT is known to degrade into DDD, DDE, and certain other metabolites in the environment. DDE is the major metabolite and may account for about 25% or more of the total DDT. DDE has been reported to be the major contributor to pesticide-induced shell-thinning in the eggs of many birds (Hazeltine 1972). DDE resulted in frequent cracking of eggs, embryo mortality, and reduction in duckling production.

In view of the widespread contamination of the environment with organochlorine insecticides and their metabolites, especially DDT, DDE, and HCH, and a dearth of studies on the fate of pesticide residues in the soil in warmer climates, the investigations reported in this paper were undertaken in northern India, particularly around Delhi.

Materials and Methods

DDT field experiments

First set

The soil of experimental plots was a sandy loam with 59.3% sand, 26.1% silt, 14.6% clay, and 0.8% organic carbon, pH 7.7, and water-holding capacity 23%. 1,1,1-trichloro-2,2-bis (4-chloro [^{14}C] phenyl) ethane (*p,p'*-DDT) with a specific activity (sp. act) 3.14 GBq/mM was obtained from Amersham International, U.K. for use in these experiments.

Three field experiments each lasting one year were started in plots located at the University of Delhi campus during summer, monsoon, and winter seasons from June 1984 to May 1985. Hard polyvinyl chloride (PVC) cylinders (length 17 cm, inside diameter 10.4 cm) were driven into the soil, with 3 cm remaining above the ground. Ten mL DDT solution containing 5 μCi ^{14}C DDT and unlabelled DDT equivalent to 1 kg a.i./ha was applied to the soil surface of each cylinder. Samples consisting of three cylinders each selected at random were dug out at suitable intervals (Fig. 1) and the soil in each cylinder was divided into an upper (7.5 cm) and two lower (3 cm each) segments.

Triplicate samples of 50 g soil each were soxhlet extracted with 150 mL methanol for 4 hours to determine the extractable residues. The methanol-extracted soil was then combusted using a wet combustion technique to determine the non-extractable 'bound' residues (Samuel et al. 1988). The solvent extracts were analysed after clean-up by gas–liquid chromatography(GLC) (Packard Series 7400) and thin-layer chromatography (TLC). Liquid scintillation counting was done using a Packard Tricarb 460 scintillation spectrometer with automatic quench correction. The details of the methods used are described by Samuel et al. (1988).

Second set

Uniformly ^{14}C-ring labelled *p,p'*-DDT (sp. act. 104 mCi/mmol) was obtained from Amersham International. The experimental set up was similar to the one described in the first set of experiments. Also, the site used was adjacent to the one used earlier. In the first experiment the soil in each cylinder was treated with 4.67 μCi ^{14}C-DDT and 1.91 mg unlabelled DDT and the experiment started in summer 1989. Another experiment using ^{14}C-DDT (sp.act. 27.95 mCi/mmol, obtained from Dupont, Boston, USA) was started in February 1990. The soil in each cylinder was treated with a 10 mL solution of 8 μCi ^{14}C DDT and 10 mg unlabelled DDT. These experiments were continued for two years. Three cylinders were dug out at each sampling time (Fig. 2) and the top 7.5 and 10 cm soil was carefully removed in the first and second experiments, respectively, for analysis of insecticide residues. The extractable residues were obtained as described above and analysed by high performance liquid chromatography (HPLC) using a Zorbax ODS C18 reverse phase column (Shimadzu LC-4A) and TLC as described by Singh and Agarwal (1992). The bound residues were determined by combusting, in a Harvey biological oxidiser Model OX-400, 500 mg soil samples before and after methanol extraction.

Third set

The ^{14}C-*p,p'*-DDT was the same as used in second set of experiments. Also, the same concentrations of labelled and unlabelled DDT were used. The experiment was set up at Dharmsala in Himachal Pradesh,

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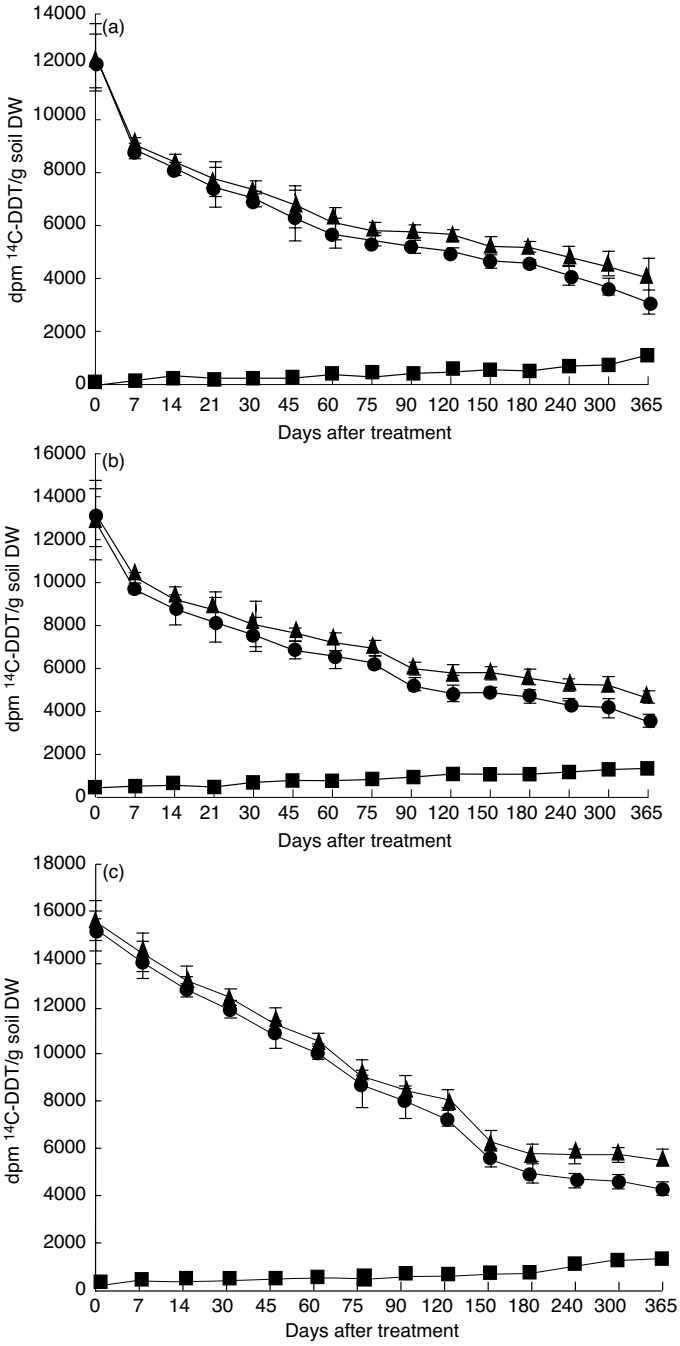

Figure 1. Dissipation of DDT from soil in (a) summer, (b) monsoon, and (c) winter: ●, extractable residues; ■, bound residues; ▲, total residues.

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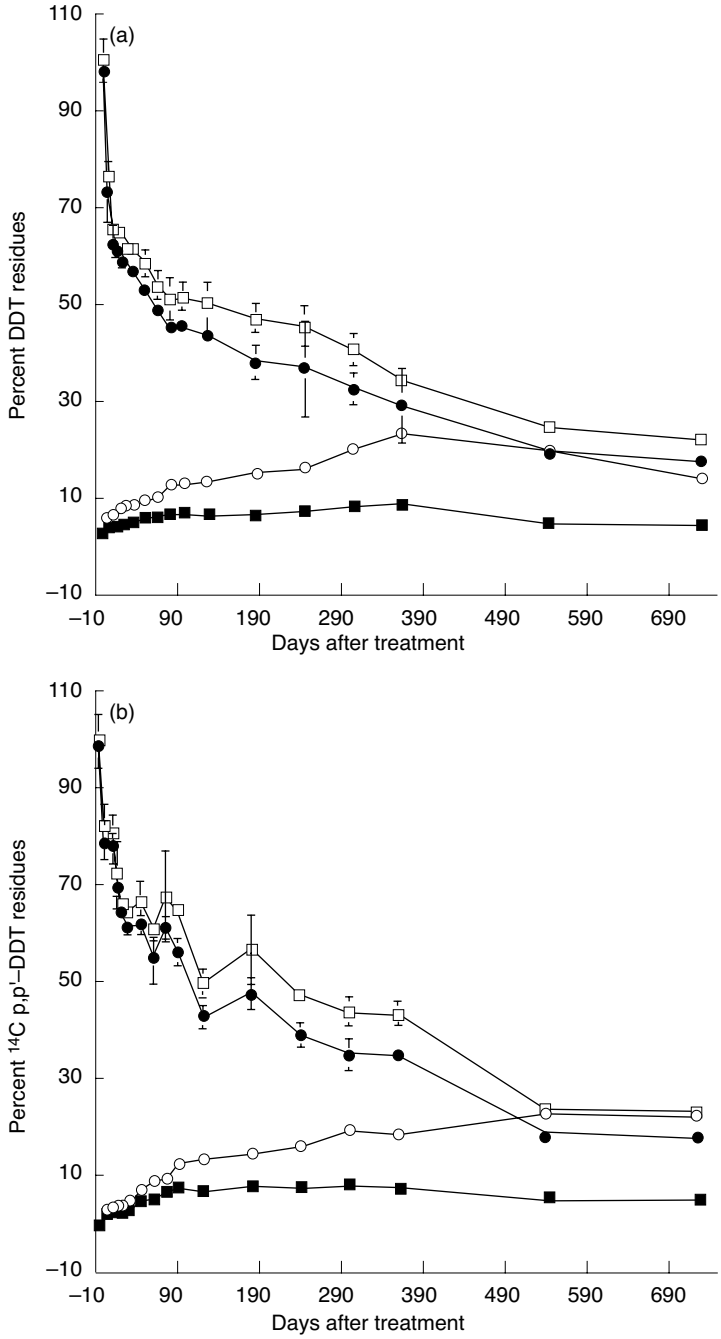

Figure 2. Persistence of ^{14}C and unlabelled DDT in field soil: (a) first experiment; (b) second experiment. Key: □, total residues; ●, extractable residues; ■, bound residues; ○, proportion of bound residues in total residues.

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India (elevation 1211 m above sea level, 32°15'N. latitude and 76°15'E. longitude). The treatment was done in October 1992. The sampling was performed at regular intervals as in the second set of experiments and the experiment was continued for one year. The soil was sandy loam, rich in organic matter and slightly acidic (pH 6.3). The soil was analysed as in the second set of experiments. The details of the methods are given by Singh and Agarwal (1995). The soil temperature and moisture content, and the amount of rainfall, were recorded regularly. The recoveries of the experimental insecticides and metabolites were determined at each stage of the processing and were generally above 93%. The data presented have been corrected for the recoveries.

Chemical release of bound residues

Fifty grams of soil extracted with methanol was left covered with sulfuric acid for 48 hours. Methanol (100 mL) was added and the mixture shaken vigorously for 30 minutes. The residues were then partitioned three times with 50 mL hexane. The pooled extracts were washed repeatedly with water to remove the acid, passed through anhydrous sodium sulfate and dried under vacuum at 40°C. The residues were then dissolved in HPLC grade methanol for analysis by HPLC.

DDT laboratory experiments

Effect of temperature

Soil samples (35 g) treated with ^{14}C (0.8 μCi) and unlabelled *p,p'*-DDT (5 μg/g) were incubated in closed 100 mL Erlenmeyer flasks at 15, 30, and 45°C for 28 days. Additional samples of treated soil were incubated under flooded conditions. Each experiment was replicated three times. Air inside the flasks was replaced after every 2 days by sucking air through the flasks for 6 minutes with a vacuum pump. Volatilised organics were trapped by polyurethane foam (PUF), while $^{14}CO_2$ was absorbed in 0.5 M KOH. The plugs were soxhlet extracted with acetone:hexane (1:1) for 2 hours and the extracts analysed for radioactivity and by GLC. KOH samples were assayed for radioactivity. At the end of the experiment the soils were extracted for the extractable residues. Bound residues were also estimated. The residues were analysed qualitatively and quantitatively as described above.

Effect of solar radiation

Soil samples (25 g), flooded and unflooded, were treated with 1.25 μCi ^{14}C and 5 μg/g unlabelled *p,p'*-DDT and exposed to summer sunlight for 42 days. The soils were held in closed dark, glass, or quartz tubes (150 mm long × 25 mm o.d.) with inlet and outlet glass tubes attached through a cork with two holes. Half the tubes (9) contained soil kept at a moisture level of 75% field capacity, while the other half contained soil covered with a 3 cm water layer (flooded). Each set composed of dark, glass and quartz tubes in triplicate. The volatilised organics and $^{14}CO_2$ were collected weekly and analysed as described above. The soil samples in the tubes were also processed and analysed at the end of the experiment as described above.

DDE field experiments

1,1-dichloro-2,2-bis-[p-chloro ^{14}C-phenyl] ethylene (*p,p'*-DDE) with a sp. act. of 3.85 M Bq/mg, was obtained from the Institute of Isotopes, Budapest, Hungary for use in these experiments. The experiments were conducted at the same site on the University of Delhi campus as the first set of DDT field experiments. Similar PVC cylinders were used. The soil in each cylinder was treated with a 10 mL hexane solution containing 8 μCi^{14}C and 10 mg unlabelled *p,p'*-DDE. All samples consisted of three cylinders which were taken out at regular intervals up to 545 days after the treatment. The top 7.5 cm soil from each cylinder was carefully removed and processed for extractable and bound residues as described in the second set of field experiments for DDT.

DDE laboratory experiments

Effect of solar radiation

One hundred grams of soil treated with 0.0187 μCi ^{14}C and 5 ppm unlabelled *p,p'*-DDE was placed in each of the experimental tubes made of quartz or glass (light and dark). The tubes were exposed to direct sunlight during the summer season for 6 weeks. Quartz tubes permitted entry of sunlight including UV, whereas glass tubes excluded most of the UV radiation. The samples in the black glass tubes were used as controls. The air inside the incubation tubes was flushed once a week for 10 minutes. ^{14}C organics volatilised were trapped in hexane, and $^{14}CO_2$ was taken up in a mixture of ethanolamine–methanol (6:4). A solution of KOH was used to remove atmospheric CO_2 before the air entered the incubation tubes. The volatilised organics, $^{14}CO_2$ and the experimental soil were processed as described in the second and third set of field experiments.

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HCH field experiments

Uniformly ring labelled ^{14}C-γHCH (1α, 2α, 3α, 4α, 5α, 6β-hexachlorocyclohexane) with a sp. act. of 2 GBq/mM, was obtained from Amersham International. The experiments were conducted at the same site as the first set of field experiments for DDT, and the soil characteristics were the same as already described. As in the case of DDT, the experiments lasted for one year and were started in summer, monsoon, and winter. The procedures used for treatment, sampling, extraction, and analyses were the same as already described for DDT (first set).

HCH laboratory experiments

Effect of temperature

Each soil sample (35 g) was treated with 0.75 μCi ^{14}C and 5 μg/g unlabelled γ-HCH in hexane solution. The soil was incubated at 15, 30, and 45°C and 30°C under flooded conditions for 28 days. The same experimental procedures including replicates were used as in case of the laboratory studies with DDT. Both mineralisation and volatilisation were monitored weekly.

Effect of solar radiation

Each soil sample (25 g) was treated with 0.45 μCi of ^{14}C and 5 μg/g unlabelled γ-HCH in hexane solution. The treated soil samples, both unflooded and flooded, were exposed to sunlight for 42 days in dark, glass, and quartz tubes. Each type of exposure to sunlight was replicated three times as before. The release of $^{14}CO_2$ due to mineralisation and the ^{14}C organic volatiles were collected weekly and processed as described earlier for studies of the effect of temperature for HCH. The soil was also analysed at the end of the experiment as above.

Results and Discussion

DDT field experiments

First set

The soil temperatures during the first two months after treatment in the summer, monsoon, and winter experiments averaged 36 ± 2°, 34 ± 2° and 11 ± 3°C, respectively. During summer, 9 to 14 weeks after treatment, 46.4 cm of rainfall was received, while during the monsoon 58.1 cm rainfall occurred between 3 and 9 weeks after treatment. In the winter, 51.5 cm rainfall was received during the 4 to 7 months after treatment.

The extractable, bound, and total residues of DDT detected in the top 7.5 cm soil in the three different treatments are shown in Figure 1 in terms of disintegrations per minute (dpm)/g soil dry weight. The data for the lower 3 cm are not given, as the amount of DDT detected in that layer of soil was very low. This may be due to the extremely low solubility of DDT in water (Sharom et al. 1980). The extractable residues initially in the summer, monsoon, and winter treatments were 12249, 12785, and 15246 dpm/g soil (dry weight), which declined to 3085, 3432, and 4262 dpm, respectively. The total residues in the summer, monsoon, and winter treatments were 12355, 13097, and 15469 dpm initially and 4054, 4592, and 5574 dpm/g soil (dry weight), respectively, after one year. Total DDT remaining in the soil after one year was 32.8, 35.1, and 36.0% of the insecticide initially applied in the summer, monsoon, and winter treatments, respectively. The overall half lives of DDT in the summer, monsoon, and winter treatments were 309, 317, and 234 days, respectively (Table 1). The rate of dissipation was rapid and linear during the initial period. A slower rate of dissipation followed in the intermediate period and it became very slow in the final period in which only about 3% of DDT was lost in 6 months in all the treatments. The soil-bound residues were almost nil in the beginning but increased gradually to reach about 8% of the DDT applied and constituted about 25% of the total residues present in the soil after one year. The extractable residues contained about 75% DDT, 20% DDE, and traces of DDD.

Second set

In the first experiment the total DDT residues present in the samples at the start of the experiment were 99.1 × 10^5 dpm which decreased to 20.4 × 10^5 dpm 730 days after treatment. The extractable ^{14}C DDT at zero-time was 97.0 × 10^5 dpm which gradually fell to 16.65 × 10^5 dpm two years later. As in the first set of experiments, the initial dissipation was very rapid. About 36% of the DDT was lost in the first 21 days. It had fallen to about 49% after 95 days and, finally, after 730 days, only about 21% of the DDT applied was left in the soil (Fig. 2a). The bound residues increased gradually with time, reaching a maximum of about 8% after one year, but declined thereafter to 3.5% after two years (Fig. 2a). The half-life of DDT in the initial phase was 33 days, and was 480 days in the final phase. The overall half-life was 319 days (Table

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Table 1. Half-lives ($t_{1/2}$) of DDT in soil under field conditions in different experiments.

Experiment no.	Experimental conditions	Phase	$t_{1/2}$ (days)	Overall $t_{1/2}$ (days)
I. (a)		Initial	70	
	Summer[a]	Intermediate	513	309
		Final	555	
(b)		Initial	78	
	Monsoon[a]	Intermediate	345	317
		Final	761	
(c)		Initial	113	
	Winter[a]	Intermediate	141	234
		Final	10 263	
II. (a)		Initial	33	
	Summer[e]	Intermediate	221	319
		Final	480	
(b)	Winter[b]	Initial	45	343
		Intermediate	484	
		Final	421	
III	Higher altitude[c,d]	Initial	34	
		Final	409	250

[a] Time of year at which experiment was initiated.
[b] Concentration of DDT five times then in II (a)
[c] Experiment conducted at an elevation of 1211 m.
[d] One-year experiments.
[e] Two-year experiment.

1). The extractable residues consisted of DDT, DDE, and DDD.

In the second experiment the concentration of unlabelled DDT was about five times that in the first experiment. The rate of dissipation was very similar to that in the first experiment. Two years after the treatment the extractable residues accounted for about 18% of the amount applied. The overall half-life of DDT was 343 days. The bound residues were maximal (about 8.2%) after one year as in the earlier experiments and declined to 5.0% after two years. DDE was the major metabolite and accounted for 28% of the extractable residues after one year, increasing to 37% after two years. DDD accounted for about 7% after one year.

Third set

The results of this high-altitude experiment are presented in Figure 3. The dissipation seems to follow a diphasic curve. The loss of DDT from the soil was rapid in the initial phase: about 35% had been dissipated within 21 days of the treatment. After one year the extractable residues accounted for about 28% of the DDT applied initially. The half-life was 34 days in the first phase and 409 days in the final phase. The overall half-life was 250 days. The extractable residues consisted of *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDMU. After one year, DDT was 28.8% of the total extractable residues. DDE increased to 24% of the initial value in one year. DDD and DDMU showed maximal values of 6.4% and 2.53% at 35 and 305 days, respectively. The soil-bound residues increased gradually to the maximum of 8.7% after one year.

The results of these studies indicate that, under Indian conditions, DDT dissipates more rapidly from the soil than it does in temperate countries. The dissipation curves obtained are more or less similar to those in earlier reports, i.e. initially the loss is rapid but it gradually becomes slower. The dissipation was generally lower in the winter months, as earlier shown by Yeadon and Perfect (1981). Half-lives of more than 2 years have been reported under temperate climates (Nash and Woolson 1967). However, shorter half-lives have been reported from Asian and African countries, e.g. 144 and 313 days in Pakistan

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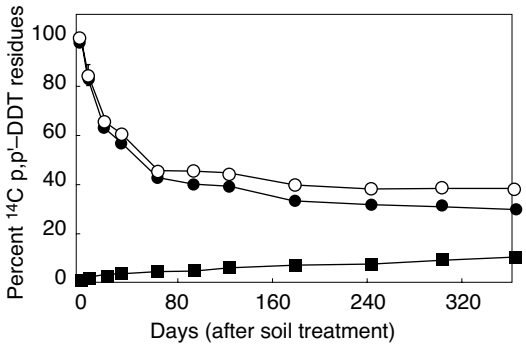

Figure 3. Persistence of DDT in soil at higher altitude: ○, total residues; ●, extractable residues; ■, bound residues

(Hussain et al. 1994), and 55 days in Egyptian soil (Zayed et al. 1994). The half-life of DDT at higher altitude was shorter than in the fields in Delhi even though the temperatures were lower. This may be attributable to higher UV radiation, rainfall, and soil moisture at higher altitude. The slightly acidic pH of the soil may also be a factor. The lack of bound residues in the initial phase may partly contribute to a rapid loss of DDT, which became much slower later in the experiment when the bound residues rose rapidly. The formation of bound residues may be useful for immobilising residues for degradation by some means or other (Kearney 1976), highlighting the practical importance of knowledge about the extent and nature of bound residues formed by DDT. In the study reported here, the major metabolites of DDT found in the extractable and bound residues released were

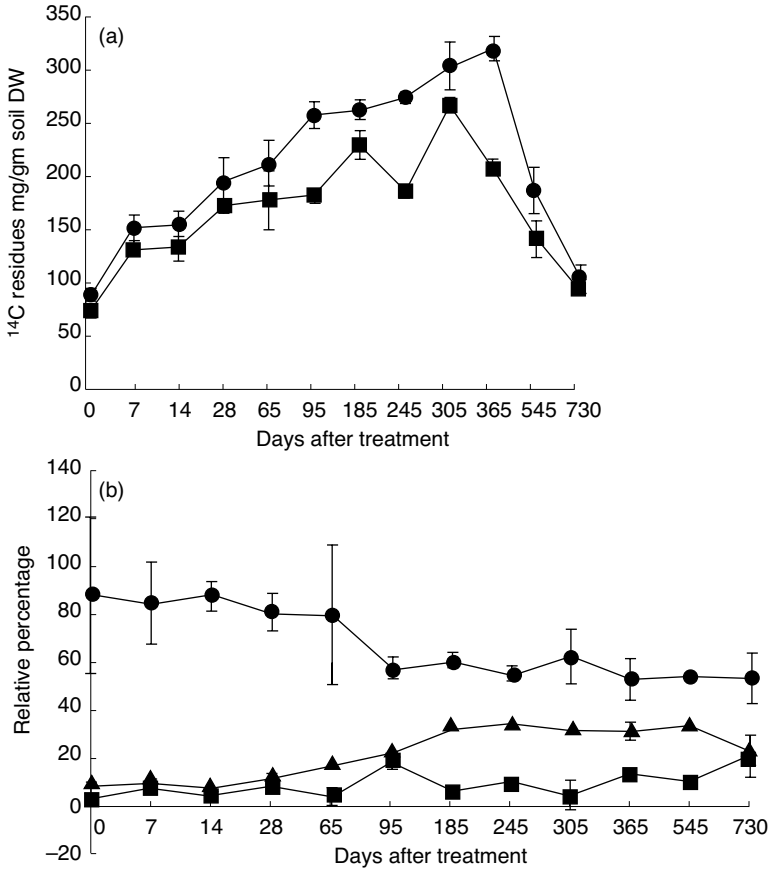

Figure 4. (a) Release of soil-bound ^{14}CDDT residues by sulfuric acid: ●, initial bound residues; ■, released by sulfuric acid. (b) Composition of soil-bound residues of DDT released by sulfuric acid: ●, DDT; ■, DDD; ▲, DDE.

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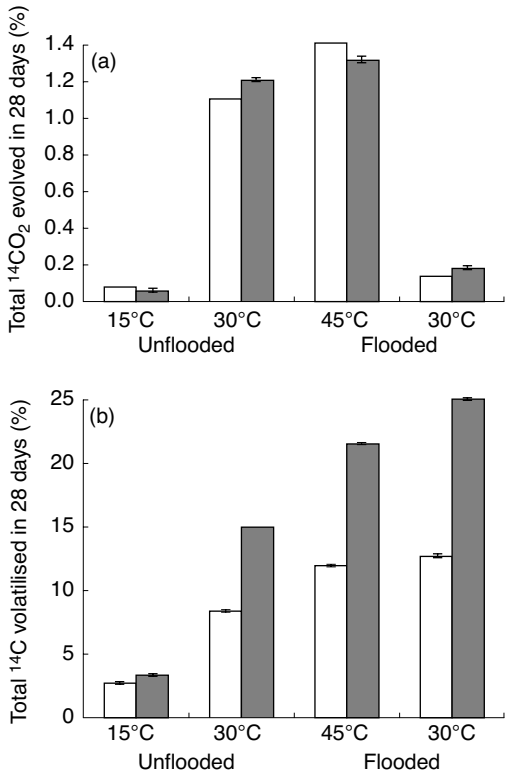

Figure 5. (a) Total $^{14}CO_2$ released from soils treated with ^{14}C DDT (unshaded bars) or ^{14}C γ-HCH (shaded bars) at different temperatures. (b) Total ^{14}C volatilised from soils treated with ^{14}C DDT or ^{14}C γ-HCH at different temperatures

DDE and DDD, confirming the findings of other researchers (e.g. Hussain et al. 1994).

Chemical release of bound residues

To determine the nature of the soil-bound residues they must first be released from the soil. To achieve this a method which uses sulfuric acid was developed. It released up to 91.5% of the bound residues (Fig. 4a). This treatment does not change the amount or composition of the residues. Similar results were reported by researchers from Egypt and Pakistan (Hussain et al. 1994; Zayed et al. 1994). The recovery of DDT and its metabolites was 86.6% for DDT, 87.9% for DDE, and 88.5% for DDD. The bound residues released by sulfuric acid treatment were found to contain predominantly *p,p'*-DDT, followed by *p,p'*-DDE and *p,p'*-DDD. The major metabolite was *p,p'*-DDE, which increased gradually to about 34% between about 245 and 545 days after treatment in the first experiment of the second set. The proportion of *p,p'*-DDD also increased gradually, but its amount was much less than that of DDE. However, by two years DDD (22%) was almost equal to DDE (Fig. 4b).

DDT laboratory experiments

Effect of temperature

The evolution of CO_2 increased with time and increase in temperature. The total CO_2 produced was maximal at 45°C and amounted to 1.4% of the initial DDT after 28 days (Fig. 5a). In the flooded sample incubated at 30°C the mineralisation accounted for only 0.13%. Volatilisation of DDT was maximal one week after treatment and declined subsequently. Volatilisation increased with temperature and was highest at 45°C (Fig. 5b). However, in the flooded samples, the volatilisation was much higher (12.6%) than in the unflooded ones (8.2%) at the same temperature in 28 days. Temperature has been shown to increase volatilisation (Farmer et al. 1972).

Effect of solar radiation

The effect of solar radiation on mineralisation from soil under unflooded and flooded conditions is shown in Figure 6. Though the rate of mineralisation is very low it increased with time. Generally, the rate of mineralisation was greater in unflooded soil.

The volatilisation of ^{14}C DDT from unflooded and flooded soils exposed to solar radiation is shown in Figure 7. The volatilisation was maximal during the first week after treatment and declined thereafter. The flooded soils showed higher volatilisation than unflooded soils. Mineralisation and volatilisation were highest in the quartz tubes and lowest in the dark tubes. These results suggest that ultraviolet radiation is the part of the solar spectrum that most stimulates mineralisation and volatilisation. As the temperatures recorded in the tubes were highest in the quartz tubes this may also have influenced the higher rates of mineralisation and volatilisation. It seems clear that the volatilisation of DDT is the major factor in dissipation of DDT from the soils, as also shown by Farmer et al. (1972) and Spencer (1991). DDE was the main metabolite, but under flooded conditions DDD was also detected. Castro and Yoshida (1971) showed that anaerobic conditions promoted the formation of DDE.

DDE field experiments

DDE is the major metabolite of DDT and is the main companion of DDT in the total residues found in the environment. The data collected on DDE dissipation are presented in Figure 8. The dissipation curve

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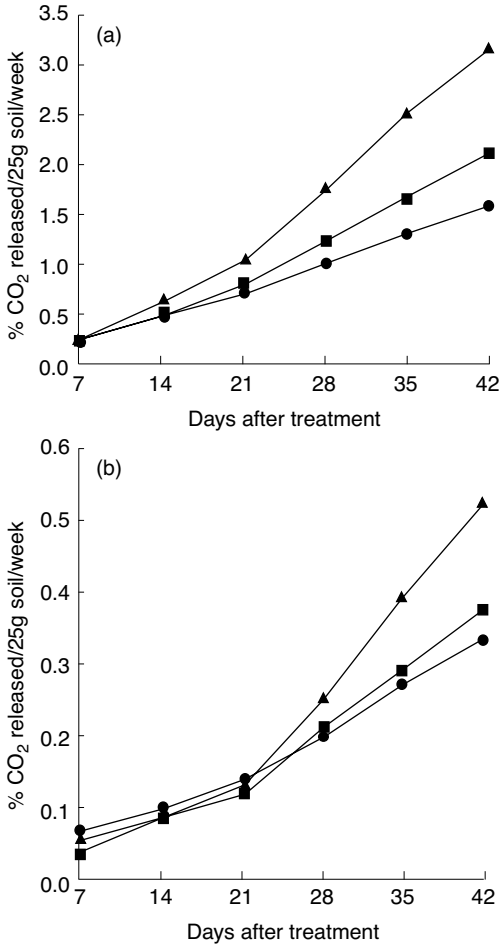

Figure 6. Cumulative percent mineralisation of ^{14}C DDT from (a) unflooded and (b) flooded soils exposed to solar radiation. Key: ▲, soil held in quartz tubes; ■, soil held in glass tubes; ●, soil held in dark tubes.

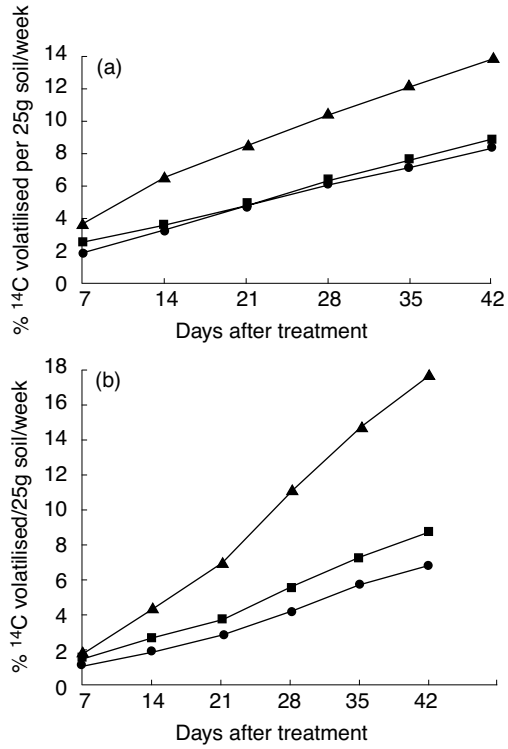

Figure 7. Cumulative percent volatilisation of ^{14}C DDT from (a) unflooded and (b) flooded soils exposed to solar radiation. Key: ▲, soil held in quartz tubes; ■, soil held in glass tubes; ●, soil held in dark tubes.

seems to be biphasic, an initial rapid phase being followed by a slower phase. About 61% DDE is lost in the first year. Ware et al. (1975) reported higher volatilisation of DDE than of DDT. Cliath and Spencer (1972) reported that the vapour pressure of *p,p'*-DDE was about eight times that of DDT. The overall half-life of DDE was 271 days, which was shorter than that of DDT at the same site.

The extractable residues also followed a similar dissipation curve. The only metabolite detected was DDMU which was about 2% of the total. As in case of DDT, the bound residues were initially very small but increased gradually to reach a maximum of about 8.7% after one year. At the end of one year about 39% of the DDE initially applied was present, more than 22% in the bound form. Like DDT it may bind to soil organic matter. It may also be trapped in soil micropores (Steinberg et al. 1987). The soil-bound residues of DDE were released by sulfuric acid treatment as already discussed in the DDT experiments. The residues released by sulfuric acid treatment contained only DDE.

DDE laboratory experiments

Effect of solar radiation

The results of these experiments are shown in Figure 9. The loss of *p,p'*-DDE by mineralisation to $^{14}CO_2$ was maximal from the soil in quartz tubes, about 8.4% after 42 days. It was only about half this in the dark tubes. Mineralisation indicates total microbial breakdown of organic compounds (Wang et al. 1985). Loss of DDE by volatilisation was almost three times that from mineralisation. Volatilisation showed the same trend as mineralisation, i.e. it was maximal

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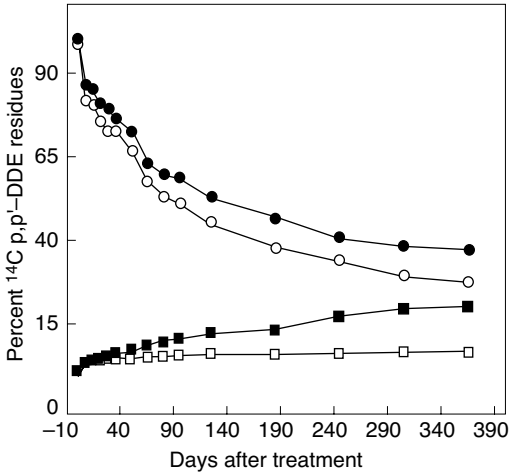

Figure 8. Persistence of ^{14}C and unlabelled DDE in field soil. Key: ●, total residues; ○, extractable residues; □, bound residues; ■, proportion of bound residues in total residues.

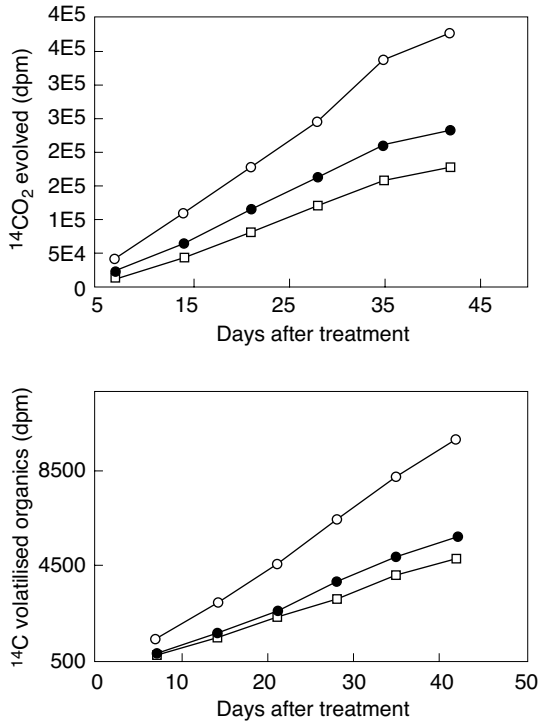

Figure 9. Cumulative (a) mineralisation and (b) volatilisation of ^{14}C and unlabelled DDE. Key: ○, soil held in quartz tubes; ●, soil held in glass tubes; □, soil held in dark tubes.

in the quartz tubes (24%), followed by the soil in glass tubes (14%), and the dark tubes (11.5%). Almost 25% of DDE was lost by volatilisation in 42 days. As in the case of DDT, the UV component of solar radiation was most stimulatory of mineralisation and volatilisation, though the higher temperatures recorded in the quartz tubes may again be a contributing factor. When the soil was extracted at the end of the experiment, the soil from quartz and light tubes was found to contain about 80% DDE and 20% DDMU. In the dark tubes, on the other hand, DDMU was only about 8%. indicating that sunlight and/or temperature increase may favour its formation.

HCH field experiments

As the ^{14}C activity present in the soil below 7.5 cm was just 3% of the initial HCH, only the top 7.5 cm soil was analysed in all samples. The dissipation of HCH followed a similar pattern as to that of DDT except that it was much more rapid (Fig. 10). After one year the total HCH residues remaining in soil were 14.7, 15.4, and 15.1% in the summer, monsoon, and winter treatments, respectively. The overall half-lives of HCH in the three treatments were 147, 144, and 124 days, in contrast with the half-life of about 1.2 years found under temperate conditions (Edwards 1966). It is therefore obvious that in north Indian conditions HCH disappears rapidly from the fields. This is apparently due primarily to volatilisation from the soil surface, which may be assisted by higher temperatures. Higher proportions of HCH than of DDT bound to the soil They were about 13% of the initial amount after one year.

At the end of one year the bound residues accounted for more than 75% of the total residues remaining in the soil. The soil-bound residues may be important in the very slow loss of HCH in the last 6 months of the experiment when less than 1% HCH was dissipated. Degradation of HCH in soil appears to be negligible as no metabolites or isomers of HCH could be detected by gas–liquid chromatography.

HCH laboratory experiments

Effect of temperature

The rate of mineralisation increased with temperature, as in the case of DDT. About 0.06% CO_2 was evolved at 15°C in 28 days, whereas at 45°C it increased to 1.31% (Fig. 5a). In the flooded soils it was much than in unflooded soils at the same temper-

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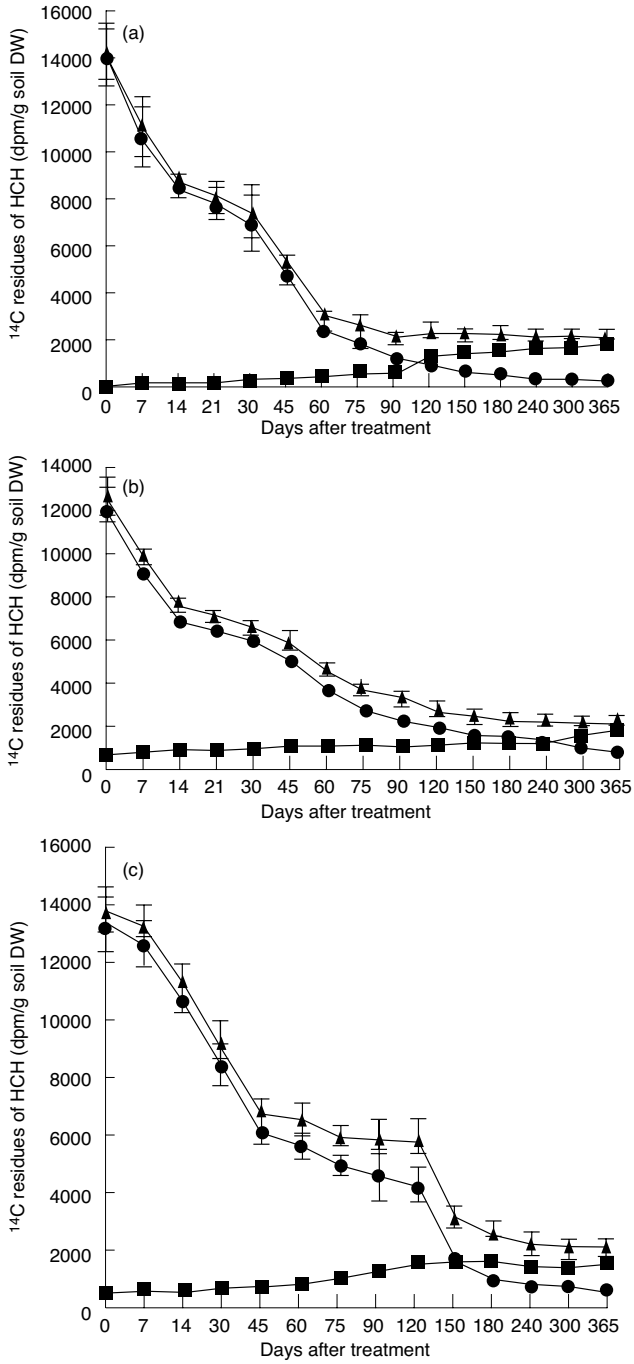

Figure 10. Dissipation of HCH from soil in (a) summer, (b) monsoon, and (c) winter: ▲, total residues; ●, extractable residues; ■, bound residues.

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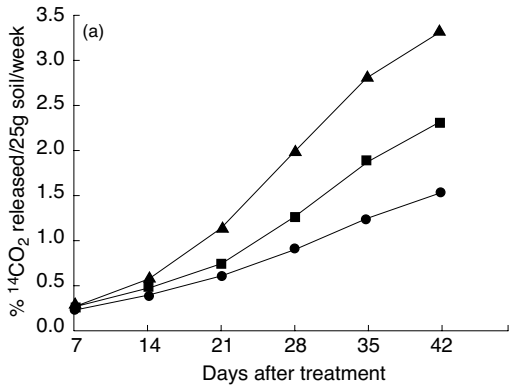

Figure 11. Cumulative percent mineralisation of ^{14}C γ-HCH from (a) unflooded and (b) flooded soil, exposed to solar radiation: ▲, soil held in quartz tubes; ■, soil held in glass tubes; ●, soil held in dark tubes.

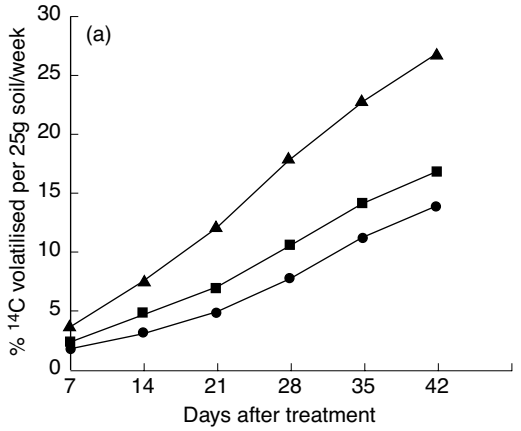

Figure 12. Cumulative percent volatilisation of ^{14}C γ-HCH from (a) flooded and (b) unflooded soils exposed to solar radiation: ▲, soil held in quartz tubes; ■, soil held in glass tubes; ●, soil held in dark tubes.

ature. The volatilisation was maximal in the first week and declined thereafter, as in case of DDT. In the unflooded soils it increased almost six-fold as the temperature rose from 15°C to 45°C. HCH volatilised much more rapidly than did DDT. In the flooded soils at 30°C about 24.9% of the initial HCH was volatilised (Fig. 5b). In the unflooded soils, the volatilised organics consisted of about 13% γ-PCCH (pentachlorocyclohexene) after 28 days in the soil exposed to 45°C. In the flooded soils at 30°C the corresponding figures for γ -PCCH were only 3% and γ -TCCH (tetrachlorocyclohexene) accounted for about 7%. The soil-bound HCH was maximal at a temperature of 45°C; it was about 16.7% after 28 days, much less than in the flooded soil (ca 4.8%).

Effect of solar radiation

The cumulative percent mineralisation in the unflooded and flooded soils under different light conditions is shown in Figure 11. The CO_2 evolution increased with time, as in the case of DDT and DDE. Again as in case of DDT and DDE, it was maximal in the quartz tubes and least in the dark tubes, suggesting that it is the UV component of the sunlight which is the most important for mineralisation.

Volatilisation was almost twice from the soils in the quartz tubes than the dark tubes (Fig. 12). Volatilisation from the flooded soil in the quartz tubes was about 26.7% in 42 days and it appeared that flooding increases volatilisation. HCH disappeared much faster from the flooded than from the unflooded soils, as also shown by Raghu and Macrae (1966). Gamma PCCH accounted for 11.5% of the volatiles in quartz tubes

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after 42 days whereas in the flooded samples it was only 2% and γ-TCCH accounted for 14.8%. Alpha HCH (about 2%) was detected from the soils in the quartz tubes only (both flooded and unflooded soils). Apparently the solar radiation affected the metabolism of HCH as more PCCH and TCCH were detected in quartz tubes. Again presence of α-HCH from the soil in quartz tubes indicated that isomerisation of HCH may be mediated by sunlight. Malaiyandi and Shah (1984) showed isomerisation of γ-HCH to α-HCH by photolysis under direct sunlight.

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Pesticide Dynamics in the Tropical Soil–Plant Ecosystem: Potential Impacts on Soil and Crop Quality

R. Naidu, R.S. Kookana, and S. Baskaran*

Abstract

Pesticides have long been used for pest control in countries throughout the world. Although the exact number and the amount of pesticides used are not clear, hundreds of different compounds are in use. For example, PESKEM 95, a database of registered pesticides in Australia, shows that there are more than 1100 pesticide products containing about 300 different active ingredients available for use. Such increasing reliance on pesticides for pest control has led to considerable degradation of soil and surface and groundwater quality in many developed and developing countries throughout the world. The lucrative export markets for produce have resulted in a marked escalation in agrochemical use for pest control in several of these countries. Pesticides can have a number of adverse impacts on the crop, soil, and water environment. These include their (i) impact on crop quality, (ii) impact on soil health, and (iii) potential to leach and contaminate surface and groundwater, especially in irrigated areas and in regions of high precipitation. All of these impacts arise from the biochemical and physical interactions between the applied pesticides and soil particles. Contamination of the environment by pesticide residues arises mainly from their application. Pesticide residues have been detected at the point of application in the field, as a result of spray drifts from aerial spraying, run-off from rain, drainage water from irrigated areas and in groundwater at selected sites throughout the world. Although much research has been conducted on the impact of pesticides in soil and water environments in the temperate region there is a dearth of information on the dynamics of pesticides in the soils of tropical regions. Humid tropical climatic conditions and agricultural practices may play a dominant role in controlling the fate and behaviour of pesticides in tropical field ecosystem. Consequently, the effect of such soil and climatic conditions on soil health and soil–plant transfer of pesticide may differ from that observed under temperate soil conditions. In this paper we focus on the dynamics of pesticides in soils under tropical climates and implications for ecotoxicological effect and availability to plants. Emphasis will be placed on the chemistry of soils in relation to pesticide adsorption–desorption behaviour controlling pesticide availability in soils.

ALONG with fertilizer applications, pesticides—a group of inorganic and organic chemicals—are increasingly being used to control pests in both rural and urban environments throughout the world. Such applications affect soil and water quality (Honeycutt and Schabacker 1994). More recent evidence suggests that pesticides can also affect crop quality (see, for example, reviews in Naidu et al. 1996a). Because of the toxicity of pesticides and their potential to move and contaminate groundwater, during the past 50 years there has been extensive research on the fate and behaviour of these chemicals and the vulnerability of groundwater to pesticide contamination. Consequently, many reviews have been published on the properties and behaviour of pesticides in soils (e.g. Weber 1994; Honeycutt and Schabacker 1994). Besides these studies, much effort has also been directed towards the role of pesticides in the storage, preservation, and quality of food grains. To this end, the Australian Centre for International Agricultural Research organised an international seminar on 'Pes-

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ticides and Humid Tropical Grain Storage Systems' in 1985 (Champ and Highley 1985). In contrast to these studies, only limited information is available on the potential for pesticide uptake by plant roots and the affect of pesticides on soil quality. This may be attributed to both the poor analytical sensitivity and the lack of appropriate techniques for pesticide extraction and analyses in plant tissues.

The importance of monitoring the effect of pesticides on non-target organisms has been emphasised. Among the non-target organisms potentially susceptible to pesticides are the soil algae, cyanobacteria, and protozoans. Some of these organisms are known to contribute to the maintenance of soil fertility and are therefore considered to be an important component of soil microflora. The impact of pesticides on soil biota, plant availability, and propensity to move and contaminate groundwater is largely related to their interactions with soil constituents. There has been much research on this topic in temperate-region soils. The chemical, mineralogical, and physical properties of such soils differ markedly from the strongly weathered soils of the tropical region (Naidu et al. 1998). In contrast to tropical soils, which are typically low in organic carbon content and high in oxidic minerals, temperate soils exhibit higher organic carbon content and are generally rich in expansive-layer silicate minerals. Because contaminant interactions occur at the soil solution–colloidal particle interface, the marked differences in surface chemical properties of the oxidic soils may not permit direct transfer of technology on pesticides from temperate region to tropical soils. Understanding pesticide interactions in soils is needed to make intelligent decisions on pesticide applications, exposure risks to soil biota, animal and human systems, and on the availability of the chemical to the plant. Tropical soils exhibit surface chemical properties that vary with ambient soil solution characteristics (Naidu et al. 1996b), and this has implications for both ionic and non-ionic pesticide interactions in soils. This review is essentially an update of pesticide interactions in soils and the influence of these to pesticide behaviour in tropical soils, plant uptake, and soil quality in the tropical region. A brief overview of the characteristics of soils from the tropical region is therefore presented.

Extent of Tropical Soils

Tropical soils are defined here as those with hyperthermic and isohyperthermic temperature regimes (U.S. Soil Survey Staff 1992) with mean annual temperatures of 22°C or higher. Based on this criterion, tropical soils cover 38% of the Earth's surface. Average annual rainfall in these regions varies from >3000 mm in tropical rainforests to < 300 mm in arid regions. Strongly weathered soils predominate in the high rainfall areas where, because of high intensity rainfall and relatively high temperatures, the weathering reactions are rapid and the strong leaching environment results in the presence of highly stable minerals such as kaolin and sesquioxides. These stable minerals have a unique effect on the surface chemical and physical properties of soils. The wide variations in rainfall and leaching environments in the tropics result in a considerable range of soils. Although soils in the tropics can range from the less-weathered Andisols (Naidu et al. 1987) to strongly weathered Oxisols, it is the highly weathered group that predominates in the humid tropics and is most important in terms of agricultural production. The discussion to follow will therefore be limited to the dominant soil orders found in the tropics, namely, acid Alfisols, Inceptisols, Oxisols, and Ultisols.

Surface Charge Characteristics of Variable Charge Soils

Interactions between contaminants and soil particles occur at the solid–solution interface. Thus, surface chemical properties of soils, particularly surface charge density, may play a dominant role in controlling the interactions between ionic pesticides and the soil surfaces. Surface charge characteristics of temperate region soils differ considerably from those of soils in the tropics. Temperate soils are generally less weathered and consist predominantly of permanently charged minerals. In such minerals, charge arises as a result of isomorphous substitution within the clay mineral lattice. In contrast, strongly weathered soils exhibit variable charge which changes in response to the composition of the ambient soil solution. Factors such as pH, ionic strength, and the major cations/anions in the soil solution determine charge magnitude in variable charge soils. The impact of pH on positively and negatively charged sites on soil surfaces is illustrated in Figure 1. Most of the minerals exhibiting variable charge have relatively high surface areas ranging from 5–40 to 60–200 to over 1000 m^2/g for kaolinite, soils goethites, and allophane, respectively. Since the surface area of a given mineral is constant, charge generation results in an increase in the particle surface charge density. There

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is only limited information on the role of surface charge on pesticide sorption in strongly weathered soils.

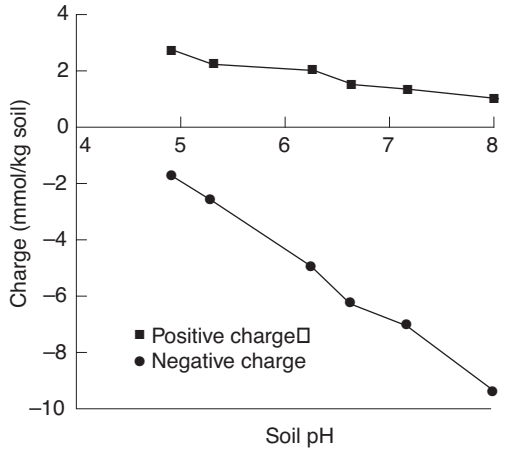

Figure 1. Effect of pH on surface negative and positive charge of an Oxisol from Fiji (Naidu 1985)

Properties and Behaviour of Pesticides

Given the widely different surfaces in tropical soils, a circumstance that arises from differences in the types and amounts of their organic matter and constituent minerals, pesticide management technology developed for temperate regions may not be transferable to pesticide management in the tropics (Ayanaba 1980). Pesticides are generally tested in temperate environments during their development but are often introduced into tropical environments without serious evaluation (Ayanaba 1980). For this reason, we include a detailed discussion on pesticide dynamics in tropical soils. Where possible, comparisons will be made between studies conducted in temperate and tropical regions. Like metal contaminants, pesticides have been shown to have adverse impacts on animal and human health. Table 1 lists some adverse impacts of pesticides on human health. The fate of pesticides in the soil environment is controlled by the properties of the compound, the soil type, and the soil environment (Weber 1994). The soil environment is determined not only by its intrinsic properties but also by external factors such as climatic conditions and agricultural practices. These factors affect the amount and periodicity of changes in the soil environment. Soil water content can affect pesticide retention and absorption by plant roots in several ways. Pesticides are usually transported to the adsorbing surfaces by water. Soil moisture content determines the accessibility of the sorption sites and affects the surface properties of the sorbent. The implications of these factors for pesticide sorption–desorption are discussed below.

Table 1. Effects of poisoning by some commonly used pesticides

Pesticide	Acute poisoning	Severe poisoning
Organophosphate e.g. diazinon	dizziness	salivation
Carbamates e.g. carbaryl	dizziness	vomiting
Organochlorines e.g. aldrin	nausea	muscle twitching
Pyrethroids e.g. permethrin	uncoordination, mental confusion	respiratory, comma, depression

The key properties controlling pesticide behaviour in soils and water include ionisation potential (pK_a), water solubility (K_{sp}), vapour pressure (VP), soil retention (K_{oc}), and persistence ($T_{1/2}$) (Weber 1994). The chemical character of a molecule affects sorption, as it determines the acidic or basic nature, its water solubility, and strength of sorption. The relative importance of van der Waals' type attraction in sorption also depends on chemical properties. All of the above properties vary considerably with the nature of the associated functional groups. Pesticides from various chemical classes and their sorption interaction with soils has been summarised in Table 2 (Weber 1994). Irrespective of the type of pesticide, the behaviour of all pesticides following entry into the soil is regulated by two simultaneous equlibria (Fig. 2).

In a closed system, at equilibrium, the concentration within each phase will be different. Mackay (1979) determined the partitioning between phases by equating the chemical potential in each phase using fugacity, which is linearly related to concentration:

$$C_i = f_i Z_i \quad [1]$$
$$= m_i / V_i \quad [2]$$

where i denotes the ith phase, C is the concentration (mol/m^3), f_i is the fugacity, m_i is the molar mass, V_i is the ith volume, and Z is the fugacity capacity ($mol/m^3/Pa$) of the phase (Mackay 1979). Fugacity has been related to the vapour pressure and pesticide

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solubility. This was illustrated by Spencer and Cliath (1970) who used soil pesticide sorption studies and Freundlich isotherms to show that the amount of lindane needed to produce a saturated soil solution was the same as that required to produce a saturated vapour pressure in the soil. These investigators concluded that the partitioning of a pesticide between the soil solution and soil air, which is related to its solubility, is influenced by pesticide sorption in soils. Based on these studies, it was concluded that the partitioning between the soil solution and soil air is governed by Henry's law constant (equation 3).

$$C_i = kP_x \quad [3]$$

where C_i is the concentration of the dissolved material, k is Henry's constant, and P is the partial pressure above the solution.

The intrinsic pesticide properties, vapour pressure, and solubility determine the dynamics of pesticide interaction with the soil matrix. The relationship between vapour pressure, solubility, and sorption can be illustrated by comparing lindane, a non-polar pesticide, and atrazine, which is a polar compound. Lindane is a chlorinated hydrocarbon with a very low water solubility (K_s < 10 mg/L). Of the 7 chlorinated hydrocarbon pesticides, three have low vapour pressure (VP < 1×10^{-6} mmHg) and four have moderate to high volatility (VP = 12×10^{-6} to 217×10^{-6} mmHg). The moderately to highly volatile lindane with low water solubility results in a much larger air–water partition coefficient than that of atrazine. Consequently, the soil retention of chlorinated hydrocarbons ranges from moderate to high (K_{oc} = 100–10^5). The lower solubility of lindane means that

Table 2. Values for K_{oc}, half-life, and soil reactivity for a number of classes of pesticides (Weber 1994)

Chemical class	Common pesticides	K_{oc} range	$t_{1/2}$ (days)	Soil reactivity
Quarternary N pesticides	Diquat and Paraquat	10^5–10^6	20–500	very high
Pyrethroid pesticides	Bifenthrin and Cypermethrin	10^4–10^6	3–30	high–very high
Dinitroaniline herbicides	Trifluralin and Pendimethalin	10^4–10^5	30–100	high
Organic As and P pesticides	Glyphosate, MSMA and DSMA	10^4–10^5	1–50	high
Organometallics fungicides	Mancozeb and Thiram	10^3–10^5	16–70	moderate–high
Chlorinated hydrocarbon pesticides	Chlorothalonil and Endosulfan	10^3–10^5	21–180	moderate–high
Phenylurea pesticides	Diuron and Linuron	10^2–10^4	10–90	low–high
Organophosphate pesticides	Chlorpyrifos, Fenamiphos and Phorate	20–10^5	1–120	very low–high
Carbamate and carbanilate pesticides	Aldicarb, Carbofuron, Methomyl and Oxamyl	5–3000	1–50	very low–moderate
Thiocarbomate herbicides	EPTC and Molinate	200–3000	7–50	low–moderate
Basic pesticides	Atrazine, Simazine, Cyanazine and Prometryn	20–2500	1–90	very low–moderate
Amide and anilide herbicides	Alachlor, Metolachlor and Napropamide	150–1500	2–40	low
Aminosulfonyl acid herbicides	Asulam, chlorsulfuron and metsulfuron	30–2000	5–90	very low–low
Hydroxy acid pesticides	Bromacil and Dinoseb	10–2000	2–150	very low–low
Carboxylic acid herbicides	2,4-D, Dicamba and MCPA	1–100	3–180	very low–low
Fumigants	Methyl bromide	10–60	1–20	very low

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it is more hydrophobic than atrazine and therefore has a higher affinity for soil organic matter (Adams 1972). This suggests that in the tropical soils with relatively low levels of organic matter, lindane retention by the mineral phase may not be as marked as is recorded in temperate-region soils. The climatic conditions prevalent in the tropical environment may have an important bearing on the volatilisation behaviour of pesticides. Volatilisation under hot and dry conditions is likely to be much higher than under cooler conditions. This has been observed, for example, for endosulfan insecticide in an Australian tropical environment (Finlayson and Silburn 1996).

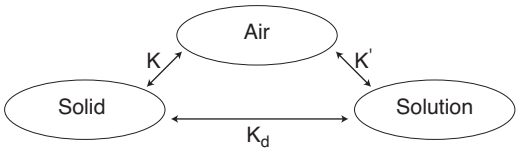

Figure 2. Pesticide equilibrium in the soil–plant–air system

In marked contrast to lindane, ionic pesticides such as the quaternary N compounds are strong bases (pK_a 9–11) with very high water solubility (K_{sp} 1–100%). These pesticides have very low potential for volatilisation (VP 0.01–0.15 × 10^{-6} mmHg at 25°C). The compounds ionise completely in aqueous solution to yield cationic species. Such ionic pesticides are readily adsorbed on the cation exchange sites, supplanting inorganic cations (Bert et al. 1972). The properties of quaternary N and non-polar pesticides are summarised by Weber (1994). Naidu and Morrison (1998) studied paraquat sorption by tropical soils with contrasting mineralogy. They found that, in these soils, the mineral fraction played a significant role in paraquat sorption which was several orders of magnitude higher in a smectite-dominated Vertisol than in an allophanic Andisol. Some acid variable charge soils in the tropics (Oxisols) can have very low CECs despite their high clay contents. The behaviour of cationic pesticides in such soils can be markedly different from the common belief that a soil with high clay content has very high affinity for bipyridillium cations such as paraquat and diquat.

Mechanism of pesticide sorption

As discussed above, the mechanism and the kinetics of pesticide sorption in soils are dependent on both the type of pesticide and the physical and chemical characteristics of soils. These factors are further influenced by hydrogeological and climatic considerations. The mechanism and kinetics of pesticide sorption can therefore involve partitioning into organic matter (Chiou 1991), electrostatic interaction (with charged external surfaces), electrostatic interaction (with charged internal surfaces), and specific interaction (leading to the formation of a covalent bond). The soil factors controlling these interactions include pH, soil solution composition, and the types of minerals present (Koskinen and Harper 1990). Different sorptive mechanisms, including ion exchange, cation bridging, charge transfer, hydrogen-bonding, and van der Waals interactions, may operate in soils depending upon the surface properties of the soils and the charge properties of the pesticides. However, these mechanisms depend on the nature of pesticides.

Pesticide–organic matter interaction

Soil organic matter is a complex polymeric mixture produced by microbial and chemical degradation, and has not yet been fully characterised. The role of organic matter on pesticide (ionic and non-ionic) retention in soils has been the subject of much research during the last 50 years. While these studies recognise the importance of soil organic matter, the mechanism of organic matter–pesticide interaction is not clear. Two major types of interactions have been postulated. These include specific interactions between soil organic matter and pesticides leading to the formation of definite bonds and partitioning. Specific interactions generally involve a rearrangement of atoms that leads to the formation of new compounds at the demand of unsatisfied valences of the surface atoms. However, such interactions are often difficult to distinguish from physical sorption because a chemisorbed layer may have a physically sorbed layer on it. This process leads to formation of compounds which are thermodynamically stable.

On the nature of the interaction with organic matter and the mode of sorption, several different hypotheses have been advanced. The most popular is that of Chiou et al. (1984), which proposes that the partitioning (solubilisation) of non-ionic organic compounds into soil organic matter is the major process of contaminant uptake by soil from water. For a detailed report on the partitioning mechanisms readers are referred to an excellent review by Chiou (1991). Partition theory is based on the miscibility of the organic phases, i.e., pesticide and organic matter, and is

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dependent on both the nature of pesticides and the size of the organic matter pool. This mechanism was proposed following consideration of sorption data in aqueous systems in which evidence for the presumed partitioning (solubilisation) of non-ionic organic compounds into soil organic matter is presented as the major process of soil uptake from water (Chiou et al. 1984). They attributed the inability of the soil mineral fraction to retain non-ionic organic compounds from aqueous systems to the strong dipole interaction of minerals with water. This action excludes the organic compounds from the portion of the soil solvated by water molecules. Horvath and Melander (1978) also attributed hydrophobic sorption of other non-polar organics on organic matter to a similar mechanism. The partition hypothesis is well supported by octanol:water partition coefficients and the solubility maxima observed in soils. Although this mechanism is strongly supported for non-ionic pesticides, the relationship between sorption of ionic pesticides and soil organic matter varies with the pH as well as with the ionisation constant of the pesticides. Generally, in low organic matter soils, pesticide sorption maxima are directly related to the mineral phase. In addition to these mechanisms, soil–pesticide interactions may also occur through van der Waals' attraction and where there is interaction through p orbitals pi bonding may also occur.

Although the partition theory has successfully explained sorption behaviour of non-ionic organic compounds in an aqueous phase, both ionic and non-ionic pesticide sorption reactions involving dry soils have shown considerable variability in the relationship between sorption mechanisms and organic carbon content. These variations have been related to the dual sorbent behaviour of soils where the organic matter functions as a partition medium (Chiou and Shoup 1985; Chiou et al. 1985) and the mineral matter functions as a conventional solid adsorbent (Hassett and Banwert 1989). Stevenson (1982) demonstrated the roles of the mineral and organic matter pools on atrazine sorption. He found that the relationship between atrazine partition coefficient and organic matter is different for soils exceeding 10% organic matter. In these soils the pesticide sorption is directly controlled by organic matter. Below 10% organic matter both mineral and OM components controlled atrazine sorption (Fig. 3). In addition to Stevenson (1976), numerous other investigators [see references in Sawhney and Brown (1989)] have reported a similar relationship between K_{oc} and sorption maxima. However, there was no attempt in any of these investigations to relate sorption maxima to organic matter on the basis of soil type. Clearly, in tropical soils that are generally low in organic matter, both the clay fraction and organic carbon may play significant roles in controlling pesticide sorption, while in less weathered soils sorption may be controlled primarily through organic matter interactions. Furthermore, the type of organic matter and its decomposition stage has a marked effect on pesticide sorption. Climatic and other edaphic factors determine the nature of organic matter in soils. For example, in grassland soils the organic matter is often rich in humic materials, whereas forest soils are dominated by fulvic acids. The differing affinities of these organic matter constituents for pesticides have long been known (e.g. Hayes et al. 1968). However, the nature of organic matter is seldom taken into consideration when pesticide behaviour is assessed for an extrapolation.

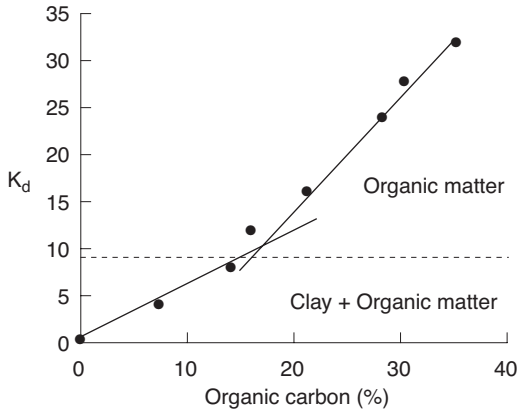

Figure 3. Relationship between atrazine partition coefficient (K_d) and organic matter and clay content (redrawn from Stevenson 1976)

Mineral–pesticide interactions

The sorption of pesticides by minerals may involve both external and internal surfaces. These interactions may also be controlled by soil solution composition and the nature of ionic pesticides. For instance, in soils with variable charge surfaces, changes in soil solution pH will lead to changes in both the colloidal surface chemical properties (see Fig. 1) and the proportion of ionic to molecular (undissociated) pesticide molecules (Fig. 4). Soil pH effects are important

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with weakly basic (triazine) and acidic (phenoxy acid) pesticides because the relative quantities in ionic form are dependent on the pK and pH of the soil system. Weakly basic pesticides become cations at low pH. In variable charge soils, such pH values lead to low surface negative charge and high surface positive charge and this results in increased sorption. In contrast, the acidic pesticides ionise to anionic form as pH increases (one or more pH units above the pKa of acid) (Weber 1993) and this may lead to reduced sorption with increasing pH. The pH dependence of surface charge on amorphous minerals is noteworthy, from the practical point of view, in that soil pH is one property which is commonly altered by soil management. Sorption depends on the unique combination of available sites on clay surfaces and the extent of dissociation of the acidic or basic pesticides at any pH. Baskaran et al. (1996) observed an increase in the 2,4-D sorption with decreasing pH. They attributed the increase in sorption to the combination of ionisation of 2,4-D molecules and modification of surface charge in an Andept soil. Werkheiser and Anderson (1996) studied the effect of soil properties and surfactant on sulfonylurea primisulfuron sorption by six soils with different organic carbon (OC), iron oxide, and clay contents. They used batch reactions at controlled pH values, and found that when pH was adjusted to values between 4 and 6.5, sorption coefficients decreased with increasing pH. At pH 4.5, sorption coefficients decreased with increasing OC and ranged from 0.6 for a soil with 0.2% OC to 15.5 for a soil with 48% OC. The effect of pH on sorption of 2,4-D by goethite, a mineral commonly found in tropical soils, has been investigated (Baskaran and Naidu, unpublished data). The results in Figure 5 indicate that, with increasing pH, the extent of 2,4-D sorption decreases, consistent with the increased surface negative charge recorded in Figure 1 for a goethite-dominated, strongly weathered soil.

Recent reviews (see Sawhney and Brown 1989) show that the acidic herbicide 2,4-D is adsorbed by organo clays (formed by intercalation) at different rates depending on the clay mineral type. They found that the sorption of this anionic herbicide was greater for high charged decylammonium-montmorillonite than for low-charged decylammonium-montmorillonite (C10M). In their most recent study, these investigators reported the binding mechanism of 2,4-dichlorophenoxyacetic acid by organo clays. This was studied using modified clays and measuring sorption at different pH values. Their studies revealed that sorption was much greater for the organic-modified vermiculite than for montmorillonite. Using X-ray diffraction and Fourier transform studies they concluded that sorption mechanisms involved sorption within the internal surfaces of the expansive minerals. While such interactions may be common in less-weathered soils, Vertisols and Mollisols that are dominated by smectitic minerals may also illustrate such behaviour in tropical regions.

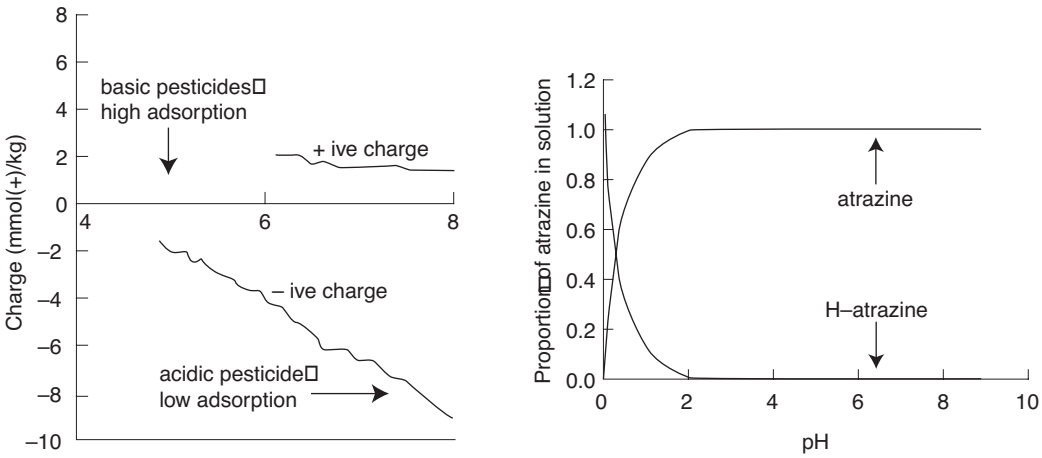

Figure 4. Effect of pH on dissociation of atrazine in realtion to surface charge characteristics

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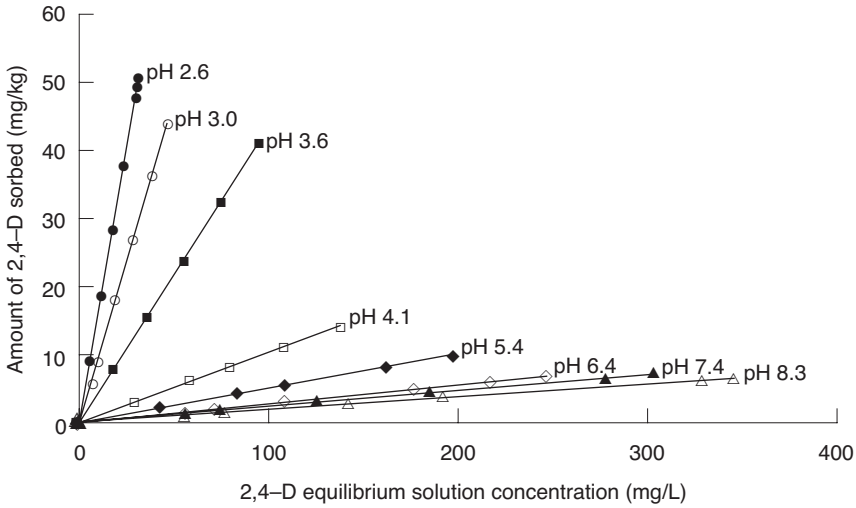

Figure 5. Effect of pH on adsorption of 2,4-D on a variable charge soil (S. Baskaran and R. Naidu, unpublishhed data)

In a comparative study on soils from temperate and tropical environments, Usoroh (1974) found quite different sorption behaviour of linuron in the two types of soils. Sorption of linuron was found to be some five times greater in temperate than in the tropical soils. Not only organic matter but other soil properties were suggested as reasons for this difference.

Ionic pesticides such as diquat and paraquat have a permanent charge, while other compounds become charged from processes such as protonation of weak bases (e.g. triazines) or by dissociation with the release of a proton from weak acids (e.g. 2,4-D, picloram). The primary sorption mechanism for ionic pesticides is ion-exchange with ions on the clay and organic matter (Burns et al. 1973).

Thus, the main processes affecting the efficiency, and ultimate fate of pesticides in the soil, are retention (sorption–desorption), transformation (biological and chemical degradation), plant uptake, and transport into the soil, atmosphere, and surface waters. These processes are more or less interdependent. Sorption, for example, may protect pesticides against microbial attack but facilitate their abiotic transformation (Bollag and Liu 1990). By inhibiting desorption (release) into the soil solution, sorption also retards pesticide transport into the soil and surface run-off (Weed and Weber 1974). The literature is replete with reviews on the fate of pesticides in soil (Bailey and White 1970; Calvet 1980; Rao and Davidson 1980; Cheng 1990; Beck et al. 1993; Kookana et al. 1998).

Pesticide desorption

The amount of pesticide desorbed is often found to decrease with an increase in soil organic carbon. It has been observed that, when sorption occurs mainly on silicate clays, near complete desorption of the pesticide is obtained, whereas when the sorption occurs mainly on the organic matter, only partial desorption is obtained (Baskaran 1994).

The concentration of pesticides remaining in the adsorbed phase decreases with increasing desorption time, and a curvilinear relationship is obtained between the cumulative time and the amount of pesticide remaining in the soil. This relationship follows a first-order reaction kinetics with respect to surface concentration (equation 4)

$$S_t = S_i \exp(-k_1 t) \quad [4]$$

where S_i and S_t are the amount of pesticide (mg/kg) in the solid phase at time 0 and t, respectively, t is the desorption period (min), and k_1 is the rate of desorption (1/min).

It has often been observed that, for a short duration, the desorption of pesticides follows first-order reaction kinetics (Smith et al. 1992). The rate of desorption decreases with an increase in the organic carbon content of the soils. An inverse relationship between rate of pesticide release and organic matter content has also been observed in solute transport studies (Brusseau and Rao 1989). This inverse relationship

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suggests that the desorption of pesticides involves the diffusion of the compounds through soil organic matter.

Temperature has a major effect on the rate and extent of pesticide desorption from the sorbed phase. Tropical ecosystems are characterised by high temperatures and moisture contents which are likely to have an effect on the release behaviour of pesticides and consequently their mobility in the environment.

Dissipation of pesticides in soils

There is increasing concern about the persistence of pesticide residues in soils and subsequent contamination of surface and groundwater. The persistence of pesticide residues in soils depends largely on the extent of sorption onto soil particles and the rate of mineralisation or degradation (Nicholls 1988; Alexander 1994). While sorption decreases the leaching of pesticide residues to groundwater, erosion of pesticide-enriched finer soil particles by run-off will lead to surface water contamination (Wauchope 1978). Erosion is one of the major soil degradation mechanism in the humid tropics where raindrop impact is high. Degradation is one of the main pathways by which pesticides are removed from soils (Alexander 1994). An understanding of the degradation of the toxic organic pesticides into less toxic or non-toxic compounds is essential for predicting pesticide persistence in the environment and developing strategies for risk management. Soil microorganisms generally play the major role in the degradation of organic pesticides.

In soils, both sorption of organic pesticides and biological activity increase with an increase in the organic matter content (Johnson and Sims 1993). These two factors have opposite effects on the degradation of pesticides. Sorption has often been shown to decrease the degradation rate of organic chemicals by reducing their availability to microbial attack (Ogram et al. 1985; Smith et al. 1992). An increase in soil microbial activity is likely to increase the rate of degradation of pesticides. The influence of microbial activity on pesticide degradation, however, has often been inferred indirectly from the effect of other factors such as temperature, moisture, and nutrient supply on degradation (Alvey and Crowley 1995).

Pesticide degradation

The rate of degradation of pesticides is measured from the amount of pesticide remaining in the soil or from the amount of CO_2 released. The rate of degradation has often been observed to follow first-order reaction kinetics (Roeth et al. 1990; Campbell et al. 1991). However, exceptions to this are also very common (Kookana et al. 1998). For the sake of simplicity and comparison between different pesticides the half-lives ($t_{1/2}$) are used as a convenient parameter.

The $t_{1/2}$ values for organic pesticides in soils vary with the type of compound and the soil and climatic conditions. This is well demonstrated for organochlorine pesticides that have been found to persist for long periods in soils from temperate regions while they are known to disappear relatively rapidly in humid tropical soils (Prasad 1996). Not only for organochlorines but for relatively modern pesticides, degradation in tropical soils has been noted to be faster than that in soils from the temperate regions. For example, Ravelli et al. (1997) found that chlorsulfuron degraded more rapidly in three Oxisols from Brazil than the rates reported for similar temperate soils. Such differences in half-lives may be related to both climatic differences and the markedly different capacities of soils to retain pesticides.

The agronomic practices and production systems in the tropics could lead to physicochemical and biological characteristics of soil which are different from those in the soils from the temperate region. Because of their aerobic–anaerobic phases, tropical soils under flooded rice systems support a diverse range of bacteria capable of degrading many pesticides. For example, under such conditions, even complex pesticide molecules (e.g. parathion) are more completely mineralised than under exclusively aerobic or anaerobic systems (Reddy and Sethunathan 1983).

There is evidence in the literature that the rate of desorption decreases with increasing sorption capacity, and the rate of desorption therefore becomes the rate limiting factor for microbial degradation (Felsot and Dahm 1979; Rijnaarts et al. 1990). Adsorbed pesticides become 'bound' or 'recalcitrant', resisting degradation and becoming more persistent in soils (Fewson 1988). Using mechanistic models, Ogram et al. (1985) showed conclusively that adsorbed 2,4-D was completely protected from biological degradation. They suggested that adsorbed 2,4-D is located sufficiently deep within the soil-organic matter matrix for bacteria to be unable to attack it. The adsorbed 2,4-D needs to be desorbed into the soil solution to undergo microbial degradation. Thus, under field conditions, depending on the rate of desorption, sorption of 2,4-D would provide a temporary

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protection from degradation. Bolan and Baskaran (1996) reported that the rate of degradation of 2,4-D, as measured by the $t_{1/2}$ value, increased with an initial increase in K_d values, but it decreased when the K_d values were greater than 8 dm^3/kg. The initial increase in $t_{1/2}$ values (decrease in the rate of degradation) was probably caused by the sorption of the pesticide onto soil. This could result in its inaccessibility to microbial attack (Smith et al. 1992) and a low concentration of the pesticide in solution. Processes which lead to a decrease in the concentration of the pesticide in the soil solution are likely to decrease the rate of degradation by the microorganisms (Alexander 1994).

Soils with high K_d values tend to have high organic matter content and microbial activity. The decrease in the concentration of pesticides in the soil solution with increasing sorption is compensated by the increased microbial activity thereby increasing the rate of degradation.

Implications for Food Quality

Although there is considerable information available on the quality of food treated with pesticides following crop harvest, only limited information is available on the soil–plant transfer of pesticides. Fungicide contamination of food is particularly prevalent because of insufficient withholding periods before crop harvest and marketing.

The limited studies conducted thus far indicate that the ability of plants to absorb nutrients varies with soil type, plant species and the type of chemical. In general, soluble pesticides accumulate much more readily into plant components than do insoluble non polar pesticides. Given the low organic matter content of many tropical soils, the retention of pesticides may be poor and this may contribute to greater bioavailability and uptake by crops. There are few instances where pesticide residues in food crops are a result of plant uptake of the pesticides from soil. However, the plant uptake of pesticides is not limited to root uptake only. Volatile pesticides may also be absorbed through plant leaves and this may be one of the major pathways of pesticide transfer from the soils.

Pesticide contamination of agricultural and horticultural crops is reported in the media from time to time. The most recent example is the pesticide contamination of Australian beef products, the result of feeding cattle cotton plants that had been sprayed with chemicals. Other examples include DDT presence in human milk (India).

The DDT accumulates in animal fat tissue and gradually with time converts to the more toxic DDE in the presence of enzymes. Such organic substances have been linked to breast cancer. Other pathways of pesticide entry into the food chain are inappropriate application rates or insufficient withholding periods before crop harvest and marketing. There are few instances where pesticide residues in food crops are a result of plant uptake of the pesticides from soil. For livestock, however, ingestion of soil contaminated with pesticides may be a major route for exposure and contamination of the food chain (Harrison et al. 1970).

Chemical residues in Australian raw food commodities are regularly surveyed under the National Residue

Table 3. Pesticide residues detected in various food commodities under National Residue Survey program in Australia.

Commodity	Number of analyses	Number of residues detected	Number of residues above MRL/MPC	Percent compliance with the Australian Standard
Wheat	4399	1693	0	100.00
Barley	1090	548	6	99.45
Sorghum, lupins, oats, field peas, chick peas and canola	1561	215	18	98.85
Flour	180	116	2	98.89
Onions	372	48	2	99.46
Macadamia nut	200	4	0	100.00
Pecan Nuts	135	0	0	100.00

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Survey (NRS) program by the Bureau of Resource Sciences. Every year around 50000 samples covering a wide range of crops and produce are analysed for a range of pesticides, other biocides, and heavy metals. The results of the survey conducted in 1996 show that 97–100% of samples from various commodity groups comply with the Australian standards for maximum permissible concentrations of chemicals. NRS results on pesticide residues in selected food grains and horticultural commodities are given in Table 3.

Conclusion

Although pesticides can have adverse impacts on soil-water and food quality, limited information is available on the chemodynamics of pesticides in the tropical soil environment. The available literature on tropical soils and the interactions of pesticides in temperate high organic matter soils suggests that the retention of pesticides by low organic matter iron and other weathered soil minerals may be low. This means that vertical movement of pesticides may be a serious issue in such soils. Such a problem may be exacerbated by the heavy tropical rainfall that may also contribute to surface transport of pesticides.

Pesticides can affect (a) soil quality through major changes in the chemistry of constituent minerals of the microecosystem, (b) surface and groundwater quality through colloidal transport and leaching, (c) crop quality through soil–plant transfer, and (d) animal and human health through contaminated crop intake. Such impacts on the quality of our environment can be reduced through development of management strategies that minimise the use of chemicals but support economically sustainable crop production.

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Tracing Aquatic Pesticide Pollution to Its Sources

Pham Hung Viet, Trinh Le Hung, Nguyen Viet Hung, Le Van Chieu, and Nguyen Hung Minh*

Vietnam is an agricultural country with 80% of its population engaged in farming. The annual average yield of rice is about 18 Mt, most of it produced in the deltas of the Mekong and Red rivers. To achieve such a high annual yield, a large amount of pesticides, estimated at about 24000 t/year, has been used. In addition, up to now, the strict regulations covering use of pesticides have not been rigorously applied.

For those reasons, the Chemical and Environmental Engineering Department (CEED) of Vietnam National University (VNU) has been conducting, since 1995, a research program to evaluate levels of pesticide residues and their distribution in the environment. This research has been focused initially on Red River Delta and in neighbouring areas along the river banks.

Many series of samples, including rice, water, sediment, and organisms, have been collected in all parts of the year. The samples were prepared by extraction, clean-up, and enrichment procedures appropriate to each type. Finally, solutions of the prepared samples were quantified by GC/ECD, with confirmation by GC/MS.

The results of these studies showed the following:

- Concentrations of organochlorine pesticide residues in water and rice in this region are still below maximum allowable limits. However, the concentrations vary widely depending on the weather, and are especially low in the wet season. This may be the result of dilution of pesticides in the water.
- For sediments, organochlorine pesticide residue concentrations in samples collected from near the mouth of the Red River Delta were higher than those in samples collected further off-shore.

THE Chemical and Environmental Engineering Department of Vietnam National University has been conducting, since 1995, a research program to evaluate levels of pesticide residues and their distribution in the environment. This research has been focused initially on Red River Delta and in neighbouring areas along the river banks.

Sample Collection and Preparation

Soil, water, and sediment samples were collected at different sites in Thai Binh Province during 1995, 1996, and 1997 as shown in Table 1. Sampling sites are shown in Figure 1.

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Three other studies were carried out, in Thai Binh (1995) and Vinh Phu provinces (1996) to monitor pesticides residue in rice, and at the mouth of the Red River in Balat to investigate biological samples.

The locations of the sites for biological sampling in Balat are shown in Figure 1, and of the five sampling sites in Thai Binh in Figure 2.

Water samples

Water samples were collected at five sites at different times of the year. They were taken from the surface layer during an incoming tide, and stored in clean, dry dark bottles in an ice box. Organochlorine samples were analysed using the reference method for marine pollution studies, with some modifications.

Water samples were passed through a glass wool filter to remove suspended particles above 0.05 mm diameter. The internal standard was added to 100 mL

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samples. Samples were then extracted with 40 mL n-hexane:diethyl ether (95:5 v/v) in a separating funnel. The funnel was shaken for 30 minutes and the contents allowed to separate. The lower layer in the funnel was similarly extracted a further three times. All extracts were then combined in a round-bottom flask and concentrated to 1–2 mL in a vacuum rotavapor. The solution was transferred to a column packed with 10 g florisil PR and overlain with 2 g of anhydrous Na_2SO_4 after priming with 20 mL of n-hexane. Pesticides were then eluted with 200 mL n-hexane:diethyl ether 75:25 as elution solvent at a flow rate of 2–3 mL/minute.

The concentrated elute (ca. 1 mL) was analysed by gas chromatography using an internal standard.

Table 1. Times and sites of sampling for pesticide residues in Thai Binh Province

		Sites for soil samples					Sites for water samples					Sites for sediment samples		
		site 1	site 2	site 3	site 4	site 5	site 1	site 2	site 3	site 4	site 5	site 1	site 2	site 3
1995	1	×	×	×	×	×	×	×	×	×	×	×	×	×
	2	×	×	×	–[a]	–	×	×	×	×	×	×	×	×
1996	3	×	×	×	×	×	×	×	×	×	×	×	×	×
	4	×	–	×	×	×	×	×	×	×	×	×	×	×
1997	5	×	×	×	×	×	×	×	×	×	×	×	×	×
	6	×	×	×	×	×	×	×	×	×	×	×	×	×

[a] No sample collected.

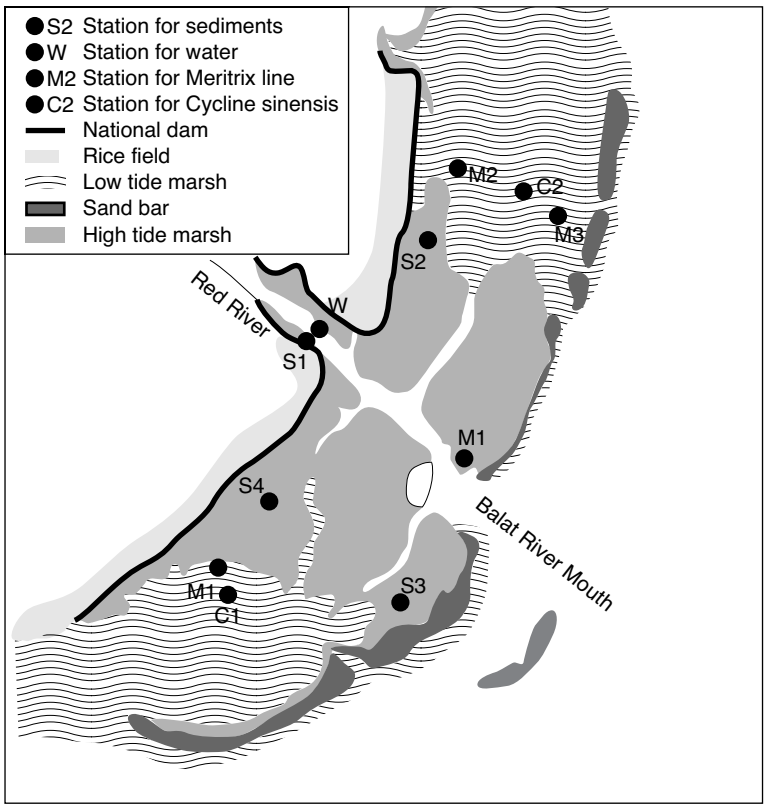

Figure 1. Sampling sites at the Balat River mouth

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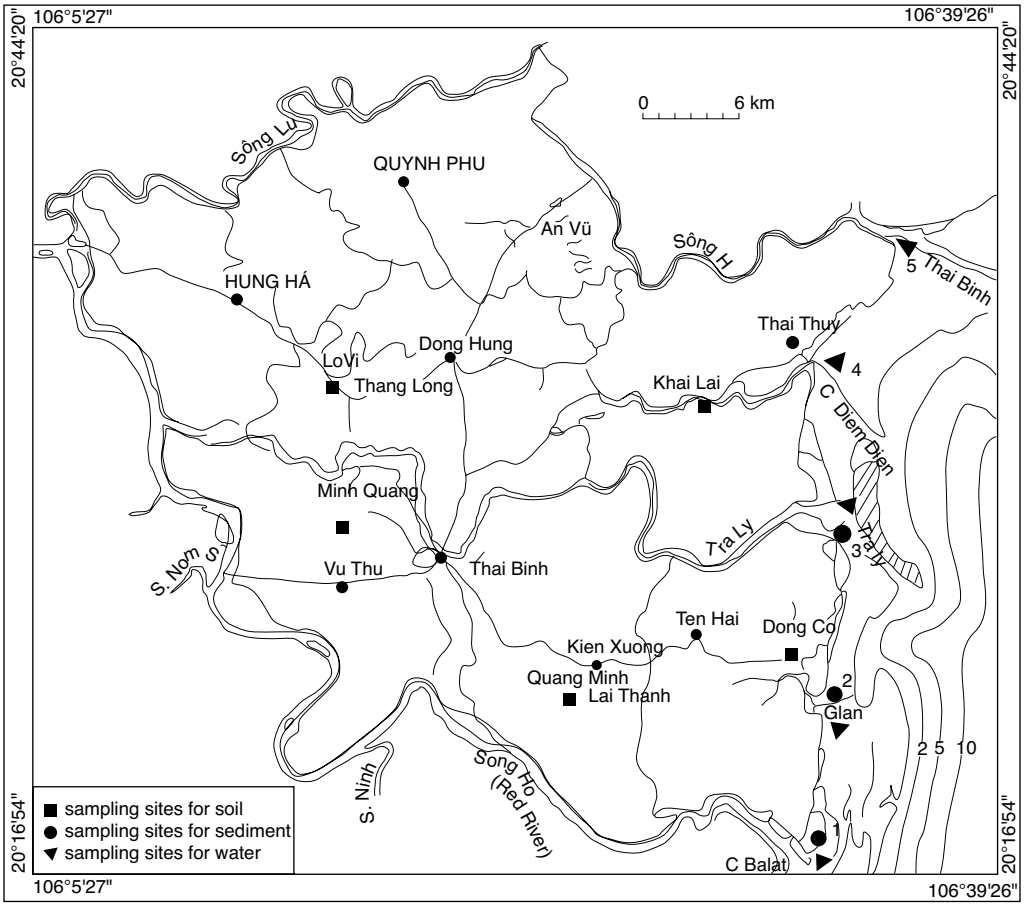

Figure 2. Samplng sites in Thai Binh Province and the surrounding area

Sediment samples

Sediment samples were collected from the surface layer using a triangular sampling grid with sides of 1 m. Samples from three points of triangle were mixed together, passed though a 0.1 mm sieve then packed in aluminium foil and stored in an ice box.

Each sediment sample was dried in air, then ground. In order to completely remove the water in samples, about 9 g of powdered anhydrous Na_2SO_4 was added to the sediment. Fifty mL of 2.5 ng/mL internal standard (TCB) was added to this mixture and extracted with 200 mL n-hexane:diethyl ether (95:5, v/v) in soxhlet apparatus for 8 hours. The extract was concentrated to 2 mL in a rotavapor apparatus at 40°C. A Florisil packed column was used to clean up the extract. Pesticides were eluted in a manner similar to that described previously for water samples.

Biological samples

Samples of two molluscs (*Meritrix line* Veneridae) and *Cycline sinesis* (Eulamellibranchia) were washed in seawater, packed in aluminium foil, and stored in an ice box. Samples of 20–25 of the molluscs were used for each determination . Dried samples were of the lyophilised tissues were dissected and ground to powder in a laboratory cutting mill. The powder obtained was further sieved through a 250 μm stainless steel sieve, only the fraction of material passing through the sieve being kept. In this way, about 0.4 kg of powder having a particle size of less than 250 μm was prepared. The powder was further homoge-

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nised by mixing in a rotating drum for 4 hours, then treated in a freeze-dryer overnight. The sample was extracted and cleaned up in a manner similar to that described for sediment samples.

Soil samples

Soil samples were taken from five different sites in Thai Binh. Samples were collected in the dry and wet seasons. Samples were dried in air, ground, and stored in clean, dry conditions before analysis. The treatment procedure for soil samples was similar to that for the sediment samples.

Rice sample

Rice samples were taken from Thai Binh Province in 1995 for organochlorine pesticides, and in Vinh Phu Province in 1996 for organophosphorous and nitrous pesticides. Samples were collected at different points and mixed together to obtain a representative sample. Samples were hulled, dried, and powdered, and stored in clean, dry conditions before analysis. The treatment procedure for rice samples was similar to that for sediment and biological samples.

Determination of moisture content of sediment and biological samples

Since these samples often contained a large amount of water, their moisture content was determined as follows. A known weight of sample was dried to constant weight in a glass beaker of known weight at 50°C. The moisture content of the sample is calculated as in equation (1) below.

Analytical Method

The organochlorine pesticide residues were analysed by gas chromatography under the conditions shown in Table 2 and as follows.

- Standard solution mixtures consisting of eight pesticides (in 1995) or 11 pesticides (in 1996; 1997): *p,p'*-DDD; *p,p"*-DDD; *p,p'*-DDE; *p,p'*;-DDE; *p,p'*-DDT; *p,p'*-DDT; lindane, dieldrin, endrin, aldrin, and trichlorobiphenyl (TCB) were prepared in acetone at different concentrations and injected into the gas chromatograph. Calibration curves for each compound was established based on the chromatograms obtained and using an integrator (CR7A-Shimadzu). The concentration of each pesticide in the samples was calculated using an internal standard.

Table 2. Gas chromatograph settings for analysis of organochlorine pesticide residues

Analytical instrument: gas chromatograph GC-14B (Shimadzu, Japan)	Temperature program: Initial oven temperature of 60°C, hold 1 min; then increase by 10°C/min to 160°C and continue increasing temperature at a rate of 4°C/min to 250°C; hold for 10 min
Temperature of detector 300°C	Temperature of injector 250°C The operating time of the splitless mode: 1 min Carrier gas: Nitrogen (99.9999%) with a flow rate of 1.2 mL/min
Make-up gas: Nitrogen (99.9999%) with a flow rate of 40 mL/min	Detector: ECD- Ni^{63}

Table 3. Recovery of trichlorobyphenyl internal standard

Kind of sample	Recovery for internal standard: TCB (%)			
	1	2	3	Average
Soil	80.4	84.6	81.3	82.1
Water	80.3	79.5	80.5	80.1
Sediment	80.1	73.2	73.3	75.5
Biological samples	76.5	79.3	75.8	77.2

- Blank and spiked samples were analysed under the same conditions. Average recoveries for water, sediment, and biological samples were 80.1%, 75.5%, and 77.2%, respectively.
- In order to further confirm the presence of above-mentioned pesticides in water, sediments, and biological samples, we have carried out the analytical procedure on a GC/MS equipment (QP-5000, Shimadzu) under conditions similar to those for gas chromatography.

$$\% \text{ moisture content} = \frac{\text{weight of initial sample} \pm (\text{weight of the last dried sample} \pm \text{weight of glass beaker})}{\text{weight of initial sample}} \times 100 \quad (1)$$

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Results and Discussion

The results of the recovery study for the internal standard (2,4,4' trichlorobiphenyl) are shown in Table 3.

The analytical results obtained show that:

- The analytical method and sample preparation procedures used are quite suitable for the separation and analysis of these chlorinated pesticides.
- However, for separation of phosphorous and nitrogen-containing pesticides, some additional pre-treatment steps are needed to obtain high recovery. It is also recommended that for these kinds of pesticides, an ultrasonic separation method should be used instead of the soxhlet method to prevent these pesticide residues decomposing. Average recoveries of all compounds were above 70%.
- The additional researches on rice samples in Thai Binh and Vinh Phu provinces found low concentrations of pesticides in some common varieties of rice (Table 4). It is considered that hulling and milling will remove most of these residues.

Table 4. Pesticides in rice samples taken in Thai Binh and Vinh Phu provinces (mg/kg dried sample).

Rice samples in Thai Binh 1995				Rice samples in Vinh Phu 1996			
Compound	CR 01	CR 203	13/2	Compound	VP-R1	VP-R2	VP-R3
HCB	–[a]	–	–	BHC	9.27	0.04	0.04
Lindane	–	–	–	Fenitrothion	0.17	0.42	0.12
Andrin	–	–	–	Malathion	0.34	–	–
DDE	19.35	25.17	20.97	Chlorpyrifos	–	–	0.05
Dieldrin	–	–	–	Endrin	0.19	0.43	–
Endrin	–	–	–	*p,p'*-DDT	0.22	0.16	0.06
DDD	3.58	–	3.87				
DDT	–	–	–				

[a] lower than detection limit

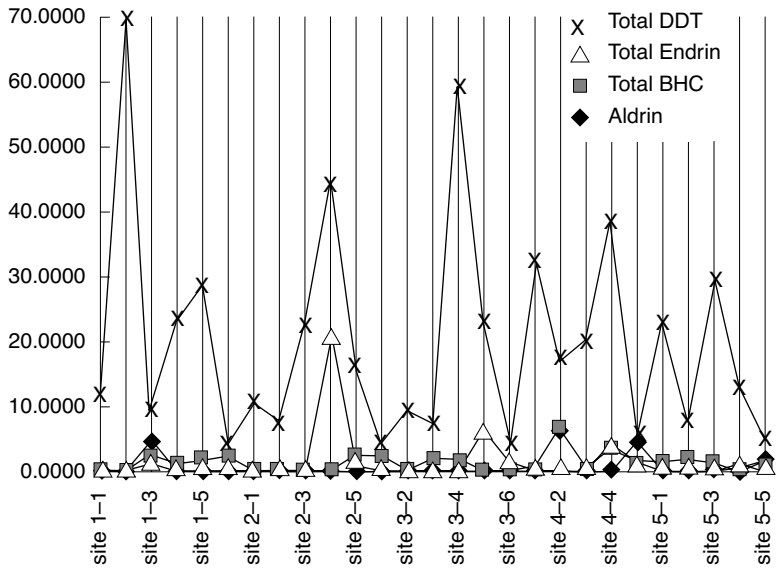

Figure 3. Variation in concentration of selected organochlorine pesticides in soil samples at 5 monitoring sites

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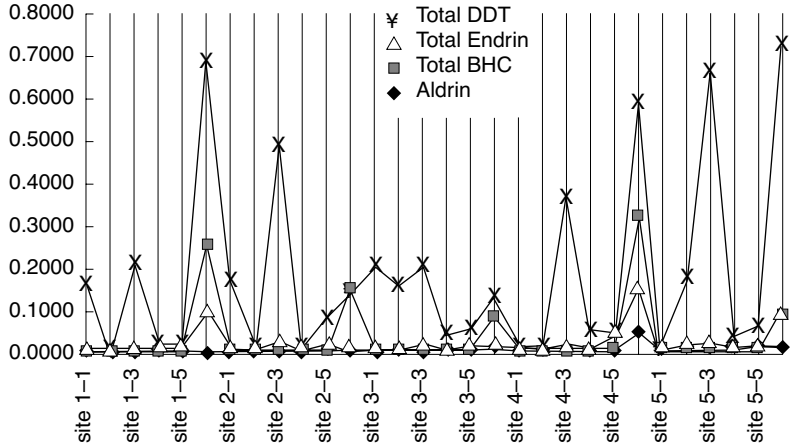

Figure 4. Variation in concentration of selected organochlorine pesticides in water samples at 5 monitoring sites

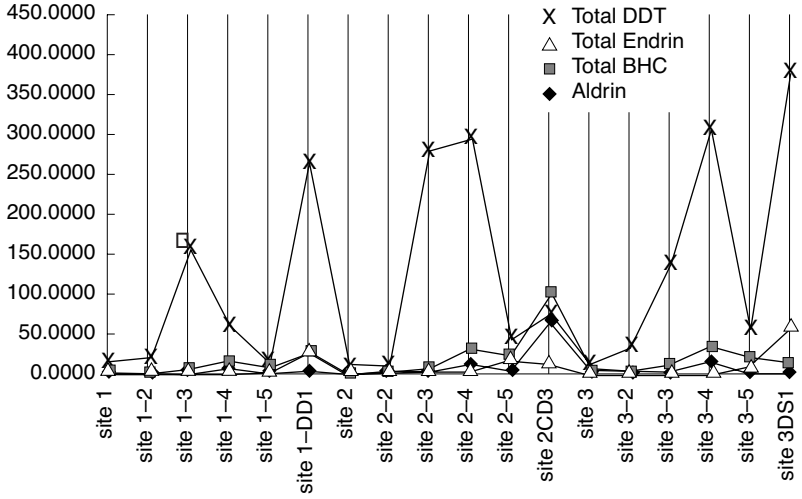

Figure 5. Variation in concentration of selected organochlorine pesticides in sediment samples at 3 monitoring sites

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- For the two biological species investigated, *Cycline sinensis* contained higher amounts of residues than *Meritrix line* (Table 5).
- In order to detect changes in the concentration of pesticides at each site within and between years, the analytical results for soil, water, and sediment samples at five monitoring sites from 1995 to 1997 are combined in temporal order in Table 5 and plotted in Figures 3,4, and 5.
- It was found that concentration of these compounds varied widely between the wet and dry seasons. It was also found that the concentration of pesticides increased at almost all sites between 1995 and 1997. This may be the result of accumulation from year to year and/or an increase in the amounts of pesticides used.
- In general, concentrations of DDT and its isomers are highest. The Government of Vietnam recently restricted use of pesticides containing DDT.

Table 5. Pesticide residues in biological samples collected at Balat, Thai Binh Province near the mouth of the Red River (mg/kg dried sample).

	ML1[a]	ML2	ML3	CS1	CS2
Lindane	44	56	42	55	149
Andrin	–[b]	–	–	–	–
DDE	16	18	12	23	66
Dieldrin	–	–	–	–	–
Endrin	–	–	–	–	–
DDT	–	–	–	–	–
DDT	9	16	8	13	26

[a] ML = *Meritrix line*; CS = *Cyline sinensis*
[b] – = below limit of detection
In order to get a outline view, in Figures 3, 4, and 5 concentrations of similar compounds are summed. Thus:
– Total BHC = total concentration of BHC and gamma-BHC
– Total endrin = total concentration of endrin and dieldrin
– Total DDT = total concentration of DDT, DDD, and DDE.

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Assessing Relative Impacts of Pesticides on Groundwater Quality Using a Simple Index

Rai S. Kookana*, Raymond L. Correll†, and Rosalind B. Miller†

Abstract

Modern agricultural production systems need to rely on pesticides to ensure high quality and quantity of produce. As a consequence of pesticide use, trace levels of their residues are present in air, water and other components of the ecosystem. This is a cause of concern from the point of view of both the ecosystem and human health. Simple but sound methods are therefore needed by pesticide users and regulators for making an assessment of pesticide impact and choosing the practices with the least detrimental impact on the environment. In this paper we present a brief overview of simple methods for the assessment of the impact of pesticides on groundwater quality as well as an alternative approach derived by the authors. This latter approach is a Pesticide Impact Ranking Index (PIRI), which represents an advance over other commonly available approaches since it can be used to

(i) rank pesticides in a cropping system, and

(ii) compare different land uses in a catchment in terms of their relative impact on water quality.

PIRI is based on a quantitative risk assessment approach and consists of three components,

- the value of the asset (water resources threatened);
- the source(s) of threat to the asset (pesticides use);
- the pathway through which the threat is released to the asset.

Each component is quantified using pesticide characteristics such as toxicity, amount used, sorption and persistence as well as soil and other site conditions such as permeability, depth of water table and water input. A description of each of the three components of PIRI is given in this paper, including an example of the application of the method.

MODERN agricultural production systems have become reliant on the use of pesticides to ensure that the produce is of high quality and quantity. Some 2.5 million tonnes of pesticides are applied to agricultural crops every year (van der Werf 1996). There is evidence from around the globe that trace levels of pesticide residues are present in air, water and other components of the ecosystem. Since these pesticide residues can adversely affect both the ecosystem and human health, there is a need to minimise their environmental impact. Pesticide impact assessment tools, which range from simple indices to complex simulation models, can be valuable in achieving this aim. Systematic methods which allow a relative assessment of pesticide impact are of great value to both pesticide users and regulators in choosing the pesticides and practices with the least detrimental impact (Levitan et al. 1995). The objective of this paper is to provide a brief overview of simple methods for assessment of pesticide impacts on water quality and also to present a newly developed Pesticide Impact Ranking Index (PIRI) for ranking pesticides in terms of their potential impact on groundwater quality.

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Table 1. Various approaches used for assessing pesticide impact on environment (after Levitan et al. 1995; van der Werf 1996)

Type of tool	Example	Parameters taken into consideration	Comments	Reference
Databases	EXTOXNET	Toxicological Ecological Environment fate Physical properties Exposure guidelines	Descriptive and numeric Toxicology and environmental chemistry information available from the pesticide information profiles	EXTOXNET (1998) http://ace.ace.orst.edu/info/extoxnet/
Single parameter assessment	WHO Classification of Pesticides by Hazard	Toxicity (LD_{50}) Pesticide formulation Route of exposure	Assesses the acute lethal dose to humans from rat/mouse tests. Hazards modified on the basis of formulation and exposure routes. No other environmental parameter included.	WHO (1988)
Multiple parameter hazard assessment	Chemical Scoring System (USEPA)	Various toxicity and exposure parameters	Screening tool for hazard assessment to humans as workers and consumers and the environment.	O'Bryan and Ross (1988)
Composite environmental impact assessment rating system	Environmental Impact Quotient (EIQ)	Environment risk (run-off, leaching) Toxicity (e.g. humans, fish, birds, bees and other biota) Application rate	Assesses exposure risk to farm workers and consumers. Ecological component includes aquatic and terrestrial effects Each parameter grouped in three hazard categories (1, 3, and 5). EIQ is calculated through a formula which, when multiplied by application rate, provides field use rating.	Kovach et al. (1992)
Assessments based on site-specific parameters	Florida Cooperative Extension Service Assessment Tool	Run-off Leaching Toxicity Soil characteristics	Risk is calculated from contamination potential of a pesticide under site-specific conditions and its toxicity. Decision making tool for choosing best pesticide option.	Hornsby (1992)
Assessment systems that include economic parameters	Fruit Growers Pesticide Index	Application rate, site, timing Persistence Efficacy Cost Toxicity Environmental effects IPM compatibility Alternatives	Rating is based on the sum of two indices, namely Potential for Residue Index and Value Index. A decision support system to reduce pesticide use and in residue management under fruit production systems	Penrose et al. (1994)
Holistic assessment systems	Planetor: Environmental and Economic Farm Planning Software	Components: soil erosion, nitrate leaching, phosphorus run-off, pesticide movement, whole farm profitability	Combines site specific environmental models with individual farm financial planning data to evaluate the impacts of reducing or changing pesticide, N, P and manure applications, tillage systems and crop rotations.	Planetor (1995)

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Assessment methods for Environmental Impact of Pesticides

Several approaches, varying in complexity and comprehensiveness, have been developed to carry out a relative assessment of pesticides on the environment, and the latest of these have been summarised in Table 1. These approaches have been recently reviewed by Levitan et al. (1995) and van der Werf (1996). Levitan et al. (1995) classified them into categories which included tabular databases, single- and multiple-parameter hazard assessments, composite impact rating systems, assessments combining economic and site-specific parameters, and holistic assessments that include agroecological impacts and pest-control practices. The objectives of these approaches may include

- an assessment of toxicity of pesticides to a particular type of organism (e.g. honeybee),
- suitability of a pesticide for an Integrated Pest Management (IPM) system or
- use as a decision making tool for choosing a pesticide with minimum potential for water contamination.

These approaches will differ greatly in terms of the parameters taken into account and the emphasis on the component of the environment, such as water quality (e.g. Hornsby 1992 and Shukla et al. 1996); farm worker and consumer health (e.g. Kovach et al. 1992); or residues in harvested produce (Penrose et al. 1994).

Assessment for pesticide impact on water quality

Several indices have been developed to assess the impact of pesticides on surface and groundwater quality. Table 2 summarises pesticide leaching assessment methods for groundwater developed by Rao et al. (1985), Gustafson (1989), Meeks and Dean (1990), Kellog et al. (1994), and Shukla et al. (1996). Rao et al. (1985) and Meeks and Dean (1990) used pesticide sorption and degradation properties with recharge rate and depth to groundwater to assess the attenuation of the applied mass in the soil profile. Gustafson (1989) developed the GUS index, which is based on a graphical plot between pesticide half-life and sorption in soil. Kellog et al. (1994) on the other hand used a simple 4 × 4 matrix using four pesticide leaching classes in combination with four soil leaching classes. These models, however, do not take the toxicity and usage of pesticide into consideration. To select priority pesticides for groundwater monitoring programs, Shukla et al. (1996) used a risk-based approach based on three components, namely the toxicity of pesticides, extent of their usage and their leaching potential. Hornsby (1992) developed Relative Leaching Potential Index and Relative Runoff Potential Index, including toxicity of pesticides and soil parameters such as permeability and hydrology. The amount of pesticide applied is not taken into consideration in these indices. Hornsby et al. (1995) developed a decision support system based on both economic and environment impact of different pest management practices. The groundwater hazard of a herbicide was estimated from the ratio of estimated pesticide concentration to the USEPA lifetime health advisory level, thereby taking toxicity of a pesticide into account.

Table 2. Simple models used for assessing pesticide mobility to groundwater

Model	Parameters used	Reference
AF	Degradation, sorption and volatilisation of a pesticide; Soil and hydrological parameters	Rao et al. (1985)
GUS	Sorption and degradation of a pesticide	Gustafson (1989)
LEACH	Degradation, sorption and volatilisation of a pesticide	Laskowski et al. (1982)
RLPI	Sorption and degradation of a pesticide. Soil and hydrological properties of the site	Hornsby (1992)
IPS	Based on leaching, toxicity and usage of a pesticide. Sorption, degradation, volatilisation, and toxicity of a pesticide. Soil and hydrological parameters	Shukla et al. (1996)

The mobility of pesticides were assessed using several of the above models. A total 'Impact Potential Score' was computed by combining three components: relative mobility index, relative toxicity index and relative use index.

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PIRI

The Pesticide Impact Ranking Index is based on a quantitative risk assessment approach (Correll and Dillon 1993). For the risk to be quantified, it is essential to identify

- The value of the asset (water resources threatened);
- The source(s) of threat to the asset (pesticides use);
- How the threat will be released to the asset (the mobility transport pathways of run-off, with sediments and through the atmosphere).

It takes into account the pesticide properties of toxicity, persistence, sorption to soil and amount applied, as well as soil parameters. The economic factor in the assessment is also included in the form of value of asset threatened.

Quantifying Water Resources as an Asset

Ideally the value of asset should be in monitory terms. The value of each aquifer and body of surface water would depend on the size of the water body, water quality, number of people and industries dependent on the water supply, and alternative sources. Water quality can range from potable and good quality drinking water to very saline water. Another assessment could include the recreational or aesthetic value of the water resource. Here the value of the asset is defined in terms of its natural aesthetic appeal, the biological diversity, presence of rare species, its uniqueness and the number of people that use the facility. Given the lack of data needed for such an assessment, PIRI uses a score system for the value parameter *V*. A water resource which is used for drinking or is of a high ecological value is given the highest score of 100, water of near drinking quality is scored at 40, water suited to industrial needs is scored at 20, irrigation water is scored at 10, stock water is scored at 5 and saline water (due to its minimum ecological significance) is given the lowest score of 1. The asset and its associated value could be the same for all land uses in a catchment.

Pesticide Load

The calculation of pesticide load requires knowledge of the amount of pesticide used under each land use in a catchment. The nature of a pesticide is equally important as the amount in determining the impact on a water resource, so a range of parameters of each pesticides are considered in the assessment in PIRI. These parameters include the toxicity of each pesticide and its half-life.

The LC_{50} for rainbow trout is used as a measure of toxicity. Toxicity values are moderated within an upper and a lower bound, because for very high or very low toxicity the impact is not proportional to the numerical values of LC_{50}.

A pesticide that is persistent is likely to accumulate in the environment, whereas a labile pesticide is unlikely to have a high concentration over a long time period. In general, the expected concentration of a pesticide in the environment would be proportional to its half life. Data are available for the half life ($t_{1/2}$) for a large number of pesticides (e.g. Hornsby et al. 1996). In PIRI, the degradation rate is assumed to be constant across sites.

The amount of pesticide applied in an area is determined from the total area of the crop (Area), the frequency of application (*f*), the dosage (*d*), the fraction of the active ingredient (*a*) and the proportion of the crop that uses that pesticide (*p*). These parameters are multiplicative, and a quantitative estimate of the amount of each pesticide applied is obtained from them. The dosage, amount of active ingredient and frequency of application is rarely known for each farm. Surrogates for these can be used from the recommendations of the manufacturer or other agencies.

The total pesticide load applied to a farm can be obtained from the above components using the formula:

$$\text{Load} = \text{Area} \sum_{\text{pesticides}} \frac{fdap}{LC_{50}} = \text{Area} \sum_{\text{pesticides}} \text{Amount applied} \quad (1)$$

Pesticide Transport

PIRI recognises different transport pathways for groundwater and surface water. Both pathways require data on the affinity of the pesticide to the organic carbon in the soil (K_{oc}). The effect of K_{oc} differs between surface water and groundwater. A pesticide with a high K_{oc} increases the amount transported off the site by sheet erosion because more pesticide would be bound in the organic carbon of the soil. A pesticide with a low value would be concentrated in the soil water. However if the K_{oc} exceeds 1000, effectively all the pesticide would be bound in the soil organic carbon, so a further increase in the K_{oc} would have essentially negligible effect on the

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amount of pesticide sorbed. The toxic load applied to the soil per year for a particular pesticide is

$$L_{pesticide} = \frac{fadp}{LC_{50}} \quad (2)$$

Transport to groundwater

The movement of pesticides through soil is retarded (i.e. slower than water) due to the sorption of pesticides to soil organic matter (K_{oc}) – the higher the organic matter or K_{oc}, the greater the retardation. The retardation factor (*RF*) can be measured from K_{oc} and other soil properties by:

$$RF = \left[1 + \frac{\rho f_{oc} K_{oc}}{\theta_{FC}}\right] \quad (3)$$

where ρ is the bulk density of soil (kg/l), f_{oc} is the organic carbon content (kg/kg soil), and θ_{FC} is the moisture content at field capacity. *RF* is in effect the reciprocal of the fraction of the pesticide that is in the water phase.

The rate of water movement in the soil profile can be represented by the quotient of the recharge rate (q) and the water content θ_{FC}. If q litres per day is input into a volume of soil containing moisture content θ_{FC}, the time for the pesticide to enter into the soil profile at depth D is D/velocity = $D\theta_{FC}/q$. Thus the residence time (t) of the pesticide in the soil profile of known depth D is

$$t = \frac{D\theta_{FC}(RF)}{q} \quad (4)$$

Loss of a pesticide through degradation will depend on the residence time in the soil. Generally, the values of the recharge rate (q) are not readily available. In such cases q can be assumed to be simply proportional to the difference between water input and evapotranspiration for the crop-growing season in question. As mentioned above, the degradation rate for PIRI is assumed to be constant across sites. The degradation of a pesticide during its transport through the soil profile can be represented in the form of attenuation factor ($T = AF$ for groundwater), given by Rao et al. (1985), as follows.

$$AF = \exp\left[\frac{\pm 0.693\, D(RF)\theta_{FC}}{q t_{1/2}}\right] = \exp\left[\frac{\log\left(\frac{1}{2}\right)t}{t_{1/2}}\right] \quad (5)$$

from equation (4). Here $t_{1/2}$ is the half life of the pesticide.

The *AF* for each pesticide is different due to its sorption (K_{oc}) and degradation rate (as measured by $t_{1/2}$) and needs to be calculated individually. Therefore the load (*L*) and the transport (*T*) components need to be multiplied to give the pollution potential (PP) for the assessment of impact on ground water. The PPs can be used to rank pesticides that are used in a particular cropping system in terms of their relative pollution potential to groundwater.

The total toxic load likely to reach ground water at a site can be calculated from the following relation:

$$T = \text{Area} \sum_{\text{pesticides}} \frac{fdap}{LC_{50}} AF = \text{Area} \sum_{\text{pesticides}} \text{PP} \quad (6)$$

Estimation of the Detriment

The general formula used for the estimation of the detriment is

$$\text{Detriment} = VLT \quad (7)$$

where *V* is the asset score, *L* the pesticide load and *T* is an average transport function. Since the transport function for the pesticides depends on the K_{oc} of the pesticide, the load on the water body has to be considered separately for each pesticide. The detriment is then the sum of the individual contributions from pesticides.

An Example of PIRI Application

PIRI can be used for two purposes, namely

1. to rank pesticides in terms of their relative pollution potential to groundwater
2. to compare different land uses in a catchment in terms of their relative impact on water quality.

Ranking pesticides

All pesticides need to be individually assessed on the basis of their chemical nature in terms of sorption, persistence and toxicity. Not only are different pesticides applied at different rates but also their frequency of use varies. Therefore the amount of pesticide applied per unit area per unit time needs to be considered. Table 3 illustrates several pesticides used in vegetable production. The attenuation factor *AF* is calculated from sorption (K_{oc}) and persistence

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($t_{1/2}$). This, together with the toxic load for that pesticide (as estimated in equation (6)), yields a pollution potential (PP) for each pesticide. The values of PP (or a score calculated from PP) can be used to rank pesticides in terms of their pollution potential – very high (score 5), high (4), medium (3), low (2) very low (1) and negligible (0). In this example, fenamiphos has the highest pollution potential (with a score of 4, and thus rated as having a high pollution potential). The major contributions to the pollution potential of fenamiphos are both its high rate of application and persistence. An alternative to using fenamiphos, which has a similar effect on the vegetable crop, but a lower pollution potential can be selected. The sum of the PP values, or score, can be used to compare various land uses in a catchment, as described below.

Ranking land uses

To rank different land uses in a catchment in terms of their impact, the detriment (equation 7) is calculated. Table 4 gives an example of the calculations and final rank for four different land uses. The total toxic load (L) is obtained from the total of the pesticides of interest for a particular industry (in this case Vegetables), as shown in Table 3 – in this example the total toxic load was 74. This is multiplied with the area (in hectares) under this particular land use (50 ha for vegetables in Table 4). The value (V) assigned to a water body in the catchment for this area was 40, the score for water of near drinking quality. As shown in the table, the ranking of land uses is possible on the basis of the detriment value shown in the last column of Table 4, which is calculated using Equation 4. In this case, vegetables are had more than twice the greater potential detriment on groundwater quality than potatoes, despite the fact that the area planted under vegetables was three times lower than that planted under potatoes.

Scope and limitations of PIRI

The PIRI model is aimed at making comparisons across a broad spectrum of crops, pesticides and water bodies or aquifers. In most cases, detailed information for modelling will not be available. This limits the accuracy of the final result. In many cases the form of the relationship between different variables is also

Table 3. An example worksheet for calculating relative contributions of different pesticides in terms of their impact on groundwater quality. (Assume: $\rho = 1.4$ g/cm^3, $\theta_{FC} = 0.4$ cm^3/cm^3, $f_{oc} = 0.005$, $q = 1$m/yr, $D = 1$m)

Pesticide	LC_{50} (fish)	Half-life (days)	K_{oc}	Active ingredient	*dfp* (kg/ha.yr)	$AF \times 100$	$\sum PP^a \times 100$	Score
Demeton-methyl	6.4	50	22	0.25	1.1	6.07	0.26	2
Trichlorfon	0.7	10	10	0.50	1.7	0.00	0.00	0
Permethrin	0.0025	30	100000	0.50	0.2	0.00	0.00	0
Chlorpyrifos	0.003	30	6070	0.50	2.0	0.00	0.00	0
Parathion	1.5	14	5000	0.50	0.7	0.00	0.00	0
Dimethoate	6.2	7	20	0.40	0.7	0.00	0.00	0
Endosulfan	0.002	50	12400	0.35	2.1	0.00	0.00	0
Metham	0.079	10	10	0.42	79.0[b]	0.00	0.29	2
Fenamphios	0.072	50	100	0.40	24.0	0.38	51.0	4
Mancozeb	2.2	70	2000	0.75	2.2	0.00	0.00	0
Matalaxyl	100.0	70	50	0.08	2.5	6.65	0.01	1
Chlorthalonil	49.0	30	1680	0.72	3.5	0.00	0.00	0
Iprodione	4.1	14	700	0.20	2.0	0.00	0.00	0
Metiram	1.1	20	500000	0.80	2.2	0.00	0.00	0
Vinclozolin	22.0	20	10000	0.50	1.0	0.00	0.00	0

[a] pollution potential $= \frac{fdap}{LC_{50}} AF$
[b] $f = 0.1$

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unknown. Because of this lack of understanding, approximate functions have been used to describe the value of assets, toxicity and the movement of pesticides in the environment. The approximations, however, are not always crucial. For example, errors in the value of the asset will not affect the relative contribution of pesticides to the overall pesticide load for each asset considered separately.

Table 4. Example calculations for ranking different landuses in terms of their impact on water quality in a catchment. ∑PP for vegetables from Table 3, other values are provisional

Land use	Area (ha)	∑PP ×100	∑PP × area (*LT*)	Value (*V*)	Detriment (*VLT*)
Potatoes	150	10.0	15.0	40	600
Vegetables	50	51.6	25.8	40	1032
Rice	200	0.9	1.8	40	72
Sorghum	100	2.2	2.18	40	87

Conclusions

Simple methods of assessing pesticide impact on groundwater quality and other components of an environment are available, which can be of major benefit in making sound decisions on pesticide use to ensure minimum possible detriment to the environment. These methods or tools vary greatly in terms of the parameters taken into consideration and the underlying approach. The user, therefore, needs to be aware of the strength and weaknesses of any method. In assessing the relative impact of a pesticide on groundwater quality, the method must include pesticide characteristics and site properties, including the often-overlooked toxicity of a pesticide and the amount used. The approach used in PIRI, which does use these variables, is therefore an advance on currently available methods.

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Application of Thin-layer Chromatography for Pesticide Residue Analysis

Árpád Ambrus*

Abstract

Thin-layer chromatography (TLC) has a long history, but has been used only to a limited extent in residue laboratories since gas–liquid chromatography (GLC) and high performance liquid chromatography (HPLC) became readily available. In recent years, there have been various developments in the quality of plate coating and in detection systems, as well as in extraction and clean-up methods. The combination of these procedures with rigid quality control make it possible to apply TLC in laboratories working in compliance with ISO 25 or good laboratory practice (GLP).

This paper reports the results of a study performed to investigate the potential of applying TLC detection in combination with the recently introduced extraction and micro clean-up methods, for providing an alternative cost-effective analytical procedure for screening and confirmation of pesticide residues in plant commodities. Cabbage, green peas, orange, and tomatoes were the sample matrices used. The method developed is intended for laboratories where irregular supply of electricity, lack of service, or a limited budget do not allow the continuous use of GLC and HPLC techniques. TLC may also be used together with GLC or HPLC detection for confirmation of the residues.

Altogether 118 pesticide active ingredients and metabolites were included in the program.

THIS study was performed to investigate the possibilities of applying thin-layer chromatography (TLC) detection in combination with the recently introduced extraction and micro clean-up methods for providing an alternative cost effective analytical procedure for screening pesticide residues in plant commodities, using cabbage, green peas, orange and tomato as representative sample matrices. The method developed is intended for laboratories where the irregular supply of electricity, lack of service, or the limited budget do not allow the continuous use of gas–liquid chromatography (GLC) and high performance liquid chromatography (HPLC) techniques. The TLC detection may also be used together with GLC or HPLC detection for confirmation of the residues.

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Altogether 118 pesticide active ingredients and metabolites were included in the program. The procedures tested were:

- extraction with ethyl acetate and an on-line extraction method applying an acetone–dichloromethane mixture;
- column chromatographic clean-up on SX-3 gel, and on an adsorbent mixture of active carbon, magnesia, and diatomaceous earth; clean-up on silica micro cartridges;
- TLC elution applying: silica gel 60 F or silica gel 60 layers with ethyl acetate, dichloromethane, benzene, cyclo-hexane:benzene:acetic acid:paraffin oil / 200:30:20:1 (volume ratio); aluminium oxide G layer with ethyl acetate; reverse phase RP-18 F-254S layer with acetone:methanol:water/30:30:30 (volume ratio); and self made silica gel 60 HF 254 layer pre-treated with paraffin oil with acetone:methanol:water/30:30:30 (volume ratio) eluents.

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- Detectability of pesticide residues was determined with chemical reagents and bioassay techniques (Ambrus et al. 1981) including:

  Method 1: o-Tolidine + potassium iodide [oTKI]
  Method 2: p-nitrobenzene–fluoroborate [NBFB]
  Method 3: p-dimethylamino benzaldehyde [pDB]
  Method 4: silver nitrate + UV exposure [AgUV]
  Method 5: photosynthesis inhibition (Hill reaction) [Hill]
  Method 6: fungal spore (*Aspergillus niger*) inhibition [FAN]
  Method 7: enzyme inhibition with cow liver extract and (β-naphthyl-acetate substrate [E β NA]
  Method 8: enzyme inhibition with pig or horse blood serum and acetylthiocholine iodide substrate [EAcI]
  Method 9: 4-p-nitrobenzyl-pyridine [4NP]

Ethyl acetate extraction in the presence of anhydrous sodium sulfate and sodium carbonate, gel chromatographic clean-up with additional purification on silica gel cartridges provided clean extracts enabling the application of 600 mg sample equivalent on the TLC plates. Detection methods 1, 6, 7, and 8 are suitable for general screening. Method 2 and to a lesser extent method 3 can be used for confirming the identity of residues. Method 9 is not suitable for residue analysis. As low as a 0.002 mg/kg limit of determination can be achieved for some organo-phosphorus, urea, and triazine type compounds, and around 0.05–0.2 mg/kg for different type of fungicides. However, the TLC detection methods available are not sensitive for detecting organochlorine pesticides at or below the current Codex Extraneous Residue Limits, synthetic pyrethroids, sulfonylurea type herbicides, and several other compounds.

The extraction and clean-up methods described can be adapted for the analysis of pesticide residues in other fruit and vegetables, as well as in soil samples. The purified extract is also suitable for GC–ECD (electron capture detector) determination.

The identification, confirmation, and quantification by TLC methods of residues in samples of unknown origin require experienced analysts and careful consideration of all available information. The repeated elutions needed take several days to complete.

When their limitations are fully recognised and the database is regularly updated, the methods described may be used successfully by experienced analysts for screening and semi-quantitative determination of a large number of pesticide resides in a wide range of food commodities in order to check their compliance with the Codex MRLs.

Methods

Preparation of portion of sample to be analysed

Chop and mince the samples preferably immediately after receipt. Use the total amount of sample and make sure that the chopped pieces are smaller than 3 mm in order to obtain representative portions of sample. Prepare 60 g portions and extract them as described below or store them individually in double-sealed plastic bags in a deep-freezer until extraction. Label each portion accurately and place the label between the two plastic bags.

Extraction

Extraction with ethyl acetate

Extract a portion of the 60 g homogenised laboratory sample with 120 mL EtAc in the presence of 15 g $NaHCO_3$ and 60 g anhydrous Na_2SO_4 using an Ultra Turrax homogeniser at around 27–30°C. Add another 60 g Na_2SO_4 to remove remaining water. Mix the slurry well. Keep the sample solvent mixture in a fume hood for about 15 to 30 minutes to let the solvent separate from the solid material. Filter the separated solvent through a small cotton wool plug into measuring cylinder to obtain 60 mL filtrate (1 mL = 0.5 g sample). Evaporate the filter in a rotary evaporator to about 2–3 mL. Transfer the concentrated extract to a calibrated conical test tube, and continue the evaporation with gentle air stream to nearly dryness. Add about 3 mL of the solvent used for the clean-up procedure, and evaporate to a few tenths of mL. Repeat changing the solvent twice. Adjust the final volume to 2 mL (equivalent to 30 g sample).

Note that the temperature during extraction must be between 25–33°C to obtain good extraction efficiency. If deep-frozen samples are processed the sample homogenate and solvent should be kept in a water bath at 30°C to reach the specified temperature range.

On-line extraction (Steinwandter 1989)

Add 200 mL acetone, about 30 g of sodium chloride, and 150 mL dichloromethane to a 100 g portion of the homogenised sample. Blend at high speed for 2 minutes. Pour the organic phase into a 400 mL beaker and dry with anhydrous sodium sulfate. Take 200 mL

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of the organic phase and reduce its volume to 2–3 mL. Add about 5 mL of solvent used for clean-up. Repeat evaporation twice. Adjust the final volume to 4 mL (equivalent of 57.14 g sample)

Note that the micro extraction method described by Steinwandter (1992) is not applicable because of two reasons: The first reason is simple: a 5 g sample is not enough to achieve, applying TLC detection methods, the required limits of determination for most of the compounds.

The second reason is general, regardless the sensitivity of detection: it was found that most of the fruit and vegetable samples cannot be minced and ground so well that their 5 g portions can be considered statistically well mixed (in other words, it is not possible to prepare a 5 g representative portion of the sample) which is necessary to estimate the error of sample preparation. Systematic studies indicate that applying the best mincing equipment available (Hobart Chopper, Stephan blender) for extraction of 100 g and 30 g portions of cabbage or lettuce the uncertainty (relative standard deviation of residues) resulting from the sample preparation step may be expected at about ±10% and 18%, respectively (Ambrus 1996). Similar uncertainty was found in case of tomatoes and apples. Consequently 5 g portions of samples should not be extracted even if the sensitivity of the detection method would be sufficient (e.g. GC with ECD or NPTID)

Clean-up procedures

Three clean-up procedures tested in this study were: column chromatography on SX-3 gel (Anderson and Ohlin 1986), mixed adsorbent (Ambrus 1981), and silica cartridges (Merck LiChrolute Si 500).

SX-3 gel column

Use: Bio-Beads SX-3 200–400 mesh gel (Bio-Rad). The elution properties are strongly depend on the manufacture process of the gel. Other styrene-diviniylbenzene polymers may not provide the necessary clean-up.

GPC apparatus: KL SX-3 gel chromatograph (supplied by Rademed Bt, Hungary) operating with constant nitrogen over-pressure of 0.5 atm. Gel column: 500 mm × 10 mm glass column. Other equipment may also be used.

Preparation of column: soak the gel in cyclohexane/dichloromethane (1+1) mixture and transfer the swollen gel into the column. Pass solvent mixture through the column to get uniform bed. Check the eluent: it should not contain any impurities giving spots on the TLC layers.

Calibration of the column: inject 1–2 mL solution of diazinon and ethion, elute pesticides with cyclohexane/dichloromethane (1+1) and collect fractions in 1 mL increments. The pesticide fraction starts at the point where about 95% of the pesticides in the test mixture are recovered. (Note: During this use of the KL SX-3 apparatus, the first 15 mL eluent was discarded, the pesticide residues were collected in the 16–36 mL fraction. The cycle, including washing after sampling, took about 60 minutes.)

Elution of extracts: run the gel chromatographic system, inject sample extract, collect the pesticide fraction determined during calibration and wash system with 50 mL solvent mixture. The system is ready for the next injection.

Evaporate eluent: to about 0.3–0.5 mL and make up to a volume of exactly 1 mL with acetone for direct application to TLC plates, or add 5 mL cyclohexane and repeat evaporation step twice in order to remove the traces of dichloromethane. Adjust the final volume of the eluent to exactly 2 mL before further clean-up on silica cartridge.

Mixed adsorbent

Mixture of 1 g active carbon, 2 g magnesia, 4 g diatomaceous earth (acid-washed).

Pre-treatment of the adsorbents:

- Active carbon: reflux 150 g active carbon in 500 mL 1 N HCl for 4 hours. Wash adsorbent with distilled water until water contains no chloride ion, and dry at 95–100°C to constant weight. Do not increase temperature above 100°C because adsorptive properties may change
- Wash magnesia in 1 litre absolute ethanol, filter, air-dry, and activate at 140°C for 4 hours. Heat diatomaceous earth at 400°C for 8 hours.

Preparation of the column: suspend 7 g pre-treated adsorbent mixture with 40 mL benzene in cylindrical glass jar. Fill 18 mm i.d. column with suspension. Rinse jar and funnel with benzene and let benzene flow through column. Add 2–5 mL extract equivalent to 20–50 g sample, and elute pesticides with 150 mL dichloromethane.

Evaporate eluent: to about 0.3–0.5 mL and make up its volume to exactly 1 mL with acetone for direct application to TLC plates, or add 5 mL cyclohexane and repeat evaporation step twice in order to remove

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traces of dichloromethane. Adjust the final volume of the eluent to exactly 2 mL before further clean-up on silica cartridge.

Silica gel cartridge

Use: LiChrolut Si 60 500 mg cartridges (Merck), or Sep-Pack silica 1000 mg cartridges (Waters).

Vacuum manifold: use a suitable vacuum manifold (e.g. Merck 1.19851.0001, or Baker 10 system to hold cartridges and facilitate elution)

Pre-wash of cartridges: wash cartridges with 10 mL toluene:cyclohexane:acetone (60:30:10 v/v) and then with 2 × 10 mL toluene/cyclohexane (15:85 v/v).

Elution of extract: take up the concentrated extract in 2 mL of cyclohexane and transfer it onto the column (10 mL syringe can be used most conveniently for transferring solvents and extracts) elute pesticides with one of the elution systems:

- System A: 15 mL toluene/cyclohexane (15:85 v/v).
- System B: 25 mL toluene/cyclohexane/acetone (60:30:10 v/v)

Concentration of the eluates: concentrate eluate to 1 mL (equivalent to 30 g sample). The concentrate is suitable for application on TLC plates.

The recovery data of pesticides on SX-3 gel column and silica gel cartridges were published, for instance, by Anderson and Ohlin (1986).

Elution of pesticides on TLC plates

The Rf values were determined in developing tanks kept in water bath held at 20°C in order to reduce the effect of temperature variation in the laboratory. The eluent was equilibrated with the vapour phase by inserting filter paper in the developing tank and waiting for at least 30 minutes before the plates were placed into the tanks. The eluent was allowed to run up to 10 ± 0.5 cm from the origin.

The Rf values (Table 1) were determined in the following systems:

Layer: silica gel 60 F-254 0.25 mm (Merck), activated at 105°C for 30 minutes before use

- System I. Silica gel 60 F – ethyl acetate
- System II. Silica gel 60 F – dichloromethane
- System III. Silica gel 60 F – benzene
- System IV Silica gel 60 F – cyclo-hexane: benzene:acetic acid:paraffin oil / 200:30:20:1 volume ratio

Layer: silica gel 60, 0.25 mm (Merck 2299161) activated at 105°C for 30 minutes before use

- System IA. Silica gel 60 – ethyl acetate

Note: this layer was used to determine the minimum detectable quantities of pesticides

Layer: aluminium oxide G (Merck) self prepared 0.25 mm layer, dried at 80°C for 45 minutes after preparation, and stored over activated silica gel until use.

- System V. aluminium oxide G – ethyl acetate

Layer: reversed phase TLC layer RP-18 F-254S (Merck), activated at 120°C for 45 minutes before use

- System VI.RP-18 F-254S – acetone: methanol:water/30:30:30 volume ratio

Layer: self made silica gel 60 HF 254 layer pretreated with paraffin oil according to Boyce and Millborrow (1965). The plates were dried at room temperature for 2 hours, then activated at 105°C for 10 minutes. The activated plates were cooled to room temperature. then impregnated by allowing a 5% solution of liquid paraffin in hexane (v/v) to run to the top of the plate followed by the evaporation of the solvent at 40°C.

- System VII. silica gel 60 HF 254 – acetone:methanol:water/30:30:30 volume ratio

Detection of Pesticides

Method 1. o-tolidine + potassium iodide [o-TKI]

Reagents:

– 0.5 g o-tolidine dissolved in 10 mL acetic acid
– 2 g KI dissolved in 10 mL distilled water

Mix two solutions and dilute to 500 mL with distilled water. The reagent can be stored in refrigerator for 2 weeks.

Detection:

Use ready-made silica gel plates. Remove eluting solvent and place the plate in developing tank saturated with chlorine for 30 sec (place a 25 mL beaker into the bottom of developing tank, add 8 g $KMnO_4$ and 10 mL concentrated HCl). Remove the excess chlorine in a well-ventilated fume hood (about 45 min), and spray the plate with the reagent solution. (Test complete removal of chlorine by spraying the upper edge of the plate. If chlorine is present the sprayed area turns blue! If no discoloration occurs spray the entire plate.)

Colour reaction: Blue, lilac or white spots on greyish-white background.

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Table 1. RRf Atrazine values of pesticides in different elution systems according to increasing order with EtAc elution

Active ingredient	System I Si-EtAc	System II Si-Dichlor	System III Si-Benze[a]	System IV Si-Mix	System V AlO-EtAc	System VI RP-18
Propamocarb	0.00	0.12	0.00	0.00	0.02	1.91
2,4-D	0.07	0.00	0.00	0.05	0.00	1.53
Haloxyflop	0.09	0.00	0.00	0.21	0.00	0.93
Omethoate	0.10	0.00	0.00	0.04	0.99	1.48
Monocrotophos	0.13	0.12	0.00	0.00	0.98	2.07
Acephate	0.15	0.08	0.00	0.01	0.95	
Ethirimol	0.20	0.00	0.00	0.07	0.02	0.83
Imazalil	0.25	0.14	0.00	0.00	0.45	0.35
Oxamyl	0.31	0.40	0.00	0.00	0.51	2.12
Phosphamidon	0.37	0.08	0.02	0.01	0.58	1.59
Trichlorfon	0.40	8.44	0.00	0.08	0.01	
Dimethoate	0.45	0.40	0.01	0.03	0.58	1.83
Chlordimeform	0.47	0.04	0.01	0.05	1.05	0.50
Carbendazim	0.49	0.06	0.00	0.05	0.29	1.30
Metoxuron	0.50	0.51	0.00	0.02	0.74	1.48
Benomyl[b]	C 0.51	4.87	C 0.02	C 0.05	C 0.27	0.41
Terbuconazol	0.55	0.95	0.00	0.11	0.55	0.45
Thiabendazole	0.55	0.62	0.00	0.00	0.72	1.20
Chloroxuron	0.57	0.61	0.01	0.04	0.94	0.64
Oxadixyl	0.60	0.69	0.00	0.00	0.40	1.57
Methomyl	0.60	0.89	0.00	0.00	0.96	1.98
DNOC	0.60	9.32	0.28	1.24	0.96	1.41
Pentachlorophenol	0.60	8.10	0.21	0.98	0.95	0.36
Diuron	0.60	1.34	0.01	0.04	0.85	0.91
Chlorotoluron	0.65	0.30	0.01	0.06	0.92	1.06
Methabenzthiazuron	0.67	1.67	0.01	0.23	0.95	1.04
Mevinphos	0.69	0.99	0.00	0.14	0.97	1.65
Fenthion-o	0.69	1.69	0.02		0.97	
Fenitrothion-o	0.70	1.27	0.00	3.82	0.00	1.18
Pirimicarb	0.74	0.54	0.00	0.00	0.97	1.12
Dioxacarb	0.75	0.54	0.00	0.07		1.76
Metalaxyl	0.76	0.49	0.02	0.09	0.91	1.14
Nuarimol	0.77	0.22	0.00	0.14	0.68	0.79
Asulam	0.78	0.56	0.00	0.01	0.00	2.20
Fenarimol[a]	0.79	0.05	0.10	0.15	0.72	0.60
Aldicarb	0.80	0.78	0.01	0.09	0.90	1.54
Dichlorvos	0.83	2.47	0.04		0.90	0.15
Diphenamid	0.85	2.17	0.01	0.24	0.97	1.00
Napropamide	0.86	1.86	0.00	0.40	1.01	0.63
Chlorfenvinphos[a]	0.90	2.24	0.00	0.61	1.03	0.51
3-CO-Carbofuran	0.91	0.47	0.00	0.15	0.95	1.59
Linuron	0.92	7.31	0.05	0.23	0.98	0.75
Bupirimate	0.92	1.62	0.00	0.10	1.01	0.54
Monolinuron	0.93	7.12	0.04	0.26	0.99	1.04
Chlorbromuron	0.94	7.63	0.05	0.27	1.02	0.70
Simazin	0.94	1.32	0.00	0.84	0.91	1.23
Thiophanat-methyl[a]	0.94	1.87	0.00	0.05	0.84	1.41
Metobromuron	0.95	5.91	0.04	0.27	0.99	1.02
Azinphos-methyl	0.96	7.19	0.06	0.45	1.01	0.83
Carbofuran	0.98	1.62	0.00	0.22		1.39
Terbutryn	0.98	1.92	0.01	0.25	1.02	0.62
Propachlor	0.99	4.39	0.04	0.38	0.99	0.99

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Active ingredient	System I Si-EtAc	System II Si-Dichlor	System III Si-Benze[a]	System IV Si-Mix	System V AlO-EtAc	System VI RP-18
Cyanazine	1.00	0.43	0.00	0.42	0.97	1.42
Carbaryl	1.00	5.52	0.04	0.24	0.96	1.24
Atrazine	1.00	1.00	0.00	1.00	1.00	1.00
Captafol	1.01	10.81	0.14	0.45	0.96	0.54
Dichloran	1.03	16.05	0.05	0.87	0.99	0.67
Metribuzin[a]	1.03	4.04	0.03	0.27	0.97	1.25
Prometryn	1.03	1.93	0.01	0.42	1.04	0.63
Folpet	1.03	15.33	0.26	0.99	1.04	0.54
Methidathion	1.04	9.07	0.15	0.70	1.02	0.81
Terbuthylazine	1.05	1.51	0.01	1.02	0.98	0.74
Triazophos	1.05	5.62	0.04	0.37	1.02	0.62
Aziprotryn	1.05	5.83	0.07	1.26	1.05	0.64
Captan	1.05	9.43	0.10	0.56	1.00	0.78
Iprodione	1.06	2.50	0.02	0.25	1.01	0.53
Lenacil[a]	1.06	0.24	0.12	0.37	0.90	1.07
Phenylphenol	1.06	12.55	0.28	0.90	0.94	0.85
Malathion	1.06	8.97	0.08	0.76	1.06	0.66
Biphenyl	1.06	19.89	0.43	2.77	1.08	0.41
Dichlofluanid[a]	1.06	14.68	0.29	1.04	0.94	0.55
Phenmedipham	1.07	1.79	0.01	0.07	0.95	1.00
Fenitrothion	1.07	15.78	0.39	1.19	1.09	0.56
Ethoxyquin	1.07	6.77	0.09	0.10	1.07	0.55
Thiometon	1.07	15.83	0.40	2.54	1.06	0.53
Procymidone	1.07	16.77	0.17	0.80	1.04	0.52
Etrimfos	1.07	8.53	0.12	1.24	1.09	0.43
Dithianon[a]	1.07	12.54	0.33	0.60	0.96	0.50
Chlorpropham	1.08	13.05	0.24	0.95	1.05	0.64
BCPE	1.08	12.13	0.18	0.79	1.06	0.39
EPTC[a]	1.08	7.88	0.13	1.57	0.97	0.51
Fenthion	1.08	15.94	0.45	1.74	0.96	0.44
Propham	1.08	10.89	0.17	0.99	0.94	1.04
Oxadiazon	1.08	13.52	0.28	1.41	1.06	0.19
Tetradifon	1.08	16.44	0.41	1.65	1.11	0.21
Bromophos-ethyl	1.09	20.09	0.60	2.81	1.10	0.10
Diazinon [a]	1.09	0.19	0.01	0.23	0.11	0.40
Prothiofos	1.09	19.96	0.58	2.90	1.00	0.08
Desmedipham	1.09	2.47	0.02	0.12	1.05	0.95
Dinobuton	1.09	15.65	0.44	1.80	1.12	0.26
Aldrin	1.10	20.32	0.64	3.02	1.09	0.07
Parathion-methyl	1.10	16.18	0.38	1.27	1.07	0.70
Bromopropylate	1.10	13.03	0.24	1.42	1.01	0.19
Nitrofen	1.10	17.52	0.56	1.89	0.97	0.21
Vinclozolin	1.10	17.00	0.39	1.35	1.05	0.50
Chlorthalonil	1.10	0.00	0.48	1.38	1.09	0.53
Chlorpyrifos[a]	1.10	19.67	0.56	2.64	0.96	1.52
Parathion	1.10	17.06	0.40	1.51	1.09	0.44
Mecarbam	1.11	9.18	0.09		1.08	
Endosulfan	1.11	17.00	0.56	2.54	1.10	0.22
Deltametrin	1.11	17.22	0.47	1.89	0.95	0.06
Chloropropylate[a]	1.11	11.68	0.27	1.30	0.97	0.23
Phosalone	1.11	15.86	0.22	0.92	1.09	0.32
Propargite	1.11	14.92	0.30	1.52	0.73	0.12

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Active ingredient	System I Si-EtAc	System II Si-Dichlor	System III Si-Benze[a]	System IV Si-Mix	System V AlO-EtAc	System VI RP-18
Lindane[a]	1.11	20.07	0.60	0.51	0.94	
Heptachlor	1.11	20.10	0.55	3.21	1.01	
Cypermethrin[b]	1.11	17.74	0.46	1.77	0.95	0.07
Tetrasul[a]	1.11	20.01	0.65	3.01	0.99	0.04
Dieldrin[a]	1.11	16.40	0.44	0.82	1.02	0.00
Butylate	1.11	9.59	0.18	0.75	1.09	
Butachlor	1.12	7.48	0.08	1.22	0.96	0.23
Cadusafos	1.12	21.20	0.69			
Benefin	1.13	19.99	0.63	2.41	0.91	0.13
Trifluralin	1.13	19.63	0.60	2.49	0.91	0.13
Phenkapton	1.13	20.23	0.58	2.47	1.10	0.09
p,p'-DDT [a]	1.13	20.39	0.14	0.13	1.08	0.10
HCB	1.14	18.76	0.59	3.17	1.00	0.07
Fenpropatrin	1.15	19.36	0.41	1.88	1.13	0.11
Chlorfenvinphos[b]			0.62			
Chloropropylate[b]					1.11	
Chlorpyrifos[b]				0.00	1.10	
Cypermetrin[b]	0.00	0.12	0.00	0.00	0.02	1.91
Deltametrin[b]					1.11	
Diazinon[b]			0.06	0.48	1.08	
Diazinon				0.69		
Dichlobenil[a]			0.14	0.80	1.02	0.80
Dichlobenil[b]			0.46	1.40		
Dichlofluanid[b]					1.04	
Dieldrin[b]				1.90		
Dithianon[b]					1.06	
EPTC[b]					1.09	
Fenarimol[b]					0.94	
Lenacil[b]		7.20	0.24	0.58		1.32
Lenacil			0.29			
Lindane[b]					1.09	
Metribuzin[b]				1.05	1.02	
p,p'-DDT[b]			0.64	0.80		
p,p'-DDT				2.88		
Tetrasul[b]					1.11	
Thiophanat-methyl[b]		0.00		0.00	1.08	0.00

Notes:
See Table 6 for RRf in System I in alphabetical list of compounds
[a] Since Rf of Atrazine is 0.00 with benzene eluent RRf cannot be calculated, consequently the Rf values are listed in this column.
[b] Benomyl is rapidly decomposing to carbendazim in standard solutions. C preceding the RRf values indicates that the two compounds could not be separated by the system

Safety precautions: o-tolidine is classified as a potential carcinogen in many countries. Use gloves for handling. Perform chlorination and removal of excess chlorine under a well-ventilated fume hood. Use gloves for placing and removing plates from chlorination tank.

Method 2. p-nitrobenzene-fluoroborate [NBFB]

Specificity: the reagent detects free phenols or phenols derived from the hydrolysis of the compounds

Reagents:

Dissolve about 0.1 g 4-nitrobenzene-diazonium-fluoroborate in a mixture of 2.5 mL ethylene glycol and 22.5 mL ethanol. The solution must be saturated, as indicated by a small portion of non-dissolved material. Prepare freshly and use the solution within 5–10 minutes.

Detection:

Use ready made silica-gel plates. Spray the air-dried layer with 1.5 M NaOH solution and place it

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into an oven at 70°C for 10 minutes. After cooling to room temperature, spray the plate with the fluoroborate reagent solution.

Colour reaction: Red, lilac, or blue spots occur on white background.

Method 3. p-dimethylamino benzaldehyde [pDB]

Specificity: compounds which can be hydrolysed to primary amines (e.g. urea herbicides) can be detected.

Reagent:

Dissolve 0.15 g p-dimethylamino-benzaldehyde in the mixture of 47.5 mL ethanol and 2.5 mL HCl. Prepare freshly!

Detection:

Use self or ready-made aluminium oxide plates (silica gel plates cannot be heated to 160°C as the layer is damaged at that temperature!)

Place the air-dried plate into oven at 160°C for 25 minutes. After cooling to room temperature spray the plate with the reagent.

Colour reaction: Yellow or (some minutes later) rose spots on white background.

Method 4. Silver nitrate + UV exposure [AgUV]

Method 4A

Specificity: non-specific, detects halogen-containing and several other compounds.

Reagent: dissolve 0.1 g $AgNO_3$ in 1 mL freshly prepared bi-distilled water (collected and stored in Pyrex glass bottle with a ground glass stopper), add 20 mL phenoxy-ethanol, 1 drop hydrogen peroxide, and make up the volume to 200 mL with acetone. Store in a brown glass bottle protected from direct UV or sunlight. The reagent can be stored about a week.

Preparation of TLC plates: Shake firmly 50 g Merck aluminium oxide G adsorbent in 55 mL freshly prepared bi-distilled water (collected and stored in Pyrex glass bottle with a ground glass stopper) in a 100 mL glass-stoppered Erlenmeyer flask for two minutes. Transfer the homogeneous slurry into the TLC spreader and draw the plates without delay. (The calcium sulfate starts to bind immediately.) The amount is sufficient to prepare five 20 × 20 cm plates with 0.25 mm thick layer. Check the uniformity of the layer. Place the good ones into the storage rack and dry them for 15 minutes at room temperature, then at 80°C for 45 minutes. Store the plates over activated silica gel in a desiccator.

Detection: Use self-made aluminium oxide layer. Dry the layer after development at room temperature, spray it uniformly with the reagent solution. Place the plate under unfiltered intensive UV light until the spots develop. (Strong bactericide lamps used for air sterilisation are the most suitable.)

Method 4B

Reagent: dissolve 0.15 g $AgNO_3$ in 15 mL freshly prepared double-distilled water (collected and stored in Pyrex glass bottle with a ground glass stopper).

Preparation of TLC plates: Shake 45 g Merck aluminium oxide G adsorbent in a 300 mL glass stoppered Erlenmeyer flask with 90 mL 0.2 % nitric acid at 300 rpm for 15 minutes.

Transfer slurry into centrifuge tubes and centrifuge it at 2500 rpm for 10 minutes.

Decant and discard the acid layer. Wash the aluminium oxide G with 3 × 50 mL double-distilled water. Centrifuge it after each wash at 2500 rpm for 10 minutes. Decant and discard water. Add 15 mL 1% $AgNO_3$ solution and 15 mL double-distilled water. Mix with a glass rod. Transfer the homogeneous slurry into the TLC spreader and draw the plates without delay.

The amount is sufficient to prepare five 20 × 20 cm plates with 0.25 mm thick layer. Place the good ones into the storage rack and dry them for 15 minutes at room temperature, then at 80°C for 45 min. Store the plates over activated silica gel in desiccator.

Detection: Use self-made aluminium oxide layer. Develop the chromatogram with appropriate eluents. Dry the layer after development for 5 minutes at room temperature. Place the plate under unfiltered intensive UV light until the spots develop. (Strong bactericide lamps used for air sterilisation are the most suitable.)

Safety precautions: Always use protective glasses with UV filtration when checking the development of spots. Do not expose skin to UV light for extended period. Strong non-filtered UV radiation can burn the skin.

Note that the time required for colour development depends on the intensity and the spectrum of the UV light source, and the distance between the lamp and the plate. The optimum distance should be determined experimentally. Normal TLC lamps used to detect spots on fluorescent layers do not provide suf-

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ficient intensity and usually cannot be used. On clear sunny days the plates may be developed under direct sunshine. Chlorine-containing compounds must be kept away from the TLC plates and distilled water must be used. If the plates have been exposed to chlorine they turn grey under the UV light. Special attention is required when other TLC detection methods applying HCl or chlorine are used in the laboratory, or the distiller is cleaned with HCl.

Colour reaction: the spots occur in greyish-black colour

Method 5. Photosynthesis inhibition (Hill reaction) [Hill]

Specificity: primarily herbicides inhibiting photosynthesis can be detected.

Reagents:

Wheat leaves: grow wheat in pots placed in the window. In good quality soil the wheat leaves can be harvested after two weeks.

Chloroplast: Cut 1–2 handfuls of wheat leaves into 2–4 mm pieces. Weigh 30 g of them into mortar. Add 3 mL glycerol, 15 mL bi-distilled water, and 5 g quartz (sea) sand. Crush the mixture with pestle until a fairly homogenous pulp is obtained. Put a 4 layer gauze over a beaker. Transfer the homogenate to the gauze, fold up like a knapsack, and press the chloroplast suspension through. Protect from light by wrapping the beaker into an aluminium foil. Store in refrigerator until use. Prepare fresh daily before use.

Borax buffer solution: mixture of 350 mL 0.05 M borax solution (9.5 g borax dissolved in 500 mL water) and 150 mL 0.1 M HCl.

DCPIP reagent: 200 mg of 2,6-dichlorphenol-indophenol Na-salt is dissolved in 500 mL borax buffer solution.

Detecting reagent: 20–25 mL wheat pressing is mixed with 10 mL of DCPIP solution, then the reagent is added drop-wise until the colour of the mixture becomes bluish-green (somewhere between the colours or pH 9–10 on an universal pH paper scale). This amount is enough for two 20 × 20 cm plates. The reagent is prepared immediately before spraying the layer.

Detection:

Use ready-made silica gel plates. Dry the eluted layer and spray uniformly with the reagent. Place the plate about 20 cm below a 60W tungsten lamp (ordinary bulb) for a few minutes. The inhibition should occur within 10 minutes. The spots are usually visible after some minutes and reach optimum after about 5 minutes. The quantification should be performed immediately after appearance of the spots as they disappear within a few minutes.

Colour reaction: blue against a greenish background

Comment: sulfonylurea type herbicides cannot be detected (limit of detection 1000 ng) under conditions which enable detection of 0.5 ng linuron.

Method 6. Fungi spore (*Aspergillus niger*) inhibition [FAN]

Specificity: selectively detects some fungicides, plant extracts usually do not interfere

Reagents:

Fungi culture media: wash about 100 g potato thoroughly in fresh water, disinfect them by soaking in 500 mL water containing 5 tablespoons of Neomagnol (Dettol™). Rinse the potatoes with fresh distilled water and peel them. Prepare fine pulp on sterilised grinder and cook 50 g portion in 250 mL distilled water for an hour. Filter the cooked liquid while it is warm through 1 layer of sterile gauze, add 5 g glucose and 5 g agar-agar. Sterilise the solution in an autoclave at 0.05 MPa (0.5 atm), at 110°C or pressure cooker over-pressure for 60 minutes.

Pour 8–10 mL of culture medium into sterilised petri dishes. Let cool them. Cover them with a another petri dish of fungal culture and with gentle tapping transfer some fungal spores to the fresh media surface. Cover the petri dish with its own lid.

Place the petri dishes in incubator containing air saturated with water at 25°C. The new culture develops within 5 days. Pack the petri dishes in plastic foil and keep them in refrigerator. The culture has to be re-inoculated at least in every 4–5 months.

Be careful not to inhale the spores! *Aspergillus niger* is a toxic fungus!

Note: Handling fungal spore cultures requires special skill and appropriate conditions. If possible, ask a trained biologist (pathologist) to maintain the culture, or seek their advice.

Suspension of fungi spores: Boil 1.5 g agar in 70 mL water. Add 1.5 g glucose, 0.3 g KNO_3 and, with the tip of a glass rod, 1.5 g malt extract. Cool to 45°C and keep the solution at that temperature. Remove the spores from the fungal culture by adding 30 mL bi-distilled water and carefully drawing with a plastic or

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glass spatula until most spores have loosened. The suspension should be dark grey from the spores. Add the suspension to the agar-agar solution. Warm to, and keep the mixture at 40°C. Filter the suspension through two layers of gauze. The fungal spore suspension should be used within one hour.

Detection:

Use factory-made silica-gel 60 plates. After elution, dry the plate with a gentle air stream. Warm up the spraying device by immersing it in a 40°C deionised water bath to prevent the agar sticking. Spray the air-dried plate with the spore suspension until the layer is thoroughly wetted, but avoid run-off, and put it immediately in an incubator or an oven held at 37°C and incubate the plate for at least 24 hours.

Note that the oven must be pre-saturated with vapour which can be achieved by placing deionised water in petri dishes on every shelf. The oven used for biotests should not be used for other laboratory activities.

Incubation for 48 hours gives better growth and the shiny inhibition zones become more visible. The spots can be better observed under narrow angle.

Comment: This detection method cannot be used in combination with developing solutions containing acetic acid.

Method 7. Enzyme inhibition with cow liver extract and β-naphthyl-acetate substrate [E βNA]

Specificity: enzyme inhibiting compounds—especially phosphoric and thio-phosphoric acid esters and carbamate pesticides—are detectable, plant extracts usually do not interfere.

Reagents:

Enzyme solution: cut fresh liver into small pieces, weigh 10 g into 90 mL bi-distilled water and homogenise it with Ultra Turrax or high speed blender. Centrifuge the homogenate at 4000 rpm for 10 minutes. Collect the supernatant in 10 or 20 mL portions and place them into a deep-freezer until used. Dilute the enzyme concentrate to 3 times with bi-distilled water before use.

β-*naphthyl-acetate:* 1.25 mg/mL solution in ethyl alcohol. It can be stored in refrigerator for extended period.

Fast blue salt (Merck Cat. No. 1.03191.0025): 10 mg salt in 16 mL double-distilled water. Prepare freshly for each use!

Substrate solution: Mixture of 10 mL β-naphthyl-acetate solution and 16 mL Echtblau-salt solution

Detection:

Air dry the ready made silica layer and treat it with bromine vapour.

Treatment with bromine: place a 25 mL beaker into the bottom of the developing tank, using a safety pipette (long measuring pipette with plunger at one end) transfer about 0.5–1 mL bromine into the baker. Cover the tank and wait for few minutes until the bromine vapour saturates the tank. Place the dry plate into the tank for 15 minutes. Remove the plate and keep it for about 45 min in a well ventilated fume hood to remove the excess bromine.

Spray the plate with enzyme solution until it gets thoroughly wet, and place it into an incubator or oven at 37°C for 30 minutes. Pre-saturate the incubator with water by placing deionised water in petri dishes on its shelves. Ensure that the plate does dry out during incubation. Remove the excess water with an air stream after incubation. Spray the plate with the substrate solution.

Colour reaction: white spots occur in a pink (bluish-red) background.

Comment: this detection method cannot be used in combination with developing solutions containing acetic acid.

Method 8. Enzyme inhibition with pig or horse blood serum and acetylthiocoline iodide substrate [EAcI]

Specificity: enzyme inhibiting compounds—especially phosphoric and thio-phosphoric acid esters and carbamate pesticides—are detectable; plant extracts usually do not interfere.

Reagents:

2,6-dichlorphenol-indophenol (0.5 mg/mL solution in distilled water)

Enzyme solution: Break the clot (coagulated blood) with a glass rod, transfer it into centrifuge tubes and centrifuge it at 4000 rpm for 10 minutes. Collect the serum in 10 mL portions and store it in a deep-freezer until use. Determine the cholinesterase activity by the Ellman method (Ellman et al. 1961). Dilute the serum with tris-buffer before use to obtain about 140 U/L activity of pig serum, and 570 U/L for horse serum. Note: If the Ellman test (see Appendix III.) cannot be carried out determine the enzyme activity (dilution rate) experimentally to obtain the best sensitivity.

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0.05 M tris-buffer (3.04 g tris(hydroxymethyl) aminomethane 500 mL bi-distilled water)

Substrate solution: acetyl thiocoline iodide in 1.5 mg/mL water solution. (It can be stored in a refrigerator at 4°C for up to 6 weeks.)

Detection:

Threat the plate with bromine and enzyme solution as in method 8. After incubation at 37°C for 30 minutes remove excess water with an air stream, spray the plate with substrate solution and incubate it again for 15 minutes.

Colour reaction: blue spots in white background

Comment: this detection method cannot be used in combination with developing solutions containing acetic acid.

Results and Discussion

Rf values of pesticides

The activity of the layers, even in the original packing, changes depending on the storage conditions. Therefore, the layers must be freshly reactivated. The differences in the activity of the layer and the saturation of the vapour phase of the developing tank can be the major sources of the variation of the Rf values. Therefore, the activation procedures described under 'Elution of pesticides on TLC plates' were strictly followed during the study, and it is strongly recommended to always use freshly activated plates and equilibrated solvent/vapour phases in the developing tanks to obtain the best reproducibility.

The Rf values were generally measured with 6–7 elutions on different layers and at different times. The Rf values determined in Systems I and VI are summarised in Table 1. The relative Rf values calculated for atrazine, carbaryl, captan, linuron, and parathion-methyl marker compounds are given in Table 2.

The effect of the eluent temperature on the Rf values was studied at 20°C and 32°C in a silica gel–ethyl acetate system. The results are shown in Table 3.

There was no significant difference between the Rf values obtained in ready-made silica gel HF and H layers. Therefore no distinction was made between these layers in regard to their elution characteristics.

Figures 1–2 show the coefficient of variation of Rf values of pesticides measured in replicate elutions in one laboratory on different days. They reflect the repeatability of elution and the expectable variation of the Rf values. Comparing the figures it can be seen that below Rf = 0.2 the coefficient of variation (CV) of the Rf values are rapidly increasing. This is mainly due to the error (the uncertainty is in the order of mm) in the visual observation of the centre of the spots and the deformation of the spots at the start resulting from the large migration velocity of the solvent at the beginning (Hargitai 1984).

The figures provide the easiest way to evaluate the performance and applicability of the elution systems tested.

System I resulted in a reasonably good spread of Rf values in the 0.05–0.7 Rf range. The majority of compounds eluted between 0.3 and 0.7. In this range the CV_{Rf} values were ±0.1, the smallest among the systems.

The compounds in System II eluted from Rf = 0 to 0.75. The Rf of about half of them was ≤0.2 with CV_{Rf} ranging from 0.15 to 2.22.

The elution patterns in System III (benzene) and System IV were similar to that obtained with System II. The CV_{Rf} values in System III were substantially higher than in System II (up to 0.25) in the higher elution range.

The Rf values were highest in System V and, unfortunately, most of them were concentrated in the 0.7–0.9 range. The CV_{Rf} values were ≤0.15. In cases of overlapping spots, benzene or petroleum ether ethyl/ether mixtures can be used for more selective separation.

System VI gave the most uniform elution pattern. The CV_{Rf} values were ≤0.15 in the 0.1– 0.8 Rf range. The reproducibility (between laboratories variation) of Rf values on ready-made RP-18 layers was very good. They were in close agreement with the values published by the Merck Company (H.E. Hauck, pers. comm. 1993).

The Rf values on self-coated paraffin oil layers (System VII) showed the greatest variation, and for the tested compounds they had the opposite retention order to that obtained on the RP-18 phase. The retention behaviour of some selected pesticides on normal and reversed phase layers is illustrated in Table 1.

Since the mechanism of elution differs between the reversed phase system and the normal phase, and the retention order on the reversed phase was different from that obtained on normal phase, separation of suspected compounds on paraffin-coated layers could provide an excellent and cheap confirmation method in the future provided that the detection of low level of pesticides can be achieved.

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Table 2. RRf values for the marker compounds in System I.

Active ingredient	Rf	Atrazine	Carbaryl	Captan	Linuron	Parathion-Me.
Acephate	0.09	0.15	0.15	0.15	0.17	0.14
Aldicarb	0.48	0.80	0.80	0.76	0.86	0.72
Aldrin	0.67	1.10	1.10	1.05	1.19	1.00
Asulam	0.47	0.78	0.78	0.74	0.84	0.71
Atrazine	0.61	1.00	1.00	0.95	1.08	0.91
Azinphos-methyl	0.58	0.96	0.96	0.92	1.04	0.87
Aziprotryn	0.63	1.05	1.05	1.00	1.13	0.95
BCPE	0.65	1.08	1.08	1.03	1.17	0.98
Benefin	0.68	1.13	1.13	1.07	1.22	1.02
Benomyl	0.31	0.51	0.51	0.48	0.55	0.46
Biphenyl	0.64	1.06	1.06	1.01	1.15	0.97
Bromophos-ethyl	0.66	1.09	1.09	1.04	1.18	0.99
Bromopropylate	0.67	1.10	1.10	1.05	1.19	1.00
Bupirimate	0.56	0.92	0.93	0.88	1.00	0.84
Butachlor	0.68	1.12	1.12	1.06	1.21	1.02
Butylate	0.68	1.11	1.12	1.06	1.21	1.01
Cadusafos	0.68	1.12	1.12	1.06	1.21	1.02
Captan	0.64	1.05	1.05	1.00	1.14	0.95
Captafol	0.61	1.01	1.01	0.96	1.09	0.92
Carbaryl	0.61	1.00	1.00	0.95	1.08	0.91
Carbendazim	0.30	0.49	0.49	0.47	0.53	0.45
Carbofuran	0.59	0.98	0.98	0.93	1.06	0.89
3-CO-Carbofuran	0.55	0.91	0.91	0.87	0.99	0.83
Chlorbromuron	0.57	0.94	0.94	0.89	1.02	0.85
Chlordimeform	0.29	0.47	0.47	0.45	0.51	0.43
Chlorfenvinphos	0.55	0.90	0.90	0.86	0.98	0.82
Chloroxuron	0.34	0.57	0.57	0.54	0.61	0.52
Chloropropilate	0.67	1.11	1.11	1.06	1.20	1.01
Chlorpyrifos	0.67	1.10	1.11	1.05	1.20	1.00
Chlorpropham	0.65	1.08	1.08	1.03	1.17	0.98
Chlorothalonil	0.67	1.10	1.10	1.05	1.19	1.00
Chlorotoluron	0.40	0.65	0.65	0.62	0.71	0.59
Cyanazine	0.60	1.00	1.00	0.95	1.08	0.91
Cypermethrin	0.67	1.11	1.11	1.06	1.20	1.01
2,4-D	0.04	0.07	0.07	0.06	0.07	0.06
p,p-DDT	0.68	1.13	1.13	1.08	1.22	1.03
Deltametrin	0.67	1.11	1.11	1.06	1.20	1.01
Desmedipham	0.66	1.09	1.09	1.04	1.18	0.99
Diazinon	0.66	1.09	1.09	1.04	1.18	0.99
Dichlofluanid	0.65	1.06	1.07	1.01	1.15	0.97
Dichloran	0.62	1.02	1.03	0.98	1.11	0.93
Dichlorvos	0.51	0.83	0.83	0.79	0.90	0.76
Dieldrin	0.68	1.11	1.12	1.06	1.21	1.01
Dimethoate	0.27	0.45	0.45	0.43	0.49	0.41
Dinobuton	0.66	1.09	1.09	1.04	1.18	0.99
Dioxacarb	0.45	0.75	0.75	0.71	0.81	0.68
Diphenamid	0.52	0.85	0.85	0.81	0.92	0.77
Dithianon	0.65	1.07	1.07	1.02	1.16	0.98
Diuron	0.37	0.60	0.60	0.58	0.65	0.55
DNOC	0.36	0.60	0.60	0.57	0.65	0.55
Endosulfan	0.67	1.11	1.11	1.05	1.20	1.01
EPTC	0.65	1.08	1.08	1.03	1.17	0.98

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Table 2. (Cont'd) RRf values for the marker compounds in System I.

Active ingredient	Rf	Atrazine	Carbaryl	Captan	Linuron	Parathion-Me.
Ethirimol	0.12	0.20	0.20	0.19	0.22	0.18
Ethoxyquin	0.65	1.07	1.07	1.02	1.16	0.97
Etrimfos	0.65	1.07	1.07	1.02	1.16	0.97
Fenarimol	0.48	0.79	0.79	0.75	0.85	0.71
Fenitrothion	0.65	1.07	1.07	1.02	1.16	0.97
Fenitrothion-o	0.42	0.70	0.70	0.67	0.76	0.64
Fenpropatrin	0.69	1.15	1.15	1.09	1.24	1.04
Fenthion	0.65	1.08	1.08	1.03	1.17	0.98
Fenthion-o	0.42	0.69	0.70	0.66	0.75	0.63
Folpet	0.62	1.03	1.03	0.98	1.12	0.94
Haloxyflop	0.05	0.09	0.09	0.08	0.10	0.08
HCB	0.69	1.14	1.14	1.09	1.24	1.04
Heptachlor	0.67	1.11	1.11	1.06	1.20	1.01
Imazolil	0.15	0.25	0.25	0.24	0.27	0.23
Iprodione	0.64	1.06	1.06	1.01	1.15	0.96
Lenacil	0.64	1.06	1.06	1.01	1.15	0.96
Lindane	0.67	1.11	1.11	1.06	1.20	1.01
Linuron	0.56	0.92	0.92	0.88	1.00	0.84
Malathion	0.64	1.06	1.06	1.01	1.15	0.97
Mecarbam	0.67	1.11	1.11	1.05	1.20	1.01
Methabenzthiazuron	0.41	0.67	0.67	0.64	0.73	0.61
Metalaxyl	0.46	0.76	0.76	0.72	0.82	0.69
Methidathion	0.63	1.04	1.04	0.99	1.13	0.95
Methomyl	0.36	0.60	0.60	0.57	0.65	0.55
Metobromuron	0.57	0.95	0.95	0.90	1.03	0.86
Metoxuron	0.30	0.50	0.50	0.47	0.54	0.45
Metribuzin	0.62	1.03	1.03	0.98	1.11	0.93
Mevinphos	0.42	0.69	0.69	0.66	0.75	0.63
Monocrotophos	0.08	0.13	0.13	0.12	0.14	0.11
Monolinuron	0.56	0.93	0.93	0.88	1.01	0.84
Napropamide	0.52	0.86	0.87	0.82	0.94	0.79
Nitrofen	0.67	1.10	1.10	1.05	1.19	1.00
Nuarimol	0.47	0.77	0.77	0.73	0.83	0.70
Omethoate	0.06	0.10	0.10	0.09	0.11	0.09
Oxadiazon	0.66	1.08	1.08	1.03	1.17	0.98
Oxadixyl	0.36	0.60	0.60	0.57	0.65	0.54
Oxamyl	0.19	0.31	0.31	0.30	0.34	0.28
Parathion	0.67	1.10	1.11	1.05	1.20	1.00
Parathion-methyl	0.67	1.10	1.10	1.05	1.19	1.00
Pentachlorophenol	0.37	0.60	0.60	0.58	0.65	0.55
Phenkapton	0.68	1.13	1.13	1.07	1.22	1.03
Phenylphenol	0.64	1.06	1.06	1.01	1.15	0.97
Phenmedipham	0.65	1.07	1.07	1.02	1.16	0.97
Phosalone	0.67	1.11	1.11	1.06	1.20	1.01
Phosphamidon	0.22	0.37	0.37	0.35	0.40	0.34
Pirimicarb	0.45	0.74	0.75	0.71	0.81	0.68
Procymidone	0.65	1.07	1.07	1.02	1.16	0.97
Prometryn	0.62	1.03	1.03	0.98	1.11	0.94
Propachlor	0.60	0.99	0.99	0.94	1.08	0.90
Propamocarb	0.00	0.00	0.00	0.00	0.00	0.00
Propargite	0.67	1.11	1.11	1.06	1.20	1.01
Propham	0.66	1.08	1.08	1.03	1.17	0.98

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Table 2. (Cont'd) RRf values for the marker compounds in System I.

Active ingredient	Rf	Atrazine	Carbaryl	Captan	Linuron	Parathion-Me.
Prothiofos	0.66	1.09	1.09	1.04	1.18	0.99
Simazine	0.57	0.94	0.94	0.89	1.02	0.85
Tebuconazol	0.33	0.55	0.55	0.53	0.60	0.50
Terbuthylazine	0.63	1.05	1.05	1.00	1.13	0.95
Terbutryn	0.60	0.98	0.98	0.94	1.06	0.89
Tetradifon	0.66	1.08	1.09	1.03	1.18	0.99
Tetrasul	0.67	1.11	1.11	1.06	1.20	1.01
Thiabendazole	0.34	0.55	0.55	0.53	0.60	0.50
Thiometon	0.65	1.07	1.07	1.02	1.16	0.97
Thiophanat-methyl	0.57	0.94	0.94	0.90	1.02	0.86
Triazophos	0.63	1.05	1.05	1.00	1.13	0.95
Trichlorfon	0.24	0.40	0.40	0.38	0.43	0.36
Trifluralin	0.68	1.13	1.13	1.07	1.22	1.02
Vinclozolin	0.67	1.10	1.10	1.05	1.19	1.00
Detection methods		o-TKI HILL	NBFB o-TKI	HILL	FAN	4NP E NA EAcI

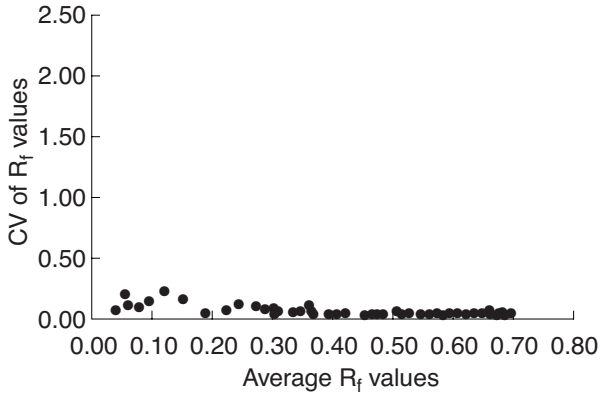

Figure 1. Relation of CV of Rfs and Rf values in system I.

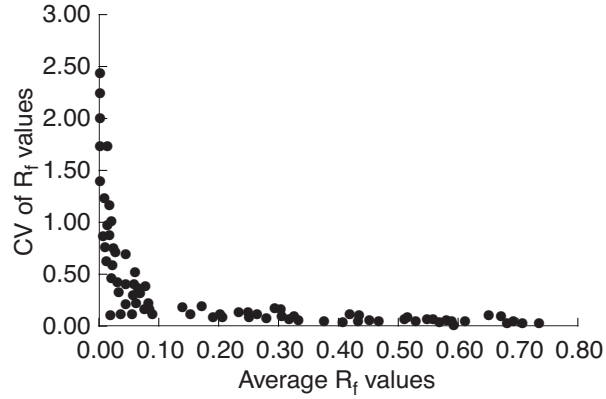

Figure 2. Relation of CV of Rfs and Rf values in system II

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Further work is required, however, for the elaboration and optimisation of the detection conditions with the methods used for normal phase layers. Until that time the reverse phase layers may be used for confirmation of the identity of residues with UV scanners.

Table 3. Effect of temperature on Rf values in system I

Active ingredient	Temperature		Decrease (%)
	20°C	32°C	
Aziprotryn	0.63	0.61	95.9
Chlorpyriphos	0.67	0.63	93.9
Dithianon	0.65	0.61	94.2
Fenarimol	0.48	0.45	93.1
Metabenzthiazuron	0.41	0.39	96.6
Monolinuron	0.56	0.53	94.8
Pirimicarb	0.45	0.42	92.9
Prometryn	0.62	0.59	94.1
Tetradifon	0.66	0.64	97.3
Average decrease in %			94.8

Systems VI and V, and to a lesser extent System III, gave very selective separation of closely related compounds, such as some of the isomers and degradation products of pesticides. Summarising the findings on the elution patterns of pesticides it was concluded that none of the systems is ideal. System I is probably the most suitable for the first screening of pesticide residues in samples of unknown origin.

Comparison of retention data obtained in System I and the silica gel–petroleum ether:diethyl ether/ 1 : 2 (Korsos and Lantos 1984) indicated similar advantages in general separation. Since the multicomponent eluents must be replaced after each plate, and taking into consideration the instability of the composition of the solvent mixture caused by the low boiling point of diethyl ether at elevated laboratory temperature, the silica gel–petroleum ether:diethyl ether/1:2 system is considered less suitable for the purpose of this study than the silica gel–ethyl acetate.

Based on the sum of our experience, System I is recommended for screening analysis. The other systems can be used to separate the suspected compounds.

Effect of sample load on Rf and RRf values

Since System I (silica gel–ethyl acetate) was found to be the most suitable for first screening, the effect of the load of various sample extracts on the Rf values was studied in that system, applying the different methods of detection. Using the sample extracts up to 30 g, portions of 10–10 μL of cleaned extracts were spotted on TLC plates representing 50, 100, 150, 300, and 600 mg (from eluent concentrated to 0.5 mL) samples, respectively. On top of the second spot, the mixture of analytical standards was spotted to see the effect of sample load on the detectability and elution characters of the system. All of the nine elution/detection systems were tested.

The results are shown in Table 4.

The within laboratory variation of Rf values of various compounds was within the expected range.

The absolute Rf values within one run were only slightly affected by the load of various sample extracts, as can be seen in Table 5. The within-plate variation caused by sample extracts was smaller than the between plate variation observed when analytical standards were applied alone.

The Rf values showed great variation between laboratories. However, the relative Rf values (RRf) measured in this study were generally in good agreement between the laboratories, as illustrated in Table 5.

The results indicate that if plates of the same make are used the RRf values would remain the same for an extended period in a laboratory, and would be very close to each other between laboratories.

Based on the results it is concluded that the RRf values can be better used for the identification of compounds than the Rf values. It is emphasised that, as with any relative retention data in chromatography, the TLC RRf values should only be used for guidance. The tabulated data must be carefully checked under the specific conditions in each laboratory condition before they are used for tentative identification of compounds. The identity of the residues must be confirmed in each case.

Minimum detectable quantities of pesticides

The minimum detectable quantity (MDQ) of a pesticide is defined as the minimum amount of analytical standard, expressed in nanograms, spotted on the plate which gives clearly visible spots after elution under average chromatographic conditions.

The average MDQ values of pesticides found with the different detection methods are summarised in Table 6. Under optimal conditions, 2–3 times lower levels may be visible. It should be noted that the spots of some pesticides rapidly disappear or become faint after reaching optimal intensity (e.g. oxamyl detected with enzyme inhibition methods, all spots detected with Hill reaction), therefore the spots should be marked and evaluated immediately after colour development. Visual observations of interferences etc. should also be recorded.

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Table 4. Cleanup requirements of sample extracts[a] with various detection methods

Methods	Tomato		Cabbage		Green pease		Orange	
	300 mg	600 mg	300 mg	600 mg	300 mg	600 mg	300 mg	600 mg
oTKI/SX-3,	yes[c]	yes	yes	yes	yes	yes	yes	yes
oTKI + Silica gel	no	no	adv.	yes	no	no	adv.	yes
oTKI/mixed a + Si gel	yes	n.a	yes	n.a.	yes	n.a.	yes	n.a.
NBFB/SX-3 + Si gel	yes	yes	yes	yes	yes	yes	yes	yes
NBFB/mixed a + Si gel	limited	n.a.	limited	n.a.	limited	n.a.o	limited	n.a.
pDB/SX-3 + Si gel	maximum 150 mg sample equivalent can be applied							
pDB/mixed a + Si gel	not tested							
pNP/SX-3[b]	yes	yes	yes	yes	yes	yes	yes	yes
NBFB/mixed a + Si gel	not tested							
AgUV/SX-3	yes	yes	yes	yes	yes	yes	yes	yes
AgUV/SX-3 + Si gel	no	no	no	no	yes	yes	yes	yes
AgUV/mixed a +Si gel	yes	yes	yes	n.a.	yes	n.a.	yes	n.a.
Hill/SX-3	no	yes	no	yes	yes	yes	no	yes
Hill/SX-3 + Si gel	no	no	no	no	no	no	no	no
Hill/mixed a	no	yes	no	yes	yes	yes	no	yes
FAN/SX-3	fa	yes	no	yes	no	yes	yes	yes
FAN/SX-3 + Si gel	no	no	no	no	no	no	no	yes
FAN/mixed a	no	yes	no	yes	no	yes	yes	yes
FAN/mixed a + Si gel	no	no	no	no	no	yes	no	yes
E NA/SX-3	yes	yes	yes	yes	yes	yes	yes	yes
E NA/SX-3 + Si gel	no	no	no	no	no	no	no	no
E NA/mixed a	no	yes	no	yes	no	yes	yes	yes
EAcI/SX-3	yes	yes	yes	yes	yes	yes	yes	yes
EAcI/SX-3 + Si gel	no	no	no	no	no	no	no	no
EAcI/mixed a	no	yes	no	yes	no	yes	yes	yes

[a] The sample equivalent transferred with the concentrated extract into the columns is given under the samples.
[b] Due to the high MDQ values the detection method is not suitable for residue analysis. It can be used, however, for identification of pesticide active ingredients in formulated products.
[c] Yes: cleanup is necessary; no: cleanup is not necessary: adv: cleanup is recommended; n.a. the cleanup procedure is not suitable for cleaning the extract; limited: certain sections of the plate contain interfering spots.

The MDQ values were also applied together with sample extracts to determine the loadability of the layers. The MDQ values reported in Table 6 could be obtained when the analytical standards were applied together with the purified sample extracts.

The selectivity of detection methods varies.

The *fungi spore inhibition* method provides the best selectivity. It detects only fungicides at residue level. It is also sensitive, and therefore one of the most suitable TLC detection method for residue analysis.

The *Hill reaction* is less selective. It detects ureas and triazines at limit of determination (LOD) of about 0.002 –0.01 mg/kg, but would also detect some other types of pesticides (not herbicide), for instance, thiabendazol (0.2 mg/kg), azinphos-methyl, triazophos and propham (LOD ≥ 1.2 mg/kg, However, *it does not detect sulfonylurea herbicides* (LOD ≥ 12 mg/kg).

Enzyme inhibition usually sensitively detects phosphorus acid and carbamate type insecticides (LOD ≥ 0.002–0.01 mg/kg level). The enzyme inhibition mechanism is different in the case of liver extract and blood serum, consequently the sensitivity of the detection can be different for some pesticides. For instance, 10–100-fold higher sensitivities were obtained in the case of carbofuran, chlorfenvinphos, diazinon, dichlorvos, fenthion, monocrotophos, and trifluralin, while the opposite trend (5–10-fold) was observed for 2,4-D, cypermethrin, dithianon, and pentachlorphenol. The latter compounds also indicate that the enzyme inhibition can be caused by non-insecticide pesticides as well. The liver and blood enzymes are complementing each other, and both should be available in a screening laboratory. The selection of enzyme source depends on the compounds to be analysed.

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Table 5. The effect of sample load[a] on the Rf values of some selected pesticides with Ethyl acetate eluent

Method/active ingredient	Tomato	Orange	Green beans	Cabbage	Mean Rf	RRf [b]	RRf[c]
pTKI							
Atrazin	0.73	0.73	0.75	0.75	0.74	1.0	1.0
Dioxacarb	0.53	0.52	0.55	0.55	0.54	0.73	0.75
Diuron	0.45	0.45	0.45	0.47	0.46	0.62	0.60
NBFB							
Dioxacarb	0.52	0.54	0.54	0.54	0.54	0.76	0.75
Carbaryl	0.74	0.7	0.71	0.7	0.71	1.0	1.0
pDB							
Chlorotoluron	0.5	0.51	0.51	0.51	0.51	0.76	0.71
Linuron	0.66	0.67	0.67	0.67	0.67	1.0	1.0
4NP							
Dimethoate	0.32	0.32	0.33	0.31	0.32	0.41	0.41
Parathion-methyl	0.77	0.76	0.78	0.75	0.77	1.0	1.0
Mevinphos	0.48	0.5	0.49	0.48	0.49	0.64	0.63
AgUV							
Dieldrin	0.76	0.76	0.75	0.76	0.76	1.0	1.0
Imazalil	0.18	0.18	0.18	0.18	0.18	0.24	0.15
Hill							
Atrazin	0.72	0.73	0.73	0.72	0.73	1.0	1.0
Diuron	0.44	0.45	0.45	0.45	0.45	0.62	0.6
Chlotoluron	0.5	0.51	0.52	0.51	0.51	0.7	0.65
FAN							
Benomyl	0.38	0.35	0.32	0.36	0.35	0.51	0.49
Captan	0.73	0.68	0.65	0.79	0.69	1.0	1.0
Thiophanate-methyl	0.68	0.6	0.58	0.6	0.62	0.9	0.9
EbNA							
Parathion-methyl	0.68	0.68	0.7	0.69	0.69	1.0	1.0
Mevinphos	0.44	0.44	0.46	0.45	0.45	0.65	0.63
Oxamyl	0.2	0.2	0.2	0.2	0.2	0.29	0.28
EAcI							
Parathion-methyl	0.72	0.7	0.7	0.7	0.7	1.0	1.0
Mevinphos	0.45	0.45	0.45	0.45	0.45	0.63	0.63
Oxamyl	0.2	0.2	0.2	0.2	0.2	0.28	0.28

Notes:
[a] The plates were loaded with 600 mg sample equivalents in extracts purified on Gel Column.
[b] Reletive Rf values measured on loaded plates in the laboratory of JNSz County.
[c] Reletive Rf values measured by spotting analytical standards on non-loaded plates in the Laboratory of Zala County.

Ortho-tolidine + KI and silver nitrate are general screening detection methods. They have a medium sensitivity for several compounds. It should be noted, however, that neither of the methods is suitable to detect organochlorine pesticide residues at the current Codex Extraneous Maximum Residue Limits.

Nitrobenzene fluoroborate is a medium sensitive reagent for detecting carbamate type pesticide residues. It can be useful for confirmation of the identity of residues.

Detection with *p-dimethylamino benzaldehyde* has limited use, mainly in confirmation of identity of some residues being present at relatively high concentrations.

The other detection methods are not sensitive enough for detecting pesticides at residue levels. They can be used, however, for identification of unknown pesticide products as part of quality control analysis of pesticides.

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Table 6. Detectability of pesticides with various detection methods

Active ingredient	$RRF_{atrazine}$ in 'Si-EtAc	o-TKI	NBFB.	AgUV	FAN	Hill	EBNA	EAcI pig	EAcI horse	pDB	4NPr	HF(254)	RP-18 F
2,4-D	0.07	5000	>5000	500		1000	500	5000		>5000		5000	1000
3-CO-Carbofuran	0.91	50	100	5000	>5000	>5000	500	50		>5000		500	100
Acephate	0.15	200	>5000	5000	>5000	5000	2500	10	100	>5000	500	5000	>5000
Aldicarb	0.80	100	>5000	1000	>5000	>5000	500	3	3	>5000		1500	100
Aldrin	1.10	2000	>5000	50		1000	5000	>5000		>5000		5000	5000
Asulam	0.78	100	5000	1000	>5000	1000	500	1000	500	100		300	100
Atrazine	1.00	25	5000	240		1	1000	5000		>5000		500	100
Azinfos-methyl	0.96	2500	>5000	1000		500	2	10	10	5000	250	750	50
Aziprotryn	1.05	100	>5000	100		100	1000	1000		>5000		250	50
BCPE	1.08	3000	>5000	100		1000	5000	2500		>5000		2000	1000
Benefin	1.13	1000	>5000	5000	>5000	>5000	>5000	2500		250		500	200
Benomyl (1)	0.51	300	5000	100	50	>5000	>5000	500	2000	>5000		1000	1000
Biphenyl	1.06	5000	>5000	5000	>5000	5000	>5000	>5000		>5000		500	50
Bromophos-ethyl	1.09	500	>5000	1000	5000	>5000	1	0.5		>5000	>5000	5000	500
Bromopropylate	1.10	5000	>5000	1000		>5000	5000	2500		>5000		1000	500
Bupirimate	0.92	50	>5000	250	500	1000	2500	1000	1000	>5000		800	250
Butachlor	1.12	5000	>5000	100	5000	1000	1000	2500		>5000		5000	1000
Butylate	1.11	300	>5000	5000		1000	1000	1000		>5000		5000	>5000
Cadusafos	1.12	>250	>250	>250	>250	>250	>250	100		>250		>250	>250
Captafol	1.01	1000	>5000	100	10	>5000	5000	1000		>5000		5000	5000
Captan	1.05	2000	>5000	100	20	5000	500	500		>5000	5000	1000	1000
Carbaryl	1.00	100	50	5000		>5000	2	10	10	>5000	>5000	1000	100
Carbendazim	0.49	1000	>5000	200	20	1000	500	500	500	>5000	>5000	2000	500
Carbofuran	0.98	500	50	>5000		>5000	1000	50	50	5000		2000	500
Chlorbromuron	0.94	50	>5000	500		1	5000	1000	1000	50	>5000	250	50
Chlordimeform	0.47	300	>5000	100	5000	>5000	500	1000		5000		500	100
Chlorfenvinphos	0.90	500	5000	100	5000	2500	1000	50		>5000	5000	1000	50
Chloropropylate	1.11	1000	>5000	200	5000	>5000	5000	2500		>5000		5000	500
Chlorothalonil	1.10	5000	>5000	100	50	250	>5000	>5000		>5000		700	100
Chlorotoluron	0.65	100	>5000	200		1	2500	1000	1000	50	>5000	500	50
Chloroxuron	0.57	100	>5000	100		1	1000	1000	1000	50	>5000	500	50
Chlorpropham	1.08	100	>5000	150		250	5000	1000	1000	100		2000	500
Chlorpyrifos	1.10	1000	>5000	250		5000	0.5	0.5	5	>5000	5000	2000	500
Cyanazine	1.00	100	>5000	250		1	5000	1000		>5000		1000	100
Cypermethrin	1.11	5000	5000	250	>5000	>5000	500	2500		>5000		2000	1000
Deltametrin	1.11	5000	5000	500		>5000	5000	5000		>5000		1000	1000

Table 6. (Cont'd) Detectability of pesticides with various detection methods

Active ingredient	$RRF_{atrazine}$ in 'Si-EtAc	o-TKI	NBFB.	AgUV	FAN	Hill	EBNA	EAcI pig	EAcI horse	pDB	4NPr	HF(254)	RP-18 F
Desmedipham	1.09	50	50	5000		1	200	500	500	100	>5000	1400	200
Diazinon	1.09	5000	>5000	1000		5000	500	0.2	10	>5000	>5000	1000	250
Dichlobenil		>5000	>5000	100	>5000	>5000	>5000	>5000		>5000		>5000	1000
Dichlofluanid	1.06	3000	5000	100	100	>5000	500	2500		5000		2500	1000
Dichloran	1.03	100	>5000	100	5000	>5000	>5000	>5000		5000		1000	250
Dichlorvos	0.83	>5000	>5000	500		>5000	20	2	5	5000	1000	>5000	>5000
Dieldrin	1.11	1500	>5000	75		>5000	>5000	>5000		>5000		5000	5000
Dimethoate	0.45	100	>5000	500		>5000	500	1000	1000	>5000	500	5000	2000
Dinobuton	1.09	1000	500	5000	500	1000	>5000	5000		>5000	5000	500	50
Dioxacarb	0.75	25	100	>5000		>5000	500	100	500	5000	>5000	5000	1000
Diphenamid	0.85	3000	>5000	5000		5000	>5000	5000		>5000		2500	1000
Dithianon	1.07	3000	1000	100	100	100	500	5000		5000	500	250	100
Diuron	0.60	30	>5000	100		1	2500	1000	2500	50	>5000	300	50
DNOC	0.60	3000	5000	5000	1500	20	>5000	5000	1000	5000		500	100
Endosulfan	1.11	300	>5000	50		>5000	5000	>5000		>5000		5000	5000
EPTC	1.08	300	>5000	5000		5000	500	500		>5000		5000	2000
Ethirimol	0.20	100	>5000	1000	5000	5000	1000	2500		>5000		1000	500
Ethoxyquin	1.07	500	5000	500	5000	1000	500	500		5000		500	100
Etrimfos	1.07	500	>5000	5000		5000	5	10	10	>5000	2500	2500	1000
Fenarimol	0.79	200	>5000	500	50	5000	>5000	500		>5000		150	500
Fenitrothion	1.07	5000	>5000	1000		>5000	5	5	10	>5000	1000	1000	100
Fenitrothion-o	0.70	50	>125	125	125	>100	10	5		>125	>125	>125	125
Fenpropatrin	1.15	5000	>5000	5000		>5000	5000	2500		5000		500	500
Fenthion	1.08	5000	>5000	100		>5000	50	5	10	>5000	1000	1000	250
Fenthion-o	0.69	>10	>12.5	12.5	>12.5	>10	>12.5	10		>12.5		>12.5	>12.5
Folpet	1.03	1500	>5000	200	30	>5000	500	500	500	>5000	5000	2500	500
Haloxyflop	0.09	1000	>5000	5000	5000	1000	500	1000		>5000		5000	500
HCB	1.14	2500	>5000	50	1000	>5000	>5000	>5000		>5000		5000	500
Heptachlor	1.11	5000	>5000	100	>5000	5000	5000	>5000		>5000	5000	>5000	>5000
Imazalil	0.25	100	>5000	100	100	1000	1000	1000		>5000		2500	2500
Iprodione	1.06	3000	>5000	1000	300	2500	5000	2500		5000		5000	1000
Lenacil	1.06	200	2500	500		1	1000	5000		>5000		1500	200
Lindane	1.11	500	>5000	50		1000	>5000	5000		>5000		5000	>5000
Linuron	0.92	300	>5000	50		1	5000	10	2500	100	5000	250	50
Malathion	1.06	1000	>5000	50		1000	10	10	10	>5000	1500	5000	2000
Mecarbam	1.11	200	>250	250	>250	250	100			>250	>250	>250	>250

Table 6. (Cont'd) Detectability of pesticides with various detection methods

Active ingredient	$RRF_{atrazine}$ in 'Si-EtAc	o-TKI	NBFB.	AgUV	FAN	Hill	EBNA	EAcI pig	EAcI horse	pDB	4NPr	HF(254)	RP-18 F
Metalaxyl	0.76	1000	>5000	500		>5000	5000	5000		>5000		5000	1000
Methabenzthiazuron	0.67	200	>5000	5000		5	5000	2500	2500	5000		250	50
Methidathion	1.04	5000	>5000	50		>5000	75	100	200	5000	1000	1000	250
Methomyl	0.60	100	300	5000		>5000	100	10	50	>5000	>5000	1500	500
Metobromuron	0.95	300	>5000	200		1	5000	1000	1000	100		480	50
Metoxuron	0.50	100	>5000	100		10	2000	1000	1000	50		480	50
Metribuzin	1.03	400	5000	5000		1	5000	2500		5000		1000	500
Mevinphos	0.69	5000	2500	5000		>5000	5	10	10	>5000	500	2500	1000
Monocrotophos	0.13	100	5000	5000		>5000	250	5	50	>5000	500	2000	500
Monolinuron	0.93	300	5000	50		5	5000	5000	5000	100		250	50
Napropamide	0.86	1000	>5000	5000	5000	1000	5000	1000		>5000		1000	250
Nitrofen	1.10	5000	>5000	100	5000	2500	5000	5000		5000		1000	250
Nuarimol	0.77	500	5000	100	100	>5000	5000	5000		>5000		600	250
Omethoate	0.10	500	5000	5000		>5000	2500	1000	1000	5000	500	5000	5000
Oxadiazon	1.08	500	>5000	100		1000	5000	5000		>5000		1000	250
Oxadixyl	0.60	2500	>5000	5000	>5000	>5000	>5000	5000		5000		2500	500
Oxamyl	0.31	100	5000	5000		>5000	10	2	5	5000		2000	500
p,p-DDT	1.13	5000	>5000	50		5000	5000	>5000		>5000		5000	200
Parathion	1.10	5000	>5000	1000		1000	0.5	1	1	>5000	2500	1000	250
Parathion-methyl	1.10	5000	>5000	1000		>5000	1	2	2	>5000	500	1500	50
Pentachlorophenol	0.60	1000	>5000	500	1000	100	500	5000		>5000	>5000	1000	250
Phenkapton	1.13	5000	>5000	200		>5000	5	5	10	>5000	2500	1000	250
Phenmedipham	1.07	100	50	5000		1	100	50	100	100		1000	250
Phenylphenol	1.06	250	50	5000	500	1000	500	500		>5000		500	500
Phosalone	1.11	5000	500	1000		>5000	5	5	5	>5000	2500	2500	500
Phosphamidon	0.37	5000	>5000	500		>5000	250	50	100	>5000	1000	2500	500
Pirimicarb	0.74	300	50	1000		5000	100	100	100	5000		500	50
Procymidone	1.07	5000	>5000	250	500	>5000	>5000	5000		5000		2000	500
Prometryn	1.03	100	5000	500		1	>5000	1500	1500	>5000		500	50
Propachlor	0.99	5000	>5000	100		>5000	>5000	5000		5000	500	2500	500
Propamocarb	0.00	100	>5000	200		>5000	5000	5000	5000	>5000		5000	>5000
Propargite	1.11	5000	5000	5000		>5000	1000	5000		>5000	1000	2500	2500
Propham	1.08	50	5000	100		500	>5000	2500	5000	500		2000	500
Prothiofos	1.09	2500	>5000	100	5000	1000	200	500		>5000		5000	1000
Simazine	0.94	50	5000	250		1	>5000	5000		>5000		800	100
Tebuconazol	0.55	2500	>5000	1000		1000	5000	5000		>5000		5000	1000

Table 6. (Cont'd) Detectability of pesticides with various detection methods

Active ingredient	$RRF_{atrazine}$ in 'Si-EtAc	o-TKI	NBFB.	AgUV	FAN	Hill	EBNA	EAcI pig	EAcI horse	pDB	4NPr	HF(254)	RP-18 F
Terbuthylazine	1.05	50	5000	250		1	5000	1000		>5000		500	100
Terbutryn	0.98	100	5000	500		1	>5000	>5000		>5000		800	50
Tetradifon	1.08	5000	>5000	200		5000	5000	>5000		>5000		250	200
Tetrasul	1.11	5000	>5000	100	5000	>5000	5000	>5000		>5000		500	50
Thiabendazole	0.55	1000	>5000	50	200	100	5000	5000		>5000		250	100
Thiometon	1.07	5000	>5000	50	5000	1000	100	5000		5000	5000	5000	1000
Thiophanat-methyl	0.94	200	5000	200	100	5000	1000	500	500	5000	>5000	250	100
Triazophos	1.05	1000	>5000	200		500	1	0.2	0.2	>5000	2500	500	100
Trichlorfon	0.40	>5000	5000	100		>5000	500	100	100	>5000	1000	5000	>5000
Trifluralin	1.13	500	5000	1000		1000	2500	50		250		800	50
Vinclozolin	1.10	1000	>5000	500	600	>5000	5000	500		500		5000	500

Application of marker compounds for internal quality control

In view of the variability of Rf values and the detection procedures, regular monitoring and control of the TLC conditions is essential for obtaining reliable analytical results. The proper elution and detection conditions can be checked on each plate by applying a mixture of analytical standards at their MDQs. If the marker compounds are well detectable and their Rf values are within the expected range the analyst can be sure, and can demonstrate it at the same time, that the method was applied properly. The Rf values of the marker compounds can also be used as reference for the RRf values (Tables 2 and 7) which greatly facilitates the identification of the spots detected on the plates.

Table 7. Recommended marker compounds for quality control of TLC procedures

	Ethyl acetate			Dichloromethane	
Marker compound	MDQ ng	RRf (Rf[1])	Marker compound	MDQ ng	RRf (Rf[1])
oTKI					
Atrazine	25	0.61	Carbaryl	100	0.19
Diuron	30	0.6	Dichloran	100	0.25
Oxamyl	100	0.31	Propham	50	1.97
NBFB					
Carbaryl	50	0.61	Carbaryl	50	0.19
Dioxacarb	100	0.73	Phenylphenol	50	2.27
Methomyl	300	0.59			
pDB					
Benefin	250	1.35	Chlorbromuron	50	2.15
Diuron	50	0.59	Diuron	50	0.26
Metoxuron	50	0.51	Metoxuron	50	0.49
4NP					
Dimethoate	500	0.41	Azinfos-methyl	250	0.45
Mevinfos	500	0.63	Dithianon	500	0.78
Parathion-methyl	500	0.67	Parathion-methyl	500	0.56
AgUV					
Dieldrin	75	0.82	Chlorfenvinpgos	400	
Endosulfan	50	1.08	Linuron	200	
Imazalil	100	0.44	Dieldrin	100	
Hill					
Atrazine	1	0.61	Desmedipham	1	0.34
Chloroxuron	1	0.65	Dithianon	100	1.71
Metoxuron	5	0.5	Linuron	1	0.25
FAN					
Captan	20	0.64	Bupirimate	500	0.17
Carbendazim	20	0.47	Captan	20	0.33
Fenarimol	50	0.75	Dichlofluanid	100	1.56
EBNA					
Dichlorvos	20	0.76	Carbaryl	10	0.34
Oxamyl	10	0.31	Etrimfos	5	0.53
Parathyon-methyl	1	0.67	Parathion-methyl	1	0.56
EAcI					
Dichlorvos	2	0.76	Parathion-methyl	2	0.56
Parathion-methyl	2	67.0	Phencapton	5	1.25
Phosphamidon	50	0.34	Triazophos	0.2	0.35

[1] The RRf values were calculated for the Rf of reference compounds underlined

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The marker compounds selected should (Ambrus 1981):

- be relatively stable in standard solutions;
- be sensitive for the detection conditions (not appearing on the plate if the conditions are not optimal);
- have reproducible Rf values.

The pesticides recommended for using as marker compounds and their MDQ values are given in Table 6.

Loadability of clean-up columns

The ethyl acetate extracts were used for testing the loadability and efficiency of the column chromatographic systems.

The LiChrolute Cartridges could only be loaded with extracts equivalent to 5 g samples. Consequently they were not used for the first clean-up of extracts.

The gel and mixed adsorbent columns were loaded with extracts of tomato, cabbage, green peas and orange equivalent to 5, 10, 15, and 30 g samples. The clean-up of extracts was carried out according to the methods described under 'Clean-up procedures'. The eluate was concentrated to 1 mL in each case. The cleaned extracts were spotted on TLC plates as described in section 3.1.1. The chromatograms were visually evaluated immediately after the appearance of the spots. The sample load did not affect the RRf values, as shown in Table 5. A second series of experiments was carried out to load the clean-up columns with sample equivalents of 60, 90, and 120 g. However, neither of the clean-up columns could provide clean extracts if they were loaded with ≥ 60 g amount of samples.

Based on the results it was concluded that the maximum permissible sample load is 30 g for these sample matrices and clean-up columns. The clean-up requirements of sample extracts are summarised in Table 4. The cleaned extract is also suitable for capillary column—ECD or other GC analyses, and, after filtration through a 0.5 μm filter, for HPLC analysis.

Summary of Principles of Good Analytical Practice in TLC Detection of Pesticide Residues

- Use freshly activated plates at all times.
- Prepare a spotting plan in advance and record in your notebook the amounts applied. Spot two indicator compounds on each plate
- Place filter paper into developing tanks for 30 minutes before eluting the plates.
- Fill the tank with developing solvent to obtain a 1 cm immersion depth for the plate.
- Place the developing tanks into a water bath and keep the temperature within ±2°C between 20 and 30°C for improving reproducibility of retention values.
- Apply 10–20 μL solutions with micro-syringe and spotting guide in spots of 3–4 mm diameter at 2 cm line from the bottom edge of the plate.
  Apply the same volume from the extracts as well as from the standard solutions.
- Elute plates up to 10 ± 0.5 cm from the origin.
- Replace solvent mixtures or adjust the volume of the single solvent mobile phase after each elution.
- Apply sufficient spraying reagents onto the plates to uniformly saturate the layer but avoid run-off of reagents.
- Evaluate the plates immediately after the full colour development. Mark the position (boundary) of the spots and record any visual observation such as colour and intensity of spots, striking spots. Record observations in your notebook for each solution applied.
- Prepare calibration curve plotting the squares of average diameter of the spots as a function of standard amounts.
- Apply a minimum of three standard concentrations (limit of detection (LOD), 4 × LOD and 8 × LOD)
- Check linearity of calibration and reduce the highest amount if the relationship is not linear.
- Dilute sample extracts if the concentration of a residue is above the linear range.
- Apply standard solution over the sample spot and check the quantity of analyte to determine the matrix effect.

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Enzyme Inhibition and Other Rapid Techniques for Pesticide Residue Detection

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Abstract

Several rapid techniques for pesticide residue detection were tested— colorimetric, enzyme inhibition, and micro thin-layer chromatography (TLC).

The simplest method adopted for organophosphate residues consists of spotting the samples on strips of filter paper pre-coated with chromogenic agent, activating over an alcohol lamp, and making the pesticide visible (blue) with an alkaline solution. Minimum detection limits are as low as 0.1 μg/g of sample.

Another method is an enzyme inhibition technique using acetylcholinesterase. The thiocholine released by hydrolysis reacts with dithionitrobenzoic acid. By spectrophotometry, a slope giving a 20% inhibition was considered the allowable error of the method, 20–50% was recommended as safe to go to market after washing the commodity, and for greater than 50% delay of harvest was advised because it exceeded the codex MRL for the pesticide tested. In another method using filter paper, a white spot over a yellow background indicates the presence of enzyme inhibitors (organophosphates and carbamates). The minimum detection limit is as low as 0.1 μg/g of sample fer certain pesticides.

For organochlorines and pyrethroids, a micro TLC method was employed using a pre-coated plastic sheet with chromogenic agent and UV flash light for visualisation. The minimum detection limit is 1 μg/g of sample.

For ethylene bis-dithiocarbamates, a 1 g sample is hydrolysed and extracted with hexane by heating in a water bath for 30 minutes. The organic layer added with the chromogenic agent turned yellow, indicating the presence of EBDC. Another technique introduced by the Taiwan Agricultural Research Institute was a rapid bioassay for fungicide using the *Bacillus thuringiensis*. The minimum detection limit is as low as 0.08 mg/kg.

TODAY, there is an increasing concern for pesticide residues remaining in foods as a consequence of crop protection. The farmers usually apply pesticides even during periods close to harvest because they may not be aware of the hazards that this practice poses to consumers. This could be the reason why several vegetables analysed for pesticide residues far exceeded the maximum residue limits (MRL) set by FAO/WHO (Magallona et al. 1979; Tejada et al. 1983; Tejada et al. 1989). As a consequence, the excessive use of these pesticides affects not only the pests but non-target organisms as well. Agricultural waste water may enter the canals and eventually empty into lake ecosystems, which may affect the aquatic organisms. The danger lies in the ability of fish to bioconcentrate the residues of these pesticides (Argente et al. 1977; Zulkifli et al. 1983; Tejada and Magallona 1985; Varca and Magallona 1987). Even animals feeding on contaminated fodders or drinking within the treated paddies have been found to contain residues of pesticides (Tejada et al. 1990; Tejada et al. 1993). The residues may also leach and contaminate the groundwater (Medina et al. 1991). Milk from lactating mothers has also been found to contain pesticide residues (Barril et al. 1977). The conventional method of detecting pesticide residues, however, is very tedious and requires expensive reagents and sophisticated equipment. Moreover, the complete

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analysis usually takes a long time. This the reason why residue data in the Philippines are generally few and far between.

A rapid bioassay technique for the detection of pesticides in fruits and vegetables is already being implemented in Taiwan. The farmers' produce is subjected to this test before before it may be traded in the market. This method is applicable only for organophosphates and carbamates which are excellent acetylcholinesterase (AChE) inhibitors (Matsumura 1975). Nevertheless, some of these insecticides are extremely toxic for short periods after application. These groups of insecticides act on the AChE in the neurosystem of insects and other animals, finally causing death. The insecticides bind to the active site of the AChE, prevent the breakdown of acetylcholine (ACh), and cause its excessive accumulation. There are many sources of acetylcholinesterase enzyme which has different sensitivity towards carbamates or organophosphates: pig liver, chicken liver, beef liver, housefly head, bee head, etc. (Mendoza et al. 1968; Guilbault et al. 1970; Mendoza and Shields 1970). The housefly head seems to be the most economical source and the enzyme from it is easy enough to produce (Chiu et al. 1991).

This study was undertaken to evaluate the rapid techniques for pesticide residue detection.

Enzyme Inhibition Test

Taiwan Agricultural Research Institute (TARI) method

The enzyme inhibition test of TARI is based on Ellman's test, in which the insecticide is assayed in vitro by reacting directly with acetylcholinesterase (Ellman et al. 1961; Casarett and Doule 1975). The thiocholine released by hydrolysis reacts with 5,5 dithio-bis (2-nitrobenzoic acid) to give the yellow anion of 5-thio-2 nitrobenzoic acid, as shown in equation 1, below.

When the AChE is partially or completely inhibited by the insecticide, the colour reaction slows down or stops (Voss 1966; Voss et al. 1971). The degree to which the reaction proceeds serves as a measure of the amount of the pesticide residue.

Assay procedure

In a glass cuvette, 6 mL phosphate-buffered saline (pH 8.0), 30 μL sample extract and 20 μLAChE were mixed for 1 min, then 100 μL DTNB and 20 μL ATCI were added to start the reaction. The increasing absorbance with time at 412 nm was automatically graphed in a recorder and compared with the insecticide-free blank for inhibition. The degree of inhibition of the crude AChE and TARI's purified AChE were compared. A 20% inhibition with AChE is considered the permissible error of the method used. TARI's purified AChE was provided by the Taiwan Agricultural Research Institute. This was purified from housefly heads using ammonium sulfate fractionation and gel chromatography following the procedure of Chiu et al. (1991).

Spectrophotometric analysis

The degree of inhibition was obtained by determining the reduction in the absorbance for a sample in comparison to the normal AChE reaction, i.e., one in which no pesticide is present and where only the substrate, acetylcholine, is acted upon by the enzyme:

$$\% \text{ inhibition} = \frac{\text{A change (blank)} \pm \text{A change (sample)}}{\text{A change (blank)}} \quad (2)$$

where A is the slope of the line. The higher the degree of inhibition the higher is the residue content. The positive response can be used as an indicator of potentially hazardous levels of pesticide residues in samples.

Of the 168 vegetable samples assayed, 11% gave more than 20% AChE inhibition, a strong indication of pesticide contamination (Table 1). It was noted that samples which gave more than 43% inhibition contained residues of organophosphates/carbamates far in excess of MRL. It is recommended that samples with <20% inhibition be allowed to go freely in the market, those with between 20 and 50% inhibition be allowed for trading after washing, and those with higher than 50% be withheld and farmers be advised to prolong the interval between application of pesticide and harvest of crops until such time that the levels are within the tolerable limits.

Gas chromatographic analysis

Gas chromatographic analysis of the vegetable samples which tested positive for pesticide residues using the enzyme inhibition revealed six different pesticides: chlorpyrifos, diazinon, malathion, methyl parathion, methomyl, and triazophos. Results of pes-

acetylthiocholine + enzyme → thiocholine + acetate thiocholine + dithio - bis nitrobenzoate
→ 5 thio - 2 - nitrobenzoic acid (yellow colour) (1)

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ticide management surveys conducted by Tejada et al. (1983, 1989) showed that farmers from Laguna, Quezon, Cavite, Bulacan, and Batangas used methyl parathion, diazinon, monocrotophos, methomyl, carbaryl and isoprocarb for vegetables like eggplant, string beans, upo, okra, and pechay.

Enzyme inhibition test kit 1

Acetylcholinesterase was dissolved in PBS while DTNB + ATCI was dissolved in water. Precoated plastic plates was treated with PBS and air dried. The enzyme inhibition assay from ARI was packaged into a kit. The sample was spotted on plastic strips using a capillary tube followed by the enzyme solution. DTNB and ATCI mixture was then added and allowed to stand for 2–3 min. A positive result was indicated by a white spot against a yellow background.

Enzyme inhibition test kit 2

Another enzyme inhibition test kit was packaged into a kit. The sensitivity of the purified acetylcholinesterase from TARI housefly head was compared with the crude pig liver enzyme. The minimum detection levels were as low as 0.0016 μg and 0.014 μg of insecticide, respectively (Table 2).

The test kit consists of the substrate, encapsulated enzyme, tris buffer acetone, enzyme substrate mixing bottle, extraction vials, and filter paper strips.

Assay

One gram of the treated sample was chopped into small pieces and extracted with 1 mL acetone. The extract was spotted on filter paper strip using a capillary tube, followed by enzyme and substrate solution. A positive result was indicated by a white spot over a blue background.

Table 1. Monitoring of pesticide residues in vegetables using the rapid bioassay (RBPR).

Vegetables and market source	RBPR test	GC Confirmation	Conc.mg/kg	Codex MRL
Baguio beans				
Calamba (1)	(+)[a] 26.4	Diazinon	0.25	
		Chlorpyrifos	0.01	0.2
Calamba (2)	(+) 40.0	Malathion	0.11	2.0
		Methomyl	0.14	
Cabbage				
Victoria (1)	(+) 23	Methyl parathion	0.48	
Pila (1)	(+) 23	Methyl parathion	0.45	
Chinese Pechay				
Calamba (1)	(+) 33	Methyl parathion	0.42	
Eggplant				
Calamba (1)	(+++) 67	Triazophos	0.70	0.1
Calamba (2)	(+) 29	Methyl parathion	0.28	1.0
LB Crossing	(++) 47	Methomyl	0.54	
Pila	(+) 21	Methomyl	0.20	
Pechay				
Victoria	(++) 43	Diazinon	0.67	
String beans				
Calamba	(++) 64	Methomyl	1.04	0.5
Tomatoes				
Calamba (1)	(+) 24	Methomyl	0.27	
Calamba (2)	(+) 25	Methomyl	0.50	
Calamba (3)	(+++) 68	Methomyl	1.60	
LB Crossing (1)	(+) 22	Methomyl	0.20	
LB Crossing (2)	(+) 25	Methomyl	0.50	
Pila	(+++)62.5	Methomyl	1.30	
Sta. Cruz	(+) 29.2	Methomyl	0.60	
Victoria	(+) 33	Methomyl	0.40	

[a] (+) = inhibited; (++) = highly inhibited; (+++) = extremely inhibited

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Colorimetric Test for Organophosphates

A rapid colorimetric method for the detection of organophosphates was packaged into a kit. This was postulated by Kramen and Gamson (1957) to be based on the reaction of the unshared pair of electrons available at the nitrogen of a pyridine ring with the available pair of electrons at the positive site of the organophosphates. A displacement takes place, with the alcohol moiety being split off, as shown below.

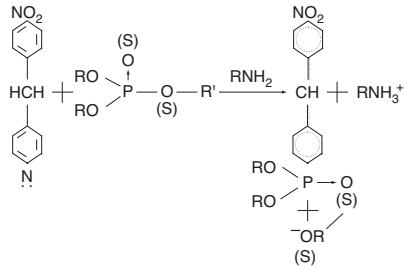

Table 2. Comparison of the sensitivity[a] of TARI housefly head AChE and crude pig liver to some organophosphate and carbamate insecticides.

Insecticide	Housefly head AChE Detection limit ($\mu g \times 10^{-2}$)	Pig liver AChE Detection limit ($\mu g \times 10^{-2}$)
Carbofuran	0.47 e[b]	24.00 f
Methomyl	2.80 d	60.40 d
Dimethoate	98.40 a	ND
Metamidophos	63.40 b	228.00 a
Monocrotophos	16.00 c	ND
Mevinphos	0.16 e	37.60 e
Parathion	2.20 d	1.80 g
Malathion	0.74 e	44.40 e
Carbaryl	0.34 e	1.40 g
Isoprocarb	0.46 e	2.60 g
Methyl parathion	2.00 d	160.60 b
Chlorpyrifos	0.92 e	97.60 c
Azinphos ethyl	0.44 e	5.60 g

[a]Average of five trials.
[b]Means followed by a common letter are not significantly different at 5% level using DMRT.
ND = not detected

Assay

A 5 g sample was extracted with 5 mL acetone. Five drops of the extract were spotted on a pretreated filter paper. This was heated for one minute and blue colour was visualised by spotting one drop of alkaline solution.

Sensitivity test

The lowest amount of pesticide that can be detected by the method was determined and compared with the other rapid test (Table 3).

Table 3. Detection limits of organophosphates and carbamates using rapid test kits.

	Enzyme inhibition		Rapid test for OP
	TARI (μg)	Test kit (μg)	(μg)
Organophosphates			
Pirimiphos methyl	0.03	0.16	0.05
Methyl parathion	0.03	0.01	0.05
Methamidophos	0.02	0.63	0.05
Fenitrothion	–	0.05	0.05
Chlorpyrifos	0.03	0.03	0.05
Malathion	0.03	0.07	0.01
Azimphos ethyl	–	0.05	0.05
Diazinon	0.03	0.05	0.05
Parathion	0.05	0.02	0.05
Triazophos	0.02	0.05	0.05
Mevinphos	0.03	0.02	–
Monocrotophos	0.02	0.16	–
Dimethoate	–	0.98	–
Acephate			
Dichloron	0.004		
Naled	0.004		
Phenthoate	0.01		
Profenofos	0.03		
Tiazorben	0.08		
Mephosfolen	0.09		
Methidathion	0.10		Rapid test for carbamates
Carbamate & other N, P cont'g compounds			
Carbofuran	0.001	0.003	0.005
Carbaryl		0.003	0.05
BPMC		0.005	0.05
Methomyl	0.06	0.03	
Thiobencarb		0.005	0.05
Isoprocarb		0.005	0.05

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Rapid Test for Organochlorines and Pyrethroids

A micro thin-layer chromatographic technique was used as a basis for the development of a method for rapid detection of chlorinated and pyrethroid residues. Several TLC systems were investigated but the simplest one adopted was the use of a pre-coated TLC plastic sheet with fluorescence detection + UV flash light

The assay was done by cutting the sample into small pieces, extracting 1g of sample with acetone, spotting, developing and exposing in UV flash light.

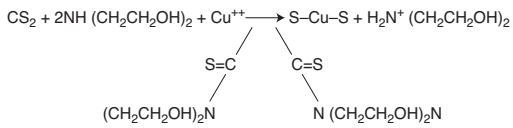
The detectable limit obtained for each pesticide ranged from 0.19 to 7 μg/kg for organochlorines and 0.2 to 0.3 for pyrethroids (Table 4).

Fungicide Test (Colorimetric)

A rapid method for the detection of ethylene bisdithiocarbamate fungicide that has been developed is based on colorimetric analysis of carbon disulfide (CS_2) evolved during acid hydrolysis of EBDC at elevated temperature.

Assay

A known amount of EBDC was hydrolysed in a screw-capped test tube using HCl in a boiling water bath. The carbon disulfide evolved during hydrolysis was absorbed in hexane layered directly above the aqueous layer. After 30 minutes, the test tube was withdrawn from the water bath and cooled. The hexane layer was transferred to another test tube where the colour reagent was added. The presence of EBDC fungicide was indicated by the formation of yellow colour on the lower layer. The limit of detection was 1 μg/g sample.



Fungicide Test (Bt)

Bacillus thuringiensis is highly sensitive to the EBDC group of fungicides.

Assay

One mL of Bt suspension was added to the sample extract which was incubated in a covered water bath shaker for 90 minutes (37°C). 100 μL of 2% 2,3,5-triphenyltetrazolium chloride was added and the mixture shaken for another 30 minutes. At the end of incubation, 1 mL of 0.5 N HCl in 5% Triton X100 detergent was added to the flasks and mixed well to stop the reaction. The minimum detection ranged from 0.008 to 2.5 ppm (Table 5).

Table 4. Detection limits of organochlorines and pyrethroids using the rapid test kits.

	Rapid test for OCLs (μg/kg)
Organochlorines	
p,p'-DDT	0.19
DDD	0.44
DDE	0.39
Aldrin	0.63
Dieldrin	5.04
2 Endosulfan	2.90
3 Endosulfan	2.50
Heptachlor	7.20
Chlordane	7.30
Pyrethroids	
Deltamethrin	0.30
Cypermethrin	0.08
Fenvalerate	0.02

Table 5. Detection limit of fungicides.[a]

Fungicides	TARI[b] (ppm)[c]	Rapid test kit (ppm)
Propineb	0.08	1
Curzate-M (50% mancozeb, 50% cymoxanil)	0.16	1
Ridomil-Mz (48% mancozeb, 10% metalaxyl)	0.31	1
Mitram	0.31	–
Maneb	0.31	1
Mancozeb	0.63	1
Zineb	1.56	1
Captafol	12.50	–
Chlorothalonil	1.25	1
Dinocap	2.50	–
Captan	2.50	1
Flopet	6.25	1

[a]Detectable sensitivity on different vegetables depends on the recovery of extraction
[b]Taiwan Agricultural Research Institute
[c]The detection limit set at 50%

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Rapid Colorimetric and Tissue Print Methods for Estimating Agrochemicals Including Benzimidazole Fungicides

Akmal Pasha*

Abstract

Colorimetric and tissue print methods are among the simplest and most accurate techniques available for the estimation of agrochemicals. Pesticides are generally colourless and hence colour is developed specifically for each pesticide by formation of ions, free radicals, π-complexation, and reaction with specific chromogenic reagents.

The most commonly used colorimetric method is the azo-coupling of aromatic diazonium salts to phenols or naphthol obtained by the hydrolysis of carbamate and organophosphate pesticides such as propoxur, sevin, carbofuran, and malathion. Reaction with specific chromogenic reagents to give coloured product is widely employed for all pesticides. Dithiocarbamates are estimated by metal-complexation and paraquat/diquat by reduction to coloured radicals. Important sensitive reagents are 4-aminoantipyrine, o-tolidine, fluorescein, N-(1-naphthyl)ethylenediamine, ammonium ceric nitrate, 4-(N,N-dimethylamino) aniline, 4-(4'-nitrobenzyl) pyridine, 2-(trichloromethyl) benzimidazole, and 2,6-dihalobenzoquinone-N-chloroimine.

This paper describes reactions standardised to develop tissue print and rapid colorimetric methods for organochlorine, organophosphate, and carbamate pesticides. Organochlorine pesticides could be detected on N,N'-diphenylbenzidine-coated paper strips at a sensitivity of 0.5 μg. Chlorinated organophosphates such as bromophos, ronnel, chlorpyrifos, chlorpyrifos-methyl, and triclopyr gave a coloured product on reaction with o-tolidine on paper strips and TLC plates. Highly sensitive methods have been developed to analyse 2-aminobenzimidazole (2-AB), the hydrolysis product of carbendazim and benomyl fungicides. 2-AB reacts with N-bromosuccinimide (NBS) to give a coloured product that is stable indefinitely on paper strips. The minimum detection limit is 20 ng as measured using a densitometer. A method was developed to analyse thiram fungicide residues using Pd and Cu(I). Cuprous thiocyanate, the most stable form of Cu(I) oxidation state, was employed to detect thiram residues in grain using paper strips coated with cuprous thiocyanate with a sensitivity of 0.1 μg. Rapid colorimetric methods were developed to analyse (i) thiram fungicide using cuprous thiocyanate (λ_{max} 420 nm; sensitivity 0.2 μg/mL) (ii) 2-aminobenzimidazole by reaction with NBS (λ_{max} 240, 260 nm; sensitivity of 0.4 μg/mL) (iii) 2,4,5-trichlorophenoxyacetic acid as silver salt in dimethylsulfoxide (λ_{max} 300, 440 nm; sensitivity 0.1 μg/mL), and other halogenated aryloxyalkanoic acid herbicides as silver salts.

AGROCHEMICAL residues are generally analysed using gas chromatography (GC) and high performance liquid chromatography (HPLC). These methods require expensive equipment, trained personnel, and involve elaborate sample preparation procedures. Hence, simple and sensitive methods are needed for the routine analysis of agrochemicals. Colorimetric and tissue print techniques are suitable for the estimation of trace quantities of contaminants. This present paper outlines these methods, sensitive chromogenic reagents, and novel colorimetric methods developed for the analysis of pesticide residues.

Pesticides are generally colourless. Hence colour is developed specifically for each pesticide by vari-

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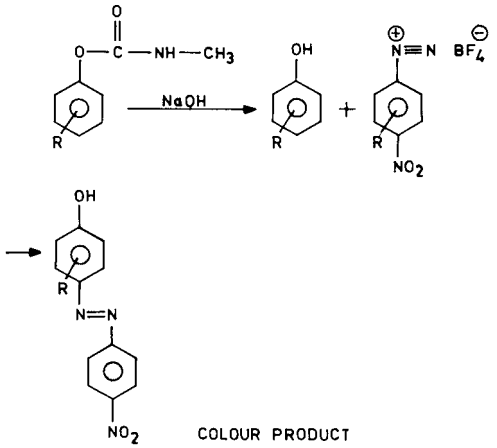
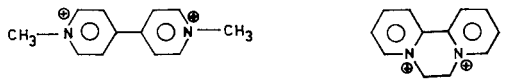
ous methods. The most commonly used method is the azo-coupling of aromatic diazonium salts to hydroxy arenes. Pesticides that give phenols (Fig. 1) (Miskus et al. 1959) or primary aromatic amines (Fig. 2) (Ercegovich and Witkontgon 1972) can be estimated by this method. Colour can also be produced by radical ion formation. Paraquat and diquat herbicides (Fig. 3), when reduced with sodium dithionite in alkaline medium, give strongly coloured radicals having absorption maxima at 600 and 377 nm, respectively.

Thiram can be estimated by colour development through π-complexation with copper and palladium. Reaction with specific chromogenic reagents to yield a coloured product is the basis for many colorimetric estimations. Important reagents are 4- aminoantipyrine for phenols (Eto et al. 1965), 4-(4-nitrobenzyl)pyridine for organophosphates (Duggan 1969), o-tolidine for organochlorines (Adamovic 1966) and pyrethroids (Pasha and Vijayashankar 1993), fluorescein for carbamates (Osselton and Snelling 1986), ammonium ceric nitrate for carbaryl (Patil and Shingare 1994), 2-(trichloromethyl) benzimidazole for heteroaromatic pesticides (Konopski 1994), 2,6-dibromo/dichloro-benzoquinone-N-chlorimine for thiocarbamate herbicides (Fodor-Csorba et al. 1992), and 4-(N,N-dimethylamino)aniline dihydrochloride for phosphorothionate and phosphorothiolothionate pesticides (Pasha et al. 1996).

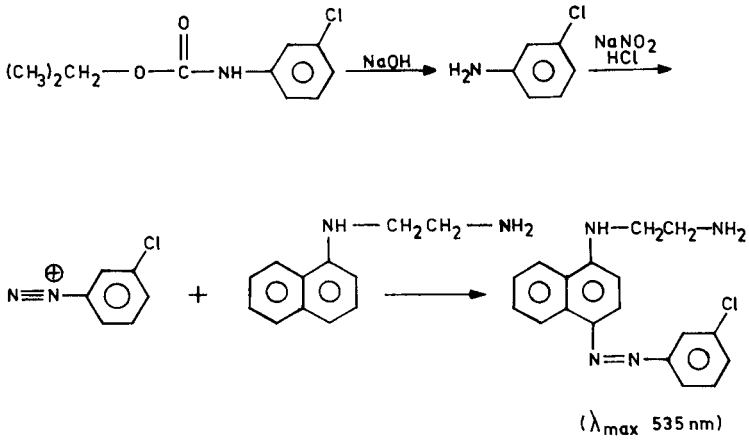


(Scheme A)



(I) (II)

Figure 3. Paraquat [I] and diquat [II]



(Scheme B)

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Newer Reagents for the Detection of Pesticides

Organochlorine pesticides are detected using silver nitrate spray and phenoxyethanol (Fodor-Csorba and Dutka 1986), o-tolidine (Adamovic 1966), or 3,3',5,5'-tetramethylbenzidine (Makhubalo et al. 1984). The first method is tedious, o-tolidine is a known carcinogen, and tetramethylbenzidine is expensive. Separation of organochlorines by thin-layer chromatography (TLC) followed by spraying with 2% N,N'-diphenylbenzidine and UV irradiation gave fine grey-pink spots on a colourless background for DDT, HCH isomers, aldrin, dieldrin, and endrin. Endosulfan could not be detected using this reagent. The sensitivity of the method was 0.5 μg.

We have already developed a very sensitive TLC method for the detection of organophosphate pesticides using 4-(N,N-dimethylamino) aniline followed by exposure to bromine vapour (Pasha et al. 1996). Another method has been developed using 2% o-tolidine spray followed by UV irradiation, specifically to detect chlorinated organophosphates such as bromophos, ronnel, chlorpyrifos, chlorpyrifos-methyl, and their metabolites. The colour varies from blue to brown and the minimum detection limit is 0.5 μg (A. Pasha and Y.N. Vijayashankar, unpublished data).

Detection of Benzimidazole Fungicides on Paper Strips

Carbendazim (1H-benzimidazol-2-yl methyl carbamate) and benomyl [methyl 1-(butylcarbamoyl) benzimidazol-2-yl carbamate] are fungicides used in agriculture and on stored fruits and vegetables, especially those for export. Hence, estimation of these fungicides is very important. Carbendazim and benomyl on hydrolysis give 2-aminobenzimidazole (2-AB) according to Scheme C (Fig. 4).

2-AB has been estimated colorimetrically using bromine (Pease and Gardiner 1969) and detected using chlorine + o-tolidine reagent on TLC plates. A very sensitive new method has been developed to analyse 2-AB residues. 2-AB was found to react with N-bromosuccinimide (NBS) to give a coloured product (Fig. 5). The colour is stable indefinitely on paper strips on a perfectly colourless background. The minimum detection limit as measured using a densitometer is 20 ng (Fig. 6).

π-Complexation of Dithiocarbamates

Thiram (tetramethyldithiuram disulfide) is a dithiocarbamate fungicide used especially for treating seed. Contaminated seed often enters poultry feed causing tibial dyschondroplasia and skeletal changes in birds. The most popular method for the analysis of thiram residues is π-complexation of thiram with copper(I) as shown in Figure 7. The reagents used are copper(I)iodide (Anon., AOAC method, 1995) or copper(I)chloride (Rangaswamy et al. 1970). These copper(I) salts are air- and moisture-sensitive. Tetraacetonitrilecopper(I)perchlorate used to estimate thiram is stable for long periods (Verma et al. 1984) but this reagent is also moisture sensitive and its preparation is tedious.

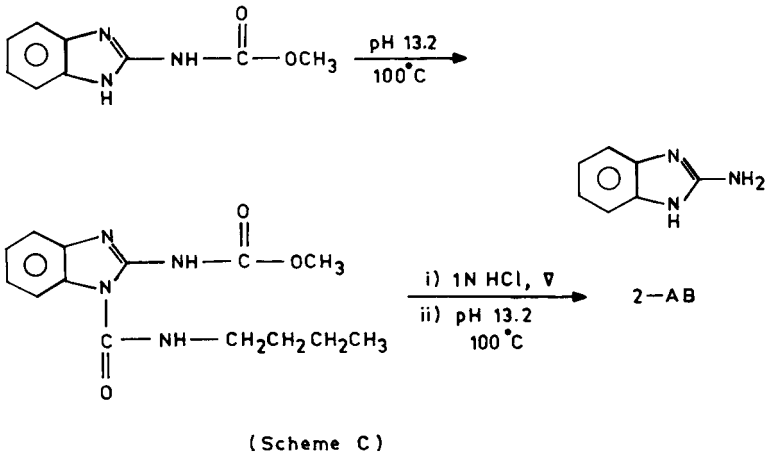

Figure 4. Scheme C: conversion of canbendazim and benomyl to 2-aminobenzimidazole

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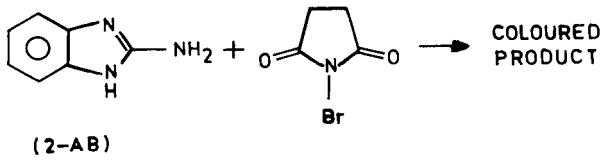

(Scheme D)

Figure 5. Scheme D: reaction of 2-aminobenzimidazole with N-bromosuccinimide

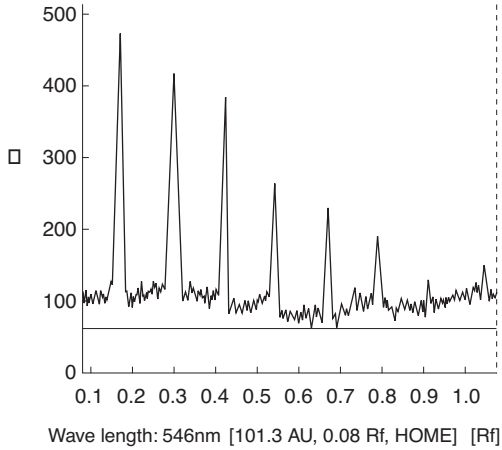

Figure 6. Assay of 2-aminobenzimidazole using a densitometer

We used copper(I)thiocyanate (CuSCN) which is the best source of an air- and light-stable copper(I) oxidation state for the estimation of thiram. It can be prepared by reacting aqueous cupric sulfate and potassium thiocyanate in the presence of ferrous sulfate. Thiram reacts with copper(I)thiocyanate instantaneously to give the π-complex .

Detection of thiram on paper strips

Filter paper strips (Whatman No. 1) dipped in a 2% solution of copper(I)thiocyanate were found to be perfectly colourless and stable when stored in polythene bags. Yellow zones were obtained on a colourless background when thiram solution was spotted onto these paper strips. The minimum detection limit of thiram was 100 ng.

Colorimetric Method of Analysis

Colorimetric methods are simple and sensitive techniques that allow estimation of a large number of pesticide residues in solution. The author has developed novel methods for the analysis of thiram, benzimidazole fungicides, and aryl and aryloxy alkanoic acids.

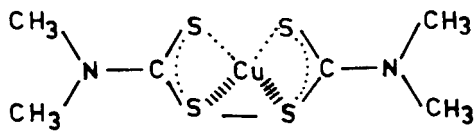

Figure 7. π-complexation of thiram fungicide with Cu(I)

Analysis of thiram residues

As noted previously, thiram reacts with copper(I)thiocyanate instantaneously.

The complex so formed was stable in solution for more than 48 hours. The maximum absorption of the complex in solution was 420 nm (Fig. 8) and Beer's Law was obeyed in the range between 0.2 g to 100 g/mL. The sensitivity of the method was 0.2 g/mL.

Thiram also formed a coloured complex with palladium. Its maximum absorption was at 382 nm.

Analysis of benzimidazole fungicides

Carbendazim and benomyl fungicide residues can be converted to 2-aminobenzimidazole. 2-AB reacts instantaneously with NBS in solution to give a coloured product having maximal absorptions at 340 and 360 nm (Fig. 9) and a molar absorptivity of 1.39×10^4 L/mol/cm (at 360 nm). The minimum detection limit was 0.4 μg/mL.

Analysis of aryl and aryloxyalkanoic acids

Aryl and aryloxyalkanoic acids are herbicides and plant-growth regulators. Residues of the phenoxy carboxylic acids are determined by GC and HPLC using fluorometric detector after tedious derivatisation (Nagata 1997).

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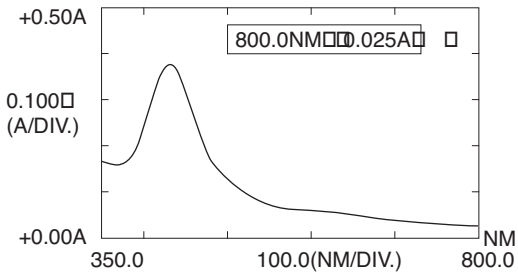


Figure 8. Absorption spectrum of thiram with copper (I) thiocyanate

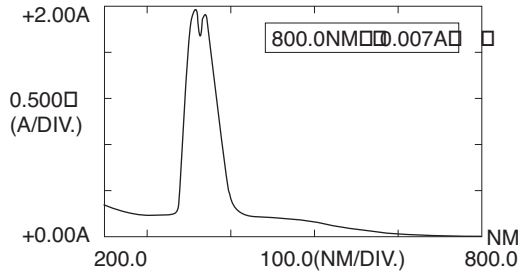
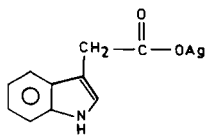
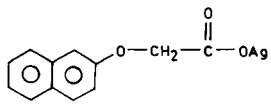
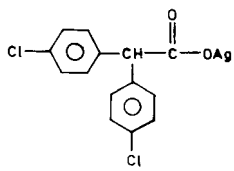
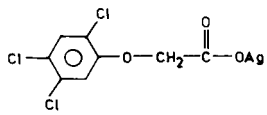
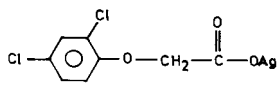
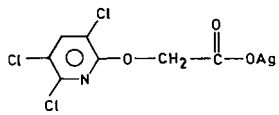


Figure 9. Absorption spectrum of the 2-AB + NBS product

Table 1. Maximum absorbance and molar absorptivity of silver salts of carboxylic acids in dimethylsulfoxide.

S1 no.	Carboxylic acid	Wavelength λ_{max} (nm)	Molar absorptivity ε (L/mol/cm)	
1.	Indole-3-acetic acid	439	3.16×10^3	
2.	2-Naphthoxyacetic acid	512	5.47×10^2	
3.	Bis(4-chlorophenyl acetic acid	465	4.45×10^2	
4.	2,4,5-Trichlorophenoxy acetic acid	488	5.80×10^2	
5.	Triclopyr	341	5.358×10^3	
6.	2,4-Dichlorophoxy acetic acid	504	5.21×10^2	

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We have found that the silver salt of aryl and aryloxyalkanoic acids when heated to 80–90°C in dimethyl sulfoxide gave a coloured product. The method may find application to estimate the residues of the alkanoic acids. The maximal absorption and molar absorptivity are given in Table 1.

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Rapid Bioassay Pesticides Residue (RBPR) Test for Monitoring Pesticide Residues in Vegetables in Ho Chi Minh City, Vietnam

Tran Van An*, Nguyen Thien†, Bui Van Thin*, and Le Van To*

Abstract

The Post Harvest Technology Institute in Vietnam has successfully applied the Taiwanese RBPR–AChE inhibition test (with some modifications) to monitor organophosphate (OP) pesticide residues in fresh vegetables. Five of the most widely used OP pesticides in Ho Chi Minh City (methamidophos, monocrotophos, azodrin, dimethoate, and dichlorvos (DDVP)) have been shown to strongly inhibit acetyl cholinesterase at the maximum residue limit authorised by Vietnamese regulation (1 ppm). This fast, simple, and economical test has efficiently served extension work for establishing safe vegetable production zones in Ho Chi Minh City.

INTENSIVE vegetables farming relies heavily on new technology such as fertilizer and pesticide application. Though some new, less toxic pesticides, such as pyrethroids have been used, about 30% of the pesticides used by vegetable growers in the suburbs of Ho Chi Minh City suburbs belong to two neurotoxic groups, the organophosphates and carbamates. A survey conducted by Ho Chi Minh City's Plant Protection Department and the University of Agriculture and Forestry showed that pesticide residues in many vegetable samples exceed the maximum residue limits of Food and Agriculture Organization (FAO)–World Health Organization (WHO) (1994) sometimes by factors of over a thousand (Table 1). So it is not surprising that in Vietnam there are now many cases of food poisoning, causing great concern for the government, the media, and consumers. Table 2 lists some statistics on, and episodes of food poisoning in Vietnam.

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According to WHO, 3 million persons worldwide suffer annually from over-exposure to pesticides, resulting in 220000 deaths. A United Nations Environment Programme study found that the proportion of agricultural workers handling hazardous pesticides is very high in Southeast Asia (in the range 30–91%). Therefore monitoring pesticide residue in vegetables is essential to protect the health of agricultural workers and consumers.

So far in Ho Chi Minh City laboratories, chemical methods (gas chromatography/mass spectrometry (GC/MS), high-performance liquid chromatography (HPLC)) are used to analyse pesticide residues. Though these methods are detailed, sensitive, and accurate, they are very time-consuming, so it is impossible to screen vegetables for pesticides residues before they reach the marketplace. Every year, we analyse a large number of samples, but we are unable to prevent residue-contaminated vegetables entering the market or to get them withdrawn.

Many developing countries are using ELISA kits for pesticide residue detection. Unfortunately, none of these kits can detect the seven pesticides currently used in Vietnam. In the Post Harvest Technology Institute we are trying to make ELISA and immuno

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affinity kits for detecting mycotoxins and pesticide residues, this will take some time and needs financial support. The pressing demands of our economy force us to have a rapid method for timely monitoring of pesticide residues in vegetables. This method must be sufficiently sensitive, rapid and accurate.

Table 1. Some data about pesticide residues in agricultural commodities in Vietnam

Commodity	Pesticide	Times exceeding MRL	Year of analysis
Tomato	Methamidophos	8	1996
	Monocrotophos	2	1996
Cove' bean	Methamidophos	71	1996
	Cypermethrin	55	1995
Melon	Methamidophos	20	1995
	Monocrotophos	3	1993
Onion	Methamidophos	186	1996
	Monocrotophos	2910	1993
	Dimethoate	252	1994
	Cypermethrin	472	1993
Eggplant	Methamidophos	400	1993
Cabbage	Methamidophos	157	1995
	Monocrotophos	605	1995
Cauliflower	Methamidophos	61	1995
	Monocrotophos	605	1993
Centella asiatica	Methamidophos	2	1993
	Thiodan	190	1993
Green cabbage	Methamidophos	12	1995
Dun' cabbage	Methamidophos	97	1996
Coleus aromaticus Benth	Methamidophos	5	1996
Stick bean	Methamidophos	6	1996
Sweet cabbage	Methamidophos	44	1996
Potato	Methamidophos	170	1995
Water morning glory	Methamidophos	90	1995
Grapes	Monocrotophos	78	1994
Black sesame	Heptachlor	2	1995
Red bean	DDT	1	1994
Linh Chi' mushroom	Methamidophos	8	1994
	Methyl parathion	2	1994

Source: Plant Protection Department (1997).

A cholinesterase (AChE) test for organophosphate pesticide residues (AOAC 1990) was developed in the 1960s. Dr E.Y. Cheng (Taiwan Agricultural Research Institute) has designed a simple method based on the same principle to monitor OP and carbamates pesticides residue in vegetables (Cheng and Chenghua Kao 1997). This method is called the rapid bioassay pesticides residue (RBPR) test.With assistance of Dr Cheng, the Post Harvest Technology Institute, in collaboration with the Department of Plant Protection, Ho Chi Minh City, has applied a modified, more sensitive variant of the RBPR method.

Table 2. Some data about food poisoning incidents in Vietnam

1	1992	The Vegetables Research Institute reported that Vietnam had 4572 reported cases of food poisoning (microorganisms, pesticide residues).
2	Jan.–May 1997	The Nutrition Research Institute reported that there were 37 cases of food poisoning, resulting in 1211 emergency hospitalisations, 17 deaths (an increase of 0.7% in comparison with the whole of 1996). The survey was conducted in 32 provinces and cities.
3	25/11/1997	17 children from the 5B kindergarten, district 5, HCMC were sick probably due to food poisoning (pesticide residues in sweet cabbage).
4	12/1997	22 persons were hospitalised in Trung Vuong Hospital HCMC, due to food poisoning.
5	6/1/1998	1000 workers of The Shoes Factory in Binh Duong Province were sick (food poisoning probably due to pesticide residues in cabbage).
6	23/12/1997	13 student-monks were hospitalised to Binh Thuan Province Hospital due to food poisoning (pesticide residues in vegetables).

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Principles of the Test

$$\text{Acetylcholinesterase(AChE)} + \text{OP / carbamates} \rightarrow \text{Inhibition} \quad (1)$$

$$\text{Acetylcholine} + H_2O \xrightarrow{\text{Free AChE}} \text{Acetic acid} + \text{Choline} \quad (2)$$

$$\text{Choline} + \text{DTNB} \longrightarrow \text{yellow colour} \quad (3)$$

The less AChE is inhibited in reaction (1), the more choline will be formed in reaction (2). The more choline reacting with the chromogen reagent (DTNB), the deeper the yellow colour occurring in reaction (3).

Materials and Methods

The crushed vegetable sample is blended with an organic solvent. A known quantity of the extract (concentrated or otherwise) is then incubated with a known quantity of AChE in a buffered solution. At the end of the incubation period, an excess of the substrate (acetylthiocholine iodine, ATCI) is added, together with the chromogen reagent DTNB. The increase in absorbance at 430 nm is then measured photometrically and compared with that of the insecticides-free blank.

The inhibition is calculated as follows:

$$\% \text{ inhibition} = [(\text{Abs. of insecticide free blank} \pm \text{Abs. of sample}) \div \text{Abs. of insecticide - free blank}] \times 100 \quad (4)$$

Inhibition above 35% is considered positive (see Tables 3 and 4)

Equipment needs are a colorimeter, a blender, an evaporator, a stop clock, and glassware.

Reagent requirements are AChE, DTNB, and ATCI, which were provided by the Taiwan Agricultural Research Institute, PBS pH = 8, and A.R. grade methanol and other solvents.

Experimental design

To check the sensitivity and accuracy of the RBPR test, we used two kinds of AChE test: one using an unconcentrated extract, the other a concentrated extract.

For each kind of test we did two experiments:

- Spiked samples containing known concentrations (0.5 and 5 ppm) pesticides
- Field-sprayed samples containing incurred residues of pesticides.

Each spiked sample was analysed twice, and the incurred residue samples three times. The seven pesticides in most common use in Ho Chi Minh City were tested:

- Organophosphates pesticides: monocrotophos; diazinon; dichlorvos; dimethoate; and methamidophos
- Carbamates pesticides: fenobucarb and isoprocarb.

Samples for which the percentage inhibition was equal to or above 35 were considered positive (following Taiwan regulations).

Results

Sensitivity and accuracy

Spiked samples

Table 3 gives the results of analyses of unconcentrated and concentrated extracts of spiked samples.

Table 3. Inhibition of cholinesterase in extracts of vegetable samples spiked at 0.5 or 5 ppm with various OP and carbamate pesticides

Pesticide	Concentration (ppm)	Inhibition (%)
A. Unconcentrated extract		
Organophosphates		
Diazinon	0.5	0
Diazinon	5	0
Dichlorvos	0.5	80
Dimethoate	0.5	0
Dimethoate	5	0
Methamidophos	0	0
Methamidophos	5	0
Monocrotophos	0.5	0
Monocrotophos	5	7
Carbamates		
Fenobucarb	0.5	0
Fenobucarb	5	66
Isoprocarb	0.5	0
Isoprocarb	5	25
B. Concentrated extract		
Organophosphates		
Diazinon	0.5	100
Dichlorvos	0.5	100
Dimethoate	0.5	69
Methamidophos	0.5	4
Methamidophos	5	40
Monocrotophos	0.5	58
Carbamates		
Fenobucarb	0.5	100
Isoprocarb	0.5	85

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Field-sprayed samples

Table 4 gives the results of analyses of unconcentrated and concentrated extracts of samples containing incurred residues

Time taken by tests

We timed the experiments and found that tests using an unconcentrated extract take 20 minutes, whereas tests on concentrated extracts take 60 minutes because of the extra time needed for the concentration step.

Conclusions

Though the test using unconcentrated extracts is very rapid, it is not sensitive enough to detect residues in vegetables at concentrations near the FAO–WHO maximum residue limits.

The modified test using concentrated extracts is sensitive enough to meet the FAO–WHO regulations for pesticides in vegetables.

The accuracy of the modified method compares favourably with chemical methods (GC/MS).

Table 4. Inhibition of cholinesterase in extracts of vegetable samples field sprayed with various OP and carbamate pesticides

Pesticide	Days after spraying	Unconcentrated extract inhibition (%)	Concentrated extract inhibition (%)	Chemical method (GC/MS) (ppm)
First experiment				
Diazinon	1	0	100	
	3	0	100	
	7	0	38	0.76
Dichlorvos	1	0	100	Not detected
	3	0	42	
	6	0	6	
Dimethoate	1	0	100	
	3	0	69	
Methamidophos	1	0	100	
	3	0	36	
	7	0	23	16.7
Monocrotophos	1	0	100	
	3	0	44	
	7	0	22	Not detected
Second experiment				
Diazinon	1	0	100	
	2	0	100	
	5	0	100	
Dichlorvos	1	0	100	
	2	0	100	
	5	0	31	
Methamidophos	1	0	100	
	2	0	100	
	5	0	6	
Monocrotophos	1	0	100	
	2	0	100	
	5	0	100	

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Table 4. (cont'd) Inhibition of cholinesterase in extracts of vegetable samples field sprayed with various OP and carbamate pesticides

Pesticide	Days after spraying	Unconcentrated extract inhibition (%)	Concentrated extract inhibition (%)	Chemical method (GC/MS) (ppm)
Fenobucarb	1	0	100	
	2	0	100	
	5	0	87	
	7	0	21	Not detected
Isoprocarb	1	0	100	
	2	0	100	
	5	0	47	
	7	0	30	Not detected
Third experiment				
Diazinon	1	21	0	
	3	0	100	
	6	-	86	
	9	-	35	0.12
Dichlorvos	1	69	100	
	3	0	38	
	6	-	20	Not detected
Dimethoate	1	6	83	
	6	-	81	
	9	-	7	0.14
Methamidophos	1	0	68	
	3	-	37	
	6	-	26	Not detected
Monocrotophos	1	12	100	
	6	-	54	
	9	-	43	
	11	-	0	Not detected
Fenobucarb	1	0	100	
	3	-	66	
	6	-	0	Not detected
Isoprocarb	1	0	92	
	3	-	41	
	6	-	26	

The modified method can be used to complement the GC/MS method. It can be used for screening vegetables for presence of pesticide residues. The positive samples can then be analysed by the GC/MS method to determine the exact concentration of pesticide.

The Service of Agriculture and Rural Development of Ho Chi Minh City intends to cooperate with the Post Harvest Technology Institute to establish a centre for monitoring pesticide residues by the RBPR method in vegetables and fruits from safe vegetable production zones (see paper by Nguyen Thien et al., these proceedings) and in wholesale markets.

We would like to propose joint research projects on the RBPR method with colleagues in Australia and Southeast Asia.

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Enzyme-linked Immunosorbent Assay (ELISA) for the Determination of Dieldrin and Atrazine Residues in Environmental Samples

Uzma Maqbool*, M. Jamil Qureshi*, Anwarul Haq and M. Zafar Iqbal†

Abstract

Enzyme-linked immunosorbent assay (ELISA) was used to determine dieldrin residues in soil and water, and atrazine residues in soil, water, and quartz sand samples. It was found that absorbance readings obtained with ELISA were inversely proportional to the concentration of the species in the sample. However, dilution resulted in an increase in the optical density values and the percentage maximal absorbance values. ELISA proved to be sufficiently sensitive for the qualitative and quantitative determination of dieldrin and atrazine residues.

THE constant application of pesticides and the growing concern about the potential contamination of different environmental matrices such as soil, groundwater, and plant materials necessitate the availability of fast screening methods. These must take into consideration legislative requirements such as the EC drinking water ordinance, with an upper limit for pesticide concentration of 0.1 μg/L for a single substance and 0.5 μg/L for the total of all pesticides (Wittman and Hock 1989).

At present, different types of pesticides, such as organochlorine insecticides and various types of herbicides, are in use for different agricultural practices. Because of their persistency, organochlorines and s-triazines are among the most critical compounds and should be continuously monitored (Huber 1985; Hussain et al. 1994a,b; Hunani et al. 1994). In addition to the classical analytical methods such as GC/MS, HPLC, and more recently, DC-automated multiple development, several enzyme immunoassays (ELISA) for the screening of cyclodienes, triazines, organochlorines, and carbamates have also appeared (Krause 1980; Huber and Hock 1984, 1985, 1986; Bushway et al. 1988; Skerritt 1995). Their results were also compared with the existing methods of determination (Thurman et al. 1990; Bradg et al. 1995). Immunoassays, being highly specific and sensitive, economical, and rapid have become a valuable tool in the field of environmental analysis, specially for screening a large number of samples within a short time interval.

The aim of the research described in this paper was to test the sensitivity and robustness of immunochemical procedures to determine different types of pesticide residues like cyclodienes and s-triazines (dieldrin, atrazine) in different environmental matrices like soil, water, and quartz sand samples and to compare these results with other instrumental techniques like GC or HPLC.

Materials and Methods

Immunoassay for dieldrin

Two types of studies have been conducted following the designs outlined in Appendixes I and II. The first study relates to the evaluation of ELISA kits provided for the determination of dieldrin contamination in unknown soil (samples provided by IAEA) and

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local water samples, to see the effects of the different matrixes. The second study was made to test the sensitivity of the immunoassay to dilution of soil extracts. The details of materials used for ELISA performed in these studies follow.

Environmental samples

Soil: Five soil samples provided by IAEA including 3 containing unknown quantities of dieldrin, 1 without dieldrin (negative control), and 1 containing 0.2 μg/g dieldrin (positive control).

Two of the 5 local soil samples were selected as having different physicochemical properties such as organic matter and mineral composition.

1. Soil-type A (Pb-70 Kasoor): EC 1.8 ds/m, pH 7.7, organic matter 1.25% (low organic matter), clay 36.6% (high mineral composition), silt 34.4%, sand 29%.

2. Soil-type B (Pb-51 Murree): EC 0.52 ds/m, pH 7.5, organic matter 2.69% (high organic matter), clay 16.4% (low mineral composition), silt 13.0%, sand 70.6%.

Water: Two of 5 water samples were selected having different physicochemical properties as under:

1. Water quality A: Water (obtained from irrigation channel, Abdullahpur, Faisalabad) contained EC 0.296 ds/m, pH 7.85, SAR 0.46, CO_3 nil and HCO_3 0.66, Cl 0.3, Na 0.5, K 0.02 and Ca+Mg 2.4 meq/L.

2. Water quality B: Water (obtained from the sub soil at NIAB Campus, Faisalabad) contained EC 1.13 ds/m, pH 8.42, SAR 1.51, CO_3 nil and HCO_3 1.66, Cl 1.0, Na 2.8, K 0.13 and Ca+Mg 6.85 meq/L.

Reagents used for dieldrin immunoassay

EnviroGard™ Plate Kit (Strategic Diagnostics Inc.) (for cyclodiene residues determination) containing:

- Microwell plates**:** coated with immobilised polyclonal antibodies raised to cyclodienes (dieldrin).
- Enzyme tracer**:** chlordane–horseradish peroxidase enzyme conjugate.
- Stock standard solution (dieldrin)**:** 1 mL stock of dieldrin (10 ppm – 10 μg/mL) in methanol diluted in a series of dilutions with nanopure water to obtain working standards, of concentration 0, 5, 10, 25, 50, 100 ng/mL dieldrin.
- Wash liquid: cool running tap water.
- Chromogen: vial of chromogen solution.
- Substrate**:** 1 vial of substrate solution.
- Stop solution: 2.5 N sulfuric acid.
- Methanol**:** analytical reagent grade, freshly distilled before use.

Experiment 1: Sensitivity of immunoassay for the determination of dieldrin residues in soil (qualitatively and quantitatively) and water matrix effect

The object of this experiment was to evaluate the provided immunoassay kits for the determination of dieldrin residues in soil and assessment of water matrix effect.

1. Five g of each soil sample in triplicate were extracted by shaking with 10 mL acetone for 2 minutes in a 50 mL conical flask. These were allowed to settle for 3 minutes and then filtered. One mL of each filtrate/supernatant was transferred to a vial and acetone was evaporated under nitrogen. Each residues was redissolved in 1 mL methanol and 4 mL distilled water.
2. 0.1 mg basic standard solution of dieldrin supplied was prepared with methanol and 100, 50, 25, 10 and 5 ng/mL dilutions were made along with a blank of water alone.
3. Using negative control soil extracts, 50, 25 and 10 ng/mL dilutions of dieldrin were prepared.
4. Two further sets of 50, 20, 10 ng/mL dieldrin solutions were made, but using two samples of unpurified water from local sources as diluent.

Experiment 2: Determination of the influence of soil matrix and dilution on the sensitivity of the immunoassay

The object of this experiment was to examine the extent to which the assay is sensitive to different soil matrices and dilution levels.

1. Five g of each soil was extracted with 10 mL acetone. The soil extracts were filtered and prepared as before.
2. One mL aliquots of the methanol: water solutions from 1 above were diluted with distilled water up to 3 and 10 mL. Thus, undiluted (u), and 3 and 10-folds dilutions of soil extract A and B were prepared.
3. Solutions containing 50, 25 and 10 ng/mL of dieldrin were made as in step 3 of Experiment 1 with each of the dilutions of the two soil extracts made in 2) above. Thus, there were 50, 25, and 10 ng/mL standards made with each of AU, BU, A3, B3, A10 and B10, a total of 18.

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Immunoassay for atrazine

Two types of studies were conducted following the design described in Appendixes III and IV. The first study related to the evaluation of the provided ELISA kit for the determination of atrazine residues in water and quartz sand samples (Appendix III) whereas the second study relates to the ELISA for the determination of atrazine residues in soil samples. Besides these soil samples, local water samples collected from different sources were also assayed (Appendix IV). Details of the material used for ELISA performed in these studies are as follows:

Environmental Samples

Water: One blank water sample (WB) without atrazine fortification, and two water samples having unknown amounts of atrazine (W1, W2) were sent by the IAEA Laboratories, Seibersdorf, Austria to be analysed. Along with these, samples collected from other sources were also assayed.

Sand: One blank quartz sand sample (QB) without atrazine fortification, and two quartz sand samples fortified with unknown amounts of atrazine (Q1, Q2) were sent by the IAEA Laboratories to be assayed.

Soil: One blank soil sample (SB) without atrazine fortification, and two soil samples fortified with unknown amounts of atrazine (SU1,SU2) were sent by the IAEA Laboratories. These samples were assayed at two different dilution levels: extracts diluted 100 times (SB-100, SU1-100, SU2-100), and extracts diluted 10,000 times (SB-10000, SU1-10000, SU2-10000).

Reagents used for atrazine immunoassay

- Microwell plates: coated with immobilised polyclonal antibodies raised against 2-aminohexane-carboxylic acid-4-isopropylamino-6-ethylamino-1,3,5,-triazine-keyhole limpet haemocyanine reconstituted in 0.05 M carbonate–bicarbonate coating buffer pH 9.6.
- Enzyme tracer: lyophilised atrazine - aminocaproic acid - horseradish peroxidase conjugate reconstituted in 100 μL nanopure water.
- Stock standard solution (Atrazine): 1 mL stock of atrazine (10 ppm – 10 μg/mL) in methanol – diluted in a series of dilutions with nanopure water to obtain working standards of concentration 0, 0.01, 0.02, 0.1, 0.2, 1.0, 2.0 pg/μL atrazine.
- Assay buffer: 0.01 M phosphate-buffered saline reconstituted to 1 L with nanopure water pH 7.4.
- Wash buffer: assay buffer diluted 1:10 (v/v) in nanopure water with 0.05% (v/v) Tween-20.
- Substrate buffer: phosphate–citrate buffer/sodium perborate in nanopure water. Prepared immediately before use and diluted with chromogen solution 1:1 (v/v).
- Chromogen solution: Three tablets of TMB in 15 mL nanopure water.
- Stop solution: 2 N H_2SO_4
- Methanol: Analytical reagent grade, freshly distilled before use.

Experiment 3:Qualitative and quantitative determination of atrazine residues in quartz sand and water samples

The design for this experiment is outlined in Appendix III.

Quartz sand extract preparation: Five g of each quartz sand sample in triplicate were extracted by shanking with 25 mL of 90% methanol for 12 hours at room temperature. The extract was filtered and 1 mL of the filtrate was added to 99 mL nanopure water for a final volume of 100 mL in 100 mL volumetric flask.

Experiment 4: Qualitative and quantitative determination of atrazine residues in soil samples

The design for this experiment is outlined in Appendix IV.

Soil extract preparation: Extraction of the soil samples was made as for quartz sand extraction and 1 mL of the aliquot was diluted to 100 mL with nanopure water as before. From this diluted extract, a further 1:100 dilution in nanopure water to a final dilution of 1:10 000 was made.

Atrazine standard solution

1. 1 mL of the standard solution supplied (1000 pg/μL) was diluted to a total volume of 10 mL with nanopure water to give a solution of 100 pg/L (S8).
2. 1 mL of S8 was diluted to 10 mL to give 10 pg/uL (S7).
3. Following working standards were made with S7.

S6: 2 mL of S7 solution to 10 mL = 2.0 pg/μL
S5: 1 mL of S7 solution to 10 mL = 1.0 pg/μL
S4: 1 mL of S6 solution to 10 mL = 0.2 pg/μL
S3: 1 mL of S5 solution to 10 mL = 0.1 pg/μL
S2: 1 mL of S4 solution to 10 mL = 0.02 pg/μL
S1: 1 mL of S3 solution to 10 mL = 0.01 pg/μL
S0: nanopure water.

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These six solutions (S1–S6) together with a blank of nanopure water alone (S0), made a series of seven working standards.

Equipment used for ELISA

- ELISA microplate reader: Porta - Reader II, Model D 602 with an interference filter of 450 nm.
- Plate washer: BIO - RAD Model 1575, Immunowash ELISA Plate washer. (Hand washing is also possible)
- Water purification system: SYBRON BARNSTEAD Nanopure - II. Water purification system (10–15 mega ohm - cm).
- Micropipettes
  - Single channel pipettes for measuring – 20-200 μL, 200–1000 μL and 1000–5000 μl (Oxford, Eppendorf, Gilson).
  - Multichannel pipettes – 8 channel system – range 50–200 μL (Socorex)
  - Fixed volume pipettes – 1 mL, 2.5 mL, 5 mL, 10 mL (Oxford, Eppendorf).
- Glassware
  - Centrifuge glass tubes or similar for diluting standard solution.
  - Glass vials (20 mL scintillation vials) for diluting enzyme conjugate.
  - Measuring cylinders and bottles (100 mL and 1000 mL) for preparing and storing buffer solutions.
- Shaker for soil extraction: Platform reciprocal / wrist-action shaker.
- Miscellaneous: Vortex mixer, refrigerator, timer, absorbent towels, marker pens (waterproof) and adhesive labels.

Immunoassay

- The strip format for all the experiments was planned as recommended in the protocol and described in Appendixes I–V.
- The ELISA microplates were used to develop competitive ELISA between sample antigen and antigen conjugated to enzyme and read by ELISA plate reader (Porta-Reader Manula-II Model No. D-306) optical densities were measured at 450 nm.
- From the observed optical density (OD) values, the average OD, along with the standard deviation and coefficient of variation, was calculated.
- Percent maximal absorbance values (% Bo) were also calculated as

$$\%\text{Bo} \frac{\text{Average OD of the sample}}{\text{Average OD of S0}} \times 100$$

- % Bo or OD values was plotted against concentration of dieldrin and atrazine standards on semilogarithmic graph paper and dieldrin and atrazine standard curves were obtained. The dieldrin and atrazine concentrations were calculated through these standard curves.
- The data were also subjected to regression/correlation analysis and regression equations were obtained. From these regression equations, the concentrations of dieldrin and atrazine residues in the various environmental samples, were also calculated.

Results and Discussion

Immunoassay for dieldrin

Experiment 1: In the 1st experiment, 5 soil samples including positive and negative controls and 3 samples (U1, U2, U3) having unknown dieldrin residues were run in conjunction with the standards. The observed optical density values are shown in Table 1. Water samples obtained from different sources were also assayed. The average optical density, the percent maximal absorbance, and dieldrin concentration were calculated (Table 2). The standard curve for dieldrin is plotted in Figure 1.

It was observed that U1 soil sample contained lowest amount of dieldrin (4.2 ppb) as compared to U2 and U3 soil samples (10.9 and 22.7 ppb). The negative control soil extract, when spiked with 10, 25, or 50 ppb of dieldrin, gave dieldrin concentrations of 12.0, 24.9, and 51.6 ppb after immunoassay. From these data, the value of the kits for qualitative screening purposes and the quantitative reproducibility of residues measurement in soil is clearly shown.

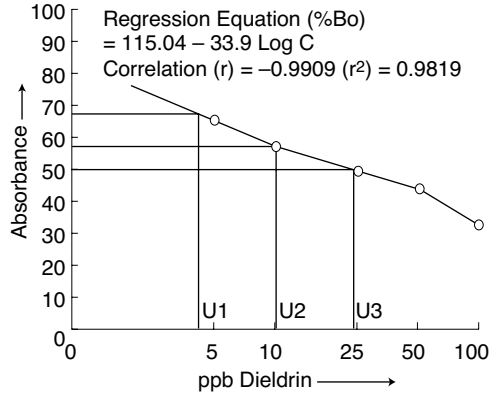

Figure 1. Dieldrin standard curve (soil analysis)

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Attempts were made to assess the effect of the water matrix when it came from different sources (Fig. 2). When the percent maximal absorbance (% Bo) of water quality A (canal water) is compared with water quality B (underground water) with respect to distilled water, it was observed that water having a high salt concentration and a heavy ionic load (underground water) showed higher values of OD or % Bo resulting in lower concentrations of dieldrin as compared with canal water which is comparatively low in salt concentration and ionic strength. Distilled water, which has negligible amounts of salts and ions, showed lowest values of OD and % Bo showing more dieldrin concentration. From these results, the sensitivity of ELISA with different water matrices for dieldrin residue detection and quantification has been clearly shown.

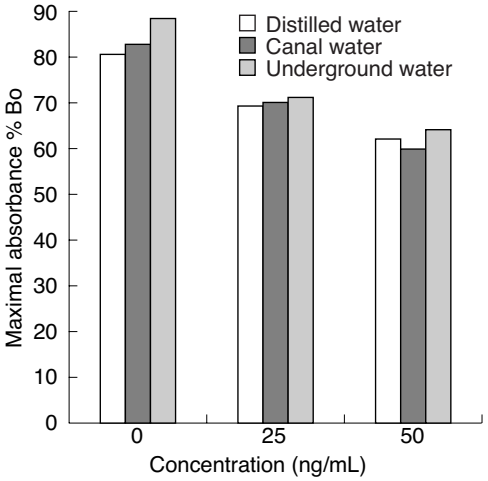

Figure 2. Water matrix effect through ELISA

Experiment 2: In the second experiment, two soil samples were assayed to determine soil matrix and dilution effects. The optical densities observed for samples in experiment 2 are shown in Table 3. The average optical density, percent maximal absorbance, and dieldrin concentration of the samples are shown in Table 4. As far as the matrix effect of the two soils is concerned, it was observed that in soil-type A, having a high mineral composition tends to assist the complex formation resulting in higher values of optical density and % Bo (Table 4), whereas the soil high in organic matter and low mineral composition resulted in lower values of optical density and % Bo (Fig. 3). The enhanced effect of high mineral composition on complex formation remains prominent even in samples diluted 3- or 10-fold. When the extracts of these two soils were subjected to immunoassay both in undiluted (U) and diluted forms (up to 3 mL and 10 mL) with or without spiking with dieldrin, it was observed that dilution results in increase in optical density values as well as percentage maximal absorbance, resulting in low concentration (Fig. 4). As absorbance inversely relates to the residual pesticide load in soil, undiluted extract having pesticide (antigen) in concentrated form (more antigen) results in lower OD values as compared with the diluted extract in which pesticide contamination becomes less due to dilution.

These results clearly indicate the sensitivity of immunoassay to determine dieldrin residues in different soil matrices having different organic matter and mineral compositions.

Immunoassay for atrazine

Experiment 3: The OD values for the water and quartz sand samples in this experiment are recorded in Table 5 according to the plate layout plan described in Appendix III. From the observed OD values, average OD values along with standard deviation (SD) and coefficient of variation (CV) were calculated and are shown in Table 6.

Percent maximal absorbance values (% Bo) and atrazine concentrations in unknown samples (both water and quartz sand samples) were also calculated (Table 6). When the %Bo values was plotted against concentration of the standards (0.01, 0.02, 0.1, 0.2, 1.0, and 0.2 pg/µL on semi-logarithmic paper the atrazine standard curve obtained for pre-trial was linear (Fig. 5). From these data, it was observed that absorbance readings obtained from ELISA were inversely proportional to the concentration of the analyte in standards. When the data were subjected to regression/correlation analysis, there was highly significant negative correlation ($r = 0.976$; $p < 0.01$) ($r = < 0.01$) between percent maximal absorbance (%Bo) and log concentration (log C) which means that increase in log C will be accompanied by a decrease in % Bo values.

The unknown concentration of atrazine residues in water and quartz sand samples were calculated by plotting their respective % Bo values in the equation (Table 6) and it was observed that unknown amount of atrazine residues (0.78 pg/μL) than unknown water sample 2 (W2) i.e. 0.43 pg/μL whereas quartz sand sample (Q1) showed higher OD values indicating low atrazine residue (0.29 pg/μL) than quartz sand sample 2 (Q2) i.e. 0.32 pg/μL.

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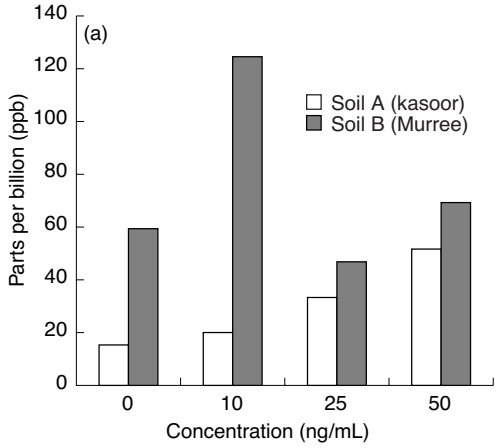

Figure 3a. Soil matrix effects (undiluted)

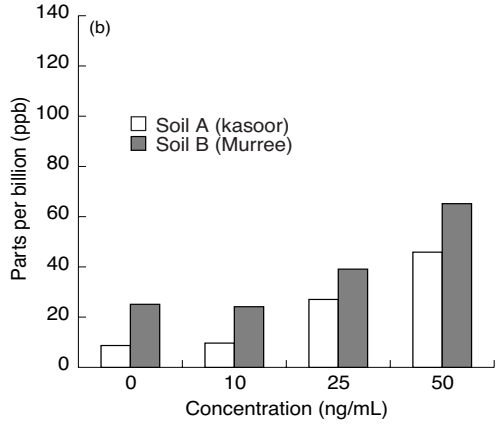

Figure 3b. Soil matrix effects (3-fold)

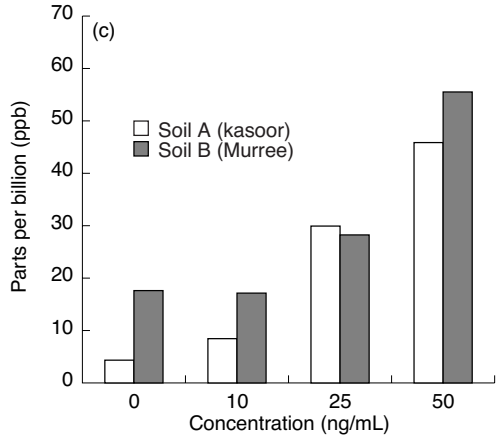

Figure 3c. Soil matrix effects (10-fold)

Values obtained from graphing are also given in the table. From these data, the qualitative and quantitative determination of atrazine residues in water and quartz sand samples through ELISA is clearly shown.

Experiment 4: In these studies, three soil samples including a blank sample without atrazine spiking and two samples having unknown amount of atrazine residues were assayed along with the series of atrazine dilution as standards according to the plate layout plan (Appendix IV).

The soil extracts were prepared at two dilution levels (100 times dilution and 10000 times dilution) in order to determine any dilution effect. The OD values for all these samples were recorded (Table 7). From the observed OD values, average OD values along with the standard deviation (SD) and coefficient of variation (CV) were calculated.

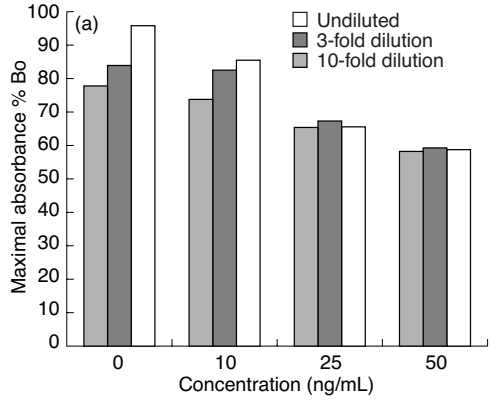

Figure 4a. Dilution effects (Soil-A)

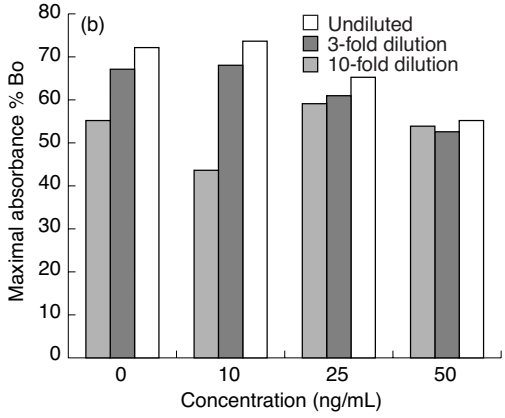

Figure 4b. Dilution effects (Soil-B)

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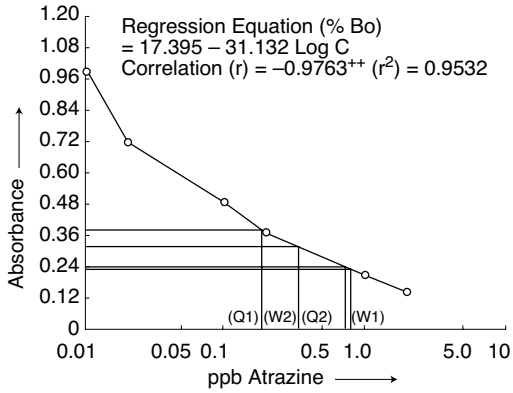

Figure 5. Atrazine standard curve (quartz sand and water analysis)

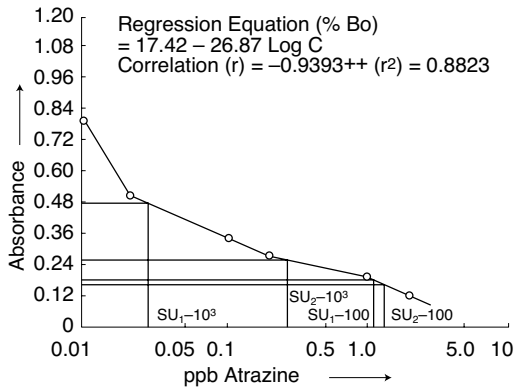

Figure 6. Atrazine standard curve (soil analysis)

Table 1. Enzyme-linked immunosorbent assay for the determination of dieldrin residues in soil and water (matrix effect).

	Optical density values											
Wells	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	-	-	-	-	-	-	-	-	-
B	-	1.2600	1.2605	1.1790	0.8681	1.2505	1.2598	0.9894	1.0369	1.1042	-	-
C	-	1.1486	1.1496	1.1976	0.8692	1.2700	1.1483	0.9862	1.0321	0.9260	-	-
D	-	1.0100	0.9897	1.1784	0.8730	1.2745	1.0183	0.8547	0.8707	0.8851	-	-
E	-	0.8634	0.8652	0.9395	-	0.4771	0.8637	0.8515	0.8681	0.8841	-	-
F	-	0.7750	0.7598	0.9909	-	0.4809	0.7692	0.7185	0.7386	0.7882	-	-
G	-	0.5773	0.5482	1.0846	-	0.4823	0.5515	0.7169	0.7348	0.7878	-	-
H	-	-	-	-	-	-	-	-	-	-	-	-

Absorbance Values taken at 450 nm.

Table 2. Qualitative and quantitative determination of dieldrin residues in soil and water through ELISA

Well contents	Average OD	+/-SD	% CV	% Bo	Dieldrin conc. (ppb)	
					R.E. Values	G. Values
S0	1.2601	0.013	1.03	100.00	-	-
S5	1.1482	0.090	7.84	91.11	-	-
S10	1.0060	0.020	1.99	79.84	-	-
S25	0.8641	0.130	15.04	68.57	-	-
S50	0.7680	0.083	10.81	60.95	-	-
S100	0.5590	0.010	1.79	44.36	-	-
U1	1.1850	0.099	8.35	94.05	4.16	4.20
U2	1.0050	0.083	8.26	79.76	10.98	10.05
U3	0.8701	0.060	6.90	69.05	22.73	24.40
NC	1.2650	0.030	2.37	100.40	-	-
PC	0.4801	0.003	0.63	38.09	-	-
SS10	0.9878	-	-	78.40	12.05	
SS 25	0.8531		-	67.71	24.90	
SS 50	0.7177		-	56.96	51.66	
SWA 10	1.0345		-	82.10	9.37	
SWA 25	0.8694		-	69.00	22.81	
SWA 50	0.7367		-	58.47	46.65	
SWB 10	1.1051		-	87.71	6.40	
SWB 25	0.8846		-	70.21	21.01	
SWB 50	0.7880			62.54	35.37	

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Table 3. Enzyme-linked immunosorbent assay for the determination of dieldrin residues in soil (dilution effect)

	Optical density values											
Wells	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	-	-	-	-	-	-	-	-	-
B	-	0.9793	1.0141	1.1476	1.3001	0.9248	1.0342	1.0342	0.5461	0.8576	0.9227	-
C	-	0.9699	1.0601	1.1920	1.3048	0.9258	1.0332	1.0960	0.5659	0.8606	0.9357	-
D	-	0.9788	1.0698	1.2610	0.8811	0.8182	0.8441	0.8201	0.7432	0.7701	0.8264	-
E	-	0.6962	0.8296	0.9204	0.8726	0.8198	0.8407	0.8231	0.7496	0.7709	0.8322	-
F	-	0.6892	0.8499	0.9188	0.7621	0.7276	0.7410	0.7385	0.6879	0.6692	0.6987	-
G	-	0.7149	0.8678	0.9028	0.7913	0.7286	0.7432	0.7399	0.6901	0.6810	0.7084	-
H	-	-	-	-	-	-	-	-	-	-	-	-

Absorbance values taken at 450 nm.

Table 4. Qualitative and quantitative determination of dieldrin residues in soil through ELISA.

Well contents	Average OD	+/-SD	% CV	% Bo	Dieldrin conc. (ppb)
AU	0.9760	0.102	10.50	77.46	12.88
BU	0.7001	0.100	14.30	55.56	56.85
A3	1.0480	0.001	0.10	83.17	8.71
B3	0.8491	0.010	1.14	67.39	25.46
A10	1.2002	0.100	8.33	95.25	3.84
B10	0.9140	0.101	11.01	72.54	17.55
AU 10	0.9253			73.44	16.87
AU 25	0.8190			65.00	29.92
AU 50	0.7281			57.79	48.84
A3 10	1.0337			82.04	9.41
A3 25	0.8424			66.86	26.37
A3 50	0.7421			58.90	45.29
A10 10	1.0651			84.53	7.91
A10 25	0.8216			65.21	29.77
A10 50	0.7392			58.67	46.01
BU 10	0.5560			44.13	123.52
BU 25	0.7464			59.24	44.27
BU 50	0.6889			54.67	67.21
B3 10	0.8591			68.18	24.11
B3 25	0.7725			61.31	38.44
B3 50	0.6751			53.58	65.01
B10 10	0.9292			73.75	16.52
B10 25	0.8293			65.82	28.31
B10 50	0.7037			55.85	55.71

Table 5. Enzyme-linked immunosorbent assay for the determination of atrazine residues in quartz sand and water samples

	Optical density values											
Wells	1	2	3	4	5	6	7	8	9	10	11	12
A	0.88	0.98	0.96	0.39	0.38	0.36	0.60	0.63	0.65	0.19	0.18	0.16
B	0.85	0.81	0.74	0.20	0.23	0.21	0.51	0.49	0.54	0.23	0.20	0.19
C	0.47	0.58	0.48	0.14	0.20	0.18	0.24	0.23	0.21	0.19	0.30	0.27
D	0.29	0.39	0.37	0.55	0.50	0.46	0.41	0.56	0.54	0.19	0.22	0.20
E	0.25	0.32	0.27	0.18	0.15	0.17	0.46	0.39	0.49	0.16	0.15	0.21
F	0.17	0.24	0.19	0.14	0.15	0.21	0.30	0.31	0.33	0.32	0.26	0.38
G	0.10	0.15	0.14	0.12	0.15	0.14	0.15	0.17	0.19	0.30	0.35	0.39
H	0.02	0.02	0.05	0.13	0.17	0.19	0.21	0.17	0.18	0.00	0.00	0.07

Absorbance values taken at 450 nm.

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Percent maximal absorbance values (% Bo) and atrazine concentrations in unknown samples were also calculated (Table 8). From these results it was observed that as the concentration of antigen in standard solutions decreased, OD values increased. When the %Bo values were plotted against concentration of the standards (0.01, 0.02, 0.2, 0.1, 1.0, and 2.0 pg/μL) on semilogarithmic paper the atrazine standard curve obtained for trial proper was linear (Fig. 6). When the data were subjected to regression/correlation analysis, there was highly significant negatived correlation ($r = 0.9393$; $p < 0.01$) between percent maximal absorbance (%Bo) concentration (log C), which means that increase in log C will be accompanied by a decrease in %Bo values.

The unknown concentrations of atrazine residues in soil samples were calculated by plotting their respective %Bo values in the regression equation (Table 8). It was observed that soil sample 1 showed higher optical density values indicating low atrazine residues (0.787 and 0.056 pg/μL) in 100 and 10000 times diluted samples, respectively, as compared to the soil sample 2 (0.944 and 0.380 pg/μL, respectively). However, dilution of the soil extracts resulted in increase in OD values but the trend of lower atrazine residues in soil sample 1 than soil sample 2 remained constant at both dilution levels.

Besides these soil samples, local water samples collected from different sources were also assayed for

Table 6. Qualitative and quantitative determination of atrazine residues in water and quartz sand through ELISA

Well contents	Average OD	+/- SD	% CV	% Bo	Atrazine conc. (pg/μL)	
					R.E. Values	G. Values
S0	1.11	0.047	4.23	100.00	-	-
S1	0.99	0.040	4.04	89.19	-	-
S2	0.72	0.640	8.88	64.86	-	-
S3	0.49	0.053	10.81	44.14	-	-
S4	0.37	0.044	11.89	33.33	-	-
S5	0.21	0.026	12.38	18.92	-	-
S6	0.14	0.025	17.86	12.61	-	-
WB	0.40	0.026	6.50	36.04	-	-
W1	0.23	0.032	13.91	20.72	0.782	0.800
W2	0.32	0.056	17.50	28.83	0.429	0.330
QB	0.48	0.086	17.91	43.24	-	-
Q1	0.38	0.041	10.79	34.23	0.288	0.190
Q2	0.24	0.045	18.75	21.62	0.732	0.770

Table 7. Enzyme-linked immunosorbent assay for the determination of atrazine residues in soil

	Optical density values											
Wells	1	2	3	4	5	6	7	8	9	10	11	12
A	1.06	1.13	1.15	0.39	0.37	0.42	0.27	0.24	0.21	0.36	0.40	0.38
B	0.95	1.03	0.98	0.20	0.25	0.19	0.31	0.33	0.34	0.07	0.07	0.04
C	0.69	0.79	0.67	0.24	0.25	0.28	0.42	0.45	0.36	0.10	0.04	0.04
D	0.43	0.51	0.53	0.51	0.59	0.55	0.36	0.33	0.35	0.08	0.09	0.08
E	0.35	0.42	0.34	0.41	0.39	0.44	0.31	0.20	0.27	0.10	0.12	0.09
F	0.19	0.24	0.20	0.23	0.19	0.24	0.36	0.41	0.43	0.03	0.09	0.06
G	0.14	0.12	0.17	0.41	0.44	0.40	0.22	0.21	0.28	0.04	0.08	0.06
H	0.05	0.05	0.05	0.05	0.07	0.03	0.05	0.01	0.03	0.02	0.01	0.01

Absorbance values taken at 450 nm.

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qualitative and quantitative determination of atrazine residues (Table 9). After the assay, it was observed that water samples collected from Abdullahpur canal showed highest amount of atrazine (1.240 pg/μL) whereas laboratory-prepared distilled water showed the lowest amount of the residues (0.183 pg/μL). Atrazine residues in other water samples being assayed ranged between these two extremes.

These results clearly indicate the sensitivity of ELISA for qualitative and quantitative determination of atrazine residues in soil and water samples.

Table 8. Qualitative and quantitative determination of atrazine residues in soil through ELISA

Well contents	Average OD	+/- SD	% CV	% Bo	Atrazine conc. (pg/μL)	
					R.E. Value	G. Values
S0	0.94	0.053	5.64	100.00	-	-
S1	0.80	0.056	7.00	85.11	-	-
S2	0.51	0.061	11.96	54.25	-	-
S3	0.35	0.053	15.14	37.23	-	-
S4	0.28	0.036	12.86	29.79	-	-
S5	0.20	0.036	18.00	21.28	-	-
S6	0.13	0.026	20.00	13.83	-	-
SB-100	0.44	0.076	17.27	46.81	-	-
SU1-100	0.19	0.029	15.26	20.21	0.787	1.100
SU2-100	0.17	0.031	18.24	18.09	0.944	1.300
SB-10,000	0.55	0.099	18.00	58.51	-	-
SU1-10,000	0.48	0.051	10.62	51.06	0.056	0.027
SU2-10,000	0.27	0.049	18.15	28.72	0.380	0.270

Table 9. Qualitative and quantitative determination of atrazine residues in water samples collected from different sources

Well contents	Average OD	+/- SD	% CV	% Bo	Atrazine conc. (pg/μL)
Canal water, Abdullahpur	.14	0.015	10.71	14.89	1.240
Tap water, Abdullahpur	0.16	0.030	18.75	17.02	1.030
NIAB – Underground water	0.17	0.020	11.76	18.09	0.944
NIAB – Tap water	0.19	0.021	11.05	20.21	0.787
Lab. Tap water					
Tissue culture	0.18	0.015	8.33	19.15	0.862
Soil salinity	0.21	0.021	10.00	22.34	0.656
Pesticide chemistry	0.25	0.057	22.80	26.60	0.455
Phytopathology	0.20	0.015	7.50	21.28	0.718
RFLP	0.17	0.032	18.82	18.09	0.944
Lab. dist. water					
Pesticide chemistry	0.32	0.060	18.75	34.04	0.241
RFLP	0.35	0.045	12.86	37.23	0.183

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Conclusion

The experiments reported here, demonstrated the robustness and sensitivity of ELISA for qualitative and quantitative determination of dieldrin and atrazine residues in soil, water, and quartz sand samples.

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Appendix I. Plan of the strip format for the determination of dieldrin residues in soil and water (matrix effect)

	1	2	3	4	5	6	7	8	9	10	11	12
A				W	A	T	E	R				
B	W	S0	S0	U1A	U3A	NCA	S0	SS10	SWA10	SWB10		W
C	A	S5	S5	U1B	U3B	NCB	S5	SS10	SWA10	SWB10		A
D	T	S10	S10	U1C	U3C	NCC	S10	SS25	SWA25	SWB25		T
E	E	S25	S25	U2A		PCA	S25	SS25	SWA25	SWB25		E
F	R	S50	S50	U2B		PCB	S50	SS50	SWA50	SWB50		R
G		S100	S100	U2C		PCC	S100	SS50	SWA50	SWB50		
H				W	A	T	E	R				

S0 =Water blank.
S5 - S10
S25 - S50 = 5, 10, 25, 50, 100 ng of dieldrin/mL dist H_20.
S100
U1A U1B U1C = Soil extract (in triplicate) containing unknown residues (U1)
U2A U2B U2C = Soil extract (in triplicate) containing unknown residues (U2)
U3A U3B U3C = Soil extract (in triplicate) containing unknown residues (U3)
NCA, NCB, NCC = Negetive control extract (in triplicate).
PCA, PCB, PCC = Positive control extract (in triplicate).
SS10, SS25 SS50 = Standards (10, 25, 50 ng of dieldrin/mL of NC extract)
SWA10 SWA25 SWA50 = Standards (10, 25, 50 ng of dieldrin/mL of water quality A (Abdullahpur).
SWB10 SWB25 SWB50 = Standards (10, 25, 50ng of dieldrin/mL of water quality B (NIAB Campus).

Appendix II. Planning of the strip format for the determination of dieldrin residues in soil (dilution effect)

	1	2	3	4	5	6	7	8	9	10	11	12
A				W	A	T	E	R				
B	W	AU	A3	A10	S0	AU10	A310	A1010	BU10	B310	B1010	W
C	A	AU	A3	A10	S0	AU10	A310	A1010	BU10	B310	B1010	A
D	T	AU	A3	A10	S25	AU25	A325	A1025	BU25	B325	B1025	T
E	E	BU	B3	B10	S25	AU25	A325	A1025	BU25	B325	B1025	E
F	R	BU	B3	B10	S50	AU50	A350	A1050	BU50	B350	B1050	R
G		BU	B3	B10	S50	AU50	A350	A1050	BU50	B350	B1050	
H				W	A	T	E	R				

AU = Soil extract A (Murree Hills)
BU = Soil extract B (Kasoor)
A3 = 1 mL of AU diluted with 3 mL
B3 = 1 mL of BU diluted with 3 mL
A10 = 1 mL of BU diluted with 10 mL
B10 = 1 mL of BU diluted with 10 mL
AU10, AU25, AU50 = AU mixed with standard (10, 25, 50 ng/mL)
A310, A325, A350 = A3 mixed with standard (10, 25, 50 ng/mL)
A1010, A1025, A1050 = A10 mixed with standard (10, 25, 50 ng/mL)
BU10, BU25, BU50 = BU mixed with standard (10, 25, 50 ng/mL)
B310, B325, B350 = B3 mixed with standard (10, 25, 50 ng/mL)
B1010, B1025, B1050 = B10 mixed with standard (10, 25, 50 ng/mL)

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Appendix III. Plate layout plan for the determination of atrazine residues in water and quartz sand

	1	2	3	4	5	6	7	8	9	10	11	12
A	S0	S0	S0	WB	WB	WB	W1	W1	W1	W2	W2	W2
B	S1	S1	S1	W1	W1	W1	W2	W2	W2	WBnt	WBnt	WBnt
C	S2	S2	S2	W2	W2	W2	QB	QB	QB	W1nt	W1nt	W1nt
D	S3	S3	S3	QB	QB	QB	Q1	Q1	Q1	W2nt	W2nt	W2nt
E	S4	S4	S4	Q1	Q1	Q1	Q2	Q2	Q2	QBnt	QBnt	QBnt
F	S5	S5	S5	Q2	Q2	Q2	WB	WB	WB	Q1nt	Q1nt	Q1nt
G	S6	S6	S6	WB	WB	WB	W1	W1	W1	Q2nt	Q2nt	Q2nt
H	B	B	B	B	B	B	B	B	B	B	B	B

B = Blank:For the caliberation of ELISA reader.
S0-S6 = Standards: Series of atrazine dilutions containing 0, 0.01, 0.02, 0.1, 0.2, 1.0 and 2.0 pg/μL, respectively.
WB = Blank(water sample without atrazine fortification)
W1, W2 = Unknown-1, Unknown-2 (Water samples 1 and 2 respectively fortified with unknown amount of atrazine).
QB = Blank (Quartz sand sample without atrazine fortification)
Q1, Q2 = Unknown-1, Unknown-2 (Quartz sand samples 1 and 2 respectively fortified with unknown amount of atrazine).
nt = No tracer (Samples being assayed without enzyme tracer)

Appendix IV.Plate layout plan for the determination of atrazine residues in soil and water

	1	2	3	4	5	6	7	8	9	10	11	12
A	S0	S0	S0	T1	T1	T1	T9	T9	T9	T17	T17	T17
B	S1	S1	S1	T2	T2	T2	T10	T10	T10	T18	T18	T18
C	S2	S2	S2	T3	T3	T3	T11	T11	T11	T19	T19	T19
D	S3	S3	S3	T4	T4	T4	T12	T12	T12	T20	T20	T20
E	S4	S4	S4	T5	T5	T5	T13	T13	T13	T21	T21	T21
F	S5	S5	S5	T6	T6	T6	T14	T14	T14	T22	T22	T22
G	S6	S6	S6	T7	T7	T7	T15	T15	T15	T23	T23	T23
H	B	B	B	T8	T8	T8	T16	T16	T16	B	B	B

B Blank (For Calibration of ELISA reader).
S0-S6 Standards (Series of atrazine dilutions containing 0, 0.01, 0.02, 0.1, 0.2, 1.0, and 2.0 pg/μL).
T1, T4 Blank (Soil samples without atrazine fortification) (100 times diluted)
T2, T5 Unknown - 1 (Soil sample 1 containing unknown amount of atrazine) (100 times diluted)
T3, T6 Unknown - 2 (Soil sample 2 containing unknown amount of atrazine). (100 times diluted)
T9, T12 Blank (Soil samples without atrazine fortification) (10000 times diluted)
T10, T13 Unknown - 1 (Soil sample-1 containing unknown amount of atrazine) (10000 times diluted)
T 11,T14 Unknown - 2 (Soil sample-2 containing unknown amount of atrazine) (10000 times diluted)

Water Samples from Different Sources
T 7, T8 Abdullahpur canal water, tap water
T 15, T16 NIAB campus under ground water, tap water.

Laboratory Tap Water
T 17 Tissue culture
T 18 Soil salinity
T 19 Pesticide chemistry
T 20 Phytopathology
T 21 RFLP

Laboratory Distilled Water
T 22 Pesticide chemistry
T 23 RFLP

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Immunoassays for Detection of Agrochemical Residues in Food and Environmental Matrices

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Abstract

Immunoassays for detection of agrochemicals offer the advantages of high throughput and low capital and per-sample costs of analysis. Over the last decade, we have developed immunoassays for more than 20 agrochemical compounds and have applied them to the analysis of food and environmental matrices. Our initial food targets were major grain protectant insecticides, including several organophosphates, carbamates, and synthetic pyrethroids. These compounds are intentionally applied to grain, but before grain can be traded and consumed, residues must have decreased to low levels. The assays have been incorporated into both laboratory and field test kits, and are marketed internationally. The assay for chlorpyrifos-methyl, an organophosphate, has official U.S. Environment Protection Agency approval as an analytical method for residues of this compound in maize, and is referenced on drum labels of the active ingredient. In late 1994, we started developing a suite of tests for organochlorines (dicofol, cyclodienes, DDT), organophosphates/carbamates (cholinesterase) and several fungicides used widely in viticulture, including benomyl/carbendazim, metalaxyl, benalaxyl, iprodione, triadimenol, fenarimol, penconazole, and propiconazole. A purpose-built immunoassay laboratory was established by the dried fruit industry and, in the 1996–97 and 1997–98 harvests, the laboratory routinely screened for organochlorines and organophosphates in dried grape samples from 90% of Australian growers. We are now developing assays for use for analysis of residues by medium and large wineries. Immunoassays have also been extensively used for environmental analysis. In the USA and Europe, they are used for analysis of persistent herbicides (e.g. triazines, acetanilides, ureas) in groundwater and of industrial chemicals (e.g. polychlorinated biphenyls, dioxins, polycyclic aromatic hydrocarbons) in soil. In contrast, our focus has been the analysis of surface water for chemicals used in irrigated rice and cotton farming. These chemicals include chlorpyrifos, diazinon, parathion-methyl, molinate, and endosulfan, and pyrethroids such as deltamethrin, cypermethrin, and cyhalothrin. In collaboration with environmental scientists, the assays have been used in studies of the drift, volatilisation, off-target loss, and loss during real and simulated rainfall. The ability of these assays to be both used on-site and to allow the analyses of high sample numbers enables dissipation processes to be much better understood than if a limited number of samples was analysed instrumentally at some later time in a remote laboratory. Other assays, such as for DDT and its metabolites, and benzoylphenylureas, will be valuable in mapping the distribution of these compounds in agricultural soils.

A WIDE range of immunoassays is available commercially (Gee et al. 1995; Skerritt 1995a,b), and several now have 'official analytical method' status. The major factor limiting the wider application in developing countries of the commercial assays that are being marketed by American and European companies is their high cost. The high costs arise largely from marketing costs and product margins, as the materials costs for these diagnostic tests are often under US$2 per test. However, several good assays have been produced and used by Asian scientists. This paper reviews the applications that we have made of immunoassays to food and environmental samples. Once the assay is developed, a critical step is application to the food or soil matrix under study.

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If the matrix produces interference, this often can be removed by further dilution of the extract, changing the extractant, using additives in the diluent, or by simple clean-up strategies. Immunoassay methods usually, but not always, require much less sample clean-up than instrumental methods. The assay should also be validated against the instrumental reference method using spiked samples and, if available, samples with incurred residues. Finally, before the ELISA can be used in other laboratories, the antibody, enzyme conjugate, and other components require stabilisation.

The most thoroughly developed applications of residue immunoassays in grain analysis are for the major mycotoxins. Typically, the synthetic chemistry for development of the assays is relatively straightforward (since many of the mycotoxins have alcohol or ketone groups that are suitable for derivatisation), and has been published, so that continued development of 'indigenous' assays in developing countries should be possible. Collaboration of experts from developed countries can assist in providing up-to-date information on test development methods, sampling, stabilisation of components, testing of assays, and the statistical and other analysis of results obtained using the new tests.

Application in Analysis of Grain Protectant Residues

Our initial focus has been on development of immunoassays for grain protectants. These insecticides include certain degradable organophosphates, carbamates, and pyrethroids which are intentionally applied to grains after harvest and before either bulk or bagged storage. Strict maximum residue limits (MRLs) or trade tolerances apply to the levels of the compounds that can be permitted to remain before the grain is marketed. Our research in this area, largely carried out between 1988 and 1992 (but with the technology transfer of tests for certain target compounds continuing), aimed to develop and apply rapid, simple, and inexpensive tests for detection of residues of key pesticides in wheat and barley grain and grain products by use of modern antibody-based and enzymic techniques. The pesticides include organophosphates (fenitrothion, chlorpyrifos-methyl, and pirimiphos-methyl), synthetic pyrethroids (bioresmethrin, phenothrin, deltamethrin, and cyfluthrin), carbaryl, and methoprene. These tests were designed to enable either rapid field testing to be done at the silo storage level, or, using microwell ELISA assays, in small company and regional laboratories where either instrumental pesticide analyses would not be feasible or equipment and staff to perform these analyses would not be available. Both formats enable quantitative pesticide analysis.

Organophosphate tests of appropriate specificity (specific for only the grain protectant under analysis) and sensitivity (detecting down to 0.1 ppm in grain) were developed for fenitrothion, chlorpyrifos-methyl, and pirimiphos-methyl. Initial experimentation with fenitrothion suggested that coupling of the agrochemical to the carrier protein via the aromatic ring did not produce antibodies of sufficient affinity or dynamic response to fenitrothion. A novel approach was developed, through synthesis of a heterobifunctional reagent, to couple organophosphates 'through the phosphate ester' (McAdam et al. 1992). This approach was also used for synthesis of haptens corresponding to the other two organophosphates. Other approaches for hapten synthesis were required for the pyrethroid insecticides, and tests detecting down to 0.02 ppm pyrethroid in grain were developed. Along with the development of simple test methods for the analysis, it was critical to develop simple methods for either laboratory or field extraction of the pesticides for use in the antibody tests. We were fortunate that most of the residue remained on the outer layers of the grain, and that earlier research had shown that mere soaking of whole grain or flour in methanol for 48 hours could quantitatively extract residues. For rapid silo (grain elevator) tests, this method was obviously too slow, but research indicated that blending ground grain for two minutes in a stainless steel Waring blender provided similar extraction efficiencies (Beasley et al. 1993; Edward et al. 1993). The assays for each agrochemical have been validated using wheat and barley grain samples with incurred pesticide residues (Edward et al. 1992; Fig. 1). In each case, good correlations have been obtained between the new test and laboratory instrumental methods.

Several of the grain protectants can persist into processed end-products, such as different bread and noodle types. Products such as wholemeal bead, which have a high bran content, also had high levels of residues, especially of the more chemically stable organophosphate pesticides such as pirimiphos-methyl. While total grain residues of synthetic pyrethroids were lower than that of organophosphates because they are applied at lower rates, the percentage of residue which persisted into end-products was

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higher. For most end-products, methods were developed for overcoming matrix interference without the need for sample clean-up. However, in order to obtain accurate results for matrices such as yellow alkaline noodles, residue determination in products had to be made with respect to standards prepared in a pesticide-free extract of the food product (Skerritt et al. 1996) .

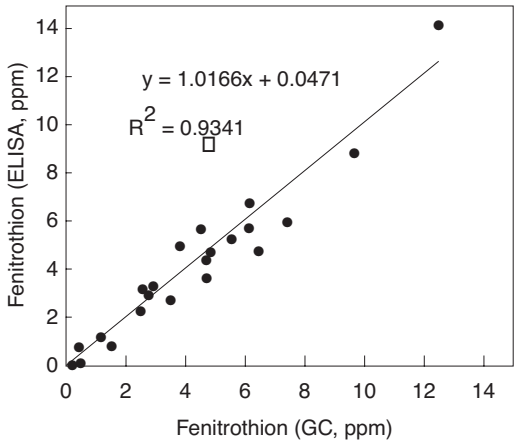

Figure 1. Analysis of incurred residues of fenitrothion in wheat grain using a rapid (10 minute) ELISA. Close correlations were obtained between the results of ELISA and GC determinations performed in independent laboratories.

Application in Analysis of Grapes, Dried Vine Fruits and Wine

The competition in export markets for dried vine fruits is fierce, so Australia has to sell 'on quality' rather than just price. Australia's European sultana (raisin) markets are a premium market with concerns about chemical residues being important. In addition, local markets are under threat from cheaper but inferior imported fruit. Grape production requires use of a range of insecticides and fungicides, integrated with non-chemical approaches for pest management. Residues of some of these compounds could be concentrated onto the dried, finished product, providing up to four-fold higher levels than in fresh grapes, so close monitoring is important. The possible presence of residues has become of major concern to Australian fruit export customers in North America and Europe. For several years, the Australian dried fruit industry tested fruit from individual growers by gas-chromatography for organochlorines only. This process was slow (as it normally takes at least 4 days for results to be returned) and expensive. Detection of only the organochlorines was an obvious weakness since residue violations of other common chemicals (e.g. those for downy mildew) could go undetected. New requirements, such as mandatory reporting of spray application details, retention of deliveries for traceback, and limitations on the range of chemicals allowed by our export customers demand more extensive monitoring to be done, but in a very cost-effective manner. We were approached by the grape industry to develop immunoassay methods to enable accurate on-site testing with results available in hours rather than days. The lower cost per test also allows screening over a wider range of problem pesticides, as well as more samples from each grower.

We have also developed protocols for the measurement of pesticide residues on dried fruit. These tests can accurately determine whether fruit is residue-free or whether residues exceed Codex MRLs, or other set limits. The tests enable on-the-spot quality control by relatively untrained staff before packing of the final dried product. Tests have been or are being developed or adapted for compounds that are (1) widely used, (2) applied late in season as a follow-on or pre-harvest spray, (3) more chemically persistent, and/or (4) are of special toxicological concern to our overseas markets. Tests are being developed or applied for the following groups of residues:

- *organochlorines* such as the permitted miticide, dicofol, as well as DDT, dieldrin, and endosulfan.
- *light brown apple moth and mealy bug insecticides*—carbaryl and organophosphate insecticides (diazinon, chlorpyrifos, and methidathion). This is carried out using a cholinesterase inhibition screening test rather than antibody-based assays.
- *powdery mildew agents*—especially fenarimol and triadimenol.
- *downy mildew agents*—especially dithiocarbamates (these may require a colorimetric test), metalaxyl, and benalaxyl.
- Botrytis *fungicides*—iprodione and benomyl.
- Phomopsis *fungicides*—dithianon.

In addition, tests for penconazole, an azole fungicide, and copper are being developed for wine and wine grape analysis. An immunoassay screening lab was established by the dried fruit industry in late 1996 in Mildura, a regional centre in Victoria, Australia, which is over 500 km away from the state capital, Melbourne, and its sophisticated residue analysis laboratories. In the initial season of operation, dried

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fruit was screened for organochlorines, organophosphates, and carbamates. Fungicide residues will be screened in subsequent seasons. To minimise the number of screening tests that had to be performed, a combined screening test was developed for the organochlorines. Alternating columns of a 96-well plate are coated with antibodies to either DDT/dicofol and cyclodiene organochlorines, such that the nature of the contamination can be determined. The test is used as a screening method rather than a quantitative assay. The results are analysed with respect to a 'low pesticide' standard, containing a mixture of 0.5 ppm dicofol, 0.05 ppm dieldrin, and 0.1 ppm DDT (after spiking into the fruit) and a 'high pesticide' standard, containing a mixture of 5 ppm dicofol, 0.5 ppm dieldrin, and 1 ppm DDT (in fruit). The spiked fruit samples are extracted in the same manner and analysed at the same time as the test samples.

One analyst in the screening facility was able to test up to 100 fruit samples daily, according to the following routine: 3:30 p.m. fruit received; 4:00 p.m. samples scooped into flasks, methanol added, and flasks stoppered; residues extracted by shaking 30 seconds then standing overnight; 9:30 a.m. next day, aliquots loaded in duplicate onto microwells for each assay; 12:00 noon, assays completed, suspect samples are quarantined. An example of data from an analysis of fruit samples and the criteria used for scoring samples is shown in Table 1. In the first year of operation, each fruit sample was also sent by courier to Melbourne for organochlorine gas chromatography analysis, and results returned 1 week later. Every sample that was positive for organochlorines by GC had already been detected as a positive by ELISA. The wine industry is also a marketer of premium products and, in most countries, is a much larger user of grapes than the drying industry. Recently, we have started to adapt the test methods to wine, grape juice, and must, and to develop new tests for agrochemicals of special interest to the wine industry. Examples of the performance of assays of wine for two fungicides, iprodione and triadimenol, are shown in Figures 2 and 3.

Approaches to the Removal of Matrix Interference

With food matrices such as most grains, grapes, dried grapes, and wine, the simplest means of accounting for sample matrix interferences involves comparison of results obtained with samples with standards prepared in an extract of a known pesticide-free sample of the same food. The methods that do not use sample treatment or clean-up include: (1) a comparison of different extraction solvents; (2) dilution of extracts before assay; (3) addition of protein and/or detergent to assay diluent; and (4) inclusion of wash steps between sample and conjugate incubation steps.

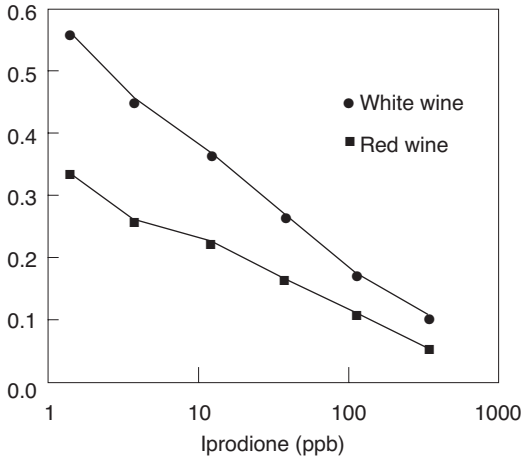

Figure 2. Standard curves for iprodione standards prepared in undiluted red wine or white wine. Dilution of the wine increased colour development in the assay.

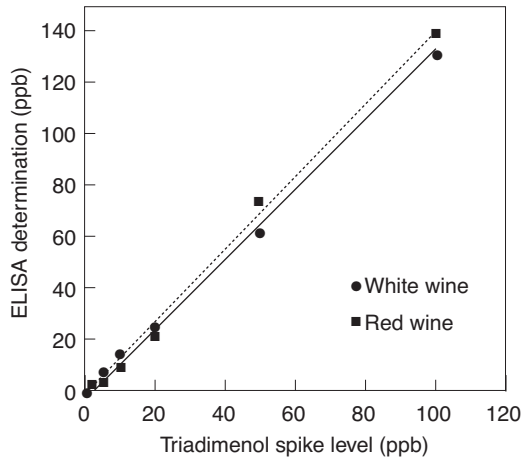

Figure 3. Recovery of spikes of triadimenol in red or white wine

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Table 1. Sample ELISA absorbance results and their interpretation of results for prototype organochlorine screening tests for dried vine fruit.

Column 1. DDT/dicofol antibody-coated	Column 2. cyclodiene antibody-coated
pesticide-free control DDT/dicofol 1.00	pesticide-free control cyclodiene 1.22
low standards 0.70	low standards 0.91
high standards 0.26	high standards 0.55
pesticide-free control DDT/ dicofol 1.05	pesticide-free control cyclodiene 1.19
sample 1 1.04	sample 1 1.17
sample 2 1.06	sample 2 1.13
sample 3 0.70*	sample 3 1.15
sample 4 0.40*	sample 4 0.88*

Sample 1. The result in column 1 is not different (> 80 %) from the control DDT/dicofol result, nor is the results in column 2 different from the control results for cyclodienes. Conclusion: the sample is free of detectable levels of these organochlorines

Sample 2. The result in column 1 is not different (> 80 %) from the control DDT/dicofol results, but the result in column 2 is significantly (< 80 %) lower than the corresponding control result for cyclodienes. Conclusion: the sample is free of detectable levels of dicofol and DDT, but has cyclodienes at about the same level as the low standard.

Sample 3. The result in column 2 is not different (> 80 %) from the control cyclodiene results, but the result in column 1 is significantly (< 80 %) lower than the corresponding control result for DDT/dicofol. Conclusion: the sample is free of detectable levels of cyclodienes, but has dicofol and/or DDT at about the same level as the low standard.

Sample 4. The results in columns 1 and 2 are significantly (< 80 %) lower than the corresponding control results for DDT/dicofol and cyclodienes. Conclusion: the sample has dicofol and/or DDT between the levels of the low and high standards and cyclodienes at about the level of the low standard.

However, for some more complex food matrices, such as spices, milk, tea, and coffee, this approach is not suitable, and steps must be added to the sample preparation procedure to remove matrix interference. With extracts that have been prepared in water-miscible solvents, approaches that can be assessed include: (1) selective removal (adsorption or partition) of interfering components; or (2) partitioning the extract to hexane or dichloromethane to remove water-soluble interfering substances; or (3) adsorption (reversed-phase) on carbon-loaded octadecylsilica, and elution with a more hydrophobic solvent. In each case, as well as confirming that interfering substances have been removed, the recovery of the target agrochemical residues needs to be checked. Approaches to the removal of sample matrix interferences have been reviewed in more detail elsewhere (Skerritt and Rani 1996).

Test Methods for Agrochemicals Used in Irrigated Agriculture

Table 2 provides a comparison of immunoassay methods and instrumental analyses. In North America and Europe, the majority of pesticide monitoring of water is carried out on groundwater, since this is a major source of water used in agriculture and also a major sink for chemicals and fertilizers used in agricultural production. In keeping with the importance of groundwater and the major crops treated (maize and soybean), many groups have developed immunoassays for active ingredients such as triazine and acetanilide herbicides. In Australia, however, most agricultural water is surface water, and this is also the most likely destination for chemical losses. Since 1991, we have had a program of research on the development, testing, and use of ELISA kits for detection in the field of pesticides in groundwater, irrigation surface run-off, and stock and domestic water supplies. The methods will contribute to safer water recycling and storage practices, especially in the cotton, rice, and horticultural industries. The project has had three main stages: development of immunoassay field tests for the range of target pesticides; validation of the assay in the laboratory with field water samples (cross-checking results obtained with data from instrumental analyses); then (with collaborators) using the methods in the field to gather information on agrochemical dissipation and water quality.

The type of applications and field testing work required depended on both the properties of the chemical, and the industry to which it is intended to apply the test. In the cotton industry, the major water management

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problem involving chemicals, is the toxicity to fish of the endosulfan and pyrethroid insecticides aerially applied for several months during maturation of the crop. In the face of increasing government, media, and public scrutiny of environmental contamination, use of certain insecticides for control of *Helicoverpa (Heliothis)* remains critical to the viability of the Australian cotton industry, as even the current genetically-engineered varieties do not provide full insect protection without use of chemical insecticides. Tailwater from irrigation is recycled on-farm, but on-farm storage capacity may not be sufficient for run-off during the severe storms that are not uncommon during the growing season, and developing enterprises may not be able to afford the investment required for on-farm recycling. The costs of storing all storm run-off on-farm make that approach unworkable. Options include: (1) retention of a portion of run-off (the 'first flush'); (2) filtering of run-off to remove sediment containing adsorbed pesticides; and (3) temporary storage, delaying discharge until the pesticide had broken down or been diluted. It is not possible to satisfactorily select among the options for particular farms or irrigation systems until more data are obtained in the field on pesticide concentrations in run-off water. It is only practicable to obtain suitable amounts of such data using a field test method, such as an immunoassay. Simple test methods will enable industry monitoring of residues after spraying and, where required, immediate clean-up of contamination from suspected escape of tailwater, and, as a spraying management tool, tailoring of spraying practices for specific properties or areas. In work carried out in a collaboration with Professor Ivan Kennedy (University of Sydney), these tests have been very useful in environmental research on the dissipation of cotton agrochemicals; immunoassay methods have been able to monitor residue run-off as it occurs, and to map the dissipation of the chemicals at high density. They have provided us with a more detailed understanding of the environmental behaviour of the chemicals than would otherwise have been possible (Lee et al. 1997).

For irrigated rice, unlike in the cotton industry, some water from paddies returns to irrigation channels during the season when pesticides are used (especially from flushing of drill-sown crops). Like much intensive cropping, the rice industry is highly depen-

Table 2. Comparisons of methods for soil and water testing

Method	Instrumental	Immunoassay
Detection	Gas chromatography–electron capture detection or mass spectroscopy. High performance liquid chromatography–UV absorption	Colour change
Sensitivity in water	0.01–1 ppb after sample extraction/concentration	0.01–1 ppb without sample preparation
Organic solvents required(?)	Yes	No
Clean-up of extracts	Water — sometimes Soil — usually	Water — rarely Soil — sometimes
Can be quantitative(?)	Yes	Yes
Simultaneous analysis(?)	Of several residues in a small number of samples	Of a few residues in many samples
Capital cost	$ 20000– 200000	$ 1000–20000
Cost per test	$ 25–200	$ 2–10
Field use	Limited	Suitable

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dent on the application of certain herbicides and insecticides. The use of these close to water can lead to their appearance in surface irrigation waters and subsequently in inland river systems, whose water is relied on by a wide range of users. The variable persistence of rice herbicides such as molinate (affected by temperature, soil type, water turbidity and rainfall) can make predictions of residues very inaccurate. The rice insecticides, chlorpyrifos, diazinon, and malathion, can damage aquatic ecosystems by affecting zooplankton, invertebrate populations, and fish, while herbicide residues can make irrigation water unsuitable for crop use. Some rice herbicides are also toxic to fish, or reduced drinking water quality. Using antisera from collaborators, we also developed tests for soil-applied uracil and urea herbicides used in citrus horticulture and vegetable gardening, and for weed control along banks of irrigation ditches. These may percolate into shallow groundwater which is recycled into the surface water system, as well as contaminate surface waters directly by run-off. Residues of these herbicides have been detected in surface waters of the Australian Murrumbidgee Irrigation Area. This probably arises from poor management of the use of ureides to control weed growth between seasons in dry irrigation channels, as well as variable release from soil used for horticulture. A summary of the various tests that we developed for insecticides and fungicides is provided in Tables 3 and 4.

Table 3. Assays developed by CSIRO for insecticides and insect growth regulators

Compound	CSIRO chemistry	Food matrices	Environmental matrices	Reference
Organophosphates				
fenitrothion	✔	grain, cereal foods		McAdam et al. 1992
parathion	✔	rice, fruit, vegetables	water	Skerritt and Lee 1996
methyl-parathion	✔	rice, fruit, vegetables[a]		Skerritt and Lee 1996
chlorpyrifos	✔		water, soil	Hill et al. 1994
chlorpyrifos-methyl	✔	grain, cereal foods		Skerritt et al. 1992b Edward et al. 1993b
pirimiphos-methyl	✔	grain, cereal foods		Skerritt et al. 1992b
diazinon	✔	fruit juices	water, lanolin	Beasley et al. 1997
malathion	✔			
Organochlorines				
cyclodienes	✔	fruit, veg, fatty foods	water, soil	Skerritt and Rani, 1996
endosulfan	✔	fruit, veg, grain, fats[a]	water, soil	Lee et al. 1995a, 1997
DDA/DDT	✔	milk, fruit, vegetables[a]	soil	Larkin et al. 1994
DDE/DDT	✔	milk, vegetables[b]	water, soil	Beasley et al. 1998a
dicofol	✔	fruit		Beasley et al 1998a
HCH metabolites	✔		water, soil	Beasley et al. 1998b
Synthetic pyrethroids				
bioresmethrin	✔	grain, cereal foods		Hill et al. 1993
phenothrin/ permethrin		grain, cereal foods		Skerritt et al. 1992a
deltamethrin	✔	grain	water, soil	Lee et al. 1998a,b
cypermethrin/ cyhalothrin	✔			Lee et al. 1998a,b
Insect growth regulators				
methoprene	✔	grain, cereal foods		Hill et al. 1991; Edward et al. 1993a
benzoylphenylureas	(Sydney Univ.)		water, soil	Wang et al. 1998

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Table 4. Assays developed by CSIRO and collaborators for herbicides and fungicides

Compound	CSIRO chemistry	Food matrices	Environmental matrices	Reference
Herbicides				
molinate			water	Guihot et al. 1996 Leet et al. 1998
diuron			water, soil	Lee et al. 1995b Lee et al. 1998
thiobencarb			water	
triclopyr	✔		water	
2,4-D	✔			
bromacil			water	
Fungicides				
benomyl/carbendazim	✔	grapes, wine		
metalaxyl	✔	grapes, wine		
triadimenol	✔	grapes, wine		
iprodione	✔	grapes, wine		
fenarimol	✔	grapes, wine		

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Developing Immunoassays in a Developing Nation: Challenges and Success in India

N.G.K. Karanth, Akmal Pasha, B.E. Amita Rani, M.B. Asha, C.G. Udayakumari, and Y.N. Vijayashankar*

Abstract

Pesticide residues have been detected in fruits, vegetables, grains, and oilseeds in India. Plantation products with high export potential, like tea, cashew nuts, coffee, sesame seeds, and groundnuts, also contain pesticide residue and face export problems. Analysis of pesticide residues by conventional methods such as GC and HPLC is a time-consuming process. It requires expertise and expensive equipment and maintenance. Hence, development of newer techniques like ELISA is important for developing countries like India.

The major organochlorine pesticide residues in India are DDT, DDE, endosulfan, and HCH. Fungicide residues of thiram, benomyl, carbendazim, and copper are also encountered. In addition, there are lesser amounts of contamination by organophosphates and pyrethroids. The challenges in developing ELISA-based systems lie in the infrastructure and expertise needed to produce haptens, protein, conjugates, and antibodies. With CSIRO expertise and ACIAR funding support, however, we have been successful in formatting assays for endosulfan, DDT, DDE, parathion, and carbendazim, with the antibodies supplied by CSIRO. This is the first laboratory to acquire such a facility in India. The initial challenge was to develop clean-up methods for the co-extractives originating from important foods. The matrix-clean-up procedure varied with commodity and pesticide and hence was labour intensive. The second challenge was to make at least one antibody for one pesticide in the extended period of 18 months. With the same collaboration, we have now been able to make three antibodies in India: for endosulfan, DDT, and carbendazim. This paper describes the challenges and success in developing ELISAs for pesticides in India.

IMMUNOASSAYS based on antibody–antigen reaction have become very useful tools in clinical tests and pharmacology laboratories. The same technology is now becoming popular for the detection of environmentally harmful chemicals. Pesticide immunoassays have, in the past two decades, made much progress and appear to be appropriate for the detection of trace quantities of residues usually present in biological samples. The method involves particular antigen–antibody reactions and hence is sensitive and specific. It is rapid and offers itself to be a method of choice especially when a large number of samples has to be analysed. The test can be performed by any laboratory technician after very little training, as the procedure involves simple steps such as dilutions, additions, washings, and reading colour intensity. Considering the need for quality assurance of export foods with respect to residue contamination on and 'lot to lot' or 'pack to pack' basis and also considering the large number of samples for analysis, the ELISA is the method of choice for use in the risk management of foods.

Until the Central Food Technological Research Institute (CFTRI) started a joint collaborative international research project with CSIRO in 1993 funded by ACIAR Australia (Project No. 9309), there was no research on the development of the ELISA method for pesticide residue analysis in India. In this Indo-

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Australian project we accepted the challenges of formatting the assay in India; initially using the antibodies developed at CSIRO to undertake labour-intensive work on studying the food matrix interference and to develop clean-up procedures, and finally to develop antibodies at CFTRI for pesticides of importance to India. The paper gives an overview of this successful project.

Materials and Methods

Most of the reagents and chemicals are of highest purity, antibodies and horseradish peroxidase enzyme conjugates for endosulfan, ethyl parathion, methyl parathion, DDT and DDE were initially procured through CSIRO Canberra, Australia.

Haptens and antibodies for three assays of Indian origin

Synthesis of haptens

Preparation of hapten-1 for carbendazim: 2-Aminobenzimidazole (1.33 g, 0.01 M) was dissolved in acetonitrile (40 mL) and succinic anhydride (1.00 g, 0.01 M) was added to the solution. The mixture was stirred at 40°C for 3 hours. The heavy precipitate was washed with distilled water and the residue was suspended in boiling methanol (50 mL), filtered, and air-dried. 2-succinimidobenzimidazole (1.48 g, 63.56%) was obtained as a fine crystalline solid.

Preparation of hapten-1 for endosulfan: Endosulfan-diol was dissolved in dry pyridine to which succinic anhydride and dimethylaminopyridine were added (Lee et al. 1991). The mixture was stirred overnight. Ethyl acetate was added to the mixture and the organic layer was successively washed with 1 M HCl, water, and brine, and dried over anhydrous magnesium sulfate. The product was analysed by thin-layer chromatography (TLC) using cyclohexane as eluent and o-tolidine as the chromogenic reagent. The solvent system was later modified to hexane: ethyl acetate:acetic acid: 50:49.9:0.1. The product contained four compounds in addition to the unreacted endosulfan diol. The R_f values were as follows:

Endosulfan-diol:	0.14
Compound 1:	0.16
Compound 2:	0.18
Compound 3:	0.21
Compound 4:	0.27

It was found by TLC that the required hapten formed in low yield was compound 3. This mixture was subjected to silica gel column chromatography using petroleum ether:ethyl acetate:glacial acetic acid: 50:49.9:0.1. Twenty-one fractions, each 10 mL, were collected, of which fractions 9–18 contained pure hapten-1.

Preparation of hapten-2 for endosulfan: Heptachlor was dissolved in acetic acid and tert-butyl acetate was added to the mixture. It was refluxed in a water bath. Crystals of the hapten precursor were obtained (mp 238°C). The hapten precursor (1.09 g) was dissolved in methanol and HCl gas was passed through the solution. The mixture was refluxed for 1 hour. HCl gas was passed again and the mixture was refluxed overnight. Methanol was evaporated off, diethyl ether added, and the mixture washed with water and sodium bicarbonate solution. The organic layer was dried over anhydrous calcium chloride and the ether removed. The product was analysed by TLC.

The hydroxy derivative (0.426 g, 1 mmol) was dissolved in pyridine (2 mL) and succinic anhydride (150 mg, 1.5 mmol) was introduced to the mixture followed by DMAP (20 mg) catalyst. The contents were stirred overnight. Ethyl acetate (30 mL) was added and the mixture was washed with 1 M HCl, water, and brine. The organic layer was dried over anhydrous magnesium sulfate and the solvent removed under vacuum. The product was obtained as colourless crystals. It was analysed by TLC (Lee et al. 1991).

Preparation of hapten-1 for DDT from DDT-OH: Bis(4-chlorophenyl)ethanol (DDT-OH) was dissolved in pyridine and succinic anhydride (10 molar excess) was added to the mixture, stirred overnight at 0°C and ethyl acetate was then added. It was washed with water, 1 N HCl, and brine. The organic solvent was dried over anhydrous sodium sulfate and solvent evaporated off to get the hapten-1. It was analysed by TLC and the structure was confirmed by ^{1}H NMR.

Preparation of hapten-2 for DDT from DDA-gamma-aminobutyric acid: 2,2-Bis(4-chlorophenyl)acetic acid (DDA) was converted to the acid chloride and dissolved in benzene. Gamma-aminobutyric acid dissolved in 1 M NaOH was added and the mixture was stirred overnight. The solvent was evaporated off. The residue was acidified with 1M HCl, and then extracted with ethyl acetate. The organic layer was washed with water and brine was dried over magnesium sulfate to get a product which was analysed by TLC using the same solvent system given before to get pure hapten-2 at a R_f value of 0.17. The yield was over 95 %. The structure was confirmed by ^{1}H NMR.

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Purification of the DDA-GABA hapten-2: The hapten was purified by silica gel column chromatography by collecting 11 fractions (10 mL each) Fractions 5 and 6 contained the unreacted DDA and pure hapten was collected in fractions 7–11. A further collection of 100 mL each of fractions 12 and 13 also had the hapten. The latter fractions were pooled to obtain pure hapten. The carboxylic acid haptens were converted to active esters by treating with N-hydroxysuccinimide in the presence of 1,2-dicyclohexylcarbodiimide in dry dichloromethane.

Preparation of hapten conjugates with carrier proteins: 2-Succinimidobenzimidazole (2 AB-hapten for carbendazim) was conjugated to two proteins viz., bovine serum albumin and ovalbumin by dissolving the 2-AB in alkali and adding to the proteins in aqueous medium followed by adding 1-ethyl-3-(3-dimethylaminopropylcarbodiimide. The conjugates were dialysed using three changes in phosphate buffer saline. The conjugation was tested by the estimation of the e-amino groups of the lysine residues by conjugating with 2,4,6- trinitrobenzenesulfonic acid and reading the absorbance at 335 nm. (Sashidhar et al. 1994). Conjugation was confirmed for both the proteins.

The other conjugates were prepared by reacting active esters with proteins in DMF.

Antibody production

One milligram equivalent of the hapten protein conjugate in Freund's complete adjuvant (1:1 ratio) was given epidermally at multiple sites to a female rabbit as a primary dose. After 30 days the primer bleed was checked for the antibodies by antibody capture assay (ACA). If the antibody titre was found satisfactory, a booster dose was given [500 μg eq. wt. protein in incomplete Freund's adjuvant (IFA)] by intramuscular injection. After 8 days, 3–5 mL blood were drawn from the marginal ear vein and checked for antibody titre. If the titre had increased, a second booster was given in IFA by intramuscular injection to thigh muscle. After 8 days, the antibody titre was again checked. Since the peak antibody production was noticed at this time, 15–20 mL blood was collected and the serum separated. Serum IgG antibody was purified using Gamma bind sepharose column (Pharmacia). The protein content was estimated spectrophotometrically at 280 nm.

Results and Discussion

Reproduction of assay format in CFTRI

After initial training at CSIRO, Canberra we could format the ELISA at CFTRI and reproduce the technique at the same level of precision for the analyses of endosulfan, heptachlor, methyl parathion, ethyl parathion, DDT, and DDE (Table 1).

CFTRI is the first institute to acquire such a facility in India. After having acquainted with the method, the challenge was to identify matrix effect of food samples and to develop clean-up procedure.

Challenge 1: Identification of food matrix effect and development of clean-up procedures

Food matrices offered challenges to develop clean-up procedures. Due to a wide range of food composition, the research became labour intensive. For the study, samples were classified into five categories: high moisture–low fat; low moisture–low fat; low moisture–high fat; high moisture–high fat; and coloured foods.

Trace amounts of pesticide residues present in food first have to be dislodged by solvent extraction. In this procedure, a number of constituents from the food (the matrix) also get partitioned along with the pesticide, which may change the assay performance

Table 1. Precision of reproduction of ELISA for pesticides at CFTRI, Mysore

	Endosulfan		Methyl Parathion		Ethyl Parathion		DDT		DDE	
	CFTRI	CSIRO	CFTRI	CSIRO	CFTRI	CSIRO	CFTRI	CSIRO	CFTRI	CSIRO
Antibody	R~KLH-Endo-diol	ditto	R~KL	ditto	R~KLH	ditto	'R~OA-DDA	ditto	R~DDE COOH - KLH	ditto
HRP conc.	1/500	1/30000	1/300000	1/100000	1/800000	1/800000	1/300000	1/200000	1/90000	1/400000
Zero OD	0.99	1.1	2.67	02.3	1.43	2.1	1.19	1.33	1.1	0.98
IC_{50} (ppb)	8	1.1	0.2	0.86	1	4.0	70	400	12	33

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either negatively or positively. This is called the 'matrix effect' (Skerritt and Rani 1996). The interference from the matrix needs to be determined and clean-up procedures developed so that ELISA for pesticide residue analysis can become an accepted official method.

Approaches to matrix clean-up

Although matrix effect determination and clean-up procedures were developed by earlier researchers, the methods were directed towards water and soil extracts. We have attempted a food matrix study. The approaches made for clean-up were derived from sample preparation methods for gas chromatographic (GC) analysis of pesticides. Of the several methods tested, the following methods were successful.

(a) Dilution of the extracts with PBS-FG [50 mM sodium phosphate - 0.9% NaCl, pH 7.2 containing 0.5% Teleostean fish skin gelatin (FG, Sigma. St. Louis, MO)]

(b) Change of extractants. Instrumental multiresidue methods have recommended methanol (Sharp et al. 1988), and acetonitrile (Storherr and Watts 1971) as initial extractants for plant food matrices.

(c) Modified Tsumura's method. The food extract was treated with the coagulating reagent (Tsumura et al. 1994) and potassium phosphate buffer (Skerritt and Rani 1996), partitioned with petroleum ether, evaporated and residue was taken in acetonitrile and was tested.

(d) One Step C18 column (Waters Sep Pak Vac) column clean-up method (Lee et al. 1991). This method has been modified to the extent that the extract in acetonitrile passed through C18 column, partitioned with petroleum ether and the residue was taken in the original extractant.

(e) Sulfonation is a well known method used for extracting the pesticides from fat by acid treatment . (Murphy 1972).

(f) Alcoholic ethanol treatment is also a method employed for extracting pesticides from fat by treatment with 10% KOH-ethanol solution (Hernandez et al. 1987).

Matrix effect and clean-up procedures

High moisture–low fat foods. Cauliflower, cabbage, tomato, green grapes, and blue grapes were studied. The routine method of methanol or acetonitrile extraction and dilution with PBS-FG did not show any matrix effect in the ELISA of all the five pesticides examined, namely endosulfan, methyl parathion, ethyl parathion, DDT, and DDE. The colour intensity and IC_{50} values of the pesticide-free methanol standard (Table 2) was comparable with that of methanol extract of the food.

Low moisture–low fat foods. Basmati rice, paddy rice, button mushrooms, and oyster mushrooms are some of the export commodities of India which were examined under this category. As many as 10 approaches, such as USDA method, column clean-up method, different solvents and their mixtures were tested with very little success. However, the clean-up was achieved using the C18 column method in the case of paddy rice and Basmati rice. Changing the extractant from methanol to petroleum ether removed the matrix effect in mushrooms, as shown in Table 3, as judged by the comparable IC_{50} value as well as colour intensity at zero pesticide concentration. However, the recovery in the spiked samples was less than 50%. A similar trend was seen with all the five pesticides studied

Low moisture–high fat foods. Cotton seed was selected for this category. Here again, change of extraction solvent, column chromatography with Cellufine, LH-20, Sephadex, Florosil, silica/charcoal (Haddad 1989) neither removed the matrix effect nor improved the sensitivity of the assay. Treatment of acetonitrile extract with concentrated sulfuric acid, washing with water (sulfonation), partitioning to petroleum ether, followed by evaporation and residue dilution with acetonitrile proved helpful, as shown in Table 4. This procedure, called the sulfonation method, was applicable to ELISA for endosulfan, methyl parathion, and ethyl parathion.

High moisture–high fat foods. Milk and butter were analysed for this group. Acetonitrile extract of milk had no matrix effect while the sulfonation/alcoholic KOH treatment resulted in good clean-up (Table 5) for butter in methyl and ethyl parathion, DDT, and DDE assays.

Coloured foods. Tea, coffee, and leafy vegetables such as spinach were studied. Methanol, acetonitrile, or acetone extracts showed matrix interference. One-step purification by passing the extract through C18 column gave good clean-up. However, this procedure was expensive compared with modified method of Tsumura. Tsumura's method was found to be equally good (Table 6) but involved more steps to achieve clean-up. However we have sufficiently reduced the number of steps as shown in Fig. 1 and is available as an appropriate method.

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Table 2. Matrix clean-up in high moisture–low fat foods

	Methanol/ acetonitrile standard		Cabbage extract	
	Zero OD	IC_{50} (ppb)	Zero OD	IC_{50} (ppb)
Methyl parathion	2.4	0.2	2.76	0.2
Ethyl parathion	1.37	2	1.11	3
DDT	1.19	37	1.14	40
DDE	1.2	15	1.09	15
Endosulfan	1.68	3	1.33	5

Table 3. Matrix clean-up in low moisture–low fat foods

	Acetonitrile standard		Paddy rice extract	
	Zero OD	IC_{50} (ppb)	Zero OD	IC_{50} (ppb)
Methyl parathion	1.5	2	0.85	3
Ethyl parathion	1.0	0.25	0.96	0.3
DDT	1.19	18	1.10	20
DDE	2.2	5	2.1	4.1
Endosulfan	1.77	12	1.8	9

Table 4. Matrix clean-up in low moisture–high fat foods

	Acetonitrile standard		Cottonseed extract	
	Zero OD	IC_{50} (ppb)	Zero OD	IC_{50} (ppb)
Methyl parathion	1.3	2	0.91	3
Ethyl parathion	0.9	0.2	0.69	0.3
Endosulfan	1.36	3	1.34	1.4

Table 5. Matrix clean-up in high moisture–high fat foods

	Methanol standard		Butter extract	
	Zero OD	IC_{50} (ppb)	Zero OD	IC_{50} (ppb)
Methyl parathion	1.2	1	1.2	1.3
Ethyl parathion	2.0	14	2.0	13
DDT	2.7	12.4	2.2	15
DDE	1.1	13	1.17	11

Table 6. Matrix clean-up in coloured foods

	Acetonitrile standard		Coffee extract	
	Zero OD	IC_{50} (ppb)	Zero OD	IC_{50} (ppb)
Methyl parathion	0.64	1.1	0.66	1.5
Ethyl parathion	2.6	0.3	2.0	0.3
Endosulfan	1.44	3.7	2.3	1

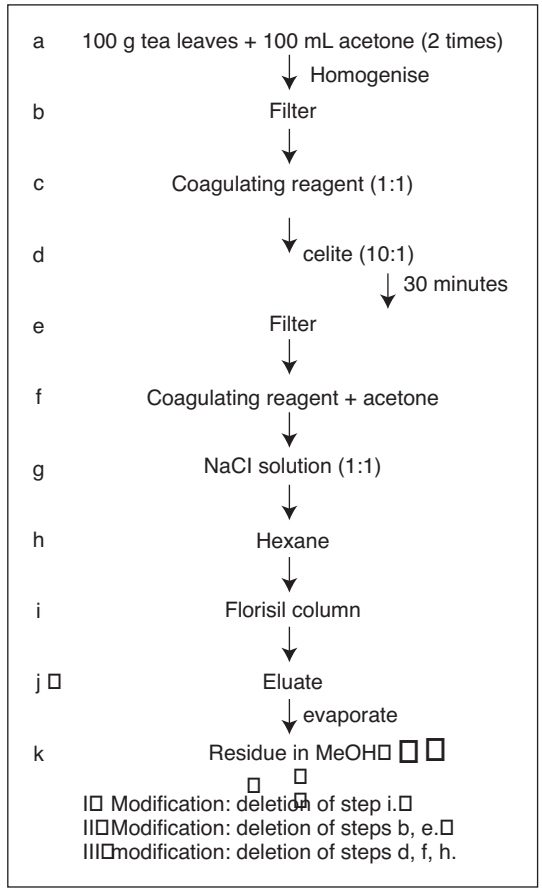

Figure 1. Tsumura's method and modifications: in step a, acetone was replaced with ACN and in h, hexane was replaced with petroleum ether.

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Table 7. Universal clean-up procedures for different classes of foods

	Endosulfan	DDT	DDE	MP	EP
High moisture	a Cauliflower	a Cauliflower	a Cauliflower	a Cauliflower	a Cauliflower
Low fat	a Cabbage	a Cabbage	a Cabbage	a Cabbage	a Cabbage
	a Blue grapes	b Blue grapes	b Blue grapes	b Blue grapes	b Blue grapes
	a Green grapes	b Green grapes	b Green grapes	a Green grapes	a Green grapes
	a Tomato	a Tomato		a Tomato	
	c Spinach	a Spinach		a Spinach	c Spinach
Low moisture	d Paddy Rice	d Paddy Rice	d Paddy Rice	d Paddy Rice	d Paddy Rice
Low fat	d Basmati rice	d Basmati rice	d Basmati rice	d Basmati rice	d Basmati rice
	b Button mushroom	b Button mushroom	b Button mushroom	b Button mushroom	b Button mushroom
	b Oyster mushroom	b Oyster mushroom	b Oyster mushroom	b Oyster mushroom	b Oyster mushroom
Low moisture High fat	e Cottonseed			e Cottonseed	e Cottonseed
High moisture		a Milk	a Milk	a Milk	a Milk
High fat		f Butter	f Butter	f Butter	f Butter
Coloured foods	c Tea	c Tea	c Tea	c Tea	c Tea
	c Coffee	c Coffee	c Coffee	c Coffee	c Coffee

a: Dilution of the extracts b: Change of extractants c: Modified Tsumura's method d: One step C18 column clean-up method
e: Sulfonation f: Alcoholic ethanol treatment

In this study, 15 food samples of five distinct categories were analysed for five pesticides. A total of 58 pesticide–matrix combinations was examined. Based on the results, a universal procedure was designed as described in Table 7.

Recovery data derived from ELISA was compared with GC data. In this study, spiked samples were extracted and involved cauliflower, cabbage, Basmati rice, tea, and coffee as shown in Figure 2. The recoveries compared well and were in the range 72–85% for both methods.

Laboratory prototype kits were assembled and used for demonstration/validation in 12 R&D centres spread throughout India. Universal clean-up procedures for removing the matrix effect of plant-derived foods have been designed. These are very simple procedures involving one step in most cases. Based on the confidence gained by the successful demonstration in different parts of the country, ELISA is now viewed as a versatile method for pesticide residue analysis that is adaptable by developing countries so that it becomes an appropriate method for on-site pesticide residue analysis, in laboratories, marketplaces, and at the farm-gate. Hence, the first challenge has become a success story.

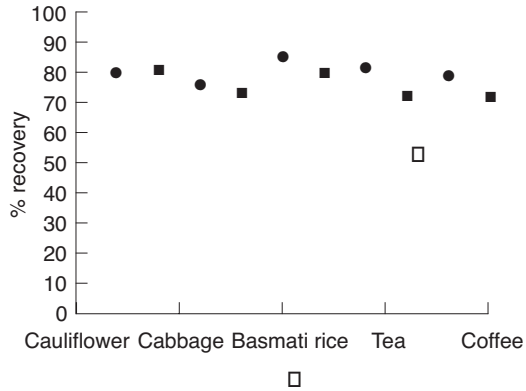

Figure 2. Comparison of endosulfan recovery by GC and ELISA: ● = ELISA; ■ = GC.

Challenge II: Development of indigenous antibodies at CFTRI for selected pesticides

It has been established that most of the food samples in India contain pesticide residues, especially DDT and DDE. Simple, rapid ELISA methods based on indigenous antibodies for pesticides were not available until the ACIAR-funded project started in India. We agreed to produce antibodies in CFTRI for pesti-

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cides of interest to India. We have produced five haptens successfully for three pesticides as described under materials and methods and have developed antibodies. We have also formatted the laboratory prototype kits based on these antibodies and tested their performance. The assays for the three pesticides viz, carbendazim, endosulfan, and DDT are comparable with those produced at CSIRO, Australia. We are aware that we have only just started in this direction and much more has to be achieved. However, we have successfully completed the challenge we accepted under the program and are confident that we can successfully undertake similar challenges.

The research funded by ACIAR, Australia and collaboration with CSIRO Plant Industry, Canberra have given us an opportunity to face the new challenges that must be met in all developing countries and India in particular. During the tenure of the project, we have gained confidence to develop immunoassays for pesticides of regional importance with, of course, the all-round support of ACIAR and CSIRO, Australia.

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Application of Competitive Enzyme Immunoassay for Screening DDT and DDE in Food and Environmental Samples: Initial Collaborative Study

B. Maestroni*, J.H. Skerritt*, and I.G. Ferris†

Abstract

DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane)) is a persistent organic pollutant that accumulates in food chains. Virtually every sample of breast milk analysed contains DDT and a persistent metabolite, DDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene). Food is the main source of contamination. In developing countries, the FAO/WHO acceptable daily intake (ADI) for DDT is often approached or exceeded, despite a global ban on DDT use in agricultural production. The FAO/WHO Codex Alimentarius Commission has established maximum residue limits (MRLs) for DDT in a range of commodities such as cereal grains, milk, meats, and eggs. The MRLs are recognised by the World Trade Organization as the international references in assessing non-tariff barriers to trade, but enforcement requires analytical facilities and trained personnel. In developing countries, analytical techniques such as gas–liquid chromatography and mass spectrometry are often too expensive or lack sufficient throughput to justify their use in all cases. ELISA (enzyme-linked immunosorbent assay) offers an alternative technique that can screen large numbers of samples, rapidly identify commodities exceeding MRLs, or identify the source of contamination.

This paper describes a joint project between the FAO/IAEA Agriculture and Biotechnology Laboratory and the CSIRO to apply an ELISA method for determining DDT/DDE in food and environmental samples. The DDT/DDE immunoassay is a polyclonal antibody-based laboratory assay for the semi-quantitative determination of residues of DDT and DDE. The antibody also detects DDD (also known as TDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane), a minor metabolite. Detection of DDA (2,2-bis-(4-chlorophenyl) acetic acid), the mammalian acidic metabolite, is 20 times less sensitive than DDT.

The assay uses competitive ELISA format where the antibody is freeze-dried onto microwell plates and the free DDT or DDE in the test sample competes with an enzyme-labelled pesticide for antibody binding. Unbound enzyme-labelled pesticide is removed by a washing buffer, and bound enzyme-labelled pesticide is activated by adding a mixture of substrate-chromogen which produces as coloured reaction product. DDT or DDE in the test sample is quantified by comparing colour development in the sample wells with that of wells containing standards. Less DDT or DDE in the test sample will result in more enzyme conjugate being bound, which leads to greater colour development.

A kit for this assay is available, complete with external quality assurance samples and a protocol which includes a list of equipment required to conduct the assay, as well as details of data acceptance and interpretation. Examples for soil and food matrices will be described, and an overview given of a collaborative research program involving participants from 17 countries.

THE Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) are helping developing countries to better monitor food contaminants in international trade through the work of their Training and Reference Center for food and pesticide control. Laboratories in member states are being assisted to implement present and future quality and safety standards set by Codex Alimentarius so that they can participate increasingly in the global marketplace.

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One mechanism of IAEA/FAO in supporting scientists in developing countries is through the so called 'Agency's Research Contract Programme'. It consists of an award given to individual scientists in order to participate in a Coordinated Research Programme (CRP). The Agency has a current CRP on 'The use of nuclear and immunochemical methods for pesticide analysis'.

This paper describes a project of the FAO/IAEA Agriculture and Biotechnology Laboratory to apply an ELISA method for determining DDT/DDE in food and environmental samples. It reports on the planning, results, and conclusions of a collaborative study for the determination of DDT in soil samples. The reason for concentrating on DDT is that DDT (1,1,1-trichloro-2,2-bis(4-chlorophenylethane)) is a persistent organic contaminant that bioaccumulates in food chains. In developing countries, the FAO/WHO acceptable daily intake (ADI) for DDT is often approached or exceeded, despite a global ban on DDT use in agricultural production. The FAO/WHO Codex Alimentarius Commission has established maximum residue limits (MRLs) for DDT in a range of commodities such as cereal grains, milk, meats, eggs. The MRLs are recognised by the World Trade Organization as the international references in assessing non-tariff barriers to trade but enforcement requires analytical facilities and trained personnel.

In developing countries, analytical techniques such as gas–liquid chromatography and mass spectrometry are often too expensive or lack sufficient sample throughput to enable their use in all cases. Literature reports in the 1980s and 1990s (Kaufman and Clower 1995) suggested that pesticide ELISA (enzyme-linked immunosorbent assay) was a cheap alternative technique that can screen large numbers of samples and rapidly identify commodities exceeding MRLs, or identify the source of contamination. Other attractions of the technique included less vulnerability to power interruptions, and high sample throughput and selectivity. The latter promised to reduce labour-intensive operations such as sample clean-up. However, pesticide ELISA remain largely untested in developing countries (Skerritt et al. 1992).

The CRP work, started in 1993, was an ideal opportunity to look at advantages and disadvantages of the ELISA method. The CRP commenced with an interlaboratory comparison of commercial cyclodiene insecticide kits. It was concluded that participants from developing countries required more training in ELISA techniques and that the commercial kit was not appropriate for counterparts in developing countries in terms of cost, ease of delivery, and simplicity of instructions. The CRP work continued with a second interlaboratory comparison using an atrazine kit developed at the Technical University in Munich. The atrazine interlaboratory comparison saw a marked improvement over the results from the previous cyclodiene kit. However, three problems were apparent: the coating of the plates required relatively large amounts of antibody; the temperature stability of the antibody was a critical issue; and there was a strong need to have a quality assurance system in place.

Objectives of the Informal Collaborative Study

For any testing laboratory it is essential that assurance can be given that test results produced are valid and reliable. It is also very important that results are comparable between different laboratories involved in similar assessments, or in other words, that there are mutually agreeable proficiency testing schemes. One of the distinct advantages of an ELISA-based system is the objectivity of reading the results and the ability to process the data using a computer. Thus, it is possible to incorporate a high level of internal quality control for every ELISA test plate used. Internal quality control has to become a routine operation for laboratories utilising FAO/IAEA ELISA-based testing systems. The main objective of this collaborative study was to start a mechanism devoted to setting up a quality assurance program for participating laboratories while promoting standardised assays and ensuring the credibility of data generated using ELISA.

Materials and Methods

The DDT/DDE immunoassay used in the collaborative study was a polyclonal antibody-based laboratory assay for the semi-quantitative determination of residues of DDT and DDE (Beasley et al. 1998). The antibody also detects DDD (also known as TDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane), a minor metabolite. Detection of DDA (2,2-bis-(4-chlorophenyl)acetic acid), the mammalian acidic metabolite, was 20 times less sensitive than DDT. The assay uses a competitive ELISA format where the antibody is freeze-dried onto microwell plates and the free DDT

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or DDE in the test sample competes with an enzyme-labelled pesticide for antibody binding. Unbound, enzyme-labelled pesticide is removed by a washing buffer, and bound enzyme-labelled pesticide is detected by addition of a mixture of substrate-chromogen which produces a coloured reaction product. DDT or DDE in the test sample is quantified by comparing colour development in the sample wells with that in wells containing standards. Less DDT or DDE in the test sample will result in more enzyme conjugate being bound, which leads to greater colour development (Stanker and Beier 1996).

A complete kit was distributed to the 19 laboratories—in America, Africa, Asia, Australia, Europe—participating in the Agency's CRP for the collaborative study. The kit was composed of: a coated plate, a freeze-dried vial of enzyme tracer, sachets of buffer salts, a vial of DDT standard, substrate and chromogen in liquid form, and a bench protocol containing instructions, operating procedures, indication of data acceptance, and troubleshooting. The bench protocol was specially designed to enable participants to reduce to a minimum level the occurrence of any errors. This approach assists with development of quality assurance and quality control systems. Seven soil samples, spiked with DDT, were distributed together with the kit. All samples were blind and coded differently according to the laboratory. Participants were asked to prepare a standard curve of DDT in 100% methanol and to analyse the unknown soil samples after extraction for 16–20 hours with 90% methanol in water using a soil:water extraction ratio of 1:5. Aqueous methanol extraction has previously been shown to be an efficient extractor of DDT residues from soils (Singh and Chalwa 1989; Dedek et al. 1991).

Results

A total of 15 laboratories carried out the analysis according to the procedure supplied with the bench protocol. All participants prepared a standard curve of DDT in methanol. In all tests, at least seven calibration points were considered and at least four of them were contained in the working range of the assay. Analyses of extracts and standards were replicated three times. Seven soil samples were distributed to the participant laboratories with nominal spiking values of 0, 0.02, 0.1, 0.2, 0.2, 0.5, and 2.0 mg/kg respectively. Table 1 presents the precision data for the collaborative study. The repeatability (*RSDr*) and the reproducibility (*RSDR*) relative standard deviations are reported as percentage values (Ambrus 1997).

Sample 1 (0 mg/kg of DDT in soil) was considered as an internal quality control sample: only one laboratory reported a detectable DDT concentration. The means from all laboratories were relatively close to the nominal spike value and the precision parameters were within tolerance according to the comparatively high analytical variability of the ELISA method at trace analyte concentrations (Ferguson et al. 1992; Skerritt and Hill 1992; Whitaker et al. 1996).

In the analysis of the results, the focus was on the identification of possible errors rather than the judgment of the performance of the laboratories. Firstly, we looked at the possibility of identifying systematic errors. We used a simplified Youden plot analysis. Such a diagram consists of a rectangular plot, on which the individual laboratory's results for two similar samples are represented with one point. Figure 1 shows an example of a Youden plot for 0.2 mg/kg DDT in soil In this study, among the total set of samples to be analysed, laboratories analysed two sam-

Table 1. Precision data for the collaborative study

Sample	Number of laboratories[a]	DDT spike (mg/kg)	Mean	RSDr[b] (%)	RSDR[c] (%)
1	13	0			
2	10	0.02	0.04	11	89
3	10	0.1	0.14	10	26
4	13	0.2	0.22	9	46
5	13	0.2	0.22	9	53
6	13	0.5	0.51	14	47
7	12	2	1.8	12	59

[a] After elimination of outliers.
[b] Relative standard deviation of repeatability (*r*).
[c] Relative standard deviation of reproducibility (*R*).

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ples of soil spiked to 0.2 mg/kg. The analysts were not aware that they were duplicates, thus they can be termed 'Youden blind pairs' (Youden and Steiner 1975). Results from some laboratories were positioned in the upper right quadrant and in the lower left quadrant: their location suggested the presence of systematic errors that lead to an increase or decrease in the recovery determined by the assay.

Secondly, we contacted the laboratories and we went through a checklist, similar to the one presented in Table 2. Once the possible errors were identified it was important to set up some procedures in order to prevent the occurrence of the errors in the next assay.

Table 2. Minimising errors by means of quality assurance

Possible errors	**Means of assurance (IQA)**
Reagents preparation, balances, purity	Internal/external quality assurance for kit utilisation
Sample extraction, solvent purity, time	QA for extractant, standard operating procedures (SOP) for extraction method
Storage conditions, temperature, humidity and light	QC of environmental conditions
Loss of activity	Refrigerated shipping Restart optimisation process (stabilisers) Data logger
Pipetting errors: Calibration Operator Random errors Contamination	 Standard operating procedures, working instructions Personnel training records Do not recycle of pipette tips
Standard preparation errors	Checklists
Balances	SOPs for balance calibration Personnel training records
Reagents, samples, standards, buffers at ambient temperature	Temperature control maintenance
Dilutions respected	SOPs for the assay Checklists for dilutions
Order of pipetting in the plate, time respected	Checklist for plate layout
Time	Timer
Temperature control of buffers	Check temperature
Not proper washing	Follow SOP for assay, checklist for flow sheet
See above for pipetting errors	
Time respected	
Light avoidance	Use of aluminium foil as per SOP
See above for pipetting errors	
Calibration of instrument	SOP Internal own checking solutions
Calculation errors: Operator Concepts errors Replicates	 Check list for analysis of results/ software Personnel training Check lists for plate layout
Acceptance/rejection/ interpretation system for results Way of presenting results, operator error	

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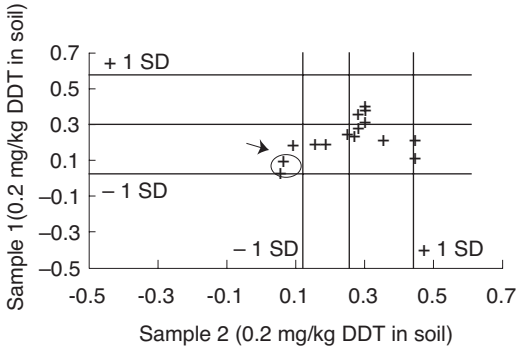

Figure 1. Simplified Youden plot analysis for samples of soil spiked to 2 mg/kg with DDT

In the analysis of some of the participants' results we wondered whether their results were affected by shipping conditions. Selected kits were accompanied by a HOBO® data logger, a simple device that records the variation of temperature during time. The conclusion was that, even if during shipping several kits stayed at high temperatures for many days, the activity of the kit was not lost.

Questionnaire Completed by Participants

As part of an external quality assurance program, a questionnaire-based survey of individual laboratories was used to investigate and monitor the presence and use of key quality elements (Joint FAO/IAEA Division 1996). The questionnaire consisted of different questions grouped under five sections: general information, shipping, laboratory, quality assurance system, and the DDT/DDE screening ELISA kit. Tables 3, 4, and 5 summarise some responses from the laboratories. It is evident that the questionnaire can be only a part of a quality assurance program. The answers given by the people do not always reflect reality, especially when the person filling in the questionnaire is different from the operator who actually did the assay. The questionnaire highlighted the problem that, for these laboratories, quality assurance is not yet a major concern, but in the future every laboratory indicated that they would like to become accredited. It is evident that some kind of quality system has to be started immediately and the Training and Reference Center can help laboratories to make a start on this.

Table 3. Questionnaire responses: kit shipping and laboratory facilities

Lab	Internet access	Delivery time (days)	Government institute	Analysts	Analysts with ELISA experience	Trained staff in ELISA	Need for training	Main laboratory analytical activity	Equipment
1	no	23	yes	9	7	no	yes	pesticide	HPLC, GC, reader, etc.
2	yes	18	yes	9	5	only 1	yes	Antibody production	HPLC, GC, reader, washer
3	yes	11	yes	3	2	yes	yes	food quality	HPLC, GC, reader
4	no	26	yes	5	2		yes	pesticide	GC
5	yes	6	no	15	6	yes	no	pesticide	GC, HPLC, reader, washer
6	no	26	yes	4	2	yes	yes	pesticide	GC, reader
7	yes	19	yes	6	2	yes	yes	pesticide	GC
8	yes	14	yes	5	2	yes	some	micronutrient	HPLC, GC, reader
9	yes	14	yes	13	3	no	yes	pesticide	TLC, HPLC, reader
10	yes	8	yes	14	10	yes	no	pesticide	reader
11	yes	29	yes	14	4	yes	no		HPLC, reader
12	yes	7	yes	40	4	yes	no	food safety	all
13	yes	3	no	7	5	yes	no	Antibody production	reader, washer

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Table 4. Questionnaire responses: quality assurance and kit

Lab	Training records	Existence of QA program	Existence of any documented procedures	Seek accreditation in future	Kit intact at receival	Suggestions for improving the bench protocol	Bench protocol easily readable	All required equipment available
1	yes	no	no	yes	yes	pre-weigh samples, no pipetting out for manual washing 3 cycles semi log paper	yes	yes
2	no	no	no	yes	yes		yes	yes
3	no	yes	no	yes	yes	sample weight, on stock solution, indicate conc.	yes	no plate washer
4	no	no	no	yes	yes	photos to end	yes	no washer, reader elsewhere
5	yes	no	no	yes	yes	send water, better lids for plates	yes	no multi-channel pipettes
6	yes	no	no	yes	yes	semi log paper, page 15.	yes	yes
7	no	no	no	no	yes		yes	no in house reader
8	yes	yes	yes	already	yes		yes	no washer
9	no	yes	yes	already	yes		yes	no washer, no pipette 1–5 mL
10	yes	no	yes	no	yes	clear indication on settling time	yes	no washer, no orbital shaker
11	yes	no	no	yes	yes		yes	yes
12	no	no	no	no	yes	reduce photos format, semilog paper	yes	yes
13	no	no	no	yes	yes		yes	yes

Conclusions

This collaborative study for DDT in soil was to assess the state-of-the-art of pesticide ELISA analysis in counterparts laboratories in order to improve the quality of their analytical data. This represented the first trial within the CRP context in which we considered quality issues. Despite two participants that were responsible for most of the outliers, the overall precision of data was acceptable. This represents an improvement over previous results. That aside, the main goal of the collaborative study was to identify errors and critical situations that can be avoided once a quality system is in place. The experience that has been gained during this interlaboratory comparison study clearly shows the need for further comparison studies between laboratories as a means to improve analytical quality in the field of pesticide ELISA analysis.

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Table 5. Questionnaire responses: pipetting and plate reading

Lab	Fixed volume pipettes	Multichannel pipettes	Calibration of pipettes	How often	Recycle tips	Calibration of reader	Last calibration
1	yes	yes	yes	once month	no	yes	before starting assay, comparing with a known solution
2	yes	yes	yes	once year	no	yes	1 year ago compared with another reader
3	adj. vol.	yes	no		no	no	next to purchase universal calibration test plate
4	adj. vol	yes	yes	once year	no	no	
5	yes	no	yes	6 month	no	no	
6	yes	yes	no		no	no	
7	no	yes	yes	6 month	no	no	
8	yes	yes	yes	every week	no	no	
9	yes	yes	no		no	no	
10	adj. vol.	yes	yes	6 month	no	no	
11	yes	no	yes	6 month	no	no	
12	adj. vol.	yes	yes	before assay	no	no	
13	adj. vol.	yes	yes	when problem	no	no	

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Development of an Immunoassay for Monitoring Sulfonylurea Herbicide Residues in China

Minghong Jia*

Abstract

An immunoassay (IA) was developed for monitoring the sulfonylurea herbicide metsulfuron-methyl (MS) residues. Hapten-protein conjugates were prepared by covalently linking 2-methoxycarbonyl phenylsulfonamide (2-MPS) moiety of metsulfuron-methyl through its amino group to proteins with an additional succinic acid spacer arm. Antibodies with high affinity and specificity against MS were yielded by hapten-BSA (bovine serum albumin) conjugate (MSH-BSA) as an immunogen coupled by using active ester method (AE). Most of other MS analogues and degradation products showed almost no cross-reactivity with the antibodies. Several other sulfonylureas with the corresponding 2-MPS moiety of MS could be recognised by the antibodies . This indicates the antibodies can also be used as essential reagent to develop other IAs for determinations of a group of sulfonylurea herbicides with the same moiety besides MS. The competitive ELISA based on immobilised hapten-protein conjugate format were established and proved available to determine free MS in aqueous media. The IC_{50} of ng/mL level were achieved in this formatted assay. The effects of pH, ionic strength and sample matrix on the ELISAs were investigated for assay optimisation. The fortified experiments provided acceptable recoveries and detection limitations. The results suggested the ELISAs could be used as an alternate and promising approach to analysis for the herbicide residue in water and soil.

Furthermore, the potential progress which would be made in China for studies and application of IAs to monitoring sulfonylurea herbicide residue were introduced briefly. A hybrid methodology referred to immunoaffinity column combined with conventional methods will also be discussed as a promising and potential approach to sulfonylurea residual monitoring.

SULFONYLUREA herbicides represent a class of aceto-lactase synthase (ALS) inhibitors and marked the beginning of the time of super-effective chemical weed-killing. Since the late 1980s, the herbicides have been studied and used in the major cereal belts of many countries (Peterson et al. 1985; Alister 1988; Blair and Martin 1988). Chlorsulfuron, metsulfuron-methyl, and chlorimuron etc. are dominant products in China, which are some of the most potent weed killers in sulfonylurea herbicide family, with very low application rates (8 g/ha for metsulfuron-methyl) (China field test network of pesticide, 1993). But unfortunately, the potential risk to crop growth from low-level residues has been identified due to their excessive persistence in soil, especially in alkaline soils, (N. Wilhelm, R. Kookana. M.H. Jia, pers. comm. 1996, 1997). Since they have higher water solubility under alkaline conditions, rapid leaching down to deep subsoil and even pollution of water sources may occur (Wang et al. 1992; Cheng et al. 1995). In addition, the single function target (ALS) and consecutive application may lead to resistance problems (Cotterman and Saari 1992), resulting in the emergence of other weeds. In cold northern China, alkaline soils are widespread. In the major cropping belts, cereals such as wheat, maize, sorghum, and grain legume are important rotational crops which supply food and feed, as well as helping to control weeds and break the disease and insect cycles in farm systems. But the existing usage pat-

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terns may present a considerable threat to crop growth and the environment. As a result, extensive cereal zones were at most risk of large crop losses. The specific environmental and cultivation conditions in China made the problems more serious and complex. The occurrence of damaged rotational crops steadily increased (C.X. Zhang et al., unpublished data 1997). Key recommendations from a recent workshop included addressing the problems of sulfonylurea herbicide residues, and seeking new analytical approaches and strategies to deal with the problem (Jia 1996, 1997).

Current analytical methodologies for sulfonylurea herbicide involve gas chromatography (GC) combined with electron capture detection (ECD) (James 1990; Cotterill 1992), high performance liquid chromatography (HPLC) with photoconductivity detection (Zahnow 1982; Hershberger and Brennan 1988; Bussmann et al. 1990). But the low use rate and relatively rapid dissipation characteristics of the herbicides complicate residue analysis. To conduct such an analysis at parts per billion (ppb) level for routine monitoring is very difficult because of the need for extremely sensitive and selective detection and for intensive sample preparation and clean-up steps which often are time-consuming and tedious. The bioassays (Hsiao and Smith 1983; Sunderland et al. 1991) that are used are usually sensitive but not specific. There is therefore a pressing need to develop more sensitive, rapid and inexpensive analytical methods.

Immunoassays (IA) have been shown to be an alternative approach to pesticide residue analysis (Hammock and Mumma 1980; Van Emon et al. 1989). The techniques are based on the ability of animals to produce highly specific antibodies to foreign materials, and they provide a sensitive, specific, and cost-effective means of analysis. Kelley et al. (1985) reported an IA of polycolonal antibody (PAb) for chlorsulfuron developed by using a diazonium derivative hapten-protein as immunogen. A competitive ELISA (Schlaeppi et al. 1992) based on a monoclonal antibody (Mab) was developed for analysis of trisulfuron residues in soil, with a sensitivity of 0.1 mg/kg. In China, good progress has been made in analytical techniques for sulfonylurea herbicide residues. The studies reported in this paper involved mainly development of ELISA methods for herbicides such as chlorsulfuron, metsulfuron-methyl, and ethametsulfuron in soil and water. The paper focuses on the development of IA for metsulfuron-methyl (MS) as a typical example. The purpose of the investigation was to establish an ELISA method to rapidly monitor and assess the residues, persistence, and fate of MS in soil and water that could be coupled with conventional methods for simplification of sample preparation. The method was also expected to be able to determine sulfonylureas as a class.

Materials and Apparatus

Materials

Metsulfuron-methyl (MS) (methyl-2-[[[[(4-methoxy-6-methyl-1,3,5,-triazin-2-yl)amino]carbonyl] amino]-sulfonyl]benzoate) and other sulfonylurea compounds were provided by the Institute for the Control of Agrochemicals, Ministry of Agriculture, PRC, or the Department of Agricultural Applied Chemistry, China Agricultural University. 2-methoxycarbonyl phenylsufonamide was synthesised in the laboratories of the Tianjin Pesticide Factory. Phosphate, citric acid, sodium carbonate, borate, Tween 20, succinic anhydride, n-tributyl amine, and triethylamine were purchased from the Beijing Chemical Reagent Company. Isobutyl chlorocarbonate, bovine serum albumin (BSA), human serum albumin (HSA), rabbit serum albumin (RSA), keyhole limpet hemocyanin (a), N-hydroxysuccinimide (NHS), and dicyclohexylcarbodimide (DCC), 1.8-diazobicyclo [5.4.0]undec-7-ene (DBU) were purchased from the Merck-Schuchardr Company. Goat anti-rabbit IgG conjugated to horseradish peroxidase (HRP), and incomplete and complete Freund's adjuvant were purchased from the Institute of Microbiology and Epidemiology, Academy of Military Medical Science of China. All of the above chemicals and the solvents including methanol, ether, ethyl acetate, N,N-dimethylformamide (DMF), dichloromethane etc. are AR grade. Water was redistilled with all glass apparatus.

Apparatus

ELISAs were carried out on 40- and 96-well polystyrene microtitre plates (Zhejiang Chemical Co., PRC) and read with a microplate reader (Jiangsu Scientific Apparatus Factory, PRC). The melting points of the haptens synthesised were determined with a melting point metre made by the Yanagimoto Manufacturing Co.(Japan). Infrared spectra (IR) were determined on a Shimadzu IR 435 spectrometer (Japan) (KBr tablet). Proton nuclear magnetic resonance spectra (NMR) were measured with a JROL

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FX#–900 90-MHz spectrophotometer (Japan) using tetramethylsilance as an internal standard. Mass spectra (MS) were obtained on a MS-50 mass spectrometer (USA) using 70-eV EI. Ultraviolet–visible spectra (UV) were obtained on a HP 8452A diode array spectrophotometer (Shimadzu, Japan). A rotary evaporator (XZ-6, Beijing, PRC) was used to remove solvents from solutions or sample extracts.

Hapten synthesis and hapten-protein conjugation

Hapten synthesis

With a spacer arm of additional succinic acid linked to the NH_2 group of 2-methoxycarbonyl phenylsulfonamide (2-MPS), the MS hapten, N-[2-methoxycarbonylphenylsulfonyl] monoamido succinic acid (MSH), was synthesised by the following steps: 4.2 g of 2-MPS (0.02 M) was mixed with 2.0 g succinic anhydride (0.02M, dissolved in 80 mL dioxane). While stirring the mixture, 6.1g DBU (0.04 M in 20 mL dioxane) was added drop by drop under slight cooled conditions. The resulting white suspension was acidified with 2 M HCl and filtered, and then dissolved in ethyl acetate. The solution was washed twice with 20 mL water, dried over anhydrous Na_2SO_4, and evaporated on a rotary evaporator. The residue was recrystallised, giving 4.8 g of white crystals (at 75% yield, m.p. 141–143°C). The resulting MSH was confirmed by IR, NMR, and MS: IR, KBr tablet : (cm^{-1}), 3248 (s, N-H), 2950 (m, O-H), 1428 (s, C-N), 1329, 1173 (s, SO_2); 1H-NMR, TMS internal standard, 90 MHz, 5 mm sample tube, 1800 Hz spectra width, DMSO solvent: δ (ppm): 8.30–7.84 (m, 4H, Ar-H), 3.92 (s, 3H, CH_3), 3.45 (s, 1H,N-H), 2.80–2.44 (m,4H, CH_2CH_2); MS, 70 ev, m/e (relative intensity): 315 (0, M^+), 316 (3, M^+ +1), 215 (8, M^++1-101, $COCH_2CH_2CH_2COOH$), 199 (100, M^+-116, $NHCOCH_2CH_2COOH$), 135 (30, M^+-180, SO_2 $NHCOCH_2CH_2COOH$), 101 (10, CO-CH_2CH_2 CO-OH), 77 (33, C_6H_5), 76 (20, C_6H_4).

Hapten-protein conjugation

The hapten (MSH) was coupled in turn with various proteins (BSA, KLH, and RSA) using the active ester method (AE) (Bauminger et al. 1980). Briefly, 7.2 mg MSH (0.033 mmol dissolved in 300 μL of DMF) reacted with excess 13.6 mg NHS (0.12 mmol in 300 μL of DMF) and 24 mg DCC (0.12 mmol in 300 μL of DMF) for 1 hour at ambient temperature and then for overnight at 4˚C. The resulting precipitate was separated by centrifugation. The active ester in the supernatant solution was then added to 5 mL protein solution (4.0 mg/mL in PBS, pH 7.0, 0.01 M) and incubated for 5 hours at 4˚C. The mixed anhydride method (Gendloff et al. 1986) was used to prepare MSH-HSA. Briefly, 16.0 mg MSH (0.05 mmol dissolved in 500 μL of DMF) was added to 20 μL n-tributyl amine and 13.6 mg isobutyl chlorocarbonate (0.1 mmol in 1.0 mL of DMF), respectively, drop by drop while stirring at 4˚C for 1 hour. The solution was then added drop by drop to 4 mL HSA (2.5 mg/mL in borate-buffer, pH 8.7, 0.05 M) and reacted for 12 hours at ambient temperature. Both final reaction solutions from AE and MA methods were dialysed intensively against PBS (0.01 M, pH 7.4) at 4˚C. The protein concentrations were determined according to the Lowry assay. The coupling rates of MSH to proteins were determined by UV absorbance at 280 nm, which corresponded approximately to the peaks of both proteins and MSH.

Preparation of antibody

Three 12-week-old New Zealand rabbits were each immunised by a series of subcutaneous and intramuscular injections or boosts in 2- or 4-week intervals with MSH-BSA conjugate (at a dose of 0.6 mg/kg body weight) mixed completely in 2 mL water with 2 mL complete Freund's adjuvant at first and 2 mL incomplete Freund's adjuvant in later series. A sample of 1 mL of blood was taken intravenously from the ear for titre test on the 7th day after each injection. When the titre was over 128000, the rabbits were bled from the collar artery and the isolated antiserum was collected and NaN_3 added to a concentration of 0.01%, then stored at –80˚C or freeze dried.

ELISA procedures

Antiserum titre test

The antiserum titre tests were carried out on 96-well microtitre plates coated with MSH-HSA at 1~5 μg/mL (100 μL/well) in sodium carbonate buffer (0.01 M, pH 9.6) and incubated at 4˚C overnight. The plate was washed with phosphate buffer (PBS, 0.2 M, pH 6.8) supplemented with 0.05% Tween 20 (PBST), and the unoccupied sites were blocked with the PBST supplemented with goat serum. Serial dilution antiserum solutions with PBST were prepared and 100 μL/well incubated at 37˚C for 1 hour. After the unbound antiserum was washed away, goat anti-rabbit IgG conjugated to HRP (diluted 1:1000 with PBST) was then incubated for 1 hour at 37˚C.

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Washed again, all wells were incubated for 15 min with 100 μL substrate solution (75 μL, 30% H_2O_2 and 40 mg OPD in 100 mL citric acid–phosphate buffer, pH 5). Colour development was terminated with 2 M HCl and the optical density was then read at 490 nm on the microplate reader.

Competitive immunoassay for MS determination

A competitive indirect format ELISA was performed for MS determination. The procedures were similar to those for the antiserum titre test. Instead of antiserum addition, the mixture of the series MS concentration standard solutions or sample extracts (50 μL) with antiserum (50 μL diluted 1:10000 to 1:40000 in PBS) was added to the wells. The antibody competitively bound either free MS in solution or the immobilised coating antigen (CAg). After unbound material was washed away, addition of goat anti-rabbit IgG-HRP revealed the presence of antibodies trapped by the CAg. The HRP enzymatic activity retained on the solid phase is inversely proportionable to the MS original concentration and can be quantified by colour development with the substrate. The standard inhibition curves prepared for this indirect competitive ELISA were analysed by computer using the half-logarithm curve-fitting procedure of MicroCal Origin 3.0 version which calculated IC_{50} values (molar inhibitor concentration for 50% maximum inhibition). Optimal CAg concentrations, antiserum titration, pH, and ionic intensity of the PBS available were confirmed by checkerboard test. In a series of cross-reaction experiments with antiserum, MS was replaced with known concentrations of related compounds and MS analogues.

Sample assay protocols

Before the ELISA determination, soil samples (30 g) were extracted with 90 mL mixture of PBS (0.02 M, pH 7.4)/methanol (2:1, v/v) for 1 hour under surging. After filtration, the solution was acidified with H_3PO_4 to pH 3~4 and extracted three times with 90 mL dichloromethane (CH_2Cl_2). The CH_2Cl_2 extract was then evaporated to dryness on a rotary evaporator. The residues were taken up in a particular volume of PBST (0.2 M, pH 6.8)/methanol (95/5) for ELISA. The water samples (100 mL) were filtered and acidified with H_3PO_4 to pH 3~4, then extracted by the same procedures as those for soil, or determined directly by the ELISA. Several extraction procedures were used to study the sample matrix effects on the assays by comparing the inhibition curves for various samples with that for the standard (PBST).

Results and Discussions

Hapten design and hapten-protein conjugation

The hapten design and hapten-protein conjugation depended on the purpose of the assay and the molecular structure of the target compound. In theory, to produce a specific antibody, the hapten-protein conjugate should be designed to preserve and enhance the determinant groups on the hapten. Any structural differences between the hapten and the target analyte— including charge, electron density, electropolarity, isomerism, and so on—may markedly affect the specificity and affinity of the antibody. In addition, to develop as selective an IA method as possible for a particular compound which happens to have a specific structure, the hapten design should ensure the linking site of the hapten to carrier is distant from the specific hapten determinant. In contrast, if there is a series of structurally related compounds, which you expect to determine simultaneously using a single IA, conjugation at a site distal from the common structure should be selected to elicit an antibody able to cross-react with all individuals of the chemical class. Moreover, the extension of the hapten away from the bulky protein carrier by way of a bridging group referred to as a spacer arm will aid the production of hapten-specific antibodies.

On this basis, we should first choose an attachment point far from the specific part of the MS molecule for a selective IA development. Unfortunately, however, neither end of the MS molecule, nor moiety of 2-MPS or the triazine ring, is structurally unique in the sulfonylurea family. Thus, whatever choice we make, we must lose at least some specificity. Considering that the other sulfonylureas that are most common in China's markets and farms are mainly chlorsulfuron and chlorimuron, specificity was assumed to be significant enough to elicit antibodies if capable of distinguishing MS from the other two, based on the structural difference in phenyl moieties of the three sulfonylurea molecules. Thus, it seemed possible to design the hapten by choice of a linking site away from phenyl moiety (2-MPS) of MS.

Unfortunately, we have failed in attempts to synthesise MS hapten with a spacer arm to link the whole MS from its heterocyclic end to carrier proteins. The ELISAs based on the antibody elicited by such means

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were neither sensitive nor selective enough to determine MS at ppb level. The failure was very likely the result of the relatively low affinity of antibody to MS as a result of the difficult conjugation of the hapten to proteins.

On the other hand, an attempt to generate Ab with high affinity and specificity to MS was successful by using 2-MPS moiety instead of the whole MS molecule to prepare hapten and hapten-protein conjugates. Succinic acid was used as an additional spacer arm to link 2-MPS to carrier proteins. It has been reported that multiple combinations of antibody and CAg may generate more sensitive and specific assays. Therefore, a series of CAgs was synthesised using different chemical approaches (AE and MA) to facilitate screening for optimal combination of antibody and CAg. The molar ratios of 16/1, 131/1, 23/1, and 19/1 for MSH-BSA, -KLH, -RSA and -HSA, respectively, were confirmed by UV determination assuming additive 280 nm absorbance values.

Confirmation of antibody and optimisation of the assay

Titration of the antibody

The antisera of three rabbits arising from MSH-BSA gave very high titres over 1.28×10^5 for CAgs of MSH-KLH, MSH-RSA, and MSH-HSA, while showing very low titres for control proteins of KLH, RSA, and HSA. This indicated the antibody could recognise the hapten determinant on the various CAgs immobilised on the plate and there were no cross-reactions with the carrier proteins of CAgs. The titre differentials on various CAgs may be due to different methods and carrier proteins used to couple with the hapten.

Affinity of the antibody

The antiserum titre tests proved very high affinities of the antibody to each CAg. A series of antibody inhibition tests was performed to check affinities of the antibody to free MS with different combinations of antibody and CAgs. All results from these tests showed the antibody can recognise free MS in aqueous solution. However, the inhibition curves based on the three combinations of antibody with MSH-HSA, MSH-KLH, and MSH-RSA gave different IC_{50}s, of 8.7, 18.8 and 107.5 μg/L, respectively, as shown in Figure 1. Differentials of IC_{50}s revealed that the affinities of the antibody to free MS vary with different CAgs used in the tests. This perhaps indicated the unspecific linking reactions arising from hapten-protein conjugation stages may contribute to the resulting antibody affinities to various CAgs, especially when prepared with a linking method the same as that for IAg. Since CAg competed to bind antibody with free MS in the assay, the higher affinity of antibody to CAg might result in a lower reactivity of antibody with free MS. As a result, the sensitivities of the ELISAs for various CAgs prepared by both MA and AE methods would correspondingly differ. MSH-HSA was prepared by a MA method different from AE method for IAg (MSH-BSA) preparation. The most appropriate combination of antibody /MSH-HSA thereby gave a most sensitive assay, suggesting different linking methods used for IAg and CAg preparations may prevent the antibody from over-binding with the CAg resulted from unspecific reactions arising from the same chemical approaches. Thus, only the combination of antibody ($1:1\times10^4$ diluted in PBST) with MSH-HSA (2.5 μg/mL) was selected for further studies. The standard inhibition curve gave a minimum detectable amounts of 0.05 ng/mL for MS and the working range of detection for MS is approximately 0.05–100 ng/mL in aqueous media.

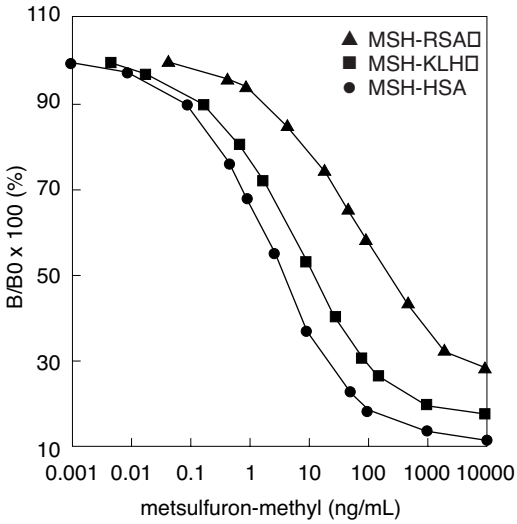

Figure 1. The standard curves of metsulfuron-methyl based on inhibition of antibody binding with various CAgs of MSH-HSA, MSH-KLH and MSH-RSA in the indirect competitive ELISA. B/B0×100 (%) represents the percentage of antibody bound to the plate.

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Specificity of the antibody

The specificity of the antibody was demonstrated by a series of cross-reactivity experiments. The results of IC_{50}s and the cross-reactivity percentage (CR%) with related compounds, as shown in Table 1, show several things. Firstly, the various heterocyclic structures of sulfonylureas, as well as the structures of triazine compounds including atrazine, simazine, and the triazine degradation products of MS (AMMT), were not cross-reactive in the tests. This corresponded with our predictions, since these structures have never been present in IAg. In contrast, when there were minor modifications of the methoxycarbonyl group or there was addition of a substituted group in the phenyl ring of the MS analogues, antibody affinities displayed considerably changes, as evidenced by chlorsulfuron, chlorimuron, Y_{35}, Y_{31}, Y_{16}, and Y_{19} with which the antibody showed very different but much lower cross-reactivities. This result suggested that the phenyl structure is indeed the antigenic determinant recognised by the antibody. Moreover, quite strong cross-reactivities of the antibody with MSH and MSHE were observed and, conversely, very low cross-reactivity of the antibody with 2-MPS (phenyl ring degradation products of MS) was also confirmed, indicating the absence of urea bridge structure may radically change the charge or electropolar distribution of the phenyl structure and thus markedly reduced the antibody affinity to this structure. It seems that an additional carbonyl aliphatic chain as an electrotaxis group linked to the nitrogen on the sulfonamide group is the prerequisite for antibody binding. A similar situation with bensulfuron supported this conclusion. Since the embedding of methylene between phenyl and sulfonamide may result in a charge or electropolar distribution shift, the recognition of antibody to bensulfuron may be influenced though its phenyl structure is the same way as that of MS. On the other hand, the relatively higher cross-reactivity of antibody with tribenuron confirmed that the influence on antibody affinity of the presence of methyl linked to the nitrogen at the urea bridge end close to heterocycle is very small. In addition, succinic acid was not recognised by the antibody. These findings suggest that the contributions of structure of the urea bridge in space to the antibody affinity and specificity are less than that of charge or electropolar effect. Once a carbonyl aliphatic chain was linked to amino group of 2-MPS, the resulting compound must be recognised by the antibody and the effects from any substituted groups of the chain on the antibody affinity and specificity may be rather limited.

In summary, the antibody is very capable of distinguishing MS from most sulfonylurea herbicides, especially chlorsulfuron and chlorimuron and so on, which are the dominant products being applied in China. On the other hand, of course, the antibody also showed very strong cross-reactions with several other sulfonylurea compounds (ethametsulfuron, tribenuron and sulfometuron, Y_9 and Y_{15} etc.) which had 2-MPS moieties similar to the hapten structure. Thus, this immunoassay was class rather than compound-specific in some cases. If the aim is to detect the total residues from several sulfonylurea herbicides with 2-MPS structure in a single assay, this antibody may present a useful method, especially when combined with some conventional methods, as discussed below.

Effect of pH

Immunoassays for non-ionic analytes usually perform satisfactorily over a wide pH range. For the potentially ionised species, however, IAs may be affected by pH values via not only the analytes and/or CAgs, but also antibodies. Since the sulfonamide hydrogen of MS (p*Ka*3.3 at 25˚C) or MSH tends to ionise under neutral or alkaline conditions, the antibody (and/or CAg) for MS will be pH sensitive. As shown in Figure 2, the affinities of antibody to MS as well as to CAg (MSH-HSA) vary markedly with pH. Maximal affinity of the antibody to both the CAg and the free MS were in the range 5.8– 6.8, as indicated by the maximal percentages of both antibody binding in the noncompetitive assay and the MS inhibition in the competitive format over this pH range, and the very low percentages of binding or inhibition in the pH ranges higher than 7.8 or lower than 4.3. This was perhaps due simply to both the ionic analyte and/or CAg. It may be that the antibody ionises differentially to produce different structural forms as the pH changes, , resulting in very different affinities of antibody to the analyte and/or CAg. Thus, it was necessary to adjust pH of the sample extract and incubation buffer (PBST) to a defined range before the assays.

Effect of ionic strength

The effect of the ionic strength of the incubation buffers on the assay result was confirmed by competitive assays using various concentrations of PBST. The higher ionic strength (increased incubation buffer concentration) significantly improved the sensitivity of the assay.

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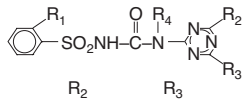
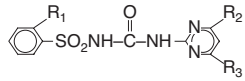
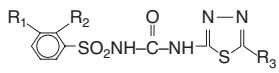
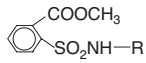
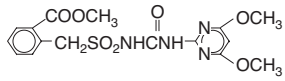
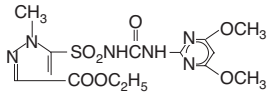
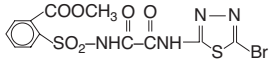
Table 1. Cross reactivity of antibody with various MS analogues and related compounds

Compounds	IC_{50} (ng/mL)[a]	CR (%)[b]	Molecular structures
			
			R_1 R_2 R_3 R_4
metsulfuron-methyl	8.7	100.00	–COO CH_3 –O CH_3 –CH_3 –H
tribenuron	38.0	14.90	–$COOCH_3$ –OCH_3 –CH_3 –CH_3
ethametsulfuron	7.1	122.25	–$COOCH_3$ –OC_2H_5 –$NHCH_3$ –H
chlorsulfuron	223.0	3.90	–Cl –CH_3 –OCH_3 –H
			
			R_1 R_2 R_3
sulfometuron	12.1	71.9	–COO CH_{3}□ –CH_{3}□ –CH_{3}□
chlorimuron	1630.0	0.53	–$COOC_2H_5$ –Cl□ –OCH_3
			
			R_1 R_2 R_3
Y_{25}	9910.0	0.09	–H –H –H
Y_{31}	8870.0	0.10	–H –H –Br
Y_{19}	≥1000	≤0.01	–Cl –H –CH_3
Y_9	17.2	50.60	–H –COO CH_3 –CH_3
Y_{16}	1970.0	0.44	–H –$COOC_2H_5$ –H
			
			R
MSH	2.0	435.00	–$CO(CH_2)_2COOH$
2-MPS	4876.0	0.17	–H
MSHE	21.3	37.60	–$COOC_2H_5$
			
			R_1 R_2 R_3
AMMT	≥10000	≤0.01	–NH_2 OCH_3 –CH_3
simazine	≥10000	≤0.01	–Cl –NHC_2H_5 –NHC_2H_5
atrazine	≥10000	≤0.01	–Cl –NHC_2H_5 –NHC_3H_7
			
bensulfuron	2950.0	0.29	
			
pyrazosulfuron	≥10000	≤0.01	
			
Y-15	64.5	13.50	
succinic acid	≥10000	≤0.01	$HOOC(CH_2)_2COOH$

[a] Inhibitor concentration for 50% maximum competitive inhibition in the ELISA.
[b] Cross-reactivity defined as (MS concentration for 50% maximum competitive inhibition/MS analogue concentration for 50% maximum competitive inhibition)×100. Y compounds represent a series of sulfonylureas synthesised.

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As illustrated in Figure 3, the IC_{50} value at a buffer concentration of 0.02 M was about 5 times that at 0.2 M. It was thought that the increase of ionic strength may inhibit the nonspecific binding which derived from heterogeneous antibodies and usually presented weaker affinity. However, if the ionic strength is too high it may cause antibody dissociation from free analyte and/or CAg and other unexpected effects on the enzyme/substrate-colour reaction. Thus, a buffer concentration of 0.2 M was recommended for further study.

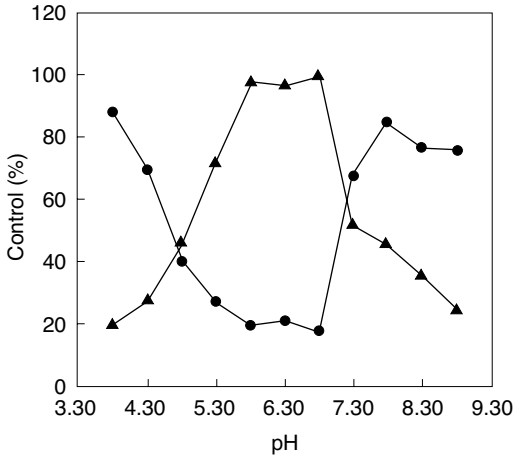

Figure 2. Effects of pH on affinities of antibody to CAg (MSH-HSA) and metsulfuron-methyl. Assays were performed by procedures similar to the titre test (noncompetitive assay) and the MS inhibition test (competitive assay). The incubation buffers used in the assays were prepared to a series of PBSTs with various pH values and the MS stock solution was diluted with these corresponding PBST to 100 μg/L. ▲, percentage of control for the points with MS added; ●, percentage control for noncompetitive assay (no MS). The data are means of four replicates. The coefficient of variation was between 4.4 and 9.7%

ELISA for MS residue in samples

Effects of sample matrix

The co-extracted materials derived from the sample matrix often considerably affect an IA. Therefore it is crucial to study matrix effects on the assay for its efficacious application in practice. Samples, including three types of soils and two types of waters, were tested for the effects of co-extracts from sample matrixes on the assay. The composition of each soil sample was analysed (Table 2). Both sorts of samples were prepared by several extraction approaches. The influences of the sample matrixes were identify by comparing the inhibition curves for various sample extracts (containing matrix materials and the series graded concentration MS in PBST buffer) with the standard curve (without matrix materials). The blank sample extracts (without MS) were used as controls.

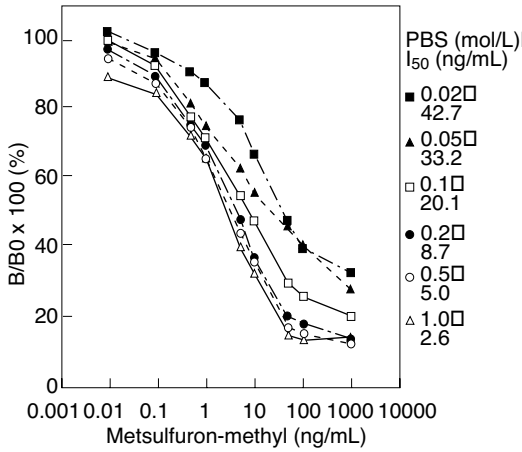

Figure 3. Effect of ionic strength on the ELISA standard curve for metsulfuron-methyl. The data are means of four replicates. Average coefficients of variation of standard curves at various incubation buffer (PBST) concentrations of 0.02, 0.05, 0.10, 0.20, 0.50, and 1.00 M are between 2.8 and 10.8%

As shown in Figure 4, when water samples were measured, whether extracted or not (directly measured), there was no significant influence on the assay. The sample inhibition curves almost replicated the standard curve, suggesting that the effects of water matrixes on the assays could be ignored.

For different soil samples, however, the extracts showed inhibitory or (in some cases) enhancing effects on antibody binding according to the extraction method used. Only the extract by the procedures outlined in the previous paragraph showed minimal influence on the assay compared with other extraction methods (Fig. 4d) we have tried. However, if deducted from the values of the blank soil sample in parallel procedures, the results of the assays gave quite acceptable inhibition curves as illustrated in Figure 4c. This suggested that organic solvent extraction was necessary for the elimination of most matrix influences to obtain a desired detection limitation.

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Table 2. Compositions of the soil samples tested

soil[a]	% water	% humus	% silt	% clay	% sand	pH
sandy	15.5	0.9	5.2	3.8	92.0	7.8
sandy loam	22.0	1.3	22.4	13.2	65.4	7.5
loamy altoll	24.0	3.6	45.6	14.4	40.0	6.5

[a] Three soils were selected respectively from Shanxi, Hebei and Heilongjiang, China

Table 3. Recovery of metsulfuron-methyl from various fortified samples

Sample[a]	Metsulfuron-methyl added (ppb)	Recovery % [b]	CV % [c]
soil			
sandy	5.0	87	6.9
	0.5	84	7.7
	0.1	79	9.7
sandy loam	5.0	88	7.3
	0.5	79	7.2
	0.1	73	21.9
loamy altoll	5.0	115	12.2
	0.5	83	9.6
	0.1	80	18.9
water extracted			
well	2.0	109	13.4
	0.2	88	9.4
	0.05	79	7.9
stream	2.0	83	10.5
	0.2	80	18.6
	0.05	78	16.1
water measured directly			
well	5.0	92	8.7
	0.5	85	17.3
stream	5.0	82	12.9
	0.5	89	20.7

[a] Soil sample: see Table 2. Well water from a well in Beijing and river water from a stream on field pathway in Herbei respectively represented groundwater and surfacewater. Fortified soil and water samples were extracted by procedures outlined in sample assay protocols.

[b] Percentage calculated according to equation: [(measured value for fortified sample – measured value for sample control)/value added] × 100.

[c] CV %, percentage of coefficient of variation in triplicate.

Recoveries from spiked samples

The recovery experiments with spiked samples were performed with the established ELISA and sample preparation procedures described previously in this paper. The MS blank samples were taken as the controls.

The recoveries averaged 85% from soil samples spiked with MS in the range 0.1–5.0 mg/kg as shown in Table 3. A sensitivity of 0.1 mg/kg for the given assay protocol and ane average coefficient of variation of 11% with all assays in triplicate were attained in these experiments.

Since the water matrix had almost no influence, the spiked assays gave similar results with and without extraction procedures. Mean recoveries were 86% and 87%, respectively. The assay with the extraction procedure was more sensitive than that without, but also more cumbersome. Minimal detection limits of about 0.05 and 0.5 ng/mL were achieved with average coefficients of variation of 12.7% and 14.9%, respectively, for the extraction and direct assay protocols. In practice, whether or not the extraction procedure is adopted depends on the possible MS concentrations in the water sample and the detection limit required.

Conclusion and Perspective

An ELISA method based on the antibody elicited by MSH-BSA conjugate has been developed and optimised for the detection of MS residues in water and soil at the parts per billion level. The simple hapten design corresponding to the 2-MPS moiety of MS molecule was proved to be a constructive approach to avoid the cumbersome chemical synthesis for the whole molecule hapten. The resulting antibody presented high specificity to MS without cross-reaction with major degradation products of MS and most of the other sulfonylurea and triazine herbicides commonly applied in many countries including China. Also the antibody may be available to develop IAs for some other MS analogues with moiety of 2-MPS. The IC_{50} value of ng/mL and minimal detection amount of 0.05 ng/mL and a wide detectable range were achieved in this assay. The effects of pH and ionic strength on antibody affinity were significant. Optimal assay occurred within a pH range of 5.8–6.8 and PBST concentration at 0.2 g/L. Average recoveries of 85.3% and 86.7–87% and sensitivities of 0.1 μg/kg and 0.05 μg/kg, respectively, were attained for soil and water samples. There were no significant matrix interferences from various samples tested according to the assay protocols described.

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In conclusion, this IA was demonstrated to be a potential analytical tool for monitoring MS at relatively low residual levels with many advantages including high sensitivity and specificity, low cost, and easy performance as compared with conventional methods such as GC and HPLC. It also ensures a more rapid and efficient assay highly applicable to mass screening with parallel sample processing rather than the sequential sampling of chromatographic methods. However, as many biotechnology-based assays are usually less precise, a two-tiered approach involving a rapid initial screening using this IA, followed by confirmatory HPLC or GC analysis only for those samples that gave positive initial responses is recommended. Alternatively, a particularly powerful analytical approach of immunoaffinity columns may facilitate the concentration and purification of sulfonylurea herbicide in samples before more precise measurements by

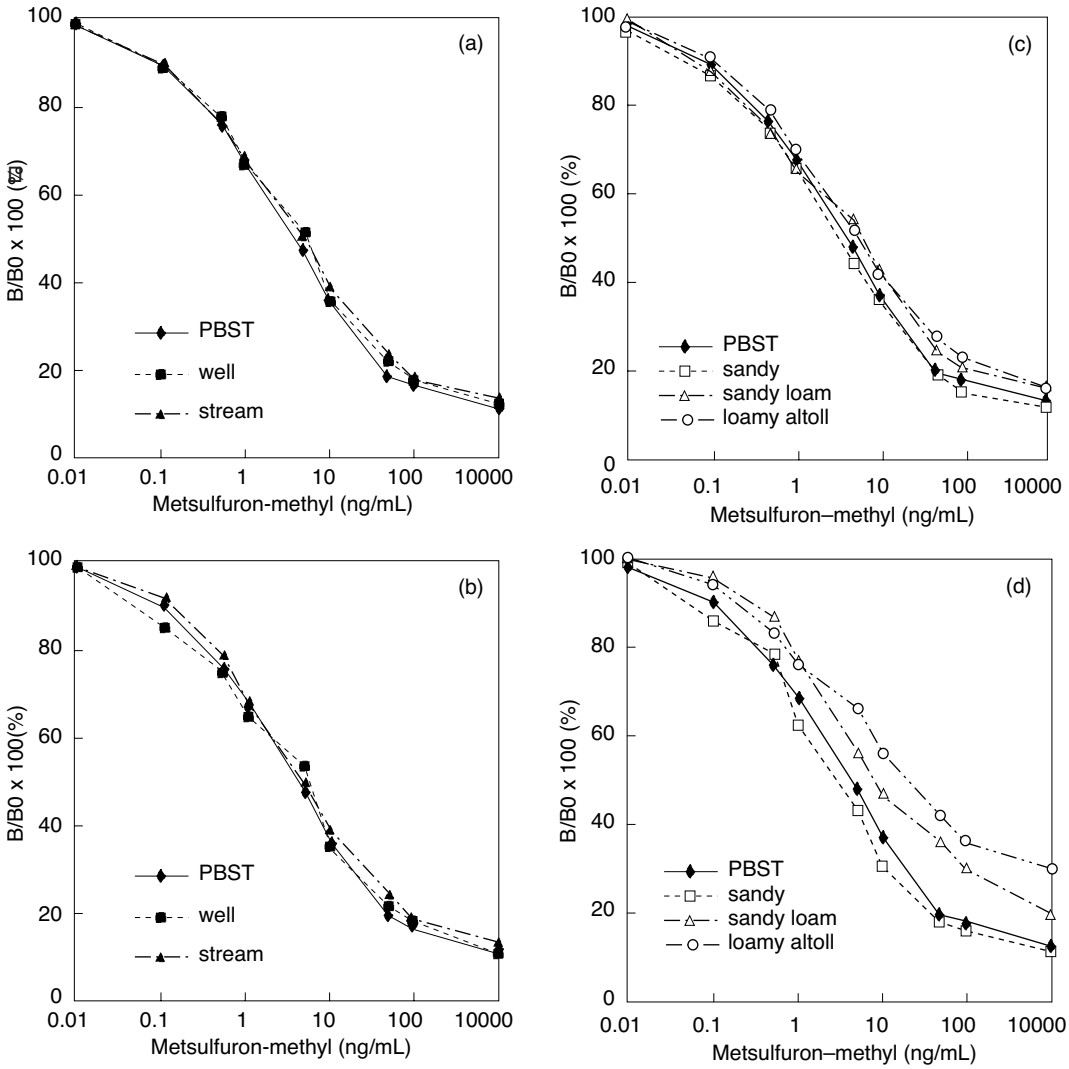

Figure 4. The effects of co-extracts from various sample matrixes on the assays. The sample matrix effects were tested by comparing inhibition curves for various samples with the standard curve (without matrix materials). The blank sample extracts (without MS) were used as controls. a, b: Water samples were extracted or directly measured. c, d: Soil sample extracts were prepared by the protocol outlined in the sample assay protocols section with PBS/methanol followed by dichloromethane and by another procedure using methanol instead.

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chromatographic techniques, thus exploiting the combined advantages of the two methods.

Several immunochemical methods for sulfonylurea herbicides have been developed successfully in China recently, and immunoassay will be used in the routine monitoring program for sulfonylurea herbicides. Further work should see the incorporation of these IAs with other methods such as chromatography and bioassay and the acceptance of the immunochemical data by management institutions and farmers.

Acknowledgments

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Abbreviations and Acronyms Used

IA, immunoassay; IC_{50}, molar inhibitor concentration for 50% maximum inhibition; ELISA, enzyme-linked immunosorbent assay; MS, metsulfuron-methyl; MSH, MS hapten with 2-MPS structure; 2-MPS, 2-methoxycarbonyl phenyl sulfonamide; ALS, aceto lactase synthase; CAg, coating antigen; IAg, immunoantigen; Ab, antibody; BSA, bovine serum albumin; KLH, keyhole limpet hemocyanin; RSA, rabbit serum albumin; HSA, human serum albumin; DMF, N,N-dimethylformamide; DMSO, dimethyl sulfoxide; DBU,1.8- diazobicyclo[5.4.0]undec-7-ene; AE: active ester method; MA: mix anhydride method; DCC, dicyclohexylcarbodimide; NHS, N-Hydroxysuccinimide; HRP, horseradish peroxidase; GC, gas chromatography; HPLC, high performance liquid chromatography; IR, infrared spectroscopy; NMR, nuclear magnetic resonance spectroscopy; UV, ultraviolet-visible spectroscopy; OD, optical density; PBS, phosphate-buffered saline solution; PBST, PBS containing 0.05% Tween 20; CV%, percentage of coefficient of variation; IC_{50}, molar inhibitor concentration for 50% maximum inhibition; CR%, 50% maximum competitive inhibition / MS analogue concentration for 50% maximum competitive inhibition; B/B0, the percentage of antibody bound to the plate.

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Immunoassays and Other Field Approaches for Environmental and Biological Monitoring of Mycotoxins, with Special Reference to Aflatoxins

R.B. Sashidhar*

Abstract

Mycotoxins are toxic fungal metabolites found to contaminate a variety of agricultural commodities meant for human consumption. These toxins are structurally diverse and are capable of causing a variety of well characterised biological and toxicological effects, both in humans and farm animals. Among the fungal toxins, aflatoxins have received worldwide attention due to their deleterious effects on human and animal health and their importance in international trade. The problem of aflatoxin and other mycotoxin contamination of foods and feeds has led to the enactment of various laws for fixing the safe limits in agricultural commodities meant for human consumption. However, in India and in many developing countries, meaningful strategies for implementing the legislation are restricted by non-availability of simple, rapid, cost-effective and reliable methods for screening and detecting aflatoxins or other mycotoxins.

Immunological methods, particularly ELISA have an edge over more-traditional methods of mycotoxin analysis, especially for field studies. Monitoring human exposure to aflatoxins has been accomplished directly by analysis of the contaminated food produce ready for consumption (environmental monitoring) or indirectly by monitoring the ingested and biotransformed toxin (biological monitoring). Aflatoxin B_1-N^7-guanine and aflatoxin B_1-albumin adducts have been identified as biomarkers for dietary exposure to aflatoxins in humans. For the first time an immunogen—bovine serum albumin (BSA)-Guanine-Aflatoxin B_1— was synthesised in our laboratory. Specific polyclonal antibodies to aflatoxin B_1-N^7-guanine adduct were produced. Further, the antibodies produced were characterised and used in establishing enzyme immunoassay for biological monitoring of aflatoxins. Development and use of ELISA methodology for molecular epidemiological studies as a measure of aflatoxin exposure are reviewed.

MYCOTOXINS are toxic chemical compounds synthesised as secondary metabolites by a variety of fungi under certain conducive environmental conditions in various agricultural produce. High risk commodities for mycotoxins include cereals and oilseeds, and their consumption poses a potential threat to human and animal health. These compounds are structurally diverse and are capable of causing a variety of biological and toxicological effects. In higher animals, these effects are influenced by sex, species, strain, environmental factors, nutritional status, and interaction with other chemicals. Most of the biological effects of mycotoxins are organ or tissue specific (WHO 1979; FAO 1979, 1988).

Among the fungal toxins, aflatoxins produced by the *Aspergillus flavus* and *A. parasiticus* group of fungi have received worldwide attention due to their harmful effects on human and animals and their importance in international trade. Almost all the agricultural commodities are susceptible to fungal attack and mycotoxin formation before and after harvest and during storage (Champ et al. 1992; Highley and Johnson 1996).

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The potentialities of aflatoxins as carcinogens, mutagens, teratogens, and immuno-suppressive agents are well documented (Yourtee et al. 1989; Robens and Richard 1992; IARC 1993). Aflatoxin B_1 is one among the most potent naturally occurring carcinogens and is classified as a Group I carcinogen (IARC 1993). High-risk agricultural commodities for aflatoxin contamination include cereals and oilseeds which form a major component of the human diet in various parts of the world. Epidemiological studies carried out in different regions have provided enough evidence to implicate dietary aflatoxins as a causative factor in various human diseases such as aflatoxic hepatitis, primary hepatocellular carcinoma, Indian childhood cirrhosis, Reye's syndrome, Kwashiorkor, certain respiratory disease and colo-rectal cancer (IARC 1993).

Human exposure to aflatoxins has been estimated by environmental monitoring (external dose) and biological monitoring (internal dose) (Wogan 1992).

Environmental and Biological Monitoring of Aflatoxins

Environmental monitoring involves detection and measurement of toxin in food samples/agricultural commodities meant for human consumption. This approach provides no information about variation in intakes or the disposition of toxin after ingestion, as dietary components play an important role in absorption of toxin. Another important shortcoming of the approach is that it relies on dietary recall for the amount and the type of food consumed and involves analysis of individual food preparations. Various analytical methods are available for environmental monitoring of aflatoxins and other mycotoxins. They include TLC, HPLC, RIA, and ELISA (FAO 1990). In general, these method can be classified as presumptive or screening , semi-quantitative, and quantitative methods, as depicted in Figure 1. The methodology involves sampling, extraction of the toxin from the food matrix, sample clean-up, and detection, determination, and chemical confirmation (Fig. 2). Sample clean-up can be achieved using adsorption chromatography or immuno-affinity chromatography. For field application, presumptive methods such as minicolumn/pressure minicolumn and dip-strip methods based on physicochemical principles, are of immense value, as these methods are rapid and simple to perform (Sashidhar et al. 1988; FAO 1990; Sashidhar 1993). These methods are useful in routine surveillance and monitoring of agricultural commodities to assess the natural occurrence of aflatoxins.

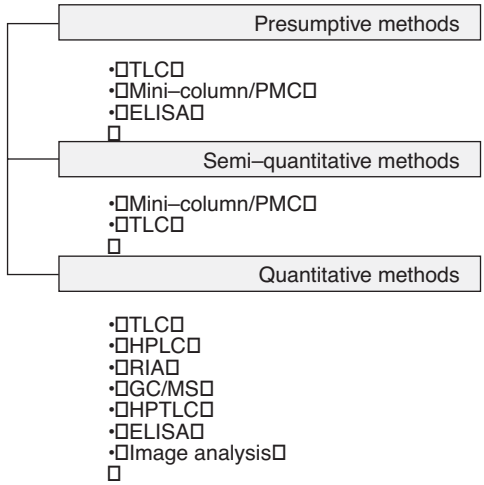

Figure 1. Methods of mycotoxin analysis

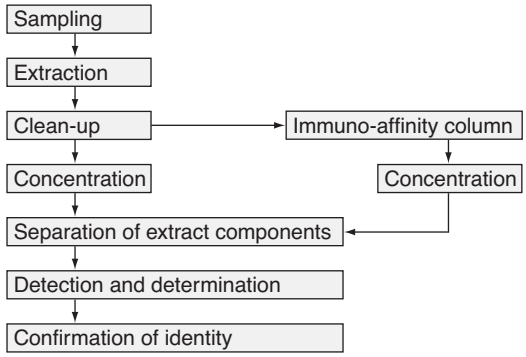

Figure 2. Typical procedure for mycotoxin analysis

Biological monitoring is a measure for the presence of the residue or bio-transformed metabolite of the ingested toxin in tissues, body fluids, or excreta. This exposure assessment at individual level is a direct measure of aflatoxin absorbed into the body from the diet. Considerable progress has been made in the recent years towards developing sensitive analytical methods to measure aflatoxin adducts formed in the body after dietary exposure to aflatoxins. In molecular epidemiological studies, aflatoxin-DNA and aflatoxin-protein adducts have been used successfully as biochemical markers for aflatoxin in the human population exposed to dietary aflatoxin. These

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methods include HPLC, ^{32}P-DNA postlabelling technique and ELISA (Groopman et al. 1992; Hemminki 1992; Shuker and Farmer 1992). However, no ELISA method was available or developed to directly assess the urinary levels of aflatoxin B_1-N^7-guanine.

Aflatoxin B_1-guanine and Aflatoxin B_1-protein Adducts Are Biochemical Markers for Biological Monitoring of Aflatoxin Exposure

Studies in experimental animals and human subjects have shown that metabolic activation of ingested aflatoxin B_1 results in the formation of a putative carcinogenic intermediate, aflatoxin B_1-8,9-epoxide, in liver via microsomal cytochrome P_{450} (1A2 and 3A4) oxidation of aflatoxin B_1. Aflatoxin B_1-8-9-epoxide is a highly reactive electrophile, which interacts covalently with cellular DNA and proteins. It attacks G–C rich regions of the DNA, resulting in the formation of the adduct at the N^7-position of guanine or interacts with serum albumin to form protein adducts. Detection of aflatoxin-guanine adduct, excreted in urine and aflatoxin-albumin adduct in blood, forms the basis of the biological monitoring of human population at risk to aflatoxin exposure. Pharmacokinetic studies in experimental animals and human subjects have demonstrated that these adducts are quantitatively related to the dose of ingested aflatoxin (Eaton and Gallagher 1994).

Development of ELISA Methodology for Detection of Aflatoxin B_1-N^7-guanine Adduct in Urine and Aflatoxin B_1-DNA Adduct in Liver

A simple, single-step procedure for the synthesis of aflatoxin B_1-guanine adduct using free guanine and *m*-chloroperbenzoic acid as the chemical oxidant for the production of aflatoxin B_1-epoxide was developed which is akin to the metabolic activation (Vidyasagar et al. 1997c). For the first time, specific polyclonal antibodies were raised in rabbits against aflatoxin B_1-guanine adduct. The immunogen, BSA-guanine-aflatoxin B_1 was synthesised in two steps: (i) initially, BSA-guanine conjugate was synthesised using dimethyl suberimidate as a linker, and (ii) the aflatoxin B_1 epoxidation reaction was carried out in the presence of BSA-guanine conjugate synthesised in the first step (Vidyasagar et al. 1997a). Further, the antibodies were characterised for their cross-reactivity with other antigens, such as poly-L-lysine-aflatoxin B_1, calf thymus DNA-aflatoxin B_1, and calf thymus DNA-(formamido-pyrimidine)-aflatoxin B_1 adducts by antibody capture assay. Assay results showed 5% cross-reactivity with poly-L-lysine-aflatoxin B_1, indicating the specificity of the antibody to the guanine-aflatoxin B_1 moiety over aflatoxin B_1 (Fig. 3).

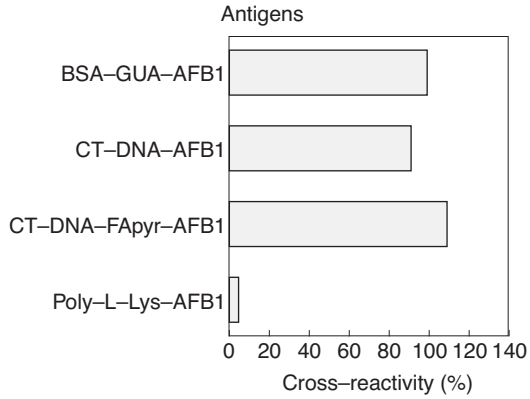

Figure 3. Cross-reaction of the antisera raised against BSA-guanine-aflatoxin B_1 (BSA-GUA-AFB1): CTA-DNA-AFB1 = calf thymus-DNA-aflatoxin B_1; CT–DNA–FApyr–AFB1 = calf thymus–DNA-(formamido-pyrimidine)-aflatoxin B_1; poly-L-Lys-AFB1 = poly-L-lysine-aflatoxin B_1

These conjugates were also synthesised by the epoxidation reaction of aflatoxin B_1 using *m*-chloroperbenzoic acid as an chemical oxidant (Vidyasagar et al. 1997b). Furthermore, the interference of free aflatoxin B_1 in the assay was less than 5% at an antiserum dilution of 1:7000. No cross-reactivity was observed when free guanine, aflatoxin G_1, and aflatoxin M_1 competed with aflatoxin B_1-guanine adduct in the assay. The ELISA procedure for biological monitoring of aflatoxins is given in Figure 4. Calf thymus DNA-Aflatoxin B_1-adduct was used as the coating antigen (containing 16 ng of aflatoxin B_1) and the indirect competitive ELISA was performed using aflatoxin B_1-guanine adduct as reference standard. To assess the tissue levels of aflatoxin B_1-DNA adduction, calf thymus DNA-aflatoxin B_1 was used as the reference standard instead of aflatoxin B_1. Alkaline phosphatase enzyme was used as a label in the assay. The working range of the ELISA method developed

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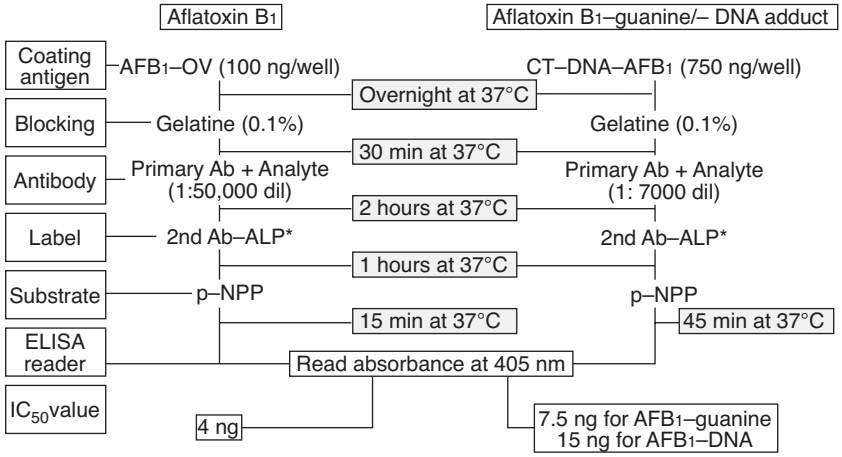

Figure 4. ELISA scheme for environmental and biological monitoring of mycotoxins: OV = ovalbumin; p-NPP = p-nitrophenyl phosphate; 2nd Ab-ALP = anti-rabbit IgG raised in goat and linked to alkaline phosphatase enzyme. Enzyme reaction was terminated with 5N NaOH.

was between 0.45 and 330 ng with an IC_{50} value of 7.5 ng and 15 ng for aflatoxin B_1-N^7-guanine and aflatoxin B_1-calf thymus DNA adduct, respectively.

The methodology developed was validated by quantification of aflatoxin B_1-guanine adduct excreted in urine from rats (Fischer 344 strain) dosed with aflatoxin B_1 (1 mg/kg body mass). Liver levels of aflatoxin-DNA adducts were also quantified in the experimental animals (Vidyasagar et al. 1997c). Figure 5 denotes the excretion of aflatoxin B_1-guanine adduct in urine, after 48 hours of dosing, along with liver tissue levels of aflatoxin B_1-DNA adduct. Interestingly, female rats excreted higher levels of adduct in urine than did male rats, while male rats had higher concentrations of aflatoxin-B_1-DNA adducts in the liver (Fig. 5). In order to detect very low levels of aflatoxin B_1-guanine adduct in human urine, an immunoaffinity clean-up column was also developed, using aflatoxin–guanine specific antibodies (IgG fraction) immobilised on to sepharose 4B gel matrix The adduct was eluted, using 70% dimethyl sulfoxide (DMSO). No interference by DMSO was observed at concentrations of less than 6% in the antibody–antigen reaction (Vidyasagar et al. 1997b). Figure 6 and Table 1 give the recoveries (92–100%) of the adduct from spiked, normal human urine and the repeatability of the procedure developed. The major advantages of the ELISA method developed, coupled with immunoaffinity column, include speed, ease of sample preparation, and low cost per analysis over the conventional methods of biological monitoring of aflatoxins.

Table 1. Repeatability studies for indirect competitive ELISA

Amount of adduct spiked (μg/mL)	Adduct quantified by ELISA (μg/mL)	% recovery
2.0	2.0 (3.3)	100
1.0	0.99 (11.6)	99
0.3	0.26 (11)	93
0.1	0.09 (17)	92
0.05	0.048 (12)	96

Values in the parentheses are coefficient of variance; n =4.

Applications of the Methodology Developed

The ELISA methodology developed, may find wide application in (i) exposure assessment, (ii) dose–response evaluation, (iii) risk management, and (iv) developing strategies for intervention of aflatoxin toxicity. Biochemically, the method can be used in screening natural and synthetic anti-carcinogenic compounds for their ability to intervene metaboli-

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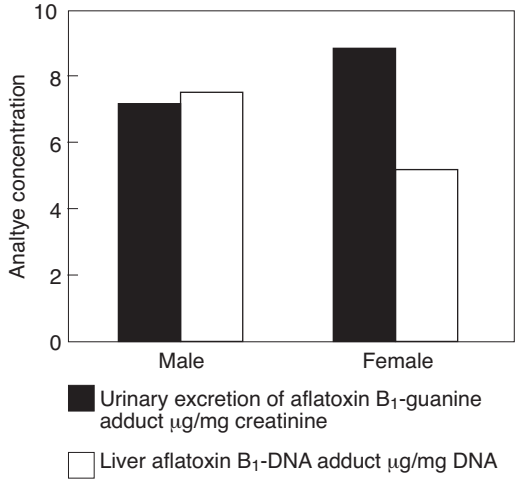

Figure 5. Biological monitoring of aflatoxins in rat urine and liver: $n = 8$ (Fischer 344 rats); aflatoxin B_1 dose = 1 mg/kg body mass.

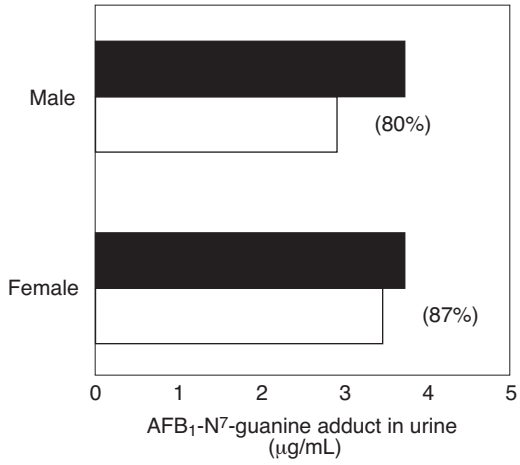

Figure 6. Recovery of spiked adduct in human urine (n = 8; 4 male, 4 female) using immunoaffinity column.

cally and reverse the genotoxic effects of dietary aflatoxins. Furthermore, the immunoaffinity clean-up column developed can be used for the single-step purification of aflatoxin-guanine adducts in HPLC analysis.

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Integration of Instrumental and ELISA Methods into Food and Human Exposure Monitoring

Tippawan Prapamontol*

Abstract

Chromatographic techniques such as high performance liquid chromatography and gas chromatography, coupled with other selective detectors, have been employed as standard methods of pesticide residue analysis for decades. Traditional analytical methods to monitor trace levels of pesticides in complex matrices, e.g. food and biological fluids, usually require thorough sample clean-up and enrichment before chromatographic analysis. Since sample preparation is rather time-consuming, and chromatographic analysis requires special training and capital investment in sophisticated apparatus, alternative and/or complementary methods have been sought. Immunochemical methods such as enzyme-linked immunosorbent assay (ELISA), based on the interactions of immunogen and antibodies, have been shown to be promising and useful in screening for pesticide residues. This paper reviews the application of instrumental and ELISA methods, and their integration to monitor pesticides in environmental and biological samples.

THERE are several techniques available for pesticide residue analysis. The traditional analytical method is chromatography which is instrumentation-orientated. Gas and liquid chromatography, coupled with selective and sensitive detection systems, have been used for decades for multiresidue analyses, using methods such as those of the U.S. Environment Protection Agency. In recent years, immunochemical-based methods such as enzyme-linked immunosorbent assay (ELISA) have been popular due to their high selectivity and sensitivity, minimal sample preparation, high sample throughput, and potentially low cost per sample. ELISA usually requires less instrumentation than chromatographic-based methods (e.g. a visible spectrophotometer as detection system) although an ELISA plate reading machine is now commercially available. Since both techniques have their own strengths and weaknesses, their integration enables combination of individual strengths to obtain cost-effective analyses. Food and body fluids are complex matrices, often lipidic and containing many interference compounds. Therefore, monitoring of pesticide residues in these matrices usually require sample treatment before analysis. This paper discusses applications of chromatographic-based and ELISA methods, and their integration to monitor pesticides in environmental and biological samples, from developing country viewpoint.

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Human Exposure Monitoring

Organochlorine pesticides

Organochlorine pesticide residues are often found in the environment and in foodstuffs, because of their persistence. They include dichlorodiphenyl trichloroethane (DDT) and its major metabolite dichlorodiphenyl dichloroethylene (DDE), and beta-hexachlorocyclohexane (β-HCH), a persistent metabolite of gamma-hexachlorocyclohexane (γ-HCH, trade name lindane). Because these compounds are present in the environment they will inevitably reach human body fluids via the food chain. Human exposure monitoring requires analytical methods to identify the contaminants in body fluid samples

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qualitatively and quantitatively. Traditionally, gas chromatography (GC), coupled with an electron capture detection (ECD) system, has been used as the primary analytical system for the determination of OCPs and other organochlorine compounds in various type of body fluids, e.g. serum, fatty tissues, and breast milk. Confirmatory tests using different detection systems, e.g. mass spectrometer (MS), or at least a different column liquid phase are also required in chromatographic methods since identification of components detected is based on the matching of their retention times against the retention times of authentic compounds (St. Louis and Hill 1990). Combined GC–MS is the most sophisticated instrumentation for identifying compounds in complex mixtures and for detecting ultratrace levels of organic compounds (Reiner and Clement 1990).

Integration of ELISA and GC methods in monitoring of human exposure to DDT— a cost effective operation of epidemiological study

We have investigated human exposure to OCPs by analysing a few hundred serum samples collected during epidemiological studies of the population of northern Thai population. DDT and its metabolites, DDE and dichlorodiphenyl dichloroethane (DDD), the latter also known as tetrachlorodiphenyl ethane (TDE), were often found in serum and milk samples, while other compounds such as dieldrin, heptachlor , and its metabolite heptachlor epoxide were rarely detected (Prapamontol et al. 1994, 1996; Wang et al. 1997). Furthermore, an epidemiological case-control study of human exposure to OCPs and their potential health risks has also been conducted. Analyses for DDT and its metabolites in about 700 serum samples collected from breast cancer and other patients have been made using capillary GC–ECD after C18-solid phase extraction of 2 mL serum. Limitations of sample throughput (low productivity of running), GC running time, and running cost per sample were obvious. Since we are interested in the health effects of particular group of OCPs, we therefore anticipate using ELISA to quantify DDT, DDE, and DDD in these serum samples with randomised confirmatory cross-checks using GC–ECD or GC–MS. However, the unit cost of environmental ELISA kits (determined by production cost and potential user demand) may restrict the use of the technique for developing country users. We can contrast ELISA kits with clinical diagnostic kits which are produced in very large numbers.

Food Monitoring

OCPs have been banned from use, particularly in agriculture, in most developed countries since in the early 1970s and they have been replaced by less persistent organophosphate (OP) and carbamate pesticides, which are now the two major classes of pesticide used in agriculture. Although OP compounds have relatively low persistence, are biodegradable, and generally do not accumulate in the food chain, their residues have been found in vegetables, probably due to overuse and/or misuse. For example, residues of organophosphates were found in vegetables during a survey by the Food and Drug Administration of Thailand (FDA 1997). Analyses of organophosphate and carbamate pesticide residues and many of their metabolites are invariably made using chromatographic techniques.

A survey of contamination by cholinesterase-inhibiting compounds, e.g. organophosphate and carbamate pesticides, in vegetables and fruits sold in Chiang Mai markets was recently conducted by a collaboration of government and non-government organisations, including the Chiang Mai Provincial Public Health Office, Chiang Mai Medical Science Centre, Chiang Mai University Research Institute for Health Sciences, and the Faculty of Agriculture. The assay kit used was developed by the Ministry of Public Health's Department of Medical Sciences. It uses a cholinesterase inhibition-based method to give a qualitative result . The test gives three categories of result: negative; positive at safe level; and positive at an unsafe level of contamination. The results of this survey showed that 17.8 % of the so-called pesticide-free vegetables (N = 28) tested positive at safe level of contamination, while 22.2 % of the general grown vegetables and fruits (N = 149) tested positive of contamination, with 2% of samples at an unsafe level (Anon., unpublished data, 1997). Due to lack of financial support and local facilities, it has not been possible to undertake confirmatory tests using traditional chromatographic-based methods.

ELISA test kits of selected pesticides in monitoring food safety: method of choice

We know that profiles of pesticide contamination in foodstuffs vary widely from place to place. Agricultural practices and pesticide use are seen to be major factors in the contamination level of pesticides in produce. Rapid tests such as ELISA and other immunochemical-based methods for selected pesticides known to contaminate vegetables and fruits

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would be useful for monitoring. Confirmatory tests, if required, can employ traditional chromatographic-based methods e.g. GC coupled with nitrogen/phosphorous detection (NPD). For example, a Thai Food and Drug Administration survey conducted from 1994 to 1995 (FDA 1997) monitored pesticide contamination in the so-called pesticide-free vegetables. Four of the pesticides found—dicrotophos, profenophos, cypermethrin, and pirimiphos-methyl—exceeded the maximum residue limits (MRL) set by Thai Codex.

Monitoring of Paraquat in Water

In northern Thailand, herbicide use has dramatically increased because of the convenience and effectiveness of these compounds. Paraquat and glyphosate are the two most popular herbicides used in orchards and vegetable fields in Chiang Mai (Chiang Mai Agrochemical Co-op., unpublished data, 1997). The impact of the overuse of paraquat on the health of sprayers and the general public is of concern.

Analytical methods available

Immunochemical-based methods for paraquat detection have been reported by a few investigators (e.g. Van Emon et al. 1986, 1987). Paraquat determination using traditional instrumental methods has also been reported. These include the determination of paraquat and diquat in crops using ion-pairing liquid chromatography with diode array detection, which was shown to be rapid and reproducible (Chichila and Walters 1991). Furthermore, spectrophotometric methods can also be used, although sensitivity and specificity may found rather poor. Special treatments such as sample enrichment using solid phase extraction can help increase sensitivity. Spectrophotometric methods and solid phase extraction (ion-pairing C8 extraction) for sample clean-up and enrichment have been modified and validated. R.M. Byanju (MSc thesis, in preparation 1998) assessed paraquat contamination of 120 shallow well and surface-water samples collected from the Sarapee District of Chiang Mai has been conducted. The sample extraction eluate was further enhanced in sensitivity using the standard addition method, which was rather time consuming and produced a large volume of chemical waste. The limit of determination of the method is 6 ng/ml with 200 mL of water sample used. From preliminary data analysis, most paraquat concentrations found exceeded the 10 mg/L standard of Canada and Britain. Using a commercial immunoassay test kit with a sensitivity of 0.02 ng/mL or 20 ppt, water samples can be measure directly without any sample treatment except that in some cases debris may need to be filtered out.

Concluding Remarks

Traditional instrumental chromatographic-based methods are suitable for multiresidue analysis and also served as a standard confirmation test.

Where interest is in a single compound only, a chromatography-based approach may not be suitable because of its cost and the time required to complete determinations. Alternative methods with larger sample throughput and lower running costs, such as immunochemical-based methods (e.g. ELISA and other formats) have been used successfully in clinical diagnostics and in environmental applications. Most environmental ELISA kits have improved sample throughput, e.g. five times that of chromatographic methods such as GC. They therefore are potentially suitable for screening of large numbers of samples from environmental monitoring, food safety and human exposure monitoring, although the cost per sample may not as low as those in the clinical diagnostics arena. However, traditional instrumentation-orientated methods such as chromatography and spectroscopy methods are required as confirmatory tests.

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Modifying Application Practice: Novartis Crop Protection Experiences in Indonesia

M. Kusnawiria*, P. Djojosumarto*, and D. Nordmeyer†

Abstract

Use of excessive amounts of sprays is common practice among farmers in developing countries. In Indonesia, some farmers believe that applying spray until it runs off will result in better control of pests. They may have little understanding of the amount of product wasted and the hazard to the environment, and have received only limited information on correct application systems. Moreover, the sprayers available on the market are not equipped with different types of nozzles.

Novartis Crop Protection is trying to solve the problem of over-use of pesticides. It has run a series of application tests in Switzerland, and has established, in a number of countries, farmer support teams (FST) to upgrade farmers' knowledge and skills on integrated pest management, and safe and effective use of Novartis products.

Based on the findings of trials in other countries, an Indonesian FST ran field demonstrations on spray volumes appropriate for potatoes, chillies, and shallots. The results showed that farmers were using too much spray, wasting money and product, and that production costs can be reduced by using correct spray volumes, with no risk of loss in yields.

To avoid loss of yield due to pests, Indonesian farmers rely on crop protection products among other measures. There were 290 active ingredients registered in Indonesia in 1997. They were labelled for the use on 53 crops (Komisi Pestisida 1997). The correct use of these products is described mainly in printed form (i.e. through leaflets and brochures) although many farmers are reluctant to read. To some extent, electronic media or oral presentations are also employed to explain the use of these products, but it is found that field demonstrations are the most effective method. Among the factors relevant to the effectiveness of crop protection products are the method of application and the sprayers themselves.

The method of application , dosage, and timing of application have been tested in a series of trials done mainly by the makers of crop protection products using their standardised sprayers. Almost all spraying systems use water as the carrier to facilitate the distribution of crop protection products onto the spray target. In fact the role of water can be minimised if the quality of distribution can be guaranteed. For example, sprayers can be equipped with specific technology that operates on ultra low volumes which need no water at all.

The sprayers used by farmers may be in a somewhat different condition to those used during the prelaunch of crop protection products. To have a standardised sprayer is costly. This is basically an outcome of not only the quality of the sprayer itself but also of quality and availability of crucial sprayer parts such as nozzles. Thornhill (1995) stated that many sprayers are not designed to accept different types of nozzles.

Considering this situation, some trials have been conducted by government research institutes on different crops to find the best application methods, especially regarding the use of water. Most of the tri-

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als used knapsack sprayers. Sukarna et al. (1974) found that spray volumes between 400 and 1200 L/ha in rice did not affect the yield as long as the recommended concentration of crop protection product was used. Koestoni and Stallen (1990) compared spray volumes of 334 and 505 L/ha to control diseases on shallots, leading to yields of 23.19 t/ha and 21.75 t/ha, respectively, i.e. no significant difference. Koestoni (1994) compared the use of the traditional hollow cone nozzle with four holes with that of a flat fan nozzle, XR11002VS. He found that there was no significant difference on the yield of shallots, but the flat fan nozzle could reduce the spray volume and pesticide cost by up to 33%.

These research findings will not readily change farmers attitude to use the traditional hollow cone nozzle. The main reason is that producers and sellers of sprayers recommend the cone nozzle with no option to use other, sometimes more suitable nozzle types. The situation is similar for power sprayers which are widely used in the uplands for potatoes. There is much less research on power sprayers than on knapsack sprayers.

This paper describes practical experiences on how to modify the available farmer sprayers—either knapsack or power—in order to reduce excessive spray volumes and, consequently, costs.

Current Farmer Practice

At the beginning of farmer support team (FST) activities, Novartis Indonesia conducted a baseline survey. Data from a highland vegetable area in Pangalengan, West Java showed that 98% farmers used knapsack sprayers for cabbage and 70% used power sprayers for potato (Marjudin 1996). In the lowland vegetable-growing areas for shallots and chillies in Brebes, Central Java, knapsack sprayers are used by all farmers. The popularity of knapsack sprayers is also in line with the statement of Thornhill (1995) that Southeast Asia, besides China, is the major market for such sprayers. Farmers use knapsack sprayers with 4-hole nozzles which are provided by sprayer manufacturers. With this nozzle, the flow rate of a knapsack sprayer at 3 Bar (300 kPa) pressure will reach 0.98 L/minute. For a power sprayer, the orifice in the nozzle used is about 2 mm in diameter. Using 5 Bar (500 kPa) pressure, the flow rate of power sprayers is 7.47 L/minute. A survey in 1993 indicated that farmers applied crop protection products with knapsack sprayers at 700 L/ha on fully-grown cabbages and at 1145 L/ha on shallots. That figure for power sprayers on potato was 1760 L/ha. These findings confirm those of Tata and Suhardi (1996).

The farmers believe that the efficacy of crop protection products is greatest when they are sprayed until run-off occurs. According to the baseline survey, Indonesian farmers are most influenced by their fellow farmers (Fig. 1) followed by extension workers and crop protection company employees. The two latter sources, in particular, should pass on information on the correct use of crop protection products. The lack of correct information on this matter caused the cost of crop protection products to be higher than necessary: by 30% on cabbages and 13.3% on shallots (Stallen and Lumkes 1990).

Actually, farmers are keen to accept new knowledge if the benefits are demonstrated to them. Subsequent efforts to deal with farmers' concerns have resulted in good acceptance of new techniques.

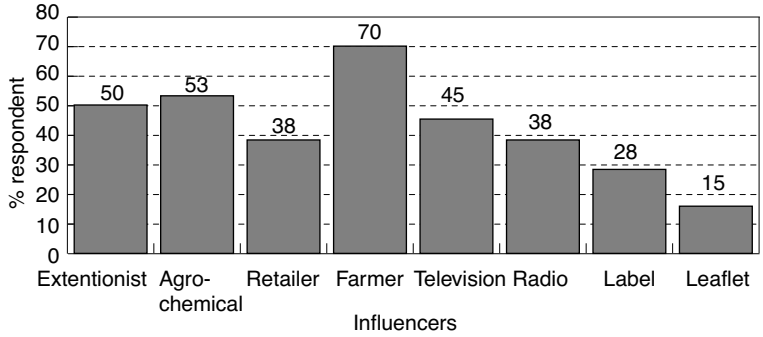

Figure 1. Source of information for farmers: survey (238 respondents) in Pangalengan, West Java, 1992.

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Novartis Efforts

Inquiries about the right spray volumes to use on different crops have come to the attention of Novartis FST. Novartis realises the importance of upgrading farmers' knowledge and skills on the use of crop protection products, not only to save them money, but also to minimise hazards to the environment and to avoid shortening the useful life of crop protection products. Novartis has conducted a series of trials on this aspect from 1980s. One important finding on deciduous fruit orchards was the formula:

$$\text{spray volume/ha} = (\text{tree volume} \times 0.02) + 200 \text{ L}$$

where the tree volume was calculated based on foliage height, tree diameter, and row spacing. The formula was verified by official bodies in Switzerland (Siegfried and Raisigl 1996). These results triggered further tests on other crops such as tomato and cucumber in glasshouses. Adapting the previously mentioned formula, these glasshouse trials generated another formula (H. Felber, unpublished data):

$$\text{spray volume} = (6 \times \text{crop height in cm}) + 200$$

Other field trials were carried out on cabbages and onions which resulted in spray volumes of 400 and 500 L/ha giving the highest recovery rates, yielding substantial economies (Anderau 1995).

These findings must be disseminated to farmers in a correct way. This is in line with the task of FST to train farmers on pest management through integrated pest management (IPM), safe and effective use of crop protection products. FST programs in Indonesia dealt with the highland vegetable crops of potatoes and cabbages for the first 2 years and were subsequently extended to the lowland vegetables, shallots and chillies. From the baseline survey, FST found that the most important concern of farmers in vegetable production were pests and diseases, followed by price of produce (Marjudin 1996). Together with implementing the other IPM components such as agronomic practice, FST adopted the outcomes from application trials carried out in Indonesia or abroad by conducting field demonstrations on potatoes, chillies, and shallots.

The objective of these demonstrations was to prove the effect of spray volumes in relation to reduction of costs using farmers' sprayers. This work was needed before the implementation of training on this aspect. On potato, spray volumes were set at 300, 600, 900, and 1200 L/ha; on chilli at 400, 600, 800, and 1000 L/ha, and on shallot at 500, 750, 1000, 1250, and 1500 L/ha. Knapsack sprayers with hollow cone nozzles were used for all demonstrations. For each crop, the same products and concentrations were used. In line with farmer practice, applications were made weekly on potato and 3 times per week on chilli and shallot. In order to achieve a uniform distribution of droplets, the applicator had to practice application of the aforementioned spray volumes by:

- practicing different walking speeds for the different spray volumes using plain water before spraying the demonstration plots.
- blocking 1–3 holes of the 4-hole nozzle, if needed.

Water sensitive paper was used to demonstrate the distribution of droplets. Pest and disease scoutings were made and yields were recorded.

The results show that there were no yield differences between different spray volumes.

Based on these demonstrations, potato farmers acknowledged that there is no benefit in applying crop protection product in excessive water. To adopt this finding to their power sprayer use, potato farmers changed their original orifice diameter from 2 mm to 1 mm. To convince farmers about the disadvantages of excessive spray volume, theoretical explanations were given also, as described in Table 1. The impact of training on this aspect has been proven by the results of the opinion surveys. Figure 2 shows a reduction of spray volume used by farmers.

There was an indication also that this modification of application has disseminated from trained to untrained farmers. Spray volume demonstrations are a vehicle to train farmers on other aspects of judicious use of crop protection products, such as preharvest interval. Combined with the reduction of application number due to implementation of IPM, i.e. application

Table 1. Pre-training spray volumes on potatoes

1.	Concentration of product A (mL/L)	0.75
2.	Spray volume (L/ha) Thus product A was used at a rate (mL/ha) of	1760 1320
3.	Retention capacity (mL/ha) before run-off, based on spray volume demonstration Thus, product A retained on the plant(mL/ha)	900 675
4.	So run-off = product loss to soil (mL/ha)	645
5.	Therefore possibility of hazard to the environment is high	

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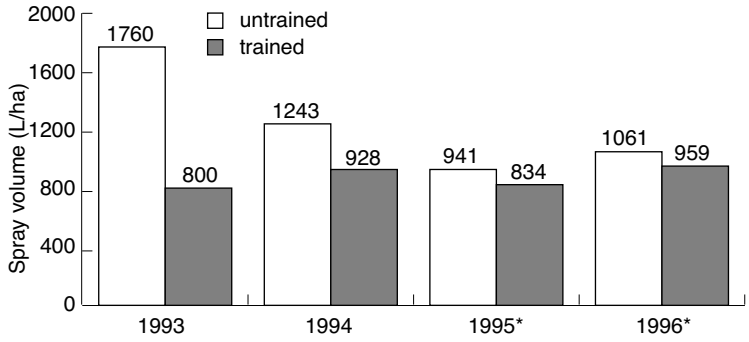

Figure 2. Reduction of spray volume on potato: opinion survey, Pangalengan, West Java.

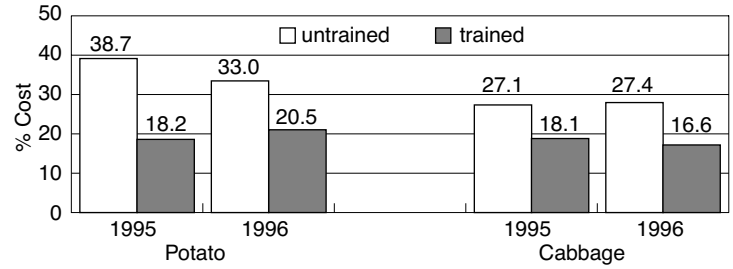

Figure 3. Input reduction in pest management: opinion survey, Pangalengan, West Java.

based on controlled threshold, pesticide cost was considerably reduced (Fig. 3), as was the likelihood of pesticide residues above maximum residue level.

Conclusion

- Farmers will implement the new application technology as long as it is practical and meets their concerns.
- Research on modification of application on other crops and training based on these findings is urgently needed to avoid excessive use of crop protection products which may create unacceptable residue levels.
- It is necessary to establish collaborative programs between government bodies and private companies to utilise resources most cost effectively.

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Reducing Pesticide Residues in Agricultural Produce through Effective Application Techniques

Dzolkhifli Omar*

Abstract

In chemical control, application of pesticide plays a crucial role in ensuring that the toxicant has the opportunity to perform its function to control pests. It influences the quantity of toxicant needed for effective field control of pests. The correct combination of spray factors and spray liquid formulations is the key to a good spray deposition and distribution, reduces wastage and increases the biological efficacy of the pesticides. This paper addresses the feasibility of placing the toxicant applied and adjusting the spray factor and formulation to obtain discriminating treatment with maximum effect against the pest. This will reduce the dosage and number of applications required thus contribute towards an effort to reduce pesticide residue.

IN chemical control, appropriate application of pesticide plays a crucial role in ensuring that the toxicant has the opportunity to perform its function to control pests. It influences the quantity of toxicant needed for effective field application, and the effectiveness of a toxicant after it has been deposited on a target surface or target pests.

The right combination of spray factors (spray droplet size, number of droplets per given area, and the concentration of toxicant in spray droplet) and spray liquid formulations is the key to good coverage, minimal wastage, and optimal biological efficacy of pesticides (Hall 1987). The pattern of toxicant placement on target will usually determine the efficiency of the toxicant in controlling the pest. This paper discusses the roles of application technique and spray formulation in obtaining maximum effect against the pest and minimal non-target effects. Proper application will help reduce the dosage and number of applications required for pest control and contribute to a reduction in pesticide residues.

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Overview of Pesticide Application Techniques

Pesticide application is a very inefficient process. Less than 1% of the toxicant intended for pest control actually reaches the target to have the intended biological effect. Pesticide application is a complex procedure. Figure 1 summarises the various processes involved. The factors influencing these processes are reviewed and discussed by Hartley and Graham-Bryce (1980).

Conventionally, pesticides are applied using hydraulic nozzles through which volumes of water are pumped to produce spray droplets of various sizes. In this application technique, coarse droplets tend to bounce off the target, while droplets of less than 50 μm are likely to drift away. Thus, only a small proportion of spray droplets is retained on the target, resulting in wastage of the chemical.

Ultra-low volume (ULV) is alternative method of application. 'ULV' refers to the use of small, total volumes of spray. It is usually defined as the application of pesticide when the minimum volume of spray is applied in a narrow spectrum of droplet sizes to achieve economic control (Matthews 1979).

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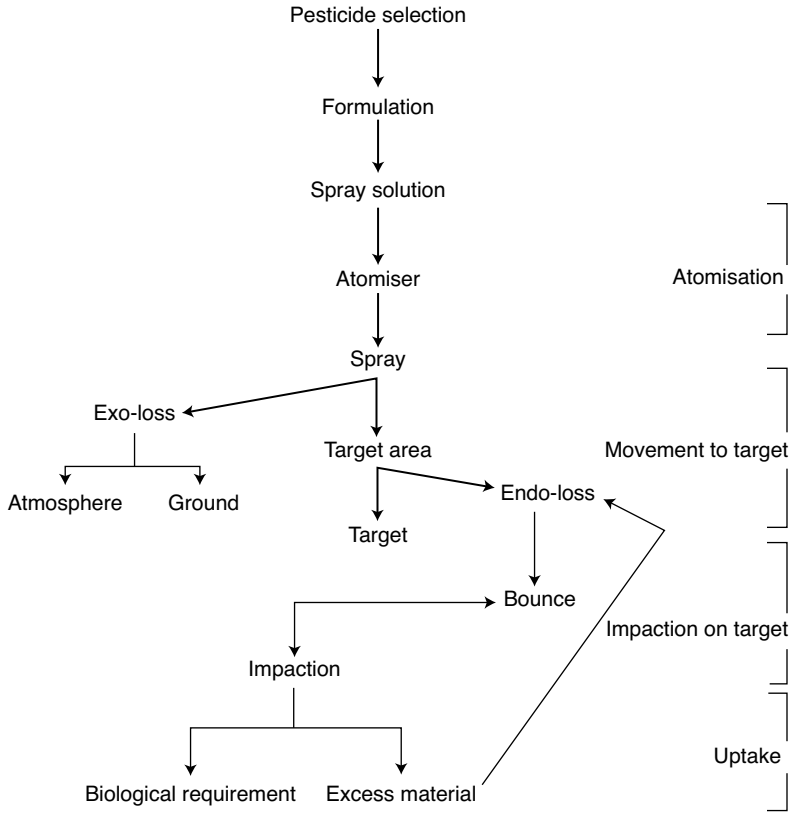

Figure 1. Processes involved in pesticide application and deposition (after Comellack 1982)

Some Factors to be Considered before Pesticide Application

Selection of chemical

Selection of the correct pesticide clearly plays a crucial part in the control of pests. Factors that influence the selection of pesticides include their mode of action, their physical and chemical properties, their persistence in the environment, and the characteristics of the target pest.

Target

The behaviour of the pest plays a crucial role in deciding which pesticide to apply. If the target is a mobile species of insect, contact with toxicant will occur as the insects move around or consume leaves. The probability of an insect coming into contact with the pesticide will depend upon factors such as the fractional cover of the treated surface, the sites of deposition on the plant, and the walking and feeding behaviour of the species (Ford and Salt 1987).

In the case of sedentary insects, the toxicant has to be placed close to them to ensure that, on spreading over the treated surface, it will come into contact with the target insect. The effectiveness of the toxicant will therefore be governed by the perimeter of the spray deposit, which determines the amount of toxicant spreading across the leaf surface (Wyatt et al. 1985).

Dosage and application variables

Normally the recommended rate of application of compounds for pest control is given as the amount of the product for a given amount of water; for example, 10–15 mL product per 4.5 L water. The recommendation neither specifies the amount of active ingredient nor the amount of spray solution that should be applied per hectare. The end-user has to guess the amount of active ingredient or volume of

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spray solution to be applied per hectare. It is thus not surprising that the dose applied is not exactly what is required. Dose should be recommended in the form of the amount of active ingredient per hectare. The applicator then decides the amount of carrier to be used. Based on the amount of carrier, the volume application rate could then be classified as given in Table 1.

Table 1. Application of pesticide according to volume application rate

Type of application	Volume of carrier (L/ha)
High volume	>600
Medium volume	200–600
Low volume	100–200
Very low volume	50–100
Ultra-low volume	<50

Upon deciding the volume to be applied, other factors that should be considered are the optimum droplet size and the number of spray droplets. Tables 2 and 3 show the optimum spray droplet sizes and distributions per given area for particular types of pesticides and targets.

Table 2. Optimum spray droplet size for pest

Target	Droplet size (μm)
Flying insect	10–50
Insects on foliage	30–50
Foliage	40–150
Herbicide	250–400

Table3. Suggested number of droplets according to pesticide type

Type of pesticides	No. of droplets per cm^2
Translocated herbicides	5–10
Pre-emergence herbicides	20–30
Post-emergence contact herbicides	30–40
Insecticides and systematic fungicides	20–30
Non-systematic fungicides	50–70

Role of spray factors in the effectiveness of pesticide

Spray factors include spray droplet size, volume application rate, concentration of active ingredient in spray droplets, and spray formulation. Spray droplet size has been shown to influence the effectiveness of pesticide deposits on the target. Spray droplets of 20 μm were more effective than 75 μm droplets in the control of greenhouse whitefly (Mboobs 1975). The dose required to maintain similar mortality on the eggs of *Tetranychus urticae* (Munthali and Wyatt 1986) and the larvae of *Trialeurodes vaporariorum* (Adams et al. 1987). In general, small droplets have been shown to be more effective in controlling the pests than coarse droplets. Small droplets distribute toxicant more efficiently than larger droplets and this increases the probability of pests coming into contact with the toxicant.

Droplet size also influences the fate of pesticides in the environment. It affects, among other things, the rate of evaporation, terminal velocity, surface tension, and spray deposition. How the spray deposits on the target depends on the physical and chemical properties of spray droplets and various plant factors (Tadros 1987). Surfactants are commonly added to spray formulations to facilitate and accentuate the emulsifying, dispersing, wetting, or other surface-modifying properties of liquids.

Selection of Application Variable for Field Application

A hydraulic nozzle fitted to a lever-operated knapsack sprayer is very popular among farmers in developing countries for applying pesticides. The nozzle produces droplets of many sizes, ranging from 1–1000 μm; the droplet spectra can be classified into fine, medium, coarse, and very coarse. Depending on the target pest and the surrounding environment, one droplet size might be preferred over others. For insect control, either fine or medium droplets would be preferred, while for weed control coarse droplets are recommended to minimise spray drift.

In a bioassay study using second instar larvae of *Plutella xylostella* (a moth pest), Chinese mustards were sprayed using a knapsack sprayer fitted with cone nozzles of varying specifications to determine the influence of the spray droplet spectrum on the effectiveness of cypermethrin. All treatments received similar volumes and application rates. It was found that the knapsack sprayer fitted with cone nozzle (HARDI) that produced smaller droplets (mean diameter = 119 μm) gave better mortality rates than bigger droplets (mean diameter = 210 μm) (Omar et al. 1991). This clearly shows that smaller droplets were more efficient in killing the larvae, through wider distribution of the pesticide on the target surface.

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In another experiment using the knapsack sprayer, it was shown that the efficacy of *Bacillus thuringiensis* endotoxin was greater using swirl plates that produced smaller droplets than with swirl plates producing coarser droplets (Table 4).

Table 4. Effect of cone nozzles on mortality of second instar larvae of *Plutella xylostella*

HARDI nozzle code	Swirl plate[a]	Mortality [b] (%)
1553–24	Blue	63.4 a
1553–18	Grey	55.8 a
1553–12	Black	46.7 b
1553–08	White	25.8 c

[a] Blue plates produce the smallest droplets and white plates the largest.
[b] Means followed by the same letter are not significantly different (P=0.05) by DMART.

A similar bioassay experiment using second instar larvae of *P. xylostella* was conducted to determine the influence of spray volume application rates on the effectiveness of cypermethrin. The HARDI cone nozzles were fitted with blue swirl plate to deliver various volume application rates of a fine droplet spectrum. All treatments were adjusted to receive similar masses of pesticide. There was no significant difference between volume application rates of 250 and 210 L/ha for both mortality and knockdown of the larvae (Table 5). It appears that, for this fine droplet spectrum, the volume application of 200 L/ha was the optimal one below which the efficacy of cypeymethrin declined.

Table 5. Effect of spray volumes of cypermethrin on mortality of second instar larvae of *P. xylostella*

Nozzle	Application rate (L/ha)	Percentage knockdown after 1 hour	Percentage mortality after 24 hours[a]
1553–24	250	58.5 a	64.0 a
1553–18	210	54.0 a	56.5 a
1553–12	140	35.0 b	43.5 b
1553–08	110	19.0 c	20.0 c

[a] Means within column followed by the same letter are not significantly different (P=0.05) by DMART (P<0.05).

In a glasshouse study on spray droplet formulations, the deposition of spray solution with and without surfactant (Pulse) on 5-week-old *Pennisetum polystachion* L. was compared using a fluorescent tracer technique. Treatments with surfactant were found to give a 20% recovery, compared with 11.7% for treatment without surfactant (Teo et al. 1997). The surfactant contributed a 71% increase in spray deposition and thus would directly contribute to increased bioefficacy of the pesticide.

Table 6. Mortality of second instar larvae of resistant and susceptible strains of *P. xylostella* to cypermethrin, with three application variables, dose, droplet size, and droplet density per unit area

Application variables of cypermethrin			Mean 24-hour mortality[a] (%)	
Dose (g a.i./ha)	VMD (μm)	No. of droplets/ cm^2	Resistant strain	Susceptible strain
15.3	64	17±3	56 a	75 a
32.3	64	36±4	84 b	90 b
97.8	133	17±3	66 a	88 b

[a] Means followed by the same letter within a column are not significantly different (P = 0.05) by DMART.

In a study of the effect of spray volume and Pulse surfactant on the efficacy of the herbicide metsulfuron-methyl for the control of *Diodia ocimifolia,* reducing spray volume from 600 to 200 L/ha without the surfactant improved the efficacy of the herbicide. Adding the surfactant also significantly enhanced the efficacy, but only at spray volumes of 400 and 600 L/ha (Ooi et al. 1997).

Application Technique

Injudicious use of pesticides has led to the development of resistance of insect pests to certain compounds, and farmers often respond to this by increasing the dose to ensure good control. This has repercussions that go beyond simply an increase in the quantity of pesticide applied to the plant.

Pesticide management has been suggested as one way to combat the resistance problem, including rotation of chemicals having different modes of action, observing economic thresholds, and not mixing insecticides (Sun 1992). Attention to application technique could also contribute to combating resistance.

A laboratory study was conducted on the mortality of resistant and susceptible strains of *P. xylostella* larvae to three application variables. The result showed that a moderate dose (32.3 g a.i./ha) applied using

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spray droplets of 64 μm and at 36 droplets/cm^2 gave the highest mortality of both resistant and susceptible strains (Table 6).

Conclusion

The placement of toxicant plays a crucial role in pest control. The spray droplet size and the number of droplets for a given area influences the biological effect of a toxicant on a pest. If these factors are taken account of in applying a pesticide its biological efficacy can be enhanced. Also, fewer applications of pesticide to agricultural produce and the environment would be needed.

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Educating Farmers for Chemical Management in Indonesia

Soeprapto Mangoendihardjo*

Abstract

Indonesian farmers have received little formal teaching in the correct use of chemicals. Although integrated pest management (IPM) has been a national priority since the 1980s, until recently farmers have not been told of its significance, or of the importance of using in their fields, the right product, at the right place, and during the right time of the cropping cycles. Some informal training in aspects of IPM has occurred, either nationally through the IPM program or locally during training programs supported by non-government organisations. Indeed, there are today many farmers who have participated in a training program, and who have substituted non-chemical products for chemicals to manage and overcome pests in the fields, but there are still many more farmers who are continuing to use chemical pesticides inappropriately.

ALTHOUGH the government is developing agroindustries and establishing many other industries, basically Indonesia remains an agricultural country. More than 50% of population of the country are farmers, but some of them are looking for employment away from their farms. Most of their children would prefer to be soldiers, governmental officers, or other professionals. That is why the work force in agriculture now consists mostly of old men or unemployed young people.

Informal education for farmers has been undertaken by the government. This began four decades ago when self-sufficiency in food become national policy. It was first implemented through 'Plot Demonstration', locally named *Demonstrasi Plot* (*DEMPLOT*) in 1957–1959. This was followed by: 'Mass Guidance in Agriculture'—*Bimbingan Massal* (*BIMAS*); 'Mass Intensification'—*Intensifikasi Massal* (*INMAS*); 'Special Intensification', *Intensifikasi Khusus* (*INSUS*); and finally *SUPRA INSUS*. Through those programs farmers were able to study and implement new technologies on their farms. During the implementation of *DEMPLOT* until *SUPRA INSUS*, farmers were trained either by field agricultural extension officers (FAEOs) or pests and diseases observers (PDO) in using fertilizers, chemical pesticides and other inputs to agricultural production. Unfortunately, during those periods the use of chemical pesticides tended to be incorrect and imprudent.

When an outbreak of brown plant hopper (*Nilaparvata lugens*) occurred in 1986, it was proven that resistance and resurgence of the pest was due to incorrect use of broad-spectrum insecticides during preceding few years. Because of this the Government of Indonesia issued a *Presidential Instruction No. 3 Year 1986* (Anon. 1986) covering the improvement of brown plant hopper (PBH) control using integrated pest management (IPM). The success of that program encouraged the government to organise informal education for farmers through a 'Field School of IPM'. Today the IPM program has been developed not only for food crops but also horticultural and estate crops. Today, non government organisations (NGOs) play an important part in accelerating the informal education.

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Governmental Policy versus Demand for Rice

Concepts of plant protection began to change when Stern et al. (1959) introduced a new concept called integrated pest control (IPC) which linked biological and chemical methods. It was based on their studies on the control of alfalfa aphid in the USA. Further stimuli to the development of the concept were the publication of Rachel Carson's classic 'Silent Spring' in 1963 and an international symposium on plant protection held by FAO in Rome in October 1965. Because it aimed to integrate all methods for managing pests, it became known as integrated pest management (IPM).

Government policy

Following emergence of the improved concept of IPM in 1966, the Government of Indonesia decided to give attention to the conservation of the environment. *Governmental Regulation No. 7 Year 1973* on 'Storage, Distribution, and Control of Pesticide Uses' was issued. Up to 1980 more than 400 kinds of pesticide trademarks were officially registered in Indonesia under this regulation (Hardjasoemantri 1997).

To encourage efforts towards environmental protection, the government issued *Law No. 4 Year 1982* (Anon. 1982), covering the 'Principles Certainties of Environmental Management', and *Presidential Instruction No. 3 Year 1986* (Anon. 1986) was issued to improve brown planthopper control by implementing IPM. During the following three years the amount of chemical pesticides used fell dramatically, from 17 300 t in 1986 to 3 000 t in 1989. In the meantime, rice production remained relatively stable. Positive results obtained during three years of implementation of (PI 3/1986) encouraged the government to stop the pesticide subsidy in 1989, which saved Rp200 billion per year (Oka 1990).

Another positive impact of the above-mentioned policies was to make farmers more careful before purchasing and applying chemical pesticides. Three years later the government issued *Law No. 12 Year 1992* (Anon. 1992) applying to the cropping system, in which IPM was included in paragraphs 20–27. The most important statement was included in point 1 paragraph 21.I.E.: 'Anybody and or corporate body is not allowed to use materials and or methods which may harm public health and or threat human safety, creating disturbance and damaging natural resources and or environment'.

To guide implementation of *Law No. 12 Year 1992*, *Governmental Regulation No. 6 Year 1995* regarding crop protection (Anon. 1995a) was issued. On 17 June 1996, the issue of *Ministerial Direction No. 473/Kpts/TP2/0/6/96*, banned the distribution and marketing of 28 chemical pesticides in Indonesia. This policy will be extended to other chemical pesticides, namely broad spectrum compounds and those harmful to the environment. To further strengthen efforts in environmental protection, the government issued *Law No. 23 Year 1997* (Anon. 1997). Facing monetary problems and the approaching free market era, in the beginning of 1998 the government issued five *Presidential Instructions*, and six *Presidential Decisions*, including PI Nos 1 and 5 to solve cropping system problems.

Demand for rice

Before 1982 Indonesia was the world's biggest rice importer. Then, in 1983, it became self-sufficient in rice, a status it maintained for 10–11 years. In 1995, however, Indonesia began importing rice again. In 1998, about 2 Mt of rice were imported.

Demand for rice has increased, partly because of changing dietary preferences. It is now the staple food for Indonesians in some regions, who formerly did not consume rice. Also, the population is increasing by about 1.6% annually (Oka 1996) and is now close to 205 million. Loss of rice-cropping areas in Java during the past 10 years due to development in non-agricultural sectors has forced the government to develop a 'mega project' for food crops in central Kalimantan: the 'One Million Hectares Project'.

A new technology called 'zero tillage farming' or *Tanpa Olah Tanah* (TOT) is widely practiced on the peat soils of Sumatra and Kalimantan. It has encouraged the large-scale use of chemical herbicides. These developments may accelerate the use of chemicals in agriculture to rapidly increase rice production, but without considering the negative effects of chemicals on the natural enemies of pests and on the environment in general. Moreover, a statement by the Chairman of Commission VI of Indonesian Parliament (*Kedaulatan Rakyat*, 16 May 1997) recommended that agrochemical companies make chemicals, such as paracol to control weeds, more readily available. This may increase the number of pesticides marketed in some provinces of Indonesia.

Some banned chemical pesticides are still being marketed, even in Gunungkidul Regency, Yogyakarta, where the price of pesticides has increased

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sharply in early 1998 because of the decline in the Rupiah exchange rate. This may be attributed to weaknesses in the control of pesticide distribution, storage, and use.

Informal Education for Farmers

During the implementation of the *DEMPLOT* to *SUPRA INSUS* programs, informal education for farmers was mostly done by extension services. During those periods, farmers were encouraged to use chemical pesticides. The pesticide subsidy applied, and in all programs pesticides were included in credit schemes for rice production.

Field school of IPM

The success in implementing IPM in rice farming stimulated the Government of the United States of America to provide aid for the acceleration of IPM through a 'Field School of IPM' (FSIPM) program. Five leading universities, i.e. IPB, UGM, USU, UNHAS, and UNIBRAW, were given a task of organising a course of training leading to a 'Diploma 1 on IPM'. The Diploma 1 holders then trained 1000 persons of FAEO and PDO all over Indonesia, who would then train farmers, in cooperation with Agricultural Extension Services in Sumatra, Java, and Sulawesi. FSIPM was then held in some provinces to train farmer groups in IPM. Farmers were trained in IPM and in the most prudent use of chemical pesticides. A popular slogan among the farming community was 'Natural Enemies are Friends of Farmers' (Anon. 1991). Training of trainers has been under way since 1989. The target for the number of trained farmers by the end of 1998 is 800 000.

Non government organisations (NGOs)

The IPM field schools stimulated the implementation of an agriculture that is not dependent on synthetic chemicals and fostered a 'back to nature' approach. Moreover, the development of organic agriculture (OA), in line with the World Trade Organization's Sanitary and Phytosanitary Protocol (Anon. 1995b) must be followed by the Government of Indonesia.

NGOs in some provinces of Indonesia played a very important role in educating farmers to carry out OA on their farms. They do not use agrochemicals, including synthetic fertilizers and pesticides. Instead of synthetic fertilizers, they use compost or green manures. The latter involves growing leguminous manure plants and ploughing them in some weeks before cropping. Many commodities are now grown under OA, including: coffee in Aceh, North Sumatra, Lampung, East Java, and East Nusa Tenggara provinces; vegetables in North Sumatra, Central Java, Yogyakarta and East Java; and rice in North Sumatra and Yogyakarta.

According to Sabirin (pers. comm. 1996) and Mangoendihardjo (1997), in Medan, North Sumatra an NGO named Bitra Indonesia has been coordinating farmers implementing OA in food crops for eight years, and is producing about 1050 t/year of rice. Another NGO called Cinta Desa is coordinating farmers in Simalungun Regency and hopes to export about 25 t/week of cabbages grown without use of pesticides (Saragih, pers. comm. 1998). Meanwhile in Yogyakarta there was a vegetable shop selling OA vegetables produced by an NGO in Salatiga District, Central Java between 1992 and 1997.

Another NGO named HPS (Hari Pangan Sedunia) in Yogyakarta (Panggih Saryoto, pers. comm. 1998) has also been coordinating farmers undertaking OA since 1991. So far the farmers participating in the OA have had informal education through the activities of farmers groups. They used leaflets and radio broadcasts on special topics, e.g. 'Village Broadcasting' (Siaran Pedesaan), through Radio Republic of Indonesia (RRI) and private radio stations. Television programs, either TVRI, or private television such as Indosiar, RCTI and SCTV, are also effective tools for transferring information.

Of the agrochemical companies operating in Indonesia, as far as is known, only CIBA-GEIGY has so far arranged programs of informal education for farmers. CIBA-GEIGY's Farmer Support Team (FST) has two projects, one located in Pengalengan, West Java which started in 1991, the other in Brebes, Central Java which started in mid 1996. In both projects the farmers were trained in IPM, including correct use of chemical pesticides. CIBA does not force the farmers to use its products, but leaves the decision with farmers as to what product they want to buy. According to Ruegg (1997) farmers participating in FST in Pengalengan may achieve higher profits in potato and cabbage production than untrained farmers. The program in Brebes is currently being evaluated.

Discussion

Though it is government policy to implement IPM and foster environmental protection, it appears that

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chemical use might actually be increasing after the issue of Law No. 12 Year 1992 (Anon. 1992), Government Regulation (GR) No. 6 Year 1995 (Anon. 1995a), and Law No. 23 Year 1997 (Anon. 1997). Theoretically, chemical use in agriculture should have fallen, as it did after the issue of PI 3/1986. It seems that the increasing amounts of chemical pesticides being used, particularly herbicides, is affected by increasing demands for food, especially rice, and uncontrolled distribution and marketing of pesticides.

It is difficult to say whether a 'pesticide Mafia' exists in Indonesia and has played an important role in increasing the amount of chemical pesticide used, a circumstance that has been claimed to apply in the USA. Perhaps the situation is not much different between the two countries. There is some evidence that a 'Pesticides Conspiracy' (van den Bosch 1980) may also be at work in this country.

Indonesian citizens may believe that after the 'Letter of Intention' between the International Monetary Fund and the Government of Indonesia was signed, some agricultural and trade problems will perhaps be solved during next 2–3 years. Based on Presidential Instruction No. 1 and No. 5 Year 1998, people may implement the 'free market' under which farmers can decide whatever crops they wish to plant on their own farms. This condition will stimulate farmers to be self-confident in producing agricultural commodities to meet market demands.

Facing the 'Free Market Era', the Government of Indonesia must continue FSIPM, and the NGOs must be encouraged to extent the area higher quality products grown. This needs practical additional knowledge, such as the use of 'effective microorganisms' (EM) (Teruo Higa 1993), 'probiotic microorganisms for agriculture' (PMA), 'cropping microorganisms' (CM) and other approaches.

The current situation in Indonesia may encourage the NGOs to play a more important role in educating farmers to accelerate their efforts in OA. Due to the limited number of NGOs dealing with OA until now, research and development cooperation between, for example, ACIAR and Indonesian institutions such as the Research Center for Biological Control at Universiti Gadjah Mada is essential. ACIAR Project No. 9110 is a good example of the type of cooperation needed.

Conclusions

- Field school on IPM run in Indonesia have provided informal training to farmers in the management of chemical use in agriculture.
- Successive governmental policies in Indonesia have not been followed by some decision-makers, agrochemicals companies, distributors, and farmers.
- A 'pesticides conspiracy' may disrupt many governmental policies in the arrangement of IPM field schools and other efforts at environmental protection.
- NGOs can play more important role in implementing organic agriculture to avoid the occurrence of pesticide residues in the crops of farmers facing a free market era.
- Development cooperation to accelerate organic agriculture is essential.

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Reduction of Pesticide Residue Contamination on Vegetables by Agro-extension Work

Nguyen Thien,* Bui Van Thin,† and Nguyen Duy Duc†

Abstract

In Ho Chi Minh City, there are nearly 10000 farmer households producing around 300000 tones of vegetables annually. However, there has been severe contamination of vegetables with residues of pesticides (especially insecticides). The causes of this are inappropriate and inaccurate use of insecticides by farmers.

To improve this situation, the Plant Protection Department of Ho Chi Minh City has carried out a program to promote the correct use of insecticides by farmers. The program has four parts:

1. Run instruction sessions for almost all farmers cultivating vegetables in the suburbs of Ho Chi Minh City, to show them how to correctly use pesticides on their vegetable fields.
2. Set up various demonstration fields using insecticides correctly, linked to field workshops teaching farmers how to apply pesticides accurately on their own vegetable fields.
3. Distribute leaflets on 'Accurate and safe use of insecticides on vegetable' to all vegetable-growers in the region.
4. Run broadcasts from the City Broadcasting Station for promoting to farmers safe and accurate application of pesticides, to protect their own health and that of consumers.

The results of a preliminary evaluation of the program are presented.

IN Ho Chi Minh City, there are around 10 000 households of farmers growing nearly 12 000 ha of various species of vegetables annually. They produce 300 000 t of vegetable product, equal to 70% of the vegetable needs of the city's 7 million people. However, according to research work monitoring pesticide residues in vegetables produced in Ho Chi Minh City, directed by the University of Agriculture and Forestry (UAF), the contamination of pesticide residue on vegetables was at a very high level, especially by several pesticides belonging to the organophosphate group. Analysis of several vegetable samples showed levels of residues many times higher than the FAO and WHO international standard (Table 1).

After investigating and analysing the situation, we concluded that the main cause of contamination was the incorrect use of pesticides by farmers.

Ways Farmers Misuse Pesticides on Vegetables

Many investigations were carried out by the Plant Protection Department of Ho Chi Minh City and by the University of Agriculture and Forestry during 1994–1995. They showed that farmers growing vegetables were using pesticides inappropriately.

1. Farmers were using pesticides as the main means to control insects in their fields. They had no knowledge of integrated pest management. This resulted in the abuse of pesticides.

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Table 1. Pesticide residues found on vegetables produced in Ho Chi Minh City and Tien Giang, Ninh Thuan, Lam Dong provinces, Vietnam.

Pesticide group	Number of samples	Percentage of samples contaminated	Percentage of samples with residue higher than the FAO standard	Pesticides found
Organophosphate	102	75	54	Methamidophos, monocrotophos, phenthoate, dimethoate
Pyrethroid	79	32	1	Cypermethrin, esfenvalerate
Total	181	55	30	

Source: University of Agriculture and Forestry, Ho Chi Minh City.

2. Farmers were using many high toxicity, persistent pesticides, including organophosphate, organochlorine, and carbamate compounds. The pesticides in most common use were methamidophos and monocrotophos (50% of total amount of pesticides and 70% of organophosphate group) (Table 2).

Table 2. Pesticides previously used by vegetable farmers in Ho Chi Minh City.

Pesticide group	% used by farmers	Compounds in most common use
1. Organophosphates	70	Methyl parathion, monocrotophos, metamidophos, phenthoate, dichlorvos, diazinon, dimethoate
2. Pyrethroids	18	Cypermethrin, fenvalerate, esfenvalerate, deltamethrin
3. Carbamates	4	Fenobucarb, isoprocarb, carbofurane, carbaryl
4. Organochlorines	2	Endosulfan
5. Insect growth regulators	0	
6. Microorganisms	2	*Bacillus thuringiensis*
7. Others	4	Cartap, fibonil, ethofenprox, etc.

3. Continuous use of particular insecticides for long periods without rotation of compounds, results in the development of resistance of insects to those pesticides. This causes farmers to increase the dosage of pesticide to many times the recommended dosage to protect their crops.
4. Applying pesticides very close to harvest time, and not observing the correct withholding period.

Five years ago, the Ministry of Agriculture and Rural Development promulgated a regulation banning the use of several very toxic pesticides on vegetables. These included methamidophos, monocrotophos, carbofuran, and endosulfan. However, farmers remained unaware of this regulation, and continued to use the banned pesticides, which were still available in the marketplace for use on crops other than vegetables, were low-priced, and had a broad-spectrum of efficacy.

Encouraging Farmers to Produce Safe Vegetables

Realising that excessive pesticide residues on vegetables ares mainly caused by incorrect use of pesticides by farmers, the Plant Protection Department of Ho Chi Minh City introduced a program entitled 'Campaign for guiding and encouraging farmers to produce safe vegetables' in June 1996. The objectives of this program are:

1. To help farmers to understand the damage caused by pesticide abuse— to the health of producers and consumers, to ecosystems, and in causing outbreaks of pests—and therefore of the need to reduce pesticide use and introduce integrated pest management.

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2. To communicate to farmers the list of pesticides banned for use on vegetables and to advise them to stop using these pesticides on vegetables.
3. To recommend to farmers that they do not use organophosphate, organochlorine and carbamate pesticides which have high toxicity and persistence, especially on vegetable crops approaching harvest.
4. To advise farmers to use instead, those insecticides which have low toxicity and short-lived residues, such as microorganisms, insect growth regulator, and pyrethroid groups chemicals.
5. To instruct farmers how to properly apply pesticides at accurate dosages and to rotate the use of different pesticide so as to prevent the development of resistance to pesticides among pests.
6. To recommend to farmers that they observe appropriate withholding periods between the application of pesticides and the harvest and sale of vegetables.

Implementation of the program

The program has been implemented as follows:

1. Organising many instruction sessions for almost all farmers growing vegetables in suburban areas of Ho Chi Minh City, to show them how to use insecticides correctly in their vegetable fields.
2. Establishing many demonstration fields in which pesticides are used correctly. Workshops for farmers are held in these fields to show them the results of safe and effective use and the need to replace the wrong use of farmers in the past.
3. Distributing many copies of a leaflet about the effective and safe use of pesticides on vegetables to almost all farmers growing vegetables in the area.
4. Using the Ho Chi Minh City radio station to run sessions instructing and encouraging all farmers to use pesticides safely and effectively so as to protect the health of consumers and themselves.

Table 3 gives statistics on these activities.

Table 3. Extension activities on safe and effective use of pesticides on vegetable crops implemented in Ho Chi Minh City between June 1996 and December 1997.

Activity	Number	Number of participating farmers
1. Farmer instruction	293 sessions	13220
2. Demonstration fields	121 fields	5366
3. Leaflet distribution	24 000 sheets	
4. Broadcasting session	15 sessions	

Source: Plant Protection Department of Ho Chi Minh City, annual reports 1996,1997.

Evaluating the program

After 18 months of activity, the Plant Protection Department of Ho Chi Minh City evaluated of the program by interviewing 150 farmers. A questionnaire used for the interviews includes many items concentrating on evaluating the change of perception, adoption, and application practice of farmers after participating in the program. In this report, we discuss the results of the evaluation of only some of the main items.

Table 4. Pesticides used by vegetable farmers in the Ho Chi Minh City area after implementation of the project.

Pesticide group	% used by farmers	Pesticides in most common use
1. Organophosphates	30	Methyl parathion, monocrotophos, metamidophos, phenthoate, dichlorvos, diazinon, dimethoate
2. Pyrethroids	38	Cypermethrin, fenvalerate, esfenvalerate, deltamethrin
3. Carbamates	2	Fenobucarb, isoprocarb, carbofuran, carbaryl
4. Organochlorines	0	Endosulfan
5. Insect growth regulators	9	Chlorfluazuron, teflubenzuron, buprofezin
6. Microorganisms	6	*Bacillus thuringiensis*
7. Others	15	Cartap, fibronil, imidachloprid, ethofenprox

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1. Table 4 summarises information provided by farmers on the kinds of pesticides they are now using.
2. The percentage of farmers still applying insecticides banned from use on vegetables is 11% (compared with 50% three years ago). The pesticides in most common use are methamidophos (7%) and monocrotophos (3%).
3. Reasons given by farmers for continued use of banned insecticides on vegetables (% of answers)

- Low price: 65%
- Rapid kill of insects 20%
- Using only early in season 12%
- Other reasons 3%

4. Perceptions of farmers about the damage caused by pesticide abuse (% of answers)

- Harm to the health of farmers 100%
- Harm to the health of consumers 100%
- Harm to the environment 85%
- Cause of outbreak of pests 28%

Responses to other items in the survey showed that farmers' approaches to protecting their vegetable crops had changed for the better.

Discussion and Conclusion

Basing on the survey results, we could confirm that the campaign had changed the perceptions and the application practices of farmers:

- Reduction in use of pesticides banned on vegetables, from 50% 3 years ago to 11% now (Fig. 1).
- Reduction in use of highly toxic pesticide groups from 76% 3 years ago to 32% now (Fig. 1).
- Increase in use of pesticides with lower toxicities and persistences (pyrethroids, microorganisms, insect growth regulators), from 20% 3 years ago to 53% now (Fig. 2).
- Increase in awareness of farmers of the problems of contamination by pesticides.

Although there has been no residue analysis work, it is assessed that the degree of pesticide residue contamination on vegetables has been improved as a result of the campaign. Nevertheless, 11% of farmers are still using prohibited insecticides and 32% are using high toxicity, persistent pesticides. So the problem has not been completely solved, and it seems that it will be only by introducing regulations to ensure

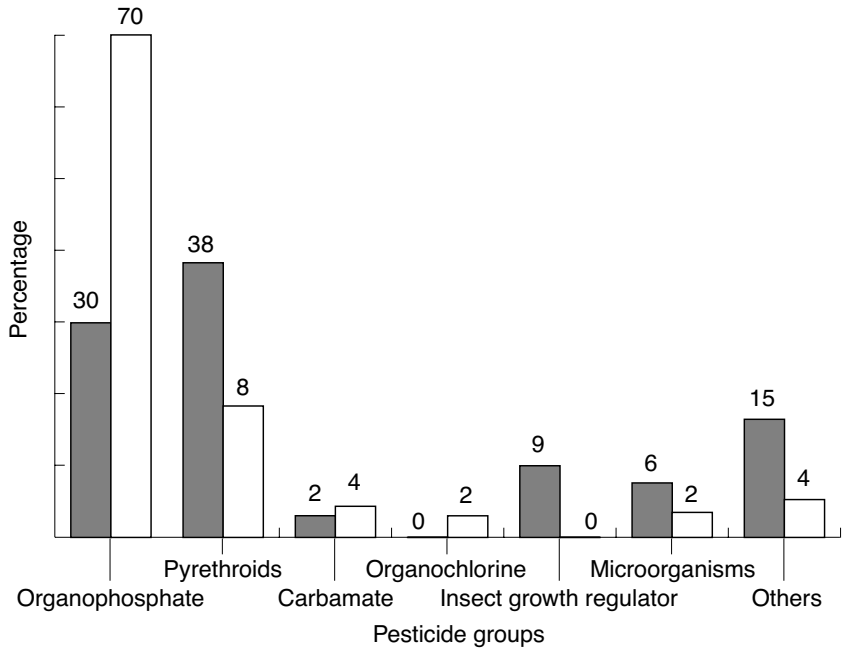

Figure 1. Comparison of banned and highly toxic pesticide use now ■ and 3 years ago □

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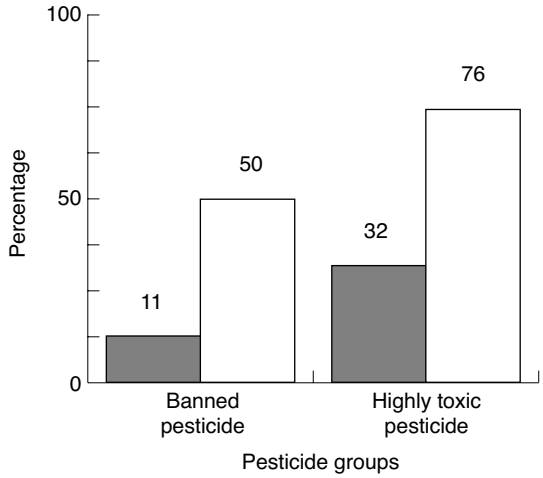

Figure 2. General comparison of characteristics of pesticide use between now (■) and 3 years ago (□): percentage use cases for each pesticide group comparing with total used.

that vegetables are not contaminated with pesticides at the time of marketing. Under such a system, vegetable wholesalers could bring pressure to bear on farmers to apply the recommended procedures for producing vegetables that are not contaminated with pesticide residues. Efforts to instruct farmers would therefore become more effective.

However, to execute such a residue management program, a rapid method for detecting the level of pesticide residues in vegetables at wholesale markets is needed. This is a problem to which we are directing much attention.

The Agriculture and Rural Development Service of Ho Chi Minh City and the Postharvest Technology Institute are cooperating in a project to establish a centre for pesticide residue analysis to support residue management activities.

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Remediation–Bioremediation and Decontamination of Residues

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Microbial Bioremediation of Organochlorines in a Rice Cropping System

N. Sethunathan*, S.K. Sahu†, and K. Raghu§

Abstract

Cultural practices commonly used in rice cultivation accelerate the degradation of certain pesticides. For instance, soil submergence, coupled with amendments with organic sources such as green manure and rice straw, can be considered as an effective option for bioremediation of hexachlorocyclohexane (HCH), an organochlorine, in the soil environment. Similarly, DDT is readily degraded in flooded soil amended with green manure or rice straw; but its metabolites DDD and DDE, which are as toxic as DDT, resist further degradation. It is now certain that HCH isomers are susceptible to very rapid degradation under both anaerobic and aerobic conditions. Consequently, the flooded rice ecosystem, with its dynamic anaerobic–aerobic interface, promotes almost complete detoxification of HCH isomers, including the thermodynamically stable β–isomer. Flooded soil also serves as an excellent medium for isolation of bacteria with an exceptional capacity to degrade HCH isomers. Strains of *Sphingomonas paucimobilis,* isolated from rice soil and sugarcane rhizosphere, were almost identical in their capacity to readily degrading α–, β–, γ–, and δ–isomers of HCH under aerobic conditions. There is scope in exploiting these bacteria for bioremediation of HCH-polluted soil and water environments, through recombinant DNA technology, by expanding their substrate range and ability to survive in the complex soil ecosystem.

HEXACHLOROCYCLOHEXANE (HCH), a broad-spectrum organochlorine insecticide, has been extensively used worldwide since the 1940s. However, because of its toxicity especially to non-target organisms, entry into the food chain through bio-magnification, and long persistence in the soil, this insecticide has been banned in developed countries for 20–30 years. Nevertheless, HCH-contaminated sites resulting from its excessive and indiscriminate use before its ban, accidental spillage of its concentrates, and discharge of wastes from HCH-manufacturing plants, are even now common and widespread in these countries. Moreover, because of its relatively low cost, HCH continues to be used on a large scale in agriculture and public health in many developing countries of the tropics and subtropics. In fact, HCH accounted for about 45% of the total pesticides used in India in 1988–89 (David 1992). Technical grade HCH (an isomeric mixture) was banned in India in 1997, but the use of γ-HCH (lindane) is still permitted. DDT, also an organochlorine and more persistent than HCH, will continue to be used in public health in India.

Commercial technical formulations of HCH used currently or until recently in many countries of the developing world, contain, theoretically, eight stereoisomers of which α-, β-, δ- and γ-isomers are the most common. Thermodynamically, β- and δ-isomers are more stable than α- and γ-isomers. There was concern in Japan over the entry of β-isomer into the food chain after regular agricultural use of technical formulations of HCH in which it was a minor constituent.

According to earlier reports, HCH isomers exhibit long persistence in aerobic soil and water environments. For instance, γ-HCH (lindane) persisted for 11 years in a temperate upland (non-flooded) soil (Licht-

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enstein and Polivka 1959), but only for 3–5 months in a tropical upland soil (IARI 1978). More recently, degradation of HCH isomers has been demonstrated also in aerobic systems (Bachmann et al. 1988a,b; Wada and Senoo 1989; Sahu et al. 1990a). This overview updates the progress made in studies on aerobic versus anaerobic instability of HCH isomers in soil and in pure cultures of soil microorganisms.

Anaerobic Degradation

Contrary to their reported long persistence in upland systems (Lichtenstein and Polivka 1959), HCH isomers were shown to undergo very rapid degradation in predominantly anaerobic ecosystems such as flooded soils and lake sediments. Anaerobic instability of γ-HCH was first demonstrated in 1966 when its residues reached low or non-detectable levels within of 3 weeks in a Philippine soil under flooded conditions (Raghu and MacRae 1966). γ-HCH, applied 3 weeks after an initial application to the flooded soil, disappeared more rapidly than that after its first application. Interestingly, α-, β- and δ–isomers of HCH were almost as short-lived as γ-HCH in a flooded soil (MacRae et al. 1969; Castro and Yoshida 1971; Sethunathan et al. 1983). Addition of organic material such as green manure (Ferreira and Raghu 1981; Zhang et al. 1982; Drego et al. 1990) or rice straw (Yoshida and Castro 1970; Siddaramappa and Sethunathan 1975) to the flooded soil can further accelerate the degradation of HCH isomers in the soil environment.

DDT, known for its extreme resistance to biodegradation in aerobic environments, undergoes very rapid degradation in predominantly anaerobic flooded soil (Castro and Yoshida 1971). Addition of organic material such as rice straw and green manure (Mitra and Raghu 1988) to the flooded soil effected a further increase in its degradation (Table 1). In contrast, under nonflooded soil conditions, degradation of DDT was not enhanced after addition of green manure (Mitra and Raghu 1988). Rapid degradation of DDT in flooded soil, especially amended with organic material, proceeded by reductive dechlorination to DDD, without enhanced formation of DDE, a dead-end metabolite formed by dehydrochlorination. Unfortunately, the DDD readily formed in flooded soils resists further degradation both in anaerobic and aerobic systems. To enable complete detoxification of DDT residues in soil and water environments, a means of enhancing the degradation of DDD and DDE is needed. Bacteria capable of degrading DDT to DDD under anaerobic conditions have been isolated from flooded rice soils (Sethunathan and Yoshida 1973), but worldwide efforts to isolate effective DDD- and DDE-degrading microorganisms have so far been unsuccessful.

Table 1. Degradation of DDT in nonflooded and flooded soil samples amended with green manure (*Glyricidia sepum*)

Moisture regime	Amendment	Percent recovery as DDT	DDD	DDE
Non flooded	Unamended	74.0	4.0	4.0
	Green manure - amended	77.0	7.0	4.5
Flooded	Unamended	25.0	26.0	3.0
	Green manure - amended	10.0	30.0	4.5

Anaerobic (obligate or facultative) bacteria were implicated in the rapid degradation of HCH isomers in anaerobic ecosystems (MacRae et al. 1969; Sethunathan et al. 1969; Sethunathan and Yoshida 1973; Jagnow et al. 1977; Haider 1979; Straube 1991). An anaerobic bacterium (MacRae et al. 1969), subsequently identified as *Clostridium sphenoides* (Heritage and MacRae 1977a), rapidly degraded α- and γ-HCH, but not β- and δ-HCH, in a mineral salts medium containing yeast extract (MacRae et al. 1969; Sethunathan et al. 1969; Heritage and MacRae 1977a,b, 1979). Both growing cells and resting cells of this bacterium readily degraded γ-HCH under anaerobic conditions. Cell-free preparations of *C. sphenoides* required reduced glutathione to metabolise γ-HCH (Heritage and MacRae 1977b). In another study, a strain of *Clostridium rectum*, isolated from paddy fields in Japan, could degrade α- and γ-HCH under anaerobic conditions, but not β- and δ-HCH (Ohisa et al. 1980, 1982), as MacRae et al. (1969) had found with *C. sphenoides* isolated in the Philippines. Besides obligate anaerobic bacteria, facultative anaerobic bacteria belonging to Bacillaceae and Enterobacteriaceae could also degrade γ-HCH under anaerobic conditions (Jagnow et al. 1977; Haider 1979).

γ-HCH was almost completely dechlorinated during anaerobic degradation by *Clostridium pasteurianum* and *Citrobacter fruendii* (Jagnow et al. 1977), *C. pasteurianum* (Jagnow et al. 1977), and *Clostridium sphenoides* (MacRae et al. 1969). Washed cell sus-

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pensions of *C. sphenoides* degraded γ-HCH to γ-tetrachlorocyclohexane (γ-TCCH) (Heritage and MacRae 1977b), as a major, but transitory, intermediate. Apart from γ-TCCH, small amounts of tri- and tetrachlorinated benzenes were formed as minor metabolites (Jagnow et al. 1977). $^{14}CO_2$ was also detected, but in very low amounts (< 0.1%) (Sethunathan et al. 1969). Benzene was detected as an end-product during rapid degradation of α-HCH in predominantly anaerobic flooded soil amended with green manure (Drego et al. 1990). More recently, bio-transformation of β-HCH to chlorobenzene and benzene by an enriched microbial consortium under methanogenic conditions has been reported (Peter et al. 1996).

Aerobic Degradation

Until the past 10 years or so, there were few reports of aerobic bacterial degradation of HCH isomers, not as a sole carbon source, in nutrient-rich broth (Tu 1976; Haider 1979). *Escherichia coli,* isolated from rat faeces, slowly degraded γ-HCH (10% in 12 days), forming γ-pentachorocyclohexane (γ-PCCH) as an intermediate (Francis et al. 1975). It is only more recently that convincing evidence for aerobic degradation of HCH isomers in a contaminated soil slurry, upland and flooded rice soils, and in pure cultures of bacteria has been provided by researchers in the Netherlands, Japan, and India. Bachmann et al. (1988a,b) were probably the first to provide evidence for substantial aerobically mediated bio-mineralisation of an isomer of HCH, viz. α-HCH in a HCH-contaminated soil slurry at a rate of 23 mg/kg/soil/day. The factors governing aerobic degradation of α-HCH in soil slurry included temperature, auxiliary carbon sources, and substrate concentration. A temperature range of 20–30°C was most favourable for biodegradation of α-HCH. Addition of auxiliary carbon sources inhibited its bio-mineralisation.

Interestingly, rapid aerobically mediated degradation of γ-HCH was noticed in an upland soil after multiple applications of the pesticide (Wada and Senoo 1989). *Pseudomonas paucimobilis* SS86 [reclassified as *Sphingomonas paucimobilis* (Senoo et al. 1992)], isolated from the upland soil treated with γ-HCH once a year for 12 years, could degrade γ-HCH under aerobic conditions (Senoo and Wada 1989, 1990). When the upland soil was inoculated with this bacterium, its population in the soil increased rapidly, reached the maximum in 2–5 weeks and declining thereafter.

Studies conducted in India showed that, under aerobic conditions, suspensions of the soil from the rhizosphere of sugarcane plants grown from sugarcane setts treated with HCH effected very rapid degradation of γ-HCH in a mineral salts medium as a sole carbon source (Sahu et al. 1990a). A species of *Pseudomonas* isolated from sugarcane rhizosphere, readily degraded γ-HCH as a sole carbon source (Sahu et al. 1990b). Moreover, this bacterium, subsequently identified as *Sphingomonas paucimobilis* (S.K. Sahu, unpublished data), readily metabolised not only α- and γ-isomers of HCH, but also thermodynamically more stable β- and δ-isomers, under aerobic conditions. Bacterial degradation of the α-, γ-, and β-isomers under aerobic conditions led to the accumulation of a transitory metabolite, γ-PCCH, and eventual release of covalently linked chlorine as chloride in stoichiometric amounts (Sahu et al. 1990b). During aerobic degradation by *Sphingomonas paucimobilis*, about 10–12% of the ^{14}C in α- and γ-HCH was accounted for as $^{14}CO_2$ as compared with 5% from β-HCH (Sahu et al. 1995). Most of the ^{14}C in α- and γ-HCH accumulated as water-soluble products, while formation of water-soluble products from β-HCH was negligible. Almost all of the ^{14}C in the three isomers of HCH was accounted for in different fractions (CO_2, chloroform-diethyl ether, water-soluble). GC–MS analysis of β-HCH residues in chloroform–diethyl ether extract suggested the formation of pentachlorocyclohexanol and tetrachlorcyclohexanediol (Fig. 1) as products of aerobic metabolism of β–HCH by *Sphingomonas paucimobilis*. This and another report from the same laboratory (Bhuyan et al. 1993) are probably the first and only instances of metabolic transformation of β-HCH in cultures of pure bacteria.

A novel bacterium isolated recently could degrade substantially high concentrations of γ–HCH (Thomas et al. 1996). Dechlorinase activity in bacterial strains capable of degrading α- and γ-isomers of HCH was attributed to the presence of extrachromosomal DNA in their cells (Karanth et al. 1984; Deo et al. 1994).

Recently, aerobic instability of HCH isomers was demonstrated in a flooded soil. Surface soil samples, not only from nonflooded rice fields, but also from predominantly anaerobic flooded rice fields, retreated with technical HCH caused aerobically mediated accelerated bio-degradation of γ-HCH (Bhuyan et al. 1992). The enhancement factor (causing accelerated degradation of γ-HCH) developed after two or three applications of HCH. It persisted for more than 12 months after the last application of HCH in non-flooded soil and for about 6

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months in flooded soil. Also, the enhancement factor was inactivated following the drying of HCH-treated fields after the rice harvest. In a follow-up study, it was found that temperature-induced moisture stress caused the inactivation of the enhancement factor, especially at 35°C (Bhuyan et al. 1993). The enhancement factor developed with almost equal ease in both rice straw-amended and unamended soil samples (irrespective of flooded or non-flooded conditions) after multiple applications of HCH. Under aerobic conditions, *Sphingomonas paucimobilis*, isolated from HCH-acclimatised flooded soil, effected rapid degradation of α-, β-, δ- and γ-isomers of HCH, added to the mineral salts medium as sole carbon source, as noted also by Sahu et al. (1990b) with *S. paucimobilis*, isolated from sugarcane rhizosphere.

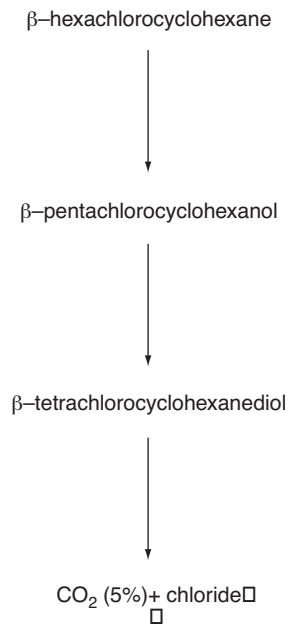

Figure 1. Pathway of aerobic metabolism of β-hexachlorocyclohexane by *Sphingomonas paucimobolis* after Sahu et al. 1995.

In catabolic transformations of a pesticide, microorganisms utilise it as an energy source for their growth. Most-probable-number estimates showed that populations of aerobic γ-HCH-degrading microorganisms in surface soil samples from rice fields distinctly and identically increased after each successive application of γ-HCH, not only in nonflooded soil, but also in predominantly anaerobic flooded soil (Table 2, Bhuyan et al. 1993). Thus, the population of γ-HCH degrading aerobes increased from 0.016×10^6/g of soil after the first application of technical HCH, to 130×10^6/g of soil after the third application under either moisture regime. This would indicate that γ-HCH served as the energy source for growth of γ-HCH-degrading aerobes under either moisture regime. In contrast, there was virtually no increase in the population of anaerobic HCH-degraders, even in predominantly anaerobic flooded soil, after three successive additions of technical γ-HCH. According to earlier reports (Ohisa and Yamaguchi 1978; Ohisa et al. 1980; MacRae et al. 1984), anaerobic microorganisms do not use γ-HCH as an energy source for their growth, but require additional energy substrates such as peptone for their proliferation, implicating co-metabolism in the anaerobic transformation of γ-HCH. This would explain why, even in flooded soils, no build-up of anaerobic γ-HCH-degraders occurred after three successive additions of HCH.

The *Sphingomonas paucimobilis* (from sugarcane rhizosphere) that readily degraded α-, δ-, β- and γ-isomers of HCH (Sahu et al. 1990b, 1992) in a mineral salts medium as a sole carbon source, was tested for its ability to degrade these isomers after their application to two soils under flooded and non-flooded conditions. Interestingly, the concentration of soil-applied α- and γ-HCH fell from the original level of 5 μg/g of soil to non-detectable levels within 10–20 days in both soils inoculated with *S. paucimobilis*. The loss of both isomers in inoculated soils was more pronounced under non-flooded than flooded conditions (Sahu et al. 1993). There was no appreciable loss of soil-applied β-HCH in inoculated soils during the corresponding period, despite the ability of the bacterium to degrade this isomer in a mineral salts medium (Sahu et al. 1990b). *Pseudomonas* sp. was able to utilise γ-HCH (Sahu et al. 1990b) and α-HCH (Sahu et al. 1993), but not β-HCH (Sahu et al. 1993) for its growth in a mineral salts medium. Thus, the population of *S. paucimobilis* increased several-fold in mineral salts medium supplemented with α- or γ-HCH as a sole carbon source, with a concomitant decrease in their concentrations to nondetectable levels during a 2-day incubation under aerobic conditions (Fig. 2). β-HCH was also degraded in the mineral salts medium by this bacterium, but without any increase in its population (Sahu et al. 1993). Evidence suggests the involvement of metabolism in the degradation of α- and γ-HCH by *S. paucimobilis* as opposed to co-metabolism of β-HCH by the bacterium under aerobic conditions.

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Table 2. Most-probable-number estimates of aerobic and anaerobic α–HCH-degrading microbial populations in flooded, and nonflooded soil samples from rice field after successive applications of technical HCH

Number of technical HCH additions	Population of aerobic γ-HCH degraders ($\times 10^6$/g soil)		population of anaerobic γ -HCH degraders ($\times 10^2$/g soil)	
	Flooded	Nonflooded	Flooded	Nonflooded
After 1st addition	0.016	0.016	0.27	0.27
After 2nd addition	2.4	2.4	0.27	0.27
After 3rd addition	130.0	130.0	0.27	0.27

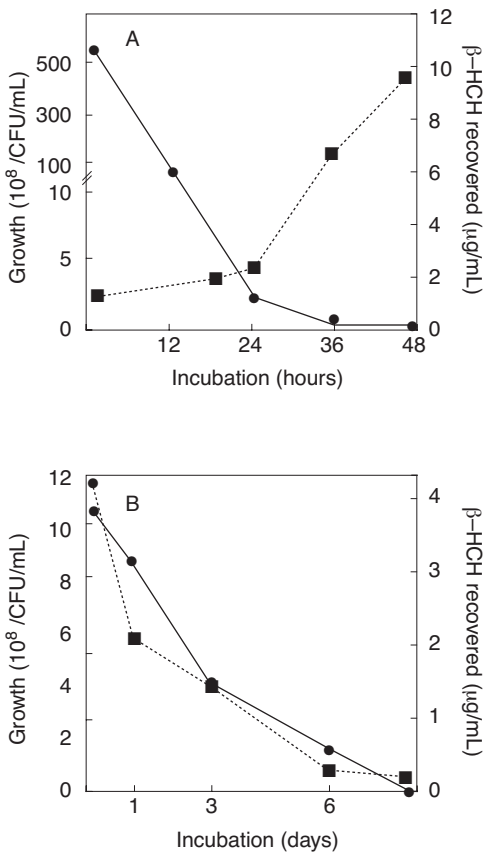

Figure 2. Growth and degradation of HCH isomers in a mineral salts medium by *Sphingomonas paucimobilis* under aerobic conditions. (a) ■, growth with α-HCH as a sole carbon source; ●, α-HCH recovered. (b) ■, growth with β-HCH as a sole carbon source; ●, β-HCH recovered (after Sahu et al. 1993).

This is probably the first published report of isomer-related metabolism versus co-metabolism of pesticides. However, soil-sorbed isomers of HCH including β-HCH were readily degraded under aerobic conditions when soils with sorbed HCH isomers were inoculated with a heavy suspension of the *S. paucimobilis* (Sreedharan 1995). This would indicate that aerobic degradation of soil-applied β-HCH can be effected by increasing the population of *S. paucimobilis*.

Rapid aerobic degradation of HCH isomers in suspensions of surface soil from HCH-treated flooded rice field merits discussion. Although a flooded soil is predominantly anaerobic within a few days after submergence, surface soil and standing water are generally in an oxidised state (Ponnamperuma 1972). Moreover, in a flooded soil planted to rice, the dynamic rhizosphere region with intense microbial activity can also be oxidised , because of the ability of rice plants to transport molecular oxygen from the atmosphere through the foliage to the root region. It appears that repeated additions of HCH to flooded soil planted to rice led to the proliferation of aerobic γ- and α-HCH-degraders in zones such as surface soil and rhizosphere where oxygen is present.

Girija (1998) has isolated from the HCH-acclimatised soils of sugarcane fields in Kerala, India, *Pseudomonas stutzeri* and a *Pseudomonas* sp. capable of degrading γ-HCH. Her studies indicated that HCH degradation in pseudomonads was plasmid-mediated.

Genetic Studies

In recent years, biotechnology has brightened the prospects of providing exciting and cost-effective approaches for solving the ecological problems of pesticide residues. With the arrival of recombinant DNA technology, it has been possible to identify and characterise the catabolic genes in microorganisms.

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There is a considerable literature on the genetic manipulation of catabolic genes responsible for the degradation of several organochlorines, 2,4-dichlorophenoxyacetic acid, chlorobenzoates, and chlorinated biphenyls. There are, however, only few genetic studies on HCH-degrading microorganisms (John et al. 1996), because aerobic microorganisms capable of degrading this organochlorine were isolated only recently.

According to Senoo and Wada (1990), genes responsible for γ-HCH degradation were encoded in plasmids and were lost when *P. paucimobilis* SS86 multiplied in the absence of γ-HCH. Alpha- and δ-isomers of HCH, but not β-isomer were aerobically degraded by this bacterium, besides γ-HCH. The initial step in the aerobic degradation of γ-HCH by *P. paucimobilis* UT26 involved dehydrochlorination to γ-PCCH (Imai et al. 1989). Subsequent studies suggested, however, that genes responsible for dehydrochlorination to γ-PCCH were located on chromosomal DNA (Imai et al. 1991).

Imai et al. (1991) constructed a genomic library of *Pseudomonas paucimobilis* UT26 [isolated from upland rice soil (Senoo and Wada 1989) and reclassified as *Sphingomonas paucimobilis* (Senoo et al. 1992)] in *Pseudomonas putida* by using the broad host-range cosmid vector pkSl3. Of 2300 clones screened, 3 showed γ-HCH-degrading capacity. Subcloning and deletion analysis showed that a fragment of 500 bp was involved in the conversion of γ-HCH to 1 ,2,4-trichlorobenzene via γ-PCCH. Mutagenesis and in vitro complementation tests showed that lin A gene (encoding γ-HCH dehydrochlorinase) was essential not only for the first step reaction (γ-HCH ➔ γ-PCCH), but also for the second step reaction (γ-PCCH to unidentified compound B) during aerobic degradation of γ-HCH by *P. paucimobilis* UT26 (Nagata et al. 1993a).

In *P. paucimobilis* UT26, the lin B gene responsible for the conversion of γ-HCH to 2, 5-DDOL was identified (Nagata et al. 1993b). Further, Nagata et al. (1994) cloned lin C gene encoding 2,5-1,4-diol dehydrogenase which converts 2,5-dichloro-2,5 dichloro-2,5 cyclohexadiene-1, 4-diol (2,5 DDOL) to 2,5-DCHQ.

Adhya et al. (1996) found that the *Sphingomonas paucimobilis* strain (Bhuyan et al. 1993) rapidly synthesised seven novel polypeptides and concomitantly developed the ability to degrade γ-HCH when exposed to γ-HCH or α-HCH. Synthesis of these proteins ceased with the disappearance of γ-HCH by degradation from the medium. In contrast, exposure of cells to β-HCH and δ-HCH did not induce the synthesis of these proteins except a 21 kDa protein. Expression of proteins was dependent on the concentration of γ-HCH. Cells, blocked in protein synthesis through addition of inhibitors of bacterial transcription (rifampicin) and translation (chloramphenicol), lost their ability to degrade γ-HCH.

Using the PCR technique, Thomas et al. (1996) characterised lin A- and lin B-like genes coding for dehydrochlorinase and dehalogenase, respectively, in an aerobic bacterium capable of degrading γ-HCH. Using the Southern hybridisation technique, Girija (1998) found that the gene responsible for degradation of γ-HCH by a *Pseudomonas* sp. isolated from sugarcane fields, shares homology with a lin A gene from *Pseudomonas paucimobilis* cloned by Imai et al. (1991). Plasmids isolated from bacteria repeatedly subcultured on peptone medium unsupplemented with γ-HCH failed to hybridise with lin A probe.

Pathways of Microbial Metabolism of HCH Isomers

Pseudomonas putida exhibited two routes of γ-HCH degradation: (i) an aerobic route involving dehydrochlorination of γ-HCH to γ-PCCH; and (ii) probably an anaerobic NAD-requiring route yielding γ-TCCH (Matsumura et al. 1976). Recent evidence suggests that both α- and γ-HCH are aerobically degraded by the same pathway of dehydrochlorination to 1,2,4-trichlorobenzene (Imai et al. 1991) via γ-PCCH (Imai et al. 1991; Adhya et al. 1996). About 10% of the ^{14}C in α- and γ-HCH was released as $^{14}CO_2$ during their degradation by *Sphingomonas paucimobilis* (Sahu et al. 1995). From their genetic studies with *Pseudomonas paucimobilis*, Nagata et al. (1994) proposed the pathway shown in Figure 3 for aerobic degradation of γ-HCH. During aerobic degradation of β-HCH by *Sphingomonas paucimobilis*, PCCH was not formed (Adhya et at. 1996) indicating a route different from that of α-HCH or γ-HCH degradation. Indeed, β-HCH was shown to be aerobically degraded by an independent route to pentachlorocyclohexanol and tetrachlorocyclohexanediol (Fig. 1; Sahu et al. 1995).

Degradation pathways of anaerobic degradation of γ-HCH in microbial cultures have been characterised. γ-HCH was degraded via γ–3,4,S,6-tetrachlorocyclohexene (γ-TCCH) by *Clostridium sphenoides* (Heritage and MacRae 1977a), *Citrobacter fruendi* (Jagnow et al. 1977), and *Clostridium rectum* (Ohisa et al. 1980). γ-TCCH was dechlorinated and dehydrochlorinated to yield monochlorobenzene (Ohisa et al. 1982).

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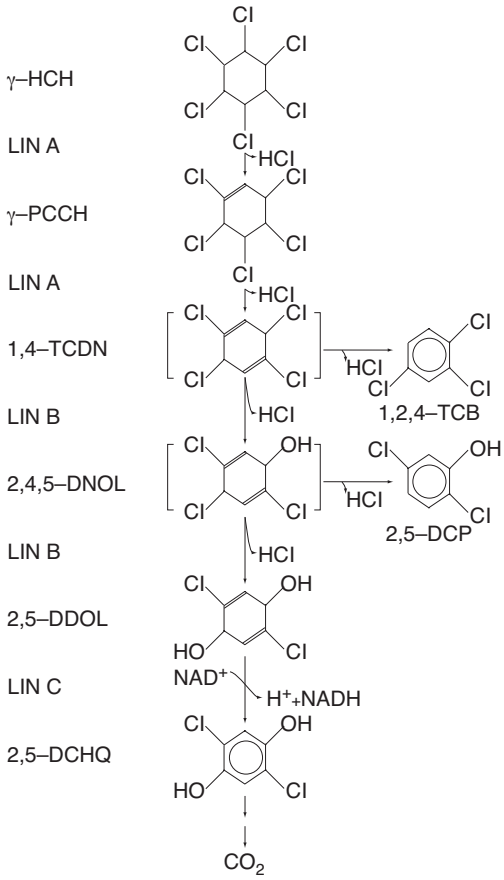

Figure 3. Proposed pathway of aerobic metabolism of γ-HCH by *Sphingomonas paucumobilis*.

Conclusions

It is now certain that HCH isomers are extremely susceptible to degradation by both aerobic and anaerobic microorganisms. As a consequence, flooded rice soil, with its dynamic oxic–anoxic interface, serves as an effective medium for almost complete detoxification of HCH isomers, including the recalcitrant β-HCH. Soil submergence, coupled with organic amendments, can be considered as a viable technology for bioremediation of HCH isomers in the contaminated sites. Bacteria, both aerobes and anaerobes, with an exceptional capacity to degrade one or more isomers of HCH have been isolated. What is particularly interesting is that almost all the aerobic γ-HCH-degrading bacterial strains, isolated from distant geographical locations (Japan and India), diverse ecosystems (upland and flooded), and different cropping systems (rice and sugarcane) were confined to strains of a single bacterium, *Sphingomonas paucimobilis* (Table 3). These aerobic bacteria need to be developed into cost-effective and 'ecofriendly' technology for bioremediation of HCH isomers through recombinant DNA technology, by expanding their substrate range and survivability in the complex heterogeneous soil contaminated with HCH residues. In developing a biotechnological approach, aerobes would be more suitable than anaerobes. Recently, the gene encoding the initial dehydrochlorination step in γ-HCH degradation has been cloned and sequenced using aerobic *Sphingomonas paucimobilis*. However, no efforts have been made on the molecular aspects of biodegradation of other HCH isomers, β-HCH in particular.

Table 3. Aerobic bacteria capable of degrading HCH isomers

Microorganism	Isolated from	Isomers degraded	Reference
Pseudomonas paucimobilis (later identified as *Sphingomonas paucimobilis*)	Upland soil	alpha-, gamma-, and delta-	Senoo and Wada (1989, 1990)
Pseudomonas sp. (later identified as *Sphingomonas paucimobilis*)	Sugarcane rhizosphere	alpha-, gamma-, delta-, and beta-	Sahu et al. (1990a)
Sphingomonas paucimobilis	Flooded rice soil	alpha-, gamma-, delta-, and beta-	Bhuyan et al. (1993)
Pseudomonas sp.	Sugarcane rhizosphere	gamma-	Girija (1998)

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There is also a need to isolate microorganisms capable of degrading DDT, ODD, and DDE, and to develop methods for bioremediation of ODD and DDE in the environment.

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Bioremediation of Organochlorine-contaminated Soil in South Australia: a Collaborative Venture

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Abstract

There are numerous sites contaminated with organochlorine pesticides in Australia. Many of these sites require remediation, and bioremediation is seen as a cost effective option. Given the recalcitrance of organochlorine compounds, co-composting of contaminated soil with green waste may be a worthwhile process, as microbial groups known to be effective in degrading these resistant chemicals are present during composting. In particular, we are examining the degradation of DDT and pentachlorophenol (PCP) during composting, because we have access to industrially contaminated soil containing these chemicals. Using controlled heating techniques we achieved over 90% removal of DDT (starting concentration 80 mg DDT per kg soil) over 40 days, the majority of degradation occurring in the thermophilic phase. Very little DDE (a dead-end metabolite of DDT) was produced, while DDD appeared to be the major transformation product. In contrast, the majority of PCP degradation during composting occurred in the cooling phase. Over 90% removal of PCP from contaminated soil was achieved and the toxicity of the soil was reduced after composting. We believe that co-composting of organochlorine-contaminated soil is an economically viable process and we are currently involved in pilot-scale field trials involving PCP-contaminated soil. These trials are being conducted at a purpose-built bioremediation plant capable of treating 1000 tonnes of contaminated soil per year.

ORGANOCHLORINES have been used extensively around the world as general pesticides. Their toxicity and persistence have led to many soil environments becoming contaminated, and it is essential that such areas be reclaimed. There are many types of organochlorine pesticides but this work focuses on DDT and pentachlorophenol (PCP), because of the substantial number of sites contaminated with these chemicals in Australia. There are estimated to be three thousand DDT-contaminated sites in Australia, most of them old cattle tick dip sites (Barzi et al. 1996). Levels of 1000 mg of DDT per kg soil are not uncommon and remediation of these areas will be difficult. PCP has seen worldwide use and its residues are a global problem. When used as a timber preservative, spillages have led to subsequent soil contamination. The persistence and high toxicity of PCP means that these sites require urgent treatment if they are to be redeveloped. We are currently undertaking remediation of 4000 tonnes of PCP-contaminated soil obtained from an old timber preservation site north of Adelaide, South Australia (McClure et al. 1997).

One method of treating such contaminated soils is bioremediation. This technique offers a cost-effective alternative to other methods such as thermal desorption. In addition, bioremediation does not destroy the soil properties or organisms present, meaning the soil is fit for re-use. Theoretically, the process produces no toxic end-products, carbon dioxide and water being the main compounds arising from microbial metabolism (Singleton 1994).

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However, to achieve a successful bioremediation the necessary microbial population and the correct environmental conditions must be present and often these conditions are not met, especially with particularly recalcitrant compounds such as organochlorine pesticides. Additional problems may include the limited bioavailability of compounds to the degrading microbes, and the presence of other toxicants.

One way of providing the optimal microbial population for bioremediation is to co-compost the contaminated soil with a suitable substrate such as green waste. During composting a succession of microbes arises, capable of degrading very complex carbon-based compounds such as lignin. The ability to degrade lignin is closely linked with the degradation of aromatic pollutants such as DDT and, indeed, composting has been used to successfully degrade polycyclic aromatic hydrocarbons, PCP, and carbaryl (Crawford et al. 1993).

Therefore, the main aim of the work reported here is to examine composting as a suitable remediation method for PCP- or DDT-contaminated soil. Work from the laboratory will be scaled-up, and the results of pilot-scale tests conducted at a newly constructed bioremediation plant will be used in full-scale treatment of 100 t of DDT-contaminated soil and 4000 t of PCP-contaminated soil.

Materials and Methods

Composting

Self -heating microcosms

Mixes of PCP-contaminated soil and green waste (50:50 mix) were added to 25-litre insulated composting vessels. Moisture content was maintained at 50% (w/w). These vessels were aerated and left to heat naturally. Temperature probes were permanently inserted into microcosms allowing continuous monitoring of temperature.

The starting PCP concentration was approximately 80 mg/kg of soil. Temperatures of 40°C were reached in these vessels after incubation for one week. Subsamples were taken weekly for analysis (see below) for a period of 6 weeks. Controls consisted of sterilised material (soil and green waste) in smaller containers (1 L) inserted inside the larger composting vessels.

Externally heated composts

DDT-contaminated soil and green waste (30:70 mix, total weight 200 g) were added to 2 L glass containers fitted with teflon-coated rubber bungs and inlet and outlet valves. Moisture content was maintained at 50% (w/w). These containers were placed into an insulated water bath allowing external temperature control. Temperature was increased from 20°C by increments of 5°C per day to 55°C. This temperature was maintained for at least two weeks. Composts were then cooled (5°C per day). Samples for DDT analysis were taken at the end of different heating phases. All composts were aerated with air passed through water to help maintain the required moisture level and sodium hydroxide solution to remove carbon dioxide. Controls consisted of sterilised soil plus green waste.

Analytical techniques

PCP analysis

Dichloromethane was used to extract PCP directly from the compost mixture (Yu and Ward 1996). To compost samples of 10 g each in 50 mL centrifuge tubes, 25 mL of dichloromethane (DCM) was added. The sample was shaken well and allowed to equilibrate for 30 minutes. After this time the sample was centrifuged at 4500 rpm for 3 minutes using a Beckman CS-15 centrifuge (Beckman Instruments). The solvent layer was retained and placed in fresh centrifuge tubes. The tubes were placed in a fume hood and the DCM allowed to volatilise until the volume was reduced to 1 mL, then dried with excess anhydrous sodium sulphate. This procedure gave a ten-fold concentration of the PCP extracted from the original soil sample.

A more stringent extraction method, modified from the method of van Leeuwen et al. (1996), was developed to remove PCP bound to the organic matrix. Samples of soil (10 g) were placed in Kjeldahl tubes containing 3 mL of 10 M KOH and 25 mL of methanol (MeOH). The samples were digested at 70°C for 3 hours. After this time samples were acidified with 2 M sulfuric acid to precipitate PCP from the aqueous phase. To prevent mixing of the DCM extract with the methanolic aqueous phase, deionised water was added to the tubes. The samples were then extracted with 25 mL of DCM. The DCM was evaporated to a final volume of 1 mL at room temperature under a fume hood.

All extracted samples were analysed by gas chromatography–flame ionisation detection (GC–FID).

DDT analysis

Compost samples (usually 10 g) were extracted with hexane:acetone (50:50) by shaking end-over-end for at least 4 hours. Samples were centrifuged and

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supernatants passed through a column containing anhydrous sodium sulfate to remove water. Samples so treated were analysed by gas chromatography–electron capture detection (GC–ECD). Metabolites of DDT (DDD and DDE) were also extracted and detected by this method. Previous examination showed that the method extracted 90% of added DDT and metabolites.

Results and Discussion

DDT degradation in composts

Over 90% removal of DDT (original soil concentration of DDT 70 mg/kg) was observed over 60 days composting (Fig. 1). Interestingly, the degradation occurred during the thermophilic phase, indicating the involvement of thermophilic microbes and the possibility that the higher temperatures increased the bioavailability of DDT. The original DDT sample contained some DDE as a contaminant, about 75% of which was degraded during incubation (Fig. 2). We suspect that DDE was also being produced as a breakdown metabolite during this time and we were seeing a concomitant production and breakdown of DDE. The significance of these results is that DDE is a suspected dead-end metabolite of DDT degradation and is therefore an unwanted product. Thus, achieving DDE degradation during composting is encouraging, and augments the finding by Aislabie et al. (1997) that DDE can be degraded DDD was also observed during composting (Fig. 3) and appears to be a major breakdown product in compost. Again this result is promising as DDD can be degraded completely (Nadeau et al. 1994). DDD is thought to be produced mainly under anaerobic conditions suggesting that the oxygen supply to the microbes during the active thermophilic composting phase may have been limited. Indeed, maximum carbon dioxide evolution was observed during that phase indicating maximal microbial activity and hence maximal oxygen uptake (results not shown). No DDT degradation was observed in sterile controls.

PCP degradation in composts

From a starting soil PCP concentration of approximately 70 mg/kg, an end point of 7 mg/kg was achieved during composting (Fig. 4). Analysis revealed that, in contrast to DDT, most of the degradation occurred during the cooling phase. This result could be a result of the different microbial populations involved in contaminant degradation or because different composting systems were used in the studies. We intend to further investigate this. Some binding of PCP to soil did occur, as evidenced by decreased recovery in controls (Fig. 4) but results from sterile controls indicated that the majority of PCP transformation was microbial in origin. In addition the toxicity of the soil:green-waste mix was greatly reduced after composting, as evidenced from plant germination studies (results not shown). This indicates that either toxic breakdown products were not formed, or, if they were, that they were degraded. Another possibility is that toxic materials became unavailable to organisms during composting as a result of binding to the compost matrix.

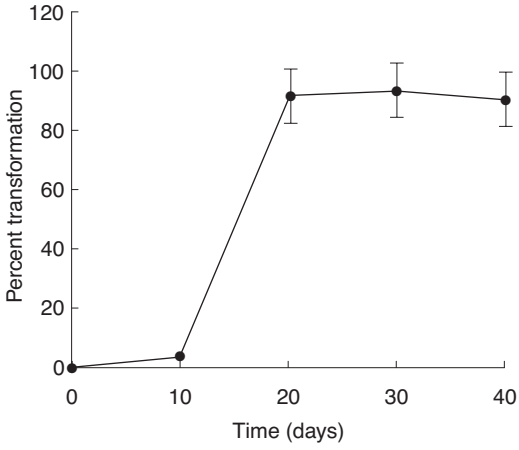

Figure 1. DDT transformation during composting.

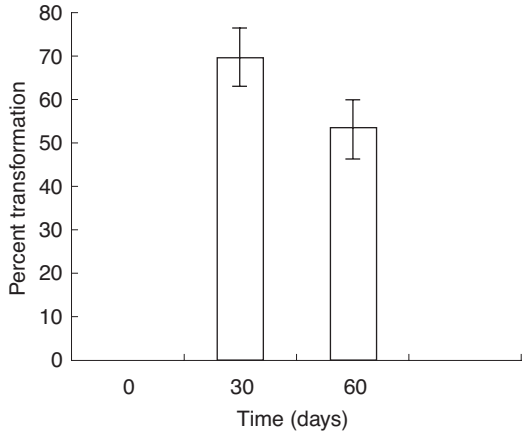

Figure 2. DDE transformation during composting

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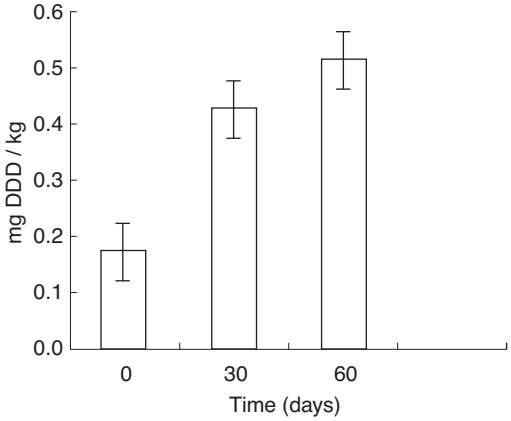

Figure 3. DDD production during composting.

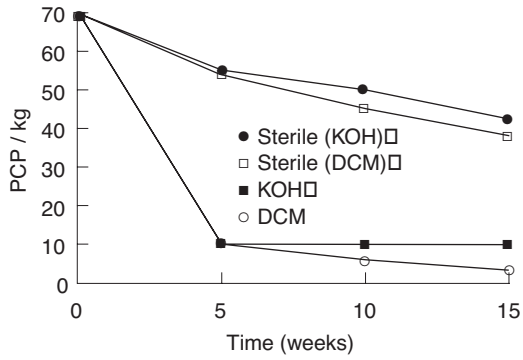

Figure 4. PCP transformation during composting

Summary and further work

Use of composting as a method to degrade recalcitrant compounds, such as chlorinated pesticides, generally appears to be very promising. Careful control of environmental conditions will be required to provide optimum degradation of these compounds. We are currently running a pilot-scale (approximately 20 t PCP-contaminated soil) compost on the PCP-contaminated soil and intend to scale-up the DDT remediation procedure. Results from pilot-scale work will be used to remediate 100 t of DDT-contaminated soil and 4000 t PCP-contaminated soil. The work on DDT may enable rehabilitation of the many sites contaminated with this compound throughout Australia. We are also isolating microbes capable of degrading DDT and PCP for possible use as microbial inoculants. These inoculants will be evaluated on both laboratory and commercial scales.

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The Need for PCB-mineralising Organisms

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Abstract

Polychlorinated biphenyls (PCBs) are a group of highly persistent and widespread contaminants in the environment, and microbial degradation is an attractive means of removing these pollutants from the environment. Most of the PCB-degrading organisms isolated from nature can only co-metabolise the compounds, and no effective PCB-mineralising organisms have yet been isolated. Chlorobenzoates are produced as dead-end metabolites during co-metabolism of PCBs. Subsequent transformation of chlorobenzoates by the general microflora results in the production of chlorocatechols. Unfortunately, only a small fraction of microorganisms able to transform chloroaromatics into chlorocatechols simultaneously contain the complete metabolic potential for mineralisation. We showed recently that these chlorocatechols are converted into a toxic antibiotic named protoanemonin which kills microorganisms. This conversion is carried out by enzymes of the 3-oxoadipate pathway, a biochemical conversion ubiquitous to soil microbes. The production of protoanemonin has important ecological consequences and negatively affects the survival of PCB co-metabolising bacteria. Hence, mineralisation rather than transformation has to be the goal of PCB bioremediation. The ecological implications of protoanemonin and the strategies to prevent protoanemonin formation are discussed.

POLYCHLORINATED biphenyls (PCBs) are a group of highly persistent and widespread contaminants in the environment that may pose a significant threat to the global ecosystem. These compounds were synthesised by direct chlorination of biphenyl. Because of their excellent stability and chemical properties, PCBs were widely used in a variety of applications, such as dielectric fluids in capacitors and transformers, and heat transfer fluids. Microbial degradation is considered to be an attractive means of removing these pollutants from the environment. A number of PCB-degrading microorganisms have been isolated from environmental samples, and the metabolic pathways and enzymes involved in the biodegradation of these compounds have been studied in isolated microorganisms.

Most of the PCB-degrading organisms isolated from nature used biphenyl as a sole source of carbon and energy, and co-metabolised a number of PCB components to chlorobenzoic acids via ring-dioxygenation and metacleavage. Subsequent transformation of chlorobenzoates by the general microflora results in the production of chlorocatechols. Mineralisation is usually achieved by chlorocatechol pathway genes. Unfortunately, only a small fraction of microorganisms able to transform chloroaromatics into chlorocatechols simultaneously contain the complete metabolic potential for mineralisation. Thus, in natural environments where chlorocatechol genes are not abundant co-metabolism will result in the formation of chlorocatechols and hence the pathways other than the chlorocatechol pathway (3-oxoadipate pathway and meta-cleavage pathway) are of considerable importance. We showed recently that these chlorocatechols are converted into a toxic antibiotic named pro-

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toanemonin which kills microorganisms. This conversion is carried out by enzymes of the 3-oxoadipate pathway, a biochemical conversion ubiquitous to soil microbes. The production of protoanemonin has important ecological consequences and negatively affects the survival of PCB co-metabolising bacteria.

Formation of Protoanemonin

When 4-chlorocatechol (4-CC) was incubated with catechol 1,2-dioxygenase (C12O 1) and muconate cycloisomerase, which are purified enzymes of 3-oxoadipate pathway from 3-chlorobenzoate degrading bacterium *Pseudomonas* sp. strain B13, protoanemonin—an unexpected novel metabolite—was formed. Although C12O 1 formed 3-chloro-cis, cis-muconate from 4-CC, the next enzyme of the 3-oxoadipate pathway, muconate cycloisomerase did not convert this product to cis-dienelactone, as does chloromuconate cycloisomerase, but to a new product. This new product was identified as protoanemonin (4-methylenebut-2-en-4-olide) by gas chromatography–mass spectrometry (GC–MS), ^{1}H-nuclear magnetic resonance, and spectrophotometric determination. Also, cell extracts of other bacteria expressing the classical 3-oxoadipate pathway (e.g. benzoate-grown *Pseudomonas* sp. B13, *Pseudomonas putida* KT2442. *Sphingomonas* sp. strain RWl, and salicylate-grown *Pseudomonas putida* RW 10) catalysed the formation of protoanemonin when incubated with 4-CC. This is indicative of a general metabolic transformation by muconate cycloisomerase enzymes. Further degradation of protoanemonin was negligible, suggesting that protoanemonin is a dead-end product of the 3-oxoadipate pathway.

Ecological Implications of Protoanemonin

Protoanemonin has been shown to be a constituent of plants of the Ranunculaceae family and to be antibiotically active against a wide variety of microorganisms. Formation of protoanemonin might thus be an important factor in the observed toxicity of chloroaromatics. Protoanemonin was found to be inhibitory to native microorganisms in soil and to several bacteria of interest to bioremediation. Addition of 75 and 150 ppm of protoanemonin to the soil decreased the dehydrogenase activity (a measure of active microbial activity) by 40 and 60%, respectively, within 2 days.

Viable counts of algae and fungi were more rapidly declined than bacteria, suggesting that eukaryotic microorganisms are more sensitive than prokaryotes. During the same period protoanemonin completely disappeared from non-sterile soils while it was fully recovered from sterile soil, suggesting microbial degradation of the compound. We observed a severe drop in the viability of bacteria when precursors of protoanemonin, such as 4-chlorobiphenyl (4-CB), 4-chlorobenzoate (4-CBA), or 4-chlorocatechol (4-CC), were applied either to isolated bacteria pre-grown on benzoate (3-oxoadipate pathway induced) or to soil microcosms in which PCB degraders were introduced. With pure cultures, protoanemonin accumulated in the supernatant as cell viability dropped.

In soil experiments only minute amounts of the antibiotic were detected. This low recovery is assumed to be due to the ability of a fraction of the natural microflora to metabolise this compound, because protoanemonin could be fully recovered from sterilised soil. Protoanemonin may, however, be present at high concentrations in the microniche where it is produced and where it exerts its ecotoxicological effects before being degraded. The decline in viable counts of PCB-degrading bacteria in soil occurred concomitantly with the decrease of the applied chloroaromatics. No toxicity was observed when the soil was either treated with a nonchlorinated analogue, sterilised before treatment, or co-inoculated with a chlorobenzoate degrader. *Pseudomonas putida* KT2442 harbouring the TOL plasmid also protected against the toxicity of 4-chlorobenzoate in non-sterile soil, thus precluding the observed toxicity to be due to 4-chlorocatechol meta-cleavage products.

These results strongly suggest that protoanemonin will always be formed if chloroaromatics are transformed into 4-chlorocatechol which in turn is metabolised by enzymes of the classical 3-oxoadipate pathway. We assume that protoanemonin formation by natural microflora is one reason for poor survival of specialised PCB degraders under environmental conditions. If a widespread problem in haloaromatic contaminated environments is the production of protoanemonin, then a possible solution will be to equip organisms able to degrade the pollutants as far as halobenzoates with the additional catabolic enzymes needed to prevent the formation and accumulation of protoanemonin. This combination not only enhances the survival of the degraders in such environments but also protects the other members of microbial community from protoanemonin poisoning. Hence, minerali-

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sation rather than transformation has to be the goal of PCB remediation.

Further details of these findings can be found in Blasco et al. (1995, 1997).

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Bioremediation of Pesticides using Enzymes

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Abstract

We consider the scope for enzymes to be used as catalytic bioremediants of pesticide residues in the environment and on the surfaces of commodities. Delivery might be ex vivo, in simple formulations, for clean-up of contaminated water or surfaces of commodities, or in vivo, in transgenic microbes or plants, for clean-up of contaminated soil. A wide range of pesticides might be amenable to the technology because many could be substantively detoxified in single-step oxidation or hydrolysis reactions and several others could be at least sequestered using transgenic plants. There are many stringent prerequisites for the enzymes, involving properties such as substrate range, cofactor requirements, kinetics, and stability, plus cheap production and formulation for ex vivo uses. However, we show that several hydrolases in particular may meet these prerequisites. Indeed, economic feasibility analyses based around a group of esterases indicate a variety of situations where effective bioremediation could be quite cheap.

SOME pesticide contamination of water, commodities, and soil is almost an inevitable consequence of many agricultural production and processing operations. Contaminated waters include irrigation wastes in horticulture, rice, and cotton, spent dip liquors in animal production, and a wide range of processing wastes, such as postharvest disinfestation dips in horticulture and the effluents from wool scouring and carpet-dye baths. Surface contamination is a major problem for commodities like fruit and vegetables if there is inadequate withholding time between treatment and marketing. Contamination of soils arises both deliberately during disinfestation of the soil, and incidentally, during broadcast spraying of crops or the operation of animal dips.

Contaminated water and soil can give rise to ecotoxicity, which in the case of water can be quite distant from the source of the contamination. Contaminated water and soil can also lead to secondary contamination of other commodities grown using those resources. Direct or indirect contamination of commodities can be a marketing problem, particularly in overseas markets where it can present serious technical barriers to trade. In extreme cases, commodity contamination also constitutes a health risk to consumers.

This paper examines the prospects for exploiting various types of enzymes for catalytic detoxification of pesticides from contaminated water and soil and contaminated surfaces of commodities. We first consider how the enzymes would need to be deployed for these purposes and the properties that they would therefore need. We then examine the types of enzymes that might fulfil these needs and the means by which such enzymes might be obtained. Finally, we comment on some specific examples of enzymes that might be suitable, showing how various of their properties would impinge on their effective deployment.

Usage Conditions and Their Implications for Enzyme Properties

The first and fundamental requirement for a bioremediation enzyme to function is effective access to the pollutant in an aqueous environment. For many con-

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taminated liquids this can be met ex vivo, by simply adding soluble enzyme formulations to the liquid. For surface contaminated fruit and vegetables, it could also be met ex vivo by adding formulated enzyme(s) to washing solutions before packing, or possibly at marketing, or even by the consumer. For some very damp soils, soluble formulations, perhaps in slow-release capsules, might also be appropriate, but for most contaminated soils a local aqueous environment would need to be created by genetically engineering the enzyme into a microbe or plant that will absorb the pesticide over time and degrade it in vivo. Bioremediation in plants has been called phytoremediation where the intention is to decontaminate the environment. Note, however, that herbicide-degrading enzymes are also often engineered into crops for a different purpose, specifically to make the crop tolerant to the doses of herbicide necessary to control cohabiting weeds.

A second general requirement for effective enzymatic bioremediation is that the pesticide is degraded to substantially less toxic products. Just how much less toxic the products must be depends on their stability, volatility, solubility, etc., but as a guide they should be at least two orders of magnitude less toxic than the pesticide. Given current technology it will also be advantageous if this level of detoxification can be achieved by a single-step reaction catalysed by a single-subunit or homopolymeric enzyme (i.e. one encoded by a single gene). The use of multiple enzymes/genes is certainly feasible in both ex vivo and in vivo applications but, particularly for ex vivo applications, will add significantly to the costs.

Note also that there is one exception to the requirement for degradation to less toxic products. This is the case of phytoremediation by sequestration, where clean-up without metabolism can be achieved if the pesticide can be transported from soil to above-ground plant material, and then harvested and removed off-site (Cunningham et al., 1996; Watanabe 1997). Bioremediation by these means may be most useful for otherwise intractable pesticides like cyclodienes or heavy metals. This might be achieved enzymically using glutathione-S-transferases (GSTs) (further discussed later in the paper).

As well as the fundamental prerequisites considered to this point, there are six key sets of enzyme properties that will determine the value of candidate bioremediation enzymes, particularly in ex vivo applications. The six properties and issues surrounding them are summarised in Table 1 and elaborated below. One of the properties which is important for formulated enzymes to be used for ex vivo bioremediation is their independence from cofactors. Some cofactors like NADPH and glutathione would be prohibitively expensive for use in ex vivo remediation. Others like metal ions might be considered pollutants in themselves. On the other hand, cofactor requirements need not be problematic for in vivo applications, where there will be ongoing cellular supplies of cofactors.

The key kinetic parameters of substrate affinity and turnover will be important determinants of the efficacy of enzymatic bioremediation. Most contamination situations will involve sub- or low ppm (mM) pesticide concentrations where reaction rates will be significantly less than maximal (K_{cat}) and substan-

Table 1. Properties required by enzymes for effective bioremediation.

Property	Potential use	Issues
Independence from cofactors	ex vivo	NADPH, glutathione prohibitively expensive; metal ions pollutants themselves
Low K_m (high substrate affinity) and high K_{cat} (turnover)	mainly ex vivo	Performance criteria are more relaxed for longer treatments
Stability	mainly ex vivo	Need resistance to conditions such as proteolysis, changes in pH and temperature, the presence of inhibitors
Broad substrate specificity	ex vivo, in vivo	Narrow substrate specificity restricts utility; multiple enzymes are costly
No undesirable side effects	mainly in vivo	Viable (plant) GMOs required in the field for mixtures of, or unidentified pesticides
Cheap production and formulation processes	ex vivo	Bacterial fermentation may not be possible with some eukaryote enzymes; substantial formulation may be necessary to stabilize enzymes in cell lysates

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tially dependent on enzyme-pesticide affinity (K_m). Satisfactory values for K_m and K_{cat} will depend greatly on the time frames in which it is practical to allow clean-up to occur. Relatively relaxed constraints might often apply in in vivo clean-up of contaminated soils where time frames of several months may be allowable and the microbe or plant will continue to produce the bioremediation enzyme throughout the period. At the other extreme, very demanding kinetic criteria will apply in some ex vivo situations where the enzyme itself will be degraded over time and not replenished. Moreover, some ex vivo situations demand clean-up in relatively short time frames. On-farm water management practices may dictate clean-up of irrigation drainage waters in a matter of a very few hours or days while clean-up of surface-contaminated fruit and vegetables might require washing periods of a few hours at the most before the immersion begins to affect the quality of the commodity. We exemplify some of the kinetic constraints around these and other applications later in this paper.

Enzyme stability is another critical parameter affecting efficacy, particularly in ex vivo applications where enzyme stocks will not be replenished. Stability in this context encompasses resistance to various biotic and abiotic challenges including biological and chemical proteolysis, loss of conformational integrity at prevailing conditions of pH and temperature, and compromised kinetics because of resident inhibitors, pH, or temperatures, etc. While many ex vivo applications will involve time frames of hours or days, the half-lives of many enzymes in the environments involved may be substantially shorter.

Two aspects of substrate specificity are important for potential bioremediation enzymes. Firstly it will generally be advantageous for the enzyme to degrade as broad a range of pesticides as possible. A narrower substrate range may restrict use to situations where the contaminant is known to be within that range, e.g. for on-farm use where the history of recent pesticide use will be known to the operator. Otherwise multiple enzymes may be necessary, adding significantly to costs. The second important constraint in respect of substrate specificity is that the enzyme should not have substrates/functions leading to undesirable side effects. Such effects are more likely in in vivo applications; even quite subtle biochemical changes in transgenic microbes or plants may have detrimental effects on their viability in the field.

A final prerequisite for ex vivo use of enzyme formulations will be a cheap production and formulation process. In practice, cheap production is likely to mean bacterial fermentation and this may be problematic for many enzymes from eukaryotic sources. Cheap formulation is likely to mean use of minimally purified enzyme from crude cell lysates. Depending on the economics of particular applications it may be important not even to dry the ferment liquor, but merely to add stabiliser to it and/or store in a cool environment for a relatively short period before application.

Types and Sources of Suitable Enzymes

Most pesticides could in theory be substantially detoxified by single-step hydrolysis or oxidation reactions. Even some exceptions, like several organochlorines and arsenic, could theoretically be sequestered by GSTs. In practice, however, with current technology the oxidation and GST sequestration options are viable essentially only in in vivo applications. As noted earlier, sequestration is probably feasible only in engineered plants and the requirement for reduced glutathione as a cofactor limits GST use to in vivo applications anyway. Similarly, with current technology, the otherwise powerful cytochrome P450 oxidation system could be deployed only in vivo, both because of its requirement for NADPH as a cofactor and because cytochrome P450 needs to be regenerated after catalysis by a second enzyme, cytochrome P450 reductase (Hodgson 1985; Agosin 1985). On the other hand, there is a wide range of hydrolase enzymes from several different multigene families. Some are metallo-enzymes but most are independent of cofactors. Organophosphates (OPs) can be detoxified by hydrolysis of their phosphoester bonds, pyrethroids and some OPs like malathion by hydrolysis of their carboxylester bonds, and diverse carbamate insecticides, herbicides, and fungicides by hydrolysis of amide or similar bonds. Most of the candidate bioremediation enzymes identified to date have been hydrolases.

The most common sources of hydrolases with bioremediation potential have so far been pesticide-tolerant soil microorganisms. These are generally bacteria that have adapted to growth on field-contaminated soils (e.g. Munnecke 1980; Serdar and Gibson 1985;Mulbry et al. 1986). Most pesticides are not particularly toxic to bacteria but they can become worthwhile nutrient sources if they are recurrently available in more than trace amounts. This is frequently the case where relatively persistent pesticides are deliberately applied to soil for treatment of soil pests or where accidental/inci-

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dental exposures regularly occur, e.g. around animal dip sites. Perhaps because the benefits to the bacteria become significant only at higher pesticide concentrations, the hydrolytic enzymes involved generally seem to have only moderate affinities for the pesticides but relatively good maximum turnover capabilities.

A smaller number of enzymes with bioremediation potential have been isolated from pesticide-resistant mutants of the (eukaryotic) organisms that the pesticides are intended to control (see e.g. Beard 1993; Newcomb et al. 1997; Campbell et al. 1998). In contrast to the pesticide-tolerant bacteria, the pesticide-resistant target organisms tend to provide enzymes with high affinity for the pesticides, albeit with often lower turnover values. This may be because even trace amounts of the pesticide would otherwise be lethal to the target organism. This is certainly the case for many 'knockdown' pesticides like most of the major classes of insecticides and some herbicides; it may be less true of insect growth regulators and many of the newer herbicides and fungicides.

Potentially at least there is a third source of candidate bioremediation enzymes, namely synthetic mutant hydrolases made in vitro by protein engineering technologies. Until recently, protein engineering has generally been accomplished by making one or a few predetermined amino acid changes by the technique of site-directed mutagenesis. It has therefore required substantial prior knowledge of sequence–structure–function relationships for the enzyme involved. Recently, however, the technique of directed evolution (based on error-prone PCR and in vitro recombination) has been devised; this allows for simultaneous selection of multiple amino acid substitutions and does not require (albeit can be expedited by) prior sequence–structure–-function information. Both techniques have been used successfully to make qualitative changes to substrate specificity and quantitative changes in kinetics for various esterases and other hydrolases (Stemmer 1994; You and Arnold 1994; Moore et al. 1997). There are no reports as yet of their use in making or improving enzymes for use in pesticide bioremediation but there is clearly an expanding capability to do so.

Some Examples

Table 2 summarises some features of four quite different hydrolase enzymes that might be considered for bioremediation. Several others might be at least as suitable (see e.g. Wang et al. 1993; Lockridge et al. 1997) but these four exemplify a range of properties

Table 2. Properties of some hydrolase enzymes under consideration for bioremediation

Enzyme	Bacterial paraoxonase	Bacterial carbamate hydrolase	Aphid esterase 4	Blowfly esterase 3
Gene	*opd*	*pcd*	*E4*	*E3*
Source	*Pseudomonas* and *Flavobacterium* species	*Arthrobacter oxydans* P52	OP resistant *Myzus persicae* and *Myzus nicotianae*	OP-resistant blowfly
Pesticide substrates	most OPs	phenmedipham, desmedipham	OPs, carbamates, pyrethroids	oxon OPs, carboxylester OPs
Kinetics	high turnover, moderate affinity	not reported	very low turnover, high affinity	slow turnover, moderate affinity
Cofactors	Zn^{2+} , Cd^{2+}, Ni^{2+} Co^{2+} or Mn^{2+}	none	none	none
Stability	protease sensitive	?	?	stable for days at room temperature
Applications (and delivery)	Soil (transgenic microbes, plants); water (membrane supports)	Soil (transgenic plants)	Water (membrane supports)	Water, commodities (crude enzyme formulation); soil (transgenic microbes, plants)

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and their consequences for efficacious deployment as bioremediants.

One of the most thoroughly characterised candidate systems is the OP degradation (*opd*) gene/enzyme system from *Pseudomonas diminuta* and *Flavobacterium* species (Serdar and Gibson 1985; Mulbry et al. 1986; Dumas et al. 1989; Serdar and Murdock 1990; Dave et al. 1993). This OP hydrolase has the important advantage of activity against the phosphoester bonds in a broad range of OPs, which it degrades with high turnover (K_{cat} for, e.g., paraoxon = 2100/s), albeit moderate affinity (K_m for paraoxon = 12 μM). One potential disadvantage is that it is a metallo-enzyme requiring metal ions such as Cd^{2+} or Zn^{2+} for activity. Another is that it is susceptible to degradation by proteases. These two disadvantages may preclude some applications and adversely affect the economics of others, particularly in ex vivo situations. Nevertheless, it is being evaluated for potential use in decontaminating diverse OPs in aqueous wastes, immobilised onto trityl agarose in a fixed bed reactor (Caldwell and Raushel 1991), or expressed on the surface of bacterial cells (Richins et al. 1997).

The genetically unrelated carbamate hydrolase (*pcd*) system of *Arthrobacter oxydans* (Pohlenz et al. 1992, 1996) contrasts markedly with *opd* in respect of substrate specificity. The carbamate hydrolase exhibits a narrow substrate specificity, hydrolysing the central carbamate linkage of the diphenylcarbamates, phenmedipham, and its close analogue desmedipham, but not carbamates with additional aryl and/or alkyl substitutions at the carbamate nitrogen. The enzyme does not require cofactors and its kinetics are sufficient to confer tenfold resistance to phenmedipham when engineered into tobacco (Streber et al. 1994). Its efficacy in ex vivo situations requiring relatively rapid action has not been reported.

The bacterial *pcd* gene is a member of the same carboxyl/cholinesterase multigene family as a number of eukaryotic enzymes with bioremediation potential. One of these is the esterase 4 (E4) enzyme, or its closely related variant, FE4, from a pesticide-resistant mutant strain of the peach-potato aphid *Myzus persicae* (Field et al. 1988). The orthologous gene/enzyme system has also been isolated from the tobacco aphid, *Myzus nicotianae* (Beard 1993). This group of enzymes has potential for a very broad range of pesticides, including OPs, pyrethroids, and carbamate insecticides. However, these compounds are more properly considered hemi-substrates than true substrates for E4; while they are avidly bound by the enzyme (K_m for paraoxon = 75 pM), their subsequent turnover is very limited indeed (K_{cat} = 0.01/min) (Devonshire 1977). Their bioremediation potential is probably therefore restricted to the sequestration of very low concentrations of problematic pesticides. This might be achieved if the contaminated water were to be passed through membranes to which the enzyme had been firmly attached, the membranes then being replaced as the enzymes' active sites become saturated with bound pesticide.

Another eukaryotic member of the carboxyl/cholinesterase multigene family with bioremediation potential is the esterase 3 (E3) enzyme from the blowfly, *Lucilia cuprina* (Hughes and Raftos 1985; Newcomb et al. 1997). Two mutant variants of this enzyme in OP-resistant strains have OP hydrolase activity for a range of oxon or carboxyl ester OPs, one preferring diethyl OPs and the other dimethyl OPs and, in particular, malathion (Campbell et al. 1998). Their combined substrate range covers about 10% of global insecticide sales. Moreover, the range would be significantly broader for in vivo applications, where the rapid conversion of thion OPs to oxon forms means that an additional 30% of the insecticides used globally become amenable to bioremediation by the enzymes.

Both the E3 variants show moderate affinity for OPs (Km for chlorfenvinphos = 21 and 126 μM, respectively), albeit not as strong as the *Mysus persicae* enzyme. On the other hand both enzymes show slow but significant turnover rates (K_{cat} = 1.7 and 43/min), with highest rates for the malathion resistance enzyme against malathion. The two variants also share another important attribute in that they are stable against a range of biotic and abiotic challenges, with half-lives in some agricultural waste streams of a number of days. In respect of ex vivo applications it is also relevant that they (like most other members of the carboxyl/cholinesterase multigene family) do not require cofactors for activity and (unusually for eukaryotic enzymes) they can be produced in active form in bacterial expression systems. For all these reasons they are being trialled for commercial development, in both in vivo and ex vivo applications.

Implementation: Issues and Examples

This section considers in more detail how enzymes with kinetics, stability, and expression characteristics like the E3 enzyme for malathion (but not necessarily the same substrate range) might actually be deployed in the field. E3 is not an ideal bioremediation

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enzyme, nor even necessarily the best currently available. However, the analyses outlined below give a useful benchmark as to how other enzymes with a range of properties might perform and how much it might cost to use them.

For ex vivo applications we believe that an enzyme like E3 would be produced by industrial-scale bacterial fermentation, followed by cell lysis (so no live GMO is released into the environment) and simple formulation, without any significant enzyme purification. On the basis of precedents with other enzymes, and allowing commercially reasonable mark-ups to the manufacturer and distributor, we suggest that the costs to end-users could be about $AU200/kg of active enzyme. Without more expensive formulation the shelf-life of the enzyme would likely be quite short, particularly if not refrigerated.

We have modelled ex vivo usage of formulated E3-like enzymes in clean-up of irrigation drainage water from cotton and rice farms under the water and pesticide management practices prevailing in Australia. Clean-up would be on-farm with the aim of adhering to tough off-site residue regulations imposed to avert downstream ecotoxicity problems. We have calculated that reductions of two orders of magnitude in residue levels in at-risk water could be achieved for the most problematic pesticides (currently endosulfan for cotton and thiocarbamate herbicides for rice) for less than $AU5 and $AU8/ha/season, respectively. These costs would reduce substantially for enzymes with better kinetics or if water management could be varied to allow longer reaction times on-farm (in the case of cotton we allowed on-farm reaction times as short as an hour after heavy rain).

There are also many potential ex vivo applications in the clean-up of recirculated water in intensive horticulture, such as market gardens, nurseries, and cut flowers. The value of the clean-up here lies in minimising both secondary contamination of commodities and contamination of leakage into groundwater around sensitive peri-urban and other catchments. Although pesticide usage is often heavy in these industries (e.g. 7–10 sprays per year) the recirculation systems allow for reaction times of many hours, with estimates of costs in the vicinity of $AU100/year to the end-user.

Another major ex vivo application is in the postharvest clean-up of surface localised residues on fruit and vegetables. As noted earlier, such residues can generate serious market access problems, particularly in respect of technical barriers to export trade. In extreme cases, fruit and vegetable residues can also pose significant consumer health issues. While internalised residues would be inaccessible to the enzymes, there are data to show that even significant proportions of pesticides that concentrate in the peel (e.g. malathion on tomatoes) are accessible (USNRC 1993). Given detergent additives to facilitate access of enzymes to the surface pesticides, we suggest that bioremediation enzymes could be usefully deployed for several fruit and vegetables that can be washed after harvest without compromising their quality. In many automated or semi-automated packing operations washing might take the form of no more than momentary sprays, giving only a few minutes reaction times while the commodity is still wet. Costs to reduce residues of a few ppm by two orders of magnitude in such cases might be prohibitively high, say $AU100/t. However, there are other cases where the commodities are or can be dipped for several hours and in these circumstances estimates of costs fall to about $AU5–10/t.

Finally, we consider an in vivo application, namely the use of an E3-like enzyme in transgenic plants to remediate contaminated soil. The plant might be a commercial commodity crop or a non-commercial off-season crop used for soil improvement. A possible disadvantage of use in a commodity crop might be compromised efficacy of pesticides needed to grow it. A possible advantage might be reduced risk of contaminated commodities. As explained earlier, the long reaction time and ongoing replenishment of enzyme make for relaxed enzyme performance criteria in transgenic plants. Costs could, in theory, be very low, since production costs are not inherently higher for a transgenic crop. Commercial issues and the need to pass on to end-users the additional developmental and regulatory expenses associated with transgenics are likely to add to costs in many circumstances. However, the nature of the commercial issues and their implications for prices will be highly variable.

Conclusions

Enzyme technologies have found increasing use over the last decade in a wide range of industrial and domestic applications. They have not as yet been commercialised for bioremediation of pesticides but we suggest that certain classes of enzymes in fact hold great potential in cleaning up pesticides in a variety of circumstances. They hold potential in several areas of waste-water treatment and commodity

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clean-up, where microbial bioremediation would be precluded by time constraints and the nutritional requirements of the microbes. Used in transgenic plants, they might also provide viable, cost-effective alternatives to microbes in the area of soil clean-up, where microbes have traditionally found most use.

However, there are also some very stringent prerequisites that potential bioremediation enzymes would need to satisfy. Breadth of pesticide substrate range is particularly important for some applications, particularly off-farm applications, where the identities of the problematic pesticides are unknown and potentially diverse. Ex vivo applications in water and commodity clean-up will impose more stringent criteria than in vivo applications in respect of enzyme kinetics, stability, and cofactor requirements. Ex vivo applications will also require cheap production and formulation processes. On the other hand in vivo applications may be more sensitive to potentially detrimental side reactions of the enzymes. A number of enzyme classes may meet the generally less demanding criteria for in vivo use. However, we propose that several groups of hydrolases may also be adequate for ex vivo use. In particular, we have shown here how esterases from at least two unrelated multigene families are variously suitable for in vivo and ex vivo applications.

The tight kinetic constraints around ex vivo uses impact strongly on the economics of deployment. Other things being equal, enzymes with higher turnover rates at low pesticide concentrations will be substantially cheaper to use. Similarly manipulation of, for example, the water management or commodity washing practices to allow for longer reaction times will also translate directly to use of less enzyme, and therefore lower cost. Nevertheless, even with the kinetics of the E3 malathion enzyme, which are significantly less than ideal, there are many circumstances where even ex vivo use is quite economical.

Since ex vivo applications do not involve the release of live genetically modified organisms (GMOs), no additional regulatory issues related to GMO release arise. However, production and formulation processes would need to meet the criteria of good industrial large-scale practice (GILSP) to avoid inadvertent release during production. Registration for most ex vivo applications would be as industrial chemicals, or agrochemicals; in neither case should registration be problematic. There is no reason to anticipate adverse ecological or occupational health and safety implications of the enzymes per se.

In vivo applications will involve the release of live GMOs and, because the GMOs are designed to degrade otherwise beneficial agrochemicals, their fate in the environment would be a significant issue. A few approvals have already been granted for the release of transgenic microbial bioremediants of other pollutants and, of course, herbicide-resistant crop plants are a widespread precedent for engineered plants for phytoremediation. In the case of the microbes, it may be necessary to build in some biological containment system to counter possible interference with the proper use of pesticides beyond the contamination requiring clean-up. However, approval in the case of the crops might be less problematic than for many other insert genes with more direct effects on the phenotype or fitness of the organisms carrying them. At least for ex vivo applications and phytoremediation we therefore feel that regulatory issues need not be problematic.

Given the promise of enzymatic bioremediation technologies as outlined here, plus the absence of many viable alternatives and the growing problems around residues issues, we suggest that the development of these technologies for chemical pesticides should be pursued vigorously. In the light of the analyses summarised here we propose that R&D priorities should lie in the development of enzymes with adequate pesticide substrate range and kinetic properties and the development of cost-effective deployment protocols.

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Remediation of Contaminated Soil and Fluid at Cattle Dip Sites in Australia

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Abstract

Cattle ticks, in particular the single-host cattle tick, *Boophilus microplus,* have presented a significant threat to the beef and dairy cattle industries in northern New South Wales and other areas throughout northern Australia. The use of tickicides such as DDT and arsenic before 1962 resulted in a significant number of contaminated sites, with soil surrounding dip baths containing up to 60 000 mg/kg DDT and 3000 mg/kg arsenic. Remediation technologies for soil contaminated with DDT and arsenic are discussed. Currently used tickicides, in particular amitraz and flumethrin, which are present in surplus dips are a risk to human health and the environment. A technology to remediate these chemicals was developed by NSW Agriculture and is being implemented at field scale. This technology involves filtration to separate the tickicides from the fluid and thermophilic composting to biodegrade the active ingredients.

NSW Agriculture conducted an extensive review of existing and emerging technologies for the clean-up of soil contaminated with DDT and arsenic. Engineered technologies such as thermal desorption, soil particle fractionation, and chemical leaching have all shown potential for the clean-up of contaminated dip soil, but their application may be limited to specific soil types or by the cost of their application. NSW Agriculture is conducting a collaborative research project with an industry partner to further develop a leaching solution which is capable of chemically degrading DDT and solubilising arsenic in an ex-situ reactor. Bioremediation has also been investigated as a lower-cost alternative remediation strategy. In particular, intrinsic bioremediation and biostimulation may provide a long-term solution to reducing the organochlorine burden in the soil.

During 1997–98, NSW Agriculture remediated over 500 000 L of dip fluid contaminated with the amitraz. The technology involved filtration and carbon sorption, followed by the bioremediation of the active ingredient. It was shown that in-situ composting could completely destroying amitraz within 26 days, and most of its metabolites within 72 days.

CATTLE have been dipped in Australia to control the single-host cattle tick, *Boophilus microplus,* since the early 1900s. More than 1600 cattle tick dip sites were constructed in northeastern New South Wales (NSW) and along the Queensland border to stop ticks being carried south into NSW (DIPMAC 1992). Most of these were built on land leased from private landholders.

Previous dip decommissioning practices by NSW Agriculture (a State-government agency) led to soil becoming highly contaminated with arsenic and DDT (McDougall 1997). Soil next to the dip bath contained up to 60 000 mg/kg DDT and 3000 mg/kg of arsenic, and nearby areas where dip sludge was disposed were found to contain DDT at even higher concentrations.

Before 1980 selected dip sites were decommissioned to a standard suitable for agricultural land use. With the increased demand for residential land on the north coast of NSW, a small number of these former dip sites were inadvertently incorporated into land for houses.

Soil contamination by persistent dip chemicals such as arsenic and DDT became a community concern in the early 1990s. In response, the NSW government formed a task force (known as 'DIPMAC') of repre-

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sentatives of government, local environmental groups, and the community. DIPMAC investigated numerous former dip sites and recommended that 35 of these, located predominantly in residential areas, be cleaned-up to minimise potential risks to human health.

DDT is a 'scheduled waste' under the *NSW Environmentally Hazardous Chemicals Act (1985)*, which means that these dip sites must be cleaned-up by excavating the contaminated material and storing it securely in a purpose-built structure.

In recent years, there has been a significant decrease in the need to dip cattle and only 236 dips remain in use. NSW Agriculture is currently decommissioning dip sites that are no longer needed or which pose a risk to either the environment or human health.

Amitraz (Taktic®), a formamidine compound, is the tickicide most in use at present. It is stabilised in dips with hydrated lime ($Ca(OH)_2$). More than 950 dips still contain this tickicide. There are 63 dips containing flumethrin (Bayticol®), a synthetic pyrethroid, and 43 containing a combination of cypermethrin and chlorfenvinphos (Barricade S®). These dips contain up to 11 000 L of active tickicide, which must be disposed of or remediated before the dip site can be decommissioned.

This paper discusses technologies NSW Agriculture has investigated for the clean-up of soil contaminated in the past by DDT and arsenic; and the technologies currently in use for the remediation of dip fluid containing amitraz.

Methodology

Methods of analysis

Organochlorine pesticides and arsenic

All analyses of DDTs and arsenic were done by commercial laboratories. The DDTs were analysed by gas chromatography (GC) using a dual column fitted with electron capture detectors. All samples were confirmed using GC/mass spectrometry (MS) in selected ion mode for DDT, DDD, and DDE.

For arsenic analyses, soils were digested using U.S. Environment Protection Agency method 3051, followed by analysis using an inductively coupled plasma mass spectrometer (ICP/MS). Filterable arsenic (liquid samples) was also analysed by ICP/MS.

Amitraz and metabolites

Samples were extracted with toluene in a method described by Machin and McDougall (1978) and Van Zwieten et al. (1997). The extract was analysed by GC using a megabore column. Amitraz, formamidine, (*N*-2,4-dimethylphenyl-*N*'-methylformamidine), aldehyde (2,4-dimethylformanilide), and DMA (2,4-dimethylaniline) were detected using flame ionisation detection.

Technologies investigated for the clean-up of DDT and arsenic

Chemical leaching

A series of solutions engineered to leach arsenic and chemically degrade DDT was supplied to NSW Agriculture by Geo2 Ltd, a remediation and mining technology company. More than 100 surfactants were tested by Geo2 Ltd before four were finally selected for further laboratory studies. The leaching solutions were tested on a red krasnozem dip soil (clay loam) contaminated with 2645 mg/kg arsenic and 5010 mg/kg DDT.

Tests were conducted using soil at a pulp density of 20% (20 g soil per 100 mL of solution). The solutions were mixed for 24 hours at room temperature in a closed stainless steel vessel. The control used for the leaching solutions was deionised water.

The soil and leach solutions were separated by filtration and stored in a refrigerator in glass vessels before analysis for DDT and arsenic.

Soil washing

Soil washing trials were conducted on two contrasting soil types: a red krasnozem (clay loam) and a sandy soil. Both soils were contaminated with DDT and arsenic. Soil (5 kg) was supplied to Metcon Laboratories, Sydney, where the soil washing trials were undertaken. The soil was divided into size fractions using mechanical screens for particle sizes greater than 75 mm, and hydrocyclones for particle sizes smaller than75 mm. The soil fractions were air dried before analysis.

A portion (< 50%) of the fractions between 75 and 2000 mm following soil washing was recombined for attritioning tests (high intensity agitation to remove the surface of the soil particles). For the attritioning tests, the recombined fraction (200 g) at 80% solids was placed in a steel vessel, and vigorously stirred using a multi-paddle impeller for 24 hours. The stirring was conducted in both the absence and presence of sodium hydroxide (1 g/kg soil). The soil was again fractionated using soil washing, and air dried before analysis for DDT and arsenic.

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Thermal desorption

Contaminated soil (25 kg) from a number of dip sites was supplied to Tox-Free Systems Ltd for the assessment of thermal desorption as a potential technology for soil clean-up. Soil was treated using a 50 kg/hour pilot plant operated by Tox-Free. This pilot plant used an indirectly fired retort that heated soil to 450–500°C (Anon. 1997).

The gases generated from the retort were passed through a hot gas filtration system and an afterburner operated at 1100°C with a gas residence time of 2 seconds. The off-gases were quenched with lime and the recondensed arsenic fumes collected in a particle filter.

The soils were analysed for arsenic and DDT before and after treatment.

Soil storage

Under an agreement with the Tweed Shire Council (northeastern NSW), NSW Agriculture has proposed a soil storage facility at a local waste disposal depot. The secure storage facility has been approved for approximately 20 000 m^3 of soil from up to 15 dip sites in the valley of the Tweed River. Because there is no community support for these types of facilities, it is unlikely that secure storage is an option for DIPMAC sites in other areas of NSW. The facility will consist of three engineered cells, each having the following features:

- A primary liner of 0.6 m of compacted clay with permeability of less than 1×10^{-9} m/second.
- A leachate detection system consisting of a 300 mm permeable granular layer with a 65 mm high density polyethylene (HDPE) collector tubes.
- A secondary lining system of a HDPE liner of 2.5 mm thickness plus a self healing geosynthetic clay liner.
- A leachate collection system of similar design to the detection system.

All leachate is drained to a plant where it is treated by a combination of oxidation by potassium permanganate and flocculation by ferric chloride for dissolved arsenic, and filtration through activated carbon for DDT.

On-site management practices will minimise and control noise, air emissions, stormwater, and soil erosion. Specially constructed enclosures will protect air, surface, and groundwater quality.

Bioaugmentation

A series of microbial inoculants and nutrient amendments obtained from Biotech Solutions Ltd and East Coast Environmental Remediation Services was tested in a microcosmic system developed by NSW Agriculture. This enabled 25–50 kg of soil to be treated under strictly controlled and monitored conditions. Temperature was maintained at 30°C, and traps were installed to collect volatile arsenic. An automatic irrigation system allowed the circulation and reuse of leachate for irrigation onto the soil. Conditions in the microcosm were engineered to emulate those expected during in-situ remediation.

Soil was removed monthly from the tanks and thoroughly mixed before taking quadruplicate samples for analysis of DDT and arsenic. Soil was replaced in the microcosms following further treatment with the microbial inoculants or nutrient mixtures. The arsenic traps were replaced and analysed fortnightly. The leachate was analysed monthly for soluble arsenic.

Intrinsic bioremediation

Soil from 13 cattle-tick dip sites was sampled and analysed for pesticide contamination, microbial activity, and intrinsic biodegradation of DDT to DDD. The sites chosen represented a range of soil types including a red krasnozem (clay-loam), sandy clay-loam, and sand. The sites were also selected on the basis of past pesticide use (e.g. DDT and/or arsenic)

Six areas within each site were sampled, covering the areas of highest pesticide contamination (closest to the dip bath) through to lowest contamination (furthest from the dip bath). Each area was separated into the top 2 cm and 2–10 cm of the soil horizon, giving twelve samples from within each site. Samples were also taken from a nearby uncontaminated area at each site in order to determine background levels of microbial activity, arsenic, and pesticide.

Samples were analysed for arsenic and DDTs, and for total microbial activity (TMA). TMA was assayed in duplicate on each sample by two different biochemical assays. The first was the hydrolysis of fluorescein diacetate (Schnurer and Rosswall 1982; Zelles et al., 1991; Fontvieille et al. 1991).The second assay monitored alkaline phosphatase activity (Tabatabai 1982).

Technologies for the field-scale clean-up of amitraz dip fluid and sludge

Field-scale dip filtration

A pilot-scale Cuno® filtration system was used for a field demonstration of a technology for amitraz dip fluid remediation (L. Van Zwieten, M. Karkkainen, and A.L. Tyler, unpublished data). The system had four fil-

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ter housings arranged in series. The dip solution was pumped through the filters at about 10 L/minute using a petrol-driven centrifugal water pump. The pore sizes of the first three filters were 150 μm, 25 μm, and 1 μm. The last filter was activated carbon.

Triplicate 250 mL samples were taken every 15 minutes (approximately every 150 L) both before and after filtration until all fluid was removed. The samples were collected in glass jars. NaOH (2 g) was added immediately to each sample to stabilise the amitraz and metabolites. The samples were stored in a refrigerator until analysed.

Bioremediation of dip sludge

Field-scale remediation of dip sludge was modelled on the Berkeley-style composting, laboratory and pilot studies of which are described by Van Zwieten et al. (1997). After the fluid was removed and remediated by the filtration system described above, the remaining sludge was bioremediated by composting. The standard compost mixture consisted of: sawdust (1 part), cow manure (1/2 part), green waste (1 part), blood and bone (50 kg), and contaminated sludge (1 part). The amount of each compost component was estimated in the field and was varied according to parameters such as moisture content, raw material quality, and potential aeration.

The compost was mixed mechanically and piled at one end of the dip bath. The compost pile was sampled a number of times to determine the rate of degradation of amitraz and metabolites. The bath was then capped with concrete but, to enable further access to compost for sampling, was not fully sealed. The concrete dip bath structure was not disturbed during, to avoid the risk of disturbing soil contaminated with DDT and arsenic.

Follow-up sampling was conducted by temporarily removing the concrete caps and taking samples in triplicate from the top and middle of the compost heap with a shovel. Samples were stored in sealed glass jars in the freezer until analysed.

Results and Discussion

Clean-up technologies for arsenic and DDT-contaminated soil

Chemical leaching technology

Collaborative research with the remediation and mining technology company Geo2 Ltd has led to the development of a leaching solution which shows promise in the clean-up of dip soil contaminated with DDT and arsenic. Details of results using the four promising leaching solutions are shown in Table 1. The soil initially contained 5010 mg/kg DDTs and 2645 mg/kg arsenic. Following treatment of the contaminated soil in the laboratory, only 118 mg/kg of DDT remained (2.4% of the original) when leach solution 4 was used and 228 mg/kg arsenic remained in the soil when leach solution 2 was employed (8.6% of the original).

There are currently no proven technologies in Australia able to simultaneously treat both DDT and arsenic in soil. The Geo2 process is thus a significant advance: it treats both the organic and inorganic contaminants in a one-stage leaching/chemical degradation process. The DDT is chemically destroyed and the arsenic is leached into solution and recovered in a downstream process. Much of the equipment required for these processes is commercially available, a factor that will facilitate the construction of pilot or field-scale units following further evaluation and development of the technology.

Although treatment using the Geo2 technology is expected to cost $250–400/t of contaminated soil, there are currently no alternatives which can match either its cost or expected performance.

This collaborative project with Geo2 Ltd is continuing with focus on:

- the establishment of collaborative agreements and funding arrangements with Geo2 Ltd;
- the determination of potential costs associated with field-scale remediation;
- increasing the efficacy of the process;
- assessing the suitability of the soil for on-site replacement; and
- engineering aspects associated with the remediation technology.

Soil washing

Soil washing has become a recognised technology for reducing the volume of contaminated soils. The process involves partitioning the wet soil through a series of screens, followed by hydrocycloning for size fractions below 75 μm (silts and clays).

Soil washing was shown to be highly effective in reducing the volume of material requiring remediation on the contaminated sandy soil type. The soil fractions between 125–500 mm, which made up 89% of the total soil weight, are free from significant contamination (Fig. 1). Soil washing, however, was

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ineffective in reducing the volume of contaminated material in a highly contaminated red krasnozem (clay-loam).

Attritioning trials (the removal of the surface of the soil particles) were undertaken on both soil types to determine whether the arsenic and DDT were bound to the surface of the soil particles. Attritioning further reduced the level of contamination in the sandy soil, but was ineffective with the red krasnozem.

In sandy soils there could thus be a significant cost saving by reducing the volume of contaminated material by soil washing ($50–$100/t) before implementing other remediation technologies which destroy the DDT and leach the arsenic from the soil ($400/t).

Table 1. Clean-up of highly contaminated dip soil using Geo2 chemical leaching solutions. The soil initially contained 5010 mg/kg DDTs and 2645 mg/kg arsenic.

Treatment	arsenic remaining in soil (mg/kg)	DDE remaining in soil (mg/kg)	DDD remaining in soil (mg/kg)	DDT remaining in soil (mg/kg)	Total DDTs (mg/kg)
Geo2 leach solution 1	271	3083	<10	413	3496
Geo2 leach solution 2	228	2346	<10	<10	2364
Geo2 leach solution 3	261	138	<10	<10	138
Geo2 leach solution 4	264	118	<10	<10	118
Control leach solution (water)	2640	256	126	4521	4903

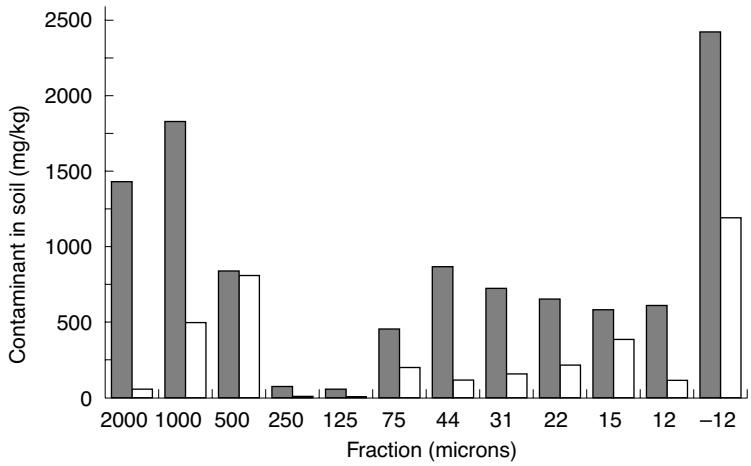

Figure 1. Concentrations of DDT and arsenic in various particle size classes of sandy soil following soil washing.

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Table 2. Clean-up of highly contaminated soil using the Tox-Free pilot-scale thermal desorption unit.

Site no.	Contaminant	Contaminant concentration in soil before treatment (mg/kg)	Contaminant concentration after treatment (mg/kg)
1	DDT	1500.0	<0.2
	DDD	52.0	<0.05
	DDE	5.8	<0.05
	Arsenic	4720.0	3670.0
2	DDT	118.0	<0.2
	DDD	21.3	<0.05
	DDE	4.3	0.21
	Arsenic	1020.0	792.0
3	DDT	264.0	<0.2
	DDD	19.4	<0.05
	DDE	6.1	<0.05
	Arsenic	3550.0	2810.0

Thermal desorption

Trials with the Tox-Free pilot thermal desorption unit showed that DDT can be removed from soil by heating to temperatures of 450–500°C (Table 2). DDT and its congeners were removed to near or below detection limits (0.2 mg/kg for DDT and 0.05 mg/kg for DDE and DDD) in three soils tested. There was some reduction in arsenic levels, but insufficient to make the technology useful for this purpose.

The estimated cost of thermal desorption treatment of dip soil contaminated with DDT only is $150–500/t (Grant Hilton, pers. comm.). As the technology is currently unable to remove adequate quantities of arsenic, alternate disposal or remediation methods for soil following treatment will increase the cost of clean-up. NSW Agriculture is collaborating with Tox-Free in a project seeking means to increase the desorption of arsenic in the retort.

Soil storage

Detailed site assessments are completed for nine of the priority dip sites in the Tweed Shire Council. The results of the assessments will allow low-level and high-level soil contamination to be separated at its source, a strategy encouraged by the NSW Environment Protection Agency under the *Waste Minimisation Act, 1997*.

The soil contains 500–2000 mg/kg arsenic and 50–5000 mg/kg DDT. The soil will be stored in three excavated cells at the secure storage facility. Soil at the storage will be remediated when suitable technologies become available.

Bioaugmentation.

Various biological and nutrient amendments were tested on dip soil contaminated with DDT and arsenic using the small-scale system described in the methods section.

There was no difference between control treatments and any of the microbial or nutrient amendments in the biodegradation of DDT in the soil. There was some loss of DDT from the system (up to 33% over 3 months), but it was concluded that the environmental conditions—including temperature, semi-anaerobic digestion, and mixing—contributed significantly to the natural biodegradation of the DDT, while bioaugmentation had little effect. No treatment induced a loss of arsenic from the soil, either by leaching or volatilisation.

Bioaugmentation is unlikely to provide a solution for the remediation of cattle tick dip sites. However, there may be natural factors which can be manipulated to enhance the natural bioremediation of contaminants, in particular, DDT. The benefits of biostimulation over bioaugmentation are discussed by Guerin (1995).

Assessment of microbial activities at dip sites and the potential for intrinsic bioremediation

Results from the sites analysed for pesticide contamination and microbial activity show that there are very low levels of microbial activity in the areas at each site containing the highest levels of arsenic and DDT contamination (directly adjacent to the dip bath).

The area within 0.5–1.5 m from the dip bath at each site generally exhibited the highest levels of microbial activity levels even though arsenic and DDT concentrations in these areas were still moderately high as compared with areas further from the dip bath. Indeed, at many of the sites, the microbial activity levels in these areas were higher than those observed in uncontaminated control sites nearby.

Statistical analyses of results have shown a near linear correlation between DDT concentration and microbial activity (see rate on Figure 2). A non-parametric regression line was fitted with the 95% confidence interval shown. Here, it is apparent that high levels are associated with significantly reduced microbial activities. By contrast, arsenic concentration and microbial

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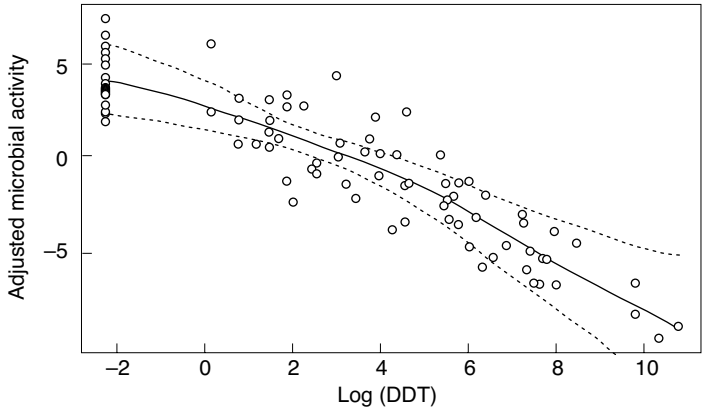

Figure 2. Plot of adjusted microbial activity against DDT concentration over 15 dip sites. Confidence intervals (95%) are included about the non-parametric regression.

Table 3. Clean-up of amitraz dip fluid using a pilot-scale Cuno® filtration system.

Sample details	Amitraz (mg/kg)	Formamidine (mg/kg)	Aldehyde (mg/kg)	DMA (mg/kg)
Pre-filter for sample 1	2±0.9	<0.5	<0.5	2±0.2
Post filter for sample 1	<0.5	<0.5	<0.5	<0.5
Pre-filter for sample 2	148±8.5	<0.5	<0.5	4±0.1
Post filter for sample 2	<0.5	<0.5	<0.5	2±0.4

activity were not correlated. The levels for microbial activity have been adjusted for differences occurring as a result of dry matter, pH, arsenic concentration, and DDT metabolites.

The primary metabolite of DDT has been shown to be DDD (Xu et al. 1994) under flooded or anaerobic conditions in soil. One site displayed comparatively high DDD levels and little DDE. The site had a sandy soil with a pH of approximately 4.6. It is situated on a flood plain, with a water table generally within 1 m of the surface, and is subject to periodic inundation. It is likely that at this site microorganisms have adapted to anaerobically degrade DDT via the metabolite DDD.

Technologies for the field-scale clean-up of amitraz dip fluid and sludge: dip decommissioning

Field-scale dip filtration

A pilot filtration system was successful in removing amitraz and DMA from dip fluid. Table 3 shows the clean-up of dip fluid which had 148 mg/L amitraz and up to 4 mg/L of the soluble metabolite, DMA. A carbon filter was required to remove the solubilised DMA from dip solution. Filtration was effective for amitraz. It has been shown to dissipate rapidly from the aqueous phase with strong adsorption to sediment (Allen and Arnold 1990). Amitraz is applied in dips as a wettable powder, and little is expected to enter solution. It has a Koc of 1000–2000 (Tomlin 1995), and a water solubility of 0.1 mg/L. Effective removal of amitraz from the dip solution was achieved by the separation of sediment and insoluble amitraz from the aqueous phase.

Optimal clean-up results were gained when the carbon filter was placed last in the filter sequence. Up to 2 mg/L of DMA passed through the pilot filtration system. However, a larger capacity carbon filtration system has been installed on the field-scale dip fluid filtration system. This is likely to further reduce the concentration of DMA in the effluent.

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Field-scale bioremediation of amitraz-contaminated sludge

Material in the trial field-scale composting trial was sampled nine times. The results in Table 4 show that rapid degradation of amitraz occurred in the in-situ bioremediation trial. The amitraz concentration in the compost was reduced from 483 mg/kg initially to 38 mg/kg within 7 days. Levels below the limit of detection were reached within 26 days. The quantities of amitraz metabolites (formamidine, aldehyde, or DMA) produced were insignificant, and were in turn biodegraded in the compost pile.

Table 4. In-situ bioremediation of amitraz-contaminated dip sludge using composting.

Days	Amitraz (mg/kg)	Forma-midine (mg/kg)	Alde-hyde (mg/kg)	DMA (mg/kg)
Initial	483±23.3	6±0.5	<0.5	48±3.8
2	304±24.3	55±9.1	11±3.8	11±2.8
7	38±13.5	81±41.7	22±8.1	14±3.1
15	5±5.2	17±1.3	9±1.3	9±1.7
26	<0.5	12±1.9	5±0.8	15±1.1
40	<0.5	5±1.1	4±1.3	4±2.8
51	<0.5	4±3.1	2±2.8	7±0.6
72	1	<0.5	<0.5	6±1.6
104	<0.5	<0.5	<0.5	4±0.7

The rate of amitraz biodegradation in the field trial was not as rapid as in the 1 m^3 pilot studies previously reported (Van Zwieten et al. 1997), because the in-situ compost pile was not turned to aid aeration. Nevertheless the procedure was successful, and the remediation technology has become a part of NSW Agriculture operating procedures for the decommissioning of cattle dip baths containing amitraz.

Conclusions

The sole option for clean-up of DIPMAC priority sites is currently the off-site secure storage of contaminated soil. This is a temporary measure for the immediate clean-up sites which may pose a risk to human health. Although there are currently no technologies available in Australia which can effectively remediate soil to appropriate standards, there are a number which show promise (Van Zwieten and Grieve 1995). These include the physicochemical technologies of chemical leaching, thermal desorption, and soil washing, and bioremediation technologies.

NSW Agriculture has a collaborative agreement with Geo2 Ltd to further develop a chemical leaching technology. This is the first remediation technology designed to treat both the inorganic contaminant arsenic and the organic contaminant DDT in a single-step process. A pilot remediation plant will be in operation by July 1999, by which time accurate costs for the full-scale implementation of this technology will be known. It is estimated that remediation will cost $250–400/t of soil.

Thermal desorption has already been shown to be effective for the treatment of DDT, but is ineffective in the treatment of arsenic in soil. NSW Agriculture is conducting collaborative trials with Tox-Free Ltd to modify the thermal desorption plant to increase the rate of arsenic volatilisation in the heated retort. The costs of remediating soil using thermal desorption are similar to those expected with chemical leaching.

Soil washing is a technology for reducing the volume of contaminated material. It was shown to be capable of reducing the volume of contaminated material in a sandy soil type by almost 90%. This technology is relatively inexpensive to implement ($100/t) and would be useful for reducing the volume of material needing remediation using higher cost technologies such as thermal desorption or chemical leaching.

The use of indigenous microorganisms for the bioremediation of DDT shows promise. Temporary flooding at one site may have led to anaerobic conditions, encouraging the biodegradation of DDT to DDD. Projects investigating the intrinsic or enhanced natural biodegradation of DDT at dip sites are continuing. It was demonstrated that bioaugmentation—the addition of microbes to soil—was unlikely to be effective in cleaning-up dip contaminants.

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Remediation technologies for amitraz in dip fluid and sludge have been developed by NSW Agriculture. The results presented here show that a combination of filtration and carbon sorption of the soluble metabolite DMA was effective in cleaning-up dip solution. Composting was used for the bioremediation of the remaining chemicals in the sludge in the dip bath. NSW Agriculture has remediated more than 50 dip sites using this technology. It has been estimated that more than 500000 L of amitraz-contaminated dip fluid has been remediated.

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Pesticides, Trade and the Environment: an Australian Perspective on Sustainable Crop Protection

Philippa Rowland*

Abstract

Uruguay Round agreements on sanitary and phytosanitary measures and technical barriers to trade provide a framework for managing trade in agricultural commodities and food. Designed to remove non-tariff harriers to trade, these agreements, together with requirements for the replacement of agricultural subsidies with tariffs, are widely predicted to provide new opportunities to expand exports of agricultural commodities and food. Capturing these benefits will require increasing attention to consumer perceptions about the safety of food and demands that commodities are produced in an environmentally sustainable manner.

A growing international trend to reduce pesticide use, supported by research into integrated crop and pest management strategies, is leading to farming systems that rely much less on inputs of synthetic pesticides than current practice. These developments hold a promise for limiting impediments to trade from the presence of pesticides and heavy metals in foods.

Australia shares global concerns about pesticides and has responded by developing a draft 'National strategy for agricultural and veterinary chemicals'. The aim of this strategy is to integrate current policies on chemical regulation and pest control methods into a coordinated framework for managing pesticide risks. Catalysts for this initiative have been the need to maintain and expand international markets, to minimise risks to human health and the environment, and to develop sustainable pest control practices in the face of increasing resistance to pesticides.

This paper describes international trends in minimising the risks posed by the use of pesticides, and gives a brief account of the development of Australia's national approach to the use of agricultural and veterinary chemicals.

URUGUAY Round agreements on Sanitary and Phytosanitary measures and Technical Barriers to Trade provide a framework for managing trade in agricultural commodities and food. Designed to remove non-tariff barriers to trade, these agreements, together with requirements for the replacement of agricultural subsidies with tariffs, are predicted to provide new opportunities to expand exports of agricultural commodities and food. Capturing these benefits will require attention to consumer perceptions about the safety of food and their increasing demands that commodities are produced in an environmentally sustainable manner.

A growing international trend to reduce pesticide use, supported by research into integrated crop and pest management strategies, is leading to farming systems that rely much less on inputs of synthetic pesticides than is current practice. These developments hold a promise for limiting impediments to trade from the presence of pesticides and heavy metals in foods.

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coordinated framework for managing pesticide risks. Catalysts for this initiative have been the need to minimise risks to human health and the environment and to maintain and expand international markets. Pesticide resistance in many pests also reinforces the need for sustainable pest control.

This paper will cover international trends in minimising the risks posed by the use of pesticides. It will also provide a history of the development of Australia's National Strategy for Agricultural and Veterinary Chemicals, including the process of stakeholder consultation.

Risks of Pesticide Use

Benefits of pesticide use—for example, increased productivity and food quality—have not been gained without risks to human health and safety, damage to the environment, and threats to trade and to agricultural sustainability. Growing recognition of these risks has led to an international trend to minimise pesticide use and to promote sustainable crop protection practices. Concern regarding pesticides is not limited to food and food safety but extends to concerns about the environment and agricultural workers (CAST 1995).

Potential and actual impacts of pesticides on human health and the environment, and growing consumer pressure for safe food have increased interest in certification and eco-labelling schemes for agricultural commodities. It is not just a matter of residues and the quality of the commodities on sale, but also the trend to discriminate between products on the basis of the production methods used, including the environmental impact of pesticides used during production. Concerned consumers lobby governments and industry for commodities with lower residues and alternative control methods for animal and plant pests. A growing emphasis on risk minimisation has led to more frequent reviews of pesticides, with increasing emphasis placed upon the prevention of unacceptable risks to the environment and ever more stringent environmental criteria for pesticide registration.

Human health and safety

Governments around the world are responding to increased public concern about chemicals in foods and the environment. Community perception of risks to human health from food exert a powerful influence, providing the motivation for changes to government regulation and marketing practices, as well as for increased scrutiny of imported products. This change occurs to some extent irrespective of whether sufficient evidence exists to verify health effects from consumption of such residues. The mere presence of residues is perceived as a major health threat, even though the actual risk of illness or death may be low (Nicholls *et al*. 1994). The present uncertainty about the effects of pesticide residues in food is partially due to the lack of epidemiological and toxicological methods for measuring long term, low dose exposure (Maskill and Harre 1994).

In Australia, calculations of acceptable daily intakes (ADIs) are undertaken by health authorities and used to establish the safe level of human lifetime exposure (ANZFA 1996). The ADI is measured in mg per kg body weight and is deemed to be the amount of chemical to which an individual can be exposed, through food, air, water or any other means, with no ill effect. Total dietary intakes from all sources are considered safe if they are below the ADI. By contrast, a maximum residue limit (MRL) is the maximum residue of a chemical allowed in food available for sale, and is measured in mg per kg of food. MRLs are designed to reflect good agricultural practice and are set on residue levels expected if pesticide label recommendations are followed. After public consultation, these MRLs are adopted into the *Food Standards Code,* which is automatically adopted by reference into all State and Territory food laws.

There is also recognition of the need to reduce the risks posed to those handling and applying chemicals or living nearby, with a growing demand for improved application techniques and management methods. Australia has drafted national occupational health and safety standards for licensed pest management operators (Worksafe Australia 1996).

Environmental risks

The effect of pesticides on biodiversity and the quality of the environment has also led to changes in patterns of pesticide use and tightened controls on the downstream or off-farm impacts of agriculture. For example, there is now widespread recognition of the need to reduce the use of chemicals that deplete the ozone layer. Declining water quality has increased pressure to change crop protection practices, with growing concern about the detection of residues in surface and groundwater (eg Wiles *et al*. 1994). The ability to detect previously unmeasurable quantities

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of pesticides has spurred the development of pesticide reduction activities in many countries. Considerable research effort is put into reducing pesticide and nitrate leaching to safeguard future drinking water supplies in Europe and North America. Recent studies in Australia are assessing the status of groundwater quality (Bauld 1996).

Pesticide assessments are frequently carried out for environmental criteria that include persistence in soil, mobility or leaching into groundwater and acute toxicity to aquatic organisms. Traditionally, ecotoxicological studies have concentrated upon aquatic ecosystems, despite the fact that some 30–60% of applied pesticides may end up as soil-bound residues. European Community workshops have been held to develop and evaluate methods to assess the effects of chemicals on the soil ecosystem (e.g. Lokke and van Gestel 1993).

Endocrine disruption

There are now indications that even small amounts of chemicals in the environment may be having previously unrecognised impacts on ecosystem health. Colborn et al. (1993) published early reports claiming that certain chemicals had the potential to act as endocrine-disrupting agents. Since then, there has been considerable debate about the validity of the concerns raised, and the scientific community has been called upon to assist in providing data to improve international understanding of endocrine disruption. The Australian Academy of Science held a national workshop on the issue in April 1998 and concluded that good communication was vital (AAS 1998). A clearing house for information is being considered.

The risks have been taken seriously in the United States. Under the requirements of the *Food Quality Protection Act* and the *Safe Drinking Water Act*, an endocrine disruptor screening and testing advisory committee (EDSTAC) has been established to develop a strategy for screening chemicals for their potential to affect endocrine functions in humans, fish and other wildlife. EDSTAC's final report was provided in July 1998: the executive summary is available at:

http://www.epa.gov/opptintr/opptendo/index.pdf

Threats to trade

Pesticide residues can pose threats to trade in agricultural products. Requirements that all food imports meet MRLs set by importing countries may become more stringent in response to continuing consumer concerns about residues. It is clear that the level of scrutiny imposed on chemicals released into the marketplace will increase with time, and it will be important for countries like Australia to maintain excellent standards in assessing agricultural and veterinary chemicals through the registration process.

All agricultural and veterinary chemicals registered for use in Australia are assessed to ensure that they meet appropriate standards for quality, safety and efficacy, and will have no adverse effects on the environment or trade. This is the responsibility of the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) which was established in 1993 to consolidate activities previously the responsibility of State governments. The NRA receives specialist toxicological advice from the Department of Health and Family Services, from the Risk Assessment Section of Environment Australia and from Worksafe Australia. The NRA is implementing an 'Existing chemicals review program' to evaluate chemicals registered many years ago to ensure that they meet contemporary standards. Five chemicals were reassessed in the first cycle: atrazine, endosulfan, mevinphos, parathion and parathion methyl.

Residue Monitoring in Australia

Australian food is of a high standard, its quality protected by a regulatory framework and supporting monitoring systems. The NRA also has responsibility for recommending MRLs for chemicals in food commodities to the Australia New Zealand Food Authority (ANZFA). ANZFA has published a framework for the assessment and management of food-related health risks, outlining general principles that underlie the protection of public health and safety (ANZFA 1996a). Australia has encouraged operators in all sectors of the food chain, from growers through to transporters, processors and retailers, to adopt a rigorous scientific approach to food safety. The hazard analysis and critical control point (HACCP) technique is one important tool (ARMCANZ 1997).

Other agencies that play a crucial role in monitoring and evaluating the risks posed by the use of agricultural and veterinary chemicals include the National Residue Survey run by the Bureau of Resource Sciences, and the Total Diet Survey (formally the Australian Market Basket Survey) run by ANZFA (ANZFA 1996b). The States have targeted

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monitoring programs to identify any breaches of good agricultural practice and to test specific commodities as required by importing countries. However, current monitoring for chemical residues and heavy metals focuses upon the food commodity/product rather than the environment. At present there are only a few programs monitoring chemicals in the Australian environment, and baseline data against which to establish trends are lacking (Rowland et al. 1997).

The National Residue Survey monitors chemical residues in Australian raw food commodities, including those used in the production of meat, grains, honey, fruit, vegetables, nuts and seafood (e.g. NRS 1997). Heavy metals and organochlorines such as DDT that may be present in the environment as a result of past use by industry are also surveyed as environmental contaminants. NRS surveys provide a snapshot of the national status of agricultural commodities. Data produced are increasingly used to validate industry quality assurance programs, providing important support for Australia's export programs.

The primary focus of the Total Diet Survey is to estimate the dietary intakes of a range of pesticides and contaminants. Diets consisting of over 70 foods are constructed, based on the most commonly eaten foods in Australia. Foods are prepared to a table-ready state and are individually screened for organophosphates, organochlorines, pyrethroids, dithiocarbamates, antimony, arsenic, cadmium, copper, lead, mercury, selenium and zinc. Other toxicologically active contaminants such as aflatoxins, herbicide residues, tin and aluminium are analysed in selected foods. This is a small survey in terms of numbers of samples, but useful because measurements are made on table-ready food.

Chemical Residues and the World Trade Organization

The move to liberalise world trade and open up international markets has been accompanied by increased scrutiny of products for residues, heavy metals and microbiological contaminants. Countries throughout Southeast Asia now implement sophisticated residue-monitoring processes at the point of entry. Hong Kong, Indonesia, Japan, Malaysia and Brunei have developed import-testing facilities, and other nations such as Singapore and Taiwan are rapidly developing similar procedures. Increasingly sophisticated detection equipment can detect residues below international MRLs. Indeed, residue tolerance levels have sometimes fallen in parallel with advances in detection and monitoring technology.

Producers from all over the world are affected by the international trading arrangements now in place under the World Trade Organization (WTO). The Agreement on Agriculture signed during the Uruguay Round imposes new disciplines on the use of export and domestic subsidies, and requires existing non-tariff barriers (import bans and quotas) to be converted into more transparent tariff equivalents. In time, implementation of the Agreement on Agriculture will significantly alter government assistance to agriculture and lead to a shift in existing patterns of production. Under these changed trading arrangements, quarantine and other technical barriers are coming under closer scrutiny all over the world.

The Sanitary and Phytosanitary (SPS) Agreement requires that quarantine measures are applied only to the extent needed to protect life or health, are based on scientific principles, and are not maintained without sufficient evidence. Countries are increasingly called upon to provide scientific risk assessments that justify existing quarantine barriers, although the SPS Agreement also brings a significant opportunity to improve trading opportunities by ensuring that products are not unjustifiably excluded from overseas markets. An SPS measure is defined as any measure applied to protect human or animal life or health within the territory of the member country from risks arising from additives, contaminants, toxins or disease-causing organisms in foods, beverages or feedstuffs, where contaminants include pesticide and veterinary drug residues.

The Agreement on Technical Barriers to Trade (TBT) deals with technical issues not covered by the SPS Agreement. These include food standards, labelling and packaging. WTO member countries have an obligation to ensure that national measures falling within the coverage of the TBT Agreement do not restrict trade by any more than is essential and have legitimate objectives. While a final position has not been taken, it is clear that under the WTO all eco-labelling schemes cannot be used as disguised barriers to trade and must be voluntary rather than mandatory.

The WTO agreements encourage member countries to base their national standards wherever possible on international standards, guidelines or recommendations, such as those developed by the Codex Alimentarius Commission, the International

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Office of Epizootics (OIE) and the International Plant Protection Convention. Codex has established several thousand MRLs for particular chemicals, including pesticides, in particular foods. This work is continuing through Codex technical committees, with support from the FAO/WHO Joint Meeting on Pesticide Residues and the Joint Expert Committee on Food Additives. Australia's position is to work multilaterally through the WTO and the Codex Alimentarius Commission to increase acceptance of a harmonised, scientifically based international approach to managing residue issues consistent with the SPS Agreement and making optimal use of Codex standards (Gebbie 1998).

Pesticides, Trade and the Environment

'Trade and the environment' is a new area of international focus with considerable potential to affect future trade in products treated with chemicals at any stage in their production process. Emerging problems such as global warming, land degradation and transboundary pollution have resulted in the rapid internationalisation of environmental issues, which are now important factors in the formulation and implementation of many domestic and trade policies.

Multilateral environmental agreements

Implementation of Agenda 21, launched at the Rio Earth Summit held in 1992 as an agreed blueprint for the next century, has led to several multilateral environmental agreements (MEAs) that influence chemical use in agriculture. These include the Montreal Protocol on Substances that Deplete the Ozone Layer, the Basel Convention on the Transboundary Movement of Hazardous Wastes and Their Disposal, and undertakings on Long Range Transboundary Air Pollution and Prior Informed Consent. Many countries are currently negotiating a Biosafety Protocol on the Transboundary Movement of Living Modified Organisms under the Convention on Biological Diversity, which will have implications for trade and movement of transgenic commodities, including herbicide-resistant crops.

The Montreal Protocol became directly relevant to agriculture when methyl bromide, a widely used broad spectrum soil fumigant, was identified as an ozone-depleting substance in 1992. This poses an enormous challenge for signatories as it is unlikely that a single replacement will be found. Australia has a comprehensive national approach to the development of alternatives to methyl bromide (Environment Australia 1997). An industry levy imposed on importers of methyl bromide is being channelled into research to develop alternatives for affected industries.

Techniques actively being pursued in Australia included solarisation, plastic mulches and biological control. An innovative example is the use of brassica crops which decompose to release isothiocynates that have been shown to inhibit the growth of soil pathogens such as take-all (*Guannomyces graminis*) (Kirkegaard and Sarwar 1998). Such biofumigation has the potential to provide biological control of pests and diseases in horticultural crops such as potatoes, by growing the brassicas as a green manure then ploughing the plant residues into the soil. However, successful adoption of alternative measures for controlling soil-borne diseases requires a concerted effort in grower education and training.

International Trend to Pesticide Risk Reduction

Awareness of the downstream impacts of pesticide use in agriculture has been growing worldwide. The last decade has seen a significant shift in international attitudes to pesticide use and a trend towards the promotion of sustainable crop protection practices that minimise any risks associated with pesticide use. The growing level of international interest in pesticide use in agriculture was reflected in the participation of 26 countries in a Pesticide Risk Reduction Workshop in October 1995. Organised by the Organization for Economic Cooperation and Development (OECD) and the Food and Agriculture Organization of the United Nations (FAO), this meeting was designed to facilitate the exchange of information and views about how to reduce risks associated with pesticide use. No formal policy was developed, but countries were encouraged to undertake further action. A subsequent OECD meeting in Copenhagen in April 1997 discussed methods of evaluating and monitoring national efforts to reduce pesticide risks.

Evans (1994) identified the following initiatives to reduce the use and/or risks of pesticides:

1. plant and animal breeding programs to incorporate resistance to pests and diseases;
2. development of biological controls;
3. development of vaccines and alternatives to synthetic pesticides;

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4. management strategies to reduce the problem of pesticide resistance in pests;
5. integrated pest management programs targeting pests, weeds and diseases;
6. programs to monitor pesticide residues in commodities and food; and
7. quarantining of properties for livestock and food production where soils have been contaminated by pesticides.

Several countries, notably Sweden, Denmark and the Netherlands, have adopted legislative targets to reduce pesticide use. Analyses of these legislative programs are given by Hurst et al. (1992), Evans (1994) and Matteson (1995). The Multi-Year Crop Protection Program in the Netherlands is comprehensive, setting pesticide reduction targets for each of ten rural industry groups and for each of four pesticide types (herbicides, fungicides, insecticides, soil fumigants). Targets were also set for reducing emissions to air, soil, and surface and groundwater. Growers may be granted temporary authorisation to use environmentally hazardous pesticides, if alternative compounds are unavailable and strict use conditions minimise impacts on the environment.

Legislative and voluntary pesticide risk reduction programs have a common focus on education, training and extension in the agricultural sector. Participatory research programs build farmers' confidence in a broad suite of pest control techniques and encourage a shift in agricultural practice away from over-dependence on chemicals. The benefits of this participatory approach are demonstrated by the successful FAO-sponsored farmer field schools in Indonesia, the Philippines and other countries.

Integrated management systems

Parallel to these moves to reduce pesticide use, there have been considerable efforts to develop national strategies that actively foster the adoption of integrated management practices. Such practices are called integrated pest management (IPM) programs in the United States and many Southeast Asian countries (see Table 1), and as integrated crop management (ICM) programs and integrated farming systems (IFS) research in Europe and elsewhere.

The world's agrochemical industries are also embracing the notion of IPM. The promotion of IPM by multinational chemical companies is a sign of the changing times in crop protection and animal health, although some detractors have said it reflects more a concept of integrated *pesticide* than *pest* management. The Global Crop Protection Federation (GCPF 1997) uses a definition of IPM given in FAO's International Code of Conduct on the Distribution and Use of Pesticides:

> A pest management system that, in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains the pest population at levels below those causing economically unacceptable damage or loss.

Table 1. Countries declaring official integrated pest management policies

Country	Year
India	1985
Malaysia	1985
Indonesia	1986
Germany	1986
Philippines	1986
Denmark	1987
Sweden	1987
Netherlands	1991
United States	1993

Source: Zadoks 1993

Integrated pest management initiative in the United States

In 1993 the Clinton Administration announced its support for sustainable agriculture, and declared that it would reduce the use of pesticides and have 75% of the arable land in the USA under IPM by 2000 (Espy et al. 1993). The IPM initiative seeks to make USDA programs more responsive to farmers' needs, by increasing local involvement in setting research priorities (Matteson 1995). It redirects and combines USDA and Land Grant University grants in a single cooperative effort, with a strong emphasis on the concept of developing partnerships and improving coordination across the country. Hoppin (1996) and Benbrook (1996) developed a measure for assessing the level of adoption of IPM that assesses the reliance on pesticides compared with a range of other techniques. They use the term 'biointensive IPM' to reflect systems that rely more on biological and cultural control and on chemical applications. Vickery (1997) has also developed methods for assessing IPM crop protection practices.

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Integrated crop management in Europe

Integrated crop management (ICM) emphasises the integration of non-chemical pest control and techniques such as choice of cultivars, crop management and environmental selection of chemicals (Fig. 1), seeking to reduce the need for off-farm inputs. ICM is promoted as the 'new direction for European agriculture' (EIF 1995), balancing economic production with environmental responsibility. Large food retailers are becoming more involved in determining agricultural practices that produce the commodities they sell. In the United Kingdom, the National Farmers' Union and a group of major retailers have produced ICM protocols for over 30 crops. Several retailers operate an accreditation scheme for a variety of fruit and vegetables based upon these protocols. Sainsbury's, one of the largest UK chain stores, announced in 1996 that all products sourced by its company would be accepted only from producers complying with guidelines and protocols laid down for ICM—irrespective of origin (Worthington 1996).

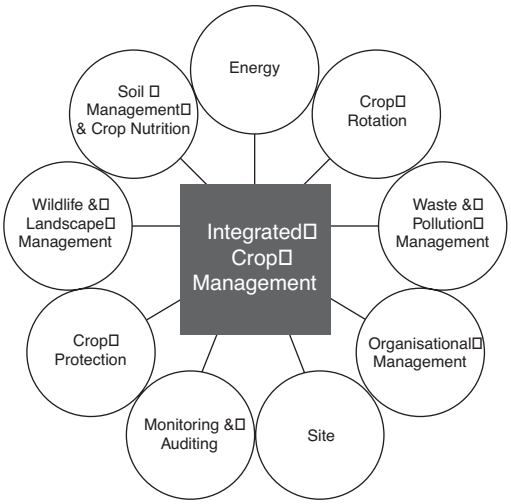

Figure 1. Integrated crop management. Source: EIF (1995)

Integrated farming systems

Research into integrated farming systems (IFS) across Europe has aimed to create production systems that meet both consumer and environmental requirements. The latest research results are integrated into arable production systems that optimise inputs at the farm level, reducing fertiliser and pesticide use without economic loss. Pesticide use reductions of up to 50% have been achieved under integrated farming systems in the Netherlands (Wijnands and Vereijken 1992). Farm-based validation of research results encourages adoption of these systems, since farmers can see that the new techniques work without risking their crops.

Increased efficiency of these systems reduces environmental damage and maximises profitability. It is possible to reduce pesticide use while adequately protecting crops from insects, weeds and pathogens, and without compromising productivity or quality. The major focus of research is to identify how crop rotations, crop damage thresholds, judicious applications of pesticides and choice of resistant cultivars can maintain yields while relying on only a fraction of the pesticide use previously required. The experimental techniques developed at Long Ashton near Bristol in the U.K. are now being trialed on several commercial farms. In Sweden, five regional plant protection centres with a total staff of 600 have been set up to promote integrated farming practices (AGROW 1996). Work at these centres concentrates on pest forecasting and warning, strategies to combat pests, weeds and diseases, and information on ways to reduce pesticide use.

Consumer Demands and the Rise of Eco-labelling

Heightened community concern about environmental degradation, ozone depletion and pesticide residues in food and the environment is being translated into consumer demands for sustainable production systems that minimise impacts on the ecosystem (Table 2). Greater consideration is being given to the environmental impact of production processes. In future, this impact on the environment is likely to become as important as the quality of the end-product. Belief in 'consumer right to know' about chemical residues and other food-borne risks in food commodities has been accompanied by increased interest in green or eco-labelling. Certification of organic produce led the way for eco-labelling in many countries and still provides a good example of rigorous standards for production methods (e.g. OPAC 1998).

Systems for rating the environmental impact of pesticides

There is a growing body of research into ratings systems, ranking the environmental impact of various

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pesticides and pest control techniques. In the United States, Kovach et al. (1992) developed an environmental impact quotient (EIQ) to assist growers using IPM to make environmentally sound pesticide choices. Wegmans Food Markets, a chain of over 200 stores in New York State and Pennsylvania, has released canned peas and maize carrying an IPM label. Wegmans supermarkets have evidently identified IPM labelling as a means of differentiating their produce in a competitive marketplace. Their label says '..Your purchase supports the efforts of growers who truly care about the environment' (Cowles 1997). The label relies on IPM elements, or protocols, developed for various crops by a partnership of Wegmans, growers, Cornell Cooperative Extension educators, and a supplier. For details see <http://www.nysaes.cornell.edu/ipmnet/ny/vegetables/elements/index.html>.

Table 2. International green labelling programs

Program	Country
Blue Angel	Germany
Environmental Choice	Canada
Eco-Mark	Japan
White Swan	Nordic Council
Green Seal	USA
Good Env. Choice	Sweden
Env. Choice NZ	New Zealand
Ecomark	India
Ecomark	Korea
Green Label Singapore	Singapore
Env. Labelling Program	European Community
Stichting Milieukeur	The Netherlands
Agri-environmental certification	The Netherlands
NF-Environnement	France

Source: Drake-Brockman (1995)

The Dutch 'environmental yardstick' for pesticides (Reus and Pak 1993) is also used for certification, providing a marketing edge for grains, fruit and vegetables. It is used as a tool to help farmers make informed choices and evaluate their progress towards environmentally sound practices, and may provide an incentive in the transition to sustainable farming (Bouwman *et al*. 1993). The Institute of Agriculture, Trade and Policy in Minnesota has been evaluating the potential of tools for assessing environmental impacts: see <http://www.iatp.org/iatp>.

In Australia, Penrose et al. (1994) have developed a pesticide rating index as an objective basis for decision-making in crop protection, and to encourage a rational approach to pesticide reduction. It provides a system for classifying pesticide use in different crops and circumstances and differs from other indices by including parameters for the 'potential for residues' and the value or importance of the pesticide in particular crop protection systems. PestDecide, a decision-support system, is designed to assist growers choose pesticide for use on apples accredited under integrated pest and disease management.

Certification and quality assurance

All exporters of agricultural products have clear incentives to stay informed about changing consumer demands and concerns about pesticide residues. Recently, Australia has seen a wave of quality assurance programs for agricultural production systems. In addition, perceptions of quality are changing to include the 'quality of production', a concept which covers the effect on the environment of the methods used to produce crops and livestock (EIF 1995).

The ISO 14000 series, new voluntary international environmental standards developed by the International Organization for Standardization, may play an important role for industries keen to obtain internationally recognised quality certification and distinguish themselves from competitors (Denton 1996). Certification under ISO 14001 does not guarantee good environmental outcomes, but it may be able to deliver a single internationally accepted standard to replace a multiplicity of registration, certification, labelling and inspections (Rowland *et al*. 1996). Although adoption has been variable, certification is perceived as an advantage to exporting (Frankel 1998). Table 3 shows the number of firms (not specifically agricultural enterprises) certified as at January 1998.

Sustainable Pest Management in Australian Agriculture

An unfortunate consequence of the outstanding success of herbicides has meant that much research has been invested in this area with other methods of weed management often being neglected.

(Lovett and Knights 1996)

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In Australia, the urgent need to conserve soil has led to the development of minimum tillage or 'conservation farming' techniques, accompanied by a significant increase in the volume of herbicides used. Widespread development of pesticide resistance in pests, particularly multiple herbicide resistance in weeds, might jeopardise the sustainability of cropping systems. Resistance has reduced the chemical tools available to farmers for the control of a wide range of pests, including insects, mites, weeds and intestinal parasites of sheep.

Table 3. Number of firms certified under ISO 14000 in 1998

Certification scheme/country	No. of certifications
ISO 14001	2800 worldwide
Japan	620
United Kingdom	440
Germany	370
Netherlands	230
Taiwan	160
Switzerland	127
Sweden	114
United States	85
EU's voluntary Eco-Management and Audit Scheme (EMAS)	1400

Source: Frankel (1998).

Resistance has emerged as a result of the selective pressure applied to populations of pests through overuse of pesticides, and the lack of appropriate strategies for preventing or retarding the emergence of resistant types. Farmers can slow the development of resistance by ensuring pesticides are used only sparingly, as part of an IPM approach that includes biological and other methods of pest control, and by adopting resistance management strategies.

In its final report, the Ecologically Sustainable Development (ESD) Working Group on Agriculture (Anon. 1991) recommended intensified research into, and monitoring of, the long term effects of herbicide use in minimum tillage systems, citing the importance of early warning for any effects threatening the objectives of ecological sustainability. Herbicide-resistant weeds are now estimated to occur over 500000 hectares or 10% of the cereal cropping area of Western Australia (Cullen et al. 1995), and multiple herbicide resistance in annual ryegrass has necessitated the development of resistance management strategies.

Lewis et al. (1997), in an address to the National Academy of Sciences (USA), put forward a cogent argument for developing farming practices that are compatible with ecological systems, and for designing cropping systems that naturally limit the elevation of an organism to pest status. The future of effective crop protection requires the development of innovative techniques and strategies that are flexible yet robust enough to effectively cope with problems that arise with the emergence of new pests and increased development of pesticide resistance.

Current levels of pesticide use in Australia

Pesticide use in Australia varies enormously across the continent, between different regions and pest/crop/climatic combinations. Figure 2 provides data on the factory gate value of pesticide sales, disaggregated by herbicides, insecticides, fungicides and animal health care products. There has been a very dramatic rise in expenditure on herbicides and animal health care products in the relatively recent past and a more modest increase in expenditure on fungicides and insecticides. What is not known is how much of the growth in expenditure was caused by an increase in the use of agricultural and veterinary chemicals and how much reflects increases in the real price of pesticides (see Figure 2).

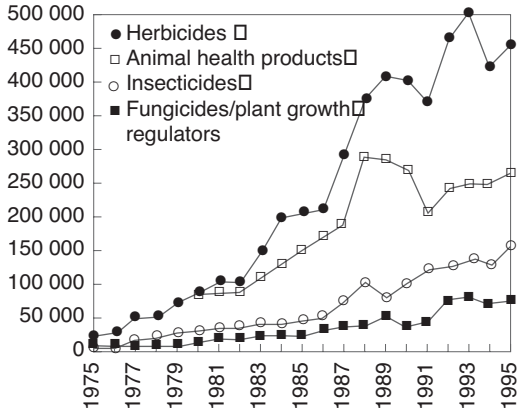

Figure 2. Expenditure on agricultural and veterinary chemicals in Australia (1987–88 dollars). Source: ABARE (1995).

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Examples of pesticide risk reduction in Australian agriculture

Activities to minimise the risks posed by pesticides are many and varied. The cotton industry has made considerable efforts to minimise the impacts on the riverine ecosystem caused by a heavy dependence on insecticides (Schofield et al. 1998). The development of transgenic cotton plants containing a gene which expresses a naturally occurring toxin produced by *Bacillus thuringiensis* may have the potential to significantly reduce pesticide use by cotton growers.

The nursery industry has developed guidelines recommending good practices in the management of chemicals, particularly as they affect water run-off from the farm (NIAA 1997). A recent national symposium (Condamine Balonne Water Committee 1998) also encouraged new thinking on pesticide management in catchments, integrating individual efforts into a regional approach. Apple growers from Batlow in New South Wales funded a research program to investigate the potential for reducing pesticide use through a supervised pest and disease control system. The work was based on defining pest population and crop damage thresholds, with strategic application of pesticides and reducing fungicide dose rates for blackspot (*Venturia inaequalis*) control (Penrose et al. 1994). The results from Batlow demonstrate growers can improve pest management with reduced pesticide use.

The wine industry is another prime example of an industry that has thoroughly researched its pesticide use. It has been motivated by the large and increasing export sales of its products and the need to maintain a reputation for quality. A comprehensive agrochemical grid has been developed, which provides a matrix that growers use to monitor and record pesticide use, keeping records and optimising spray regimes to meet the requirements of diverse and highly competitive world markets. The Horticulture 2000 Group (1998) has developed a residue management plan as a blueprint for ways in which horticultural industries can minimise chemical residue incidents. Queensland fruit and vegetable growers (QFVGA 1998) are developing a code of practice to meet requirements of due diligence under the *Environment Protection Act (1994)*.

The development of comprehensive 'codes of good agricultural practice' and public demand for more sustainable production systems have led to a situation in which growers must document their on-farm practices if they wish their produce to be accepted, providing clear evidence of the purchasers' considerable power and influence over producers. The requirements for transparency and documentation have become universal.

Education and training

Investing more funds in research and development into alternative crop protection and animal health systems that reduce reliance on agricultural and veterinary chemicals may have the potential to achieve the same level of production, quality and economic productivity achieved by conventional pesticide dependent agriculture. Bearing in mind that an integrated approach requires education:

> Pest management through applied ecology requires a high-quality learning process that provides farmers with the principles, knowledge and skills necessary to manage their own farm ecosystems.
>
> (Matteson 1995)

Training producers in the responsible use of agricultural and veterinary chemicals is a key element in the production of high quality food and the reduction of pesticide risk. The opportunities to improve chemical use patterns have also been recognised by producers and processors, by the farm chemicals industry, by educators and by industry peak bodies, all of whom have responded with initiatives to promote the production of high quality food that is free from unacceptable residues of agricultural and veterinary chemicals.

Initiatives include the Agsafe accreditation scheme, which trains all chemical resellers in the safe, effective and legal use of chemicals and the National Farmcare course (previously known as the Farm Chemical Users' Training Program) conducted through Technical and Further Education institutions under the supervision of individual State committees. In South Australia, Farmcare has undertaken successful pilot programs targeting safe chemical use by primary producers from non-English speaking backgrounds (Georg 1997).

Australia's National Strategy for Agricultural Chemicals

Managing agricultural and veterinary chemicals safely is of vital concern to Australia as a major producer and exporter of primary produce, and for the protection of human health and the environment. A national strategy covering their use has therefore been developed by the Standing Committee on Agriculture and Resource Management (SCARM), on behalf of the peak minis-

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terial council on agricultural and resource matters, the Agriculture and Resources Management Council of Australia and New Zealand (ARMCANZ).

SCARM consulted widely in preparing the strategy and considered the recommendations and views of a national pesticide risk reduction workshop run by the Bureau of Resource Sciences in April 1997. It brought together many of the key stakeholders in the production and use of agricultural and veterinary chemicals in April 1997. Since then, comments were sought from over 700 stakeholders, and responses from nearly one hundred have been considered in the preparation of the final document, endorsed by ARMCANZ in February 1998.

'Management of agricultural and veterinary chemicals: a national strategy' (ARMCANZ 1998) sets out how Australia manages and intends to manage agricultural and veterinary chemicals. It is hoped that all stakeholders will help contribute to a vision of the use of agricultural and veterinary chemicals in a way which minimises the risks to health, the environment and trade; ensures the long-term sustainability of agricultural productivity; and best contributes to national prosperity.

The nine broad objectives underpinning the vision and goal are given below. The first two relate to the need to use chemicals in particular circumstances and their place relative to other pest management practices. The remaining seven follow a lifecycle approach to the regulation of chemicals and relate to mechanisms to reduce risks once the decision has been made to use chemicals.

1. *Integrated farm and natural resource management*—to promote the further development and adoption of integrated farm, forest and natural resource planning and management systems that minimise adverse impacts and use chemicals only as needed.
2. *Reducing reliance on chemicals*—to reduce reliance on chemicals through the development and implementation of Integrated Pest Management (IPM) programs and alternatives to chemicals.
3. *Assessment, approval and availability of chemicals*—to increase the efficiency and effectiveness of chemical assessment and approval processes in making available efficacious products that have minimal risks to health, the environment and trade.
4. *Risk reduction in the application of chemicals*—to reduce the risks associated with handling and applying agricultural and veterinary chemicals—to the environment; to those using the chemicals; to people living in the environs of application; to handlers of produce; and to consumers—through best practice management of chemical application.
5. *Minimise risks to human health*—to understand the potential impacts of chemical use on human health, and reduce the adverse effects, through best practice procedures to identify and minimise the risks, and to monitor, assess and act on outcomes.
6. *Minimise risks to the environment*—to better understand the potential impacts of chemical use on the environment, and reduce adverse effects, through best practice procedures to identify and minimise the risks, and to monitor, assess and act on outcomes.
7. *Residues in food and fibre*—to produce and market food and fibre that meets the needs of customers and enhances Australia's reputation as a supplier of safe, high quality food and fibre, through the adoption of production, processing and marketing systems that assure customers (both domestic and overseas) about quality and ensure that primary produce exported from Australia complies with the requirements of importing countries.
8. *Trade and market access*—to enhance market access for Australian primary produce through the identification and management of potential trade risks related to chemical use, and through constructive engagement with major trading partners and in international forums, to ensure Australia's trade interests are protected.
9. *Safe disposal of unwanted chemicals and containers*—to minimise the risks presented by unwanted farm and household agricultural and veterinary chemicals, and their containers, through measures designed to minimise future wastes and ensure the safe collection and destruction of unwanted chemicals and chemical containers.

SCARM's next task will be to encourage and consult further with key stakeholders on developing the means to implement the strategy and achieve the shared vision.

Conclusions

There is no doubt that the development of cohesive national strategies has helped to promote pesticide

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risk reduction activities in many countries. Such strategies provide a strong focus for all stakeholders and give the impetus and motivation required to move forward and find new solutions, building farmers' confidence in a broad suite of pest control practices and encouraging the shift towards more sustainable crop protection systems.

There are many examples of successful and innovative research that consolidates the development of farming systems relying substantially on good management and enlightened crop protection techniques and less on routine applications of pesticides. More encouraging still is the involvement of farmers at an early stage of research, ensuring results can be validated on a commercial scale and that new crop protection techniques are practical and do not jeopardise either productivity or farm income. There is enormous value in this innovative participative approach into the development of sustainable agricultural production systems.

Crop protection practices and residue monitoring need to be considered as part of a package to guarantee that agricultural commodities can be produced with minimal reliance on pesticides and can reliably meet increasing demands for food quality and safety. Feedback is required so growers can get a sense of how they are performing, and understand the market implications of their on-farm crop protection practices. Credible, well documented certification systems will assist farmers to gain the market benefits arising from the adoption of such practices.

Monitoring can also assist in the assessment of 'good agricultural practice' and is becoming more important as industry commitment to quality management and quality assurance expands. Feedback is required to give growers a sense of how they are performing and the market implications of their on-farm crop protection practices. The underlying challenge for Australia's agricultural industries is to consider crop protection practices and residue monitoring as part of an overall program. The long term aim is to guarantee the sustainability of Australia's rural industries and to find a means to reduce the health, trade and environmental risks posed by pesticide use.

Present technologies provide some answers to pest control dilemmas—future practices will have to provide for the needs of succeeding generations. Much can be learnt from drawing on the considerable experiences of other countries in the region and their are mutual benefits to be gained from sharing expertise in reducing risks posed by use of pesticides. It is crucial to success that there be cooperation and collaboration between all stakeholders—all levels of government, industry, academia and growers themselves.

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Pesticide Residues on Some Vegetables, and Reductions Possible by Integrated Pest Management

A.L.H. Dibiyantoro*

Abstract

Pesticides are a basic tool for pest management in Indonesia. They are used by farmers growing vegetables to ensure high yields. When properly used, pesticides can be successfully combined with other pest control method in many instances.

An investigation was made of the pesticide residues occurring on vegetables such as shallots and hot pepper that are part of the daily diet, and on lowland vegetables such as cucumber, yardlongbeans, eggplant, and amaranth. A wide range of insecticides is used in lowland areas, most commonly organophosphates, BHC, organochlorines, synthetics pyrethroids, and insect growth regulators (IGR). Levels of residues of BHC found in cucumber and shallots were in the range 0.007–0.017 mg/kg, whereas the stipulated maximum residue limit (MRL) is 0.001 mg/kg. Lindane residues were detected in hot pepper at 0.007 mg/kg. The details of pesticides used and their residues in West Java and Central Java provinces are discussed briefly in this paper

Spodoptera exigua, Thrips tabaci, and *T. parvispinus* are major pests of shallots polycultured with hot pepper. Treatments that are environmentally less damaging than insecticides are already available and fully accepted by farmers. These include use of *S. exigua* nuclear polyhedrosis viruses (NPVs) and *S. exigua* CVs, and a cheap trap for *Thrips* spp. Use of NPVs and CVs in conjunction with sex-pheromone traps could reduce insecticide spraying by 85%. Using an action threshold control approach, insecticides use was reduced by an average of 63% compared with twice weekly spraying. Indigenous thrips predators belonging to the Coccinellidac *(Coccinella transversalis* and *Cheilomenes sexmaculata),* Scymninae, Chilocorinae, Reduviidae, and others described in the text, were verified on unsprayed peppers. New thrips species were investigated, identified as *T. pallidus* and *T. taiwanus,* in the Brebes area, Central Java.

THRIPS species and *Spodoptera exigua* Hbn. armyworms are major factors limiting production of shallots and hot peppers, especially in tropical regions. In Java the yield loss caused by *Thrips tabaci* and *S. exigua* on shallots may range from 57 to 100%; and *Thrips parvispinus* on hot peppers may cause yield losses of 35–55% (Dibiyantoro 1996). The extent of damage caused by thrips to shallots and hot peppers, though very high, is yet to be successfully managed. Little research has been done on *Thrips* because rearing and observation of these insects is very tedious. Lack of accurate control strategies for both pests on these crops has been highlighted in crop management programs. Over-use of pesticides on shallots and hot pepper crops not only has a negative influence on farmers' economic status but also is of great concern on environmental grounds.

In the Brebes area of Central Java, where shallots and hot peppers are grown on almost 70000 ha, farmers have to face problems with *S. exigua* ('ulat bawang') and *Thrips tabaci* and *T. parvispinus* ('kemreki troubles'). In 1994 there was widespread damage that was initially thought to have been caused by *S. exigua*. Further observations revealed, however, that a new species of armyworm, *S. exempta,* had attacked more than 6000 ha. Shallots and hot peppers

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are high-value crops for farmers and traders. This has stimulated farmers to grow them all year round, rotating 5–6 crops of shallots and hot peppers. The overlapping generations of armyworms and thrips make these pests even more difficult to control.

Modern agriculture has brought costs and benefits. The nation has to face increasing soil, water and food contamination due to excessive pesticide use (INEM 1997), as well as the possibility of problems in maintaining food/rice self-sufficiency. Although integrated pest management (IPM) is just part of the answer to sustainable agriculture, it was an important first step that could pave the way for other sustainability measures.

Vegetables generally need only 65–90 days to reach harvest, and during this very short period may be exposed to high pesticide use. Thus, the product is very likely to contain significant pesticide residues, mainly of insecticides. Information about insecticide residues on vegetables was collected in West and Central Java from the central production system. Among vegetables growers, pesticides played a role as an insurance for a successful harvest (Woodford et al. 1981). In lowland cultivation of shallots, pesticides were the second most costly input, after shallot-tubers for planting (Basuki and Koster 1990; Suwandi et al. 1993). Woodford et al. (1981) reported that 30–50% of the cost of production goes on pesticides and foliar fertilizers.

Review of Insecticide Residues on Vegetables

Several studies have revealed high levels of pesticides in vegetables grown in various parts of Java.

Insecticide residues on lowland and medium elevation vegetables

Along the north coast of Central Java, especially around Brebes, farmers tended to use pesticide 'cocktails'. Pesticide companies were active in the area, stimulated by higher market demand. Insecticide dosages sometimes tripled if crop damage appeared. The most commonly used insecticides were organophosphates—protiofos, metamidofos, profenofos, chlorpyrifos, and diazinon. The spraying frequency was every 2–3 days, depending on the level of damage. Profenofos residues on produce from the Brebes area were in the range 0.194–0.481 ppm, and chlorpyrifos residues were up to 0.612 ppm (Soeriaatmadja et al. 1993).

In Yogyakarta the highest residues detected were of chlorpyrifos (1.4 ppm) in hot peppers, and prophenofos (1.7 ppm) in shallots. Residues of chlorpyrifos of 0.024 ppm were found in amaranth. Residues of BHC found in cucumber and shallots were in the range 0.007–0.017 ppm, whereas the maximum residues limit (MRL) is 0.001 ppm. Lindane was detected in hot pepper at 0.007 ppm (Soeriaatmadja et al. 1993).

In the Sukabumi area of West Java, pesticide spraying of vegetable crops was intensive. Eggplant samples contained residues of diazinon of 0.285 ppm, and 0.03 ppm of carbaryl. Around Serang, decamethrin residues of 0.106 ppm and diazinon residues of 0.202 ppm were found on yardlongbeans. Diazinon was also found in hot peppers, in the range 0.015–0.03 ppm. From Tangerang, carbaryl residues the range 1.79–17.54 ppm were found in yardlongbeans, Chinese cabbage, and eggplant. At the eastern part of West Java, namely Indramayu, pesticides were used as intensively as on the north coast of Central Java. BHC residues in cucumber and hot peppers were in the range 0.017–0.037 ppm (Dibiyantoro et al. 1989).

Detection of insecticide residues on highland vegetables

Reported here are the results of Rustaman (1988), who analysed residues in tomatoes and cabbages in the Lembang district, Pangalengan, and Cisurupan-Garut in West Java. Profenofos residues of 0.11 ppm were found in tomatoes from Lembang, and 0.41 ppm in cabbage. In tomatoes from Pangalengan, profenofos residues of 0.03 ppm were found. Cabbages from Cisurupan-Garut had residues of 0.07 ppm profenofos. Tomatoes from Lembang had the highest decamethrin residues (0.20 ppm). The insecticides applied to tomatoes in Lembang were cypermethrin (0.56 ppm), decamethrin (0.07 ppm), and permethrin (0.16 ppm). In Cisurupan-Garut, smaller amounts of insecticide were applied than in Lembang and Pangalengan. Farmers applied *Bacillus thuringiensis* (Bt) insecticide in Lembang, Pangalengan, and Cisurupan, as Dipel, Thuricide or Bactospeine, but no Bt residues were found.

Nurmalah (1992) carried out similar work in collaboration with the Horticultural Research Institute. Residues in carrots, cabbages, and tomatoes from Lembang and Pangalengan district were analysed. In these areas farmers sprayed their crops with profenofos, chlorpyrifos, synthetic pyrethroids and teflubenzuron insect growth regulator (IGR). The residues found were: profenofos, 6.11 mg/kg; deltamethrin,

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7.73 mg/kg; chlorpyrifos, 2.81 mg/kg; teflubenzuron, 2.89 mg/kg; and permethrin, 1.80 mg/kg.

'Kangkong' *(Ipomoea aquatica* Forsk.) is a very popular vegetable, cultivated in ponds. In the southern part of Bandung, some ponds are polluted by wastewater from textile manufacturers, suspected of containing heavy metals. Using the baseline data of van Lishout (1992), it is predicted that by 2000 annual demand for kangkong will be 1.3 Mt, greater than the predicted demand for either potatoes or cabbage in that year. The University of Indonesia (1994) reported that leaves of 'kangkong darat' *(Ipomoea aquatica* var. *reptans* Poir.)cultivated in the Pub Mas–Jakarta region within 5–10 m of roadways contained residues of lead of 29.9 ppm, and stems 15.5 ppm. Residues were lower in plants grown further away from the road.

Some Achievements of Integrated Pest Management

Initially, IPM was aimed at rice fields only, but has been extended to include other grains and vegetables since 1992. By 1992, 300000 rice farmers in 20 provinces and 2450 'highland' vegetable farmers had been trained in IPM. The program also trained more than 8000 agricultural extension workers. The training applied the adult participatory education methodology, using the field as an educational medium. These IPM programs have been extended, so that a target of training for 800,000 farmers and 16,000 agricultural extension workers can be achieved (Kusumaatmadja 1994).

At the policy level, Indonesia has tried to attain the objectives of IPM through *Act No. 12/1992* on the Plant Cultivation System. The Act states that plant cultivation must be based on use, conservation, and sustainability, supporting the tenets of Agenda 21. At the implementation level, Indonesia has made good progress in IPM programs (Kusumaatmadja 1994). IPM activities such as workshops were conducted to solve IPM-related problems and to disseminate the principles and practices of IPM to a wider audience. Up to February 1995, IPM dissemination had reached 90% of its target output. The expectations are that those involved in the activity could train 15 million other farmers in IPM.

The technology of IPM needs to be improved, in order to pave the way for sustainable agriculture by optimising use of indigenous natural enemies. Moreover, Indonesia is a country very rich in biological diversity, and is home to an estimated 17% of the total number of species in the world (INEM 1997). As such, Indonesia is keen to conserve biodiversity for sustainable development. One direct benefit of species richness is the existence of beneficial organisms/microorganisms that are natural enemies of pests in the vegetable ecosystem. These can play a role in the natural regulation of insect pests.

Soetarya in 1993, and Dibiyantoro and Soetarya in 1994 (cited in Dibiyantoro 1998) investigated a nuclear polyhedrosis virus (NPV) for suppressing *Spodoptera exigua* on shallots in Pacet-Ciparay, West Java. Carner and Suryawan (1993) found a similar NPV in Cimacan, West Java. Additionally, in 1997 Dibiyantoro, Aunu Rauf and Azirin implemented a joint-experiment on Farmers Participatory Research (FPR) to apply NPV for pest control in Brebes (Dibiyantoro et al. 1997).

Other beneficial viruses investigated include *S. exempta* NPV (SexNPV), *S. exigua* CV (SeCV), *Helicoverpa armigera* BV (HeBV) (Dibiyantoro 1996) *Plusia chalcites* BV(PcBV) and suspected BV on *Plutella xylostella* (PxBV). Of those beneficial viruses, only SeNPY has been widely applied at a field scale, while SeCV has been tried on a small scale. Others are being studied in the laboratory and will subsequently be tested in the field during the dry season.

Selections of Control Measures as an Alternative Technology

Devising an IPM program should raise the question 'How do we design and implement IPM that fully addresses farmers' problems'. According to Dent (1995), an appropriate IPM program should have the following attributes:

- provide effective control of the pest;
- be economically viable;
- be simple and flexible;
- use compatible control measures;
- be sustainable; and
- have a minimum harmful impact on the environment .

An alternative technology appropriate to alliums and hot peppers is predator manipulation. Since little information was available on the possibilities of manipulating thrips populations, Dibiyantoro (1996) investigated some beneficial predators of thrips on hot peppers: they included coccinellids, *Cheilomenes sexmaculata, Coccinella transversalis, C. repanda, Scymnus* spp., and some species of the Reduviidae.

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Another technology which has had some success is use of SeNPV against armyworm caterpillars on shallots. Armyworms are serious pests of shallots and hot peppers grown either in monocultured or polycultured systems (Dibiyantoro 1998a,b; Suwandi et al. 1990; Suriaatmadja and Omoy 1990).

Shallot farmers who usually intercropped with hot peppers and used SeNPY, SeCV, and S1NPV rather than insecticide produced greener and fresher shallots.

Farmers can see the advantages of using viral agents for insect pest problems. Not only are the agents easy to prepare, but also their quality is relatively constant, and they are cheaper than insecticides. Farmers can collect and multiply viral materials from the field at any time. When the insect populations reaches a predetermined level, the viruses may be applied as an alternative to insecticide spraying. Use of sex pheromone traps may provide a means to increase the effectiveness of NPVs. Decoy adults of *S. exigua* or S. *litura* (Dibiyantoro 1988b) treated with BV/NPVs (Dibiyantoro et al. 1997) could be used in the traps. After they mate, they will lay infected eggs. These eggs will hatch as infected larvae that cannot survive and reproduce, but will spread the infection. Thus, use of sex-pheromone-baited traps serves a double function: for mass trapping and as a means of introducing viral infections.

The reliability of viruses against armyworm caterpillars is widely known, and explains their acceptability to farmers as observed around Brebes, on the north coast of Java (Dibiyantoro et al. 1997). In the early dry season (June–July) of 1997, four farmers who had collaborated by using NPV Arin were able to harvest their crops, while most farmers in Klampok and Keboledan failed to achieve a harvest, because of severe attack by armyworms. The highest peak of *S. exigua* numbers occurred 36-44 days after planting, but from 48 to 52 days after planting the population was falling. This is the time when the presence of *S. exempta* could affect the bulb harvest. According to Sutarya (1993), Smit (1987), Whitlock (1986), and Briese (1982) pathogenic viruses could be as efficacious as insecticides in controlling certain pests. Smit (1987) and Whitlock (1986) reported that most younger noctuid moth larvae were more sensitive to NPVs than older larvae. Also, the effect of NPV in suppressing numbers of caterpillars fell with days after planting. Bt treatment and deltamethrin plots suppressed numbers by 39.9% and 35.5%, respectively, compared with control plots. By using sex-pheromone-trap caught individuals to manipulate the dispersal of NPV, insecticide use to control *S. exigua* was reduced by 85%.

Similar results have been achieved by Dibiyantoro and Sutarya (1993), with NPV showing similar efficacy to the Bts and deltamethrin. According to Lambert et al. (1981), the persistence of NPV, because it inhibits the endonuclease system on insects, makes it more reliable than other competitors. Subsequently, Smit (1987) reported that, in California, NPV epidemically infested *S. exigua* year around, indicating that the viruses were stable under variable conditions of humidity, temperatures, acidity, and drought. Sutarya (1993) reported that virus deposits remained stable and effective after 72 hours their application. Arifin et al. (1992) studied the persistence and efficacy of Sl-NPV *(S. litura)* against the soybean armyworm and found no differences related to time of application.

SexNPV was applied to small shallot plants in a Klampok field, and the results compared with check plots, non NPV and non insecticide, and deltamethrin treatment. Results showed that SexNPV was able to consistently control the *S. exempta* under a range of 24–39%. Problems with SexNPV occur when there is more than one species of armyworms present and *S. exempta* is not the dominant species.

Conclusions

- To reduce insecticide residues on vegetables, manipulation of entomopathogenic viruses that are indigenous to Indonesia should be promoted for eco-efficiency issues, and to foster development of a useful industry.
- Investigation of viruses that are acceptable to farmers and safe to use, will lead to reduced costs for pesticides. NPV and BV were as effective as deltamethrin or Bt applications at suppressing populations and damage, reducing damage to about 50% of that on control plots.
- As pesticide use falls, the impact of natural enemies increases.
- Horticultural products with minimal or no residues command a higher price on open markets.

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Immunochemical Technology for Analysis of Pesticide Residues

Uzma Maqbool*, M. Jamil Qureshi*, Anwarul Haq*, and M. Zafar Iqbal

Abstract

Enzyme-linked immunosorbent assay (ELISA) is an effective means of determining environmental pollutants such as pesticide residues. Detection limits for dieldrin residues using ELISA were around 5 ppb, those for atrazine residues were about 0.01 ppb and those for DDT residues 0.02 ppm. From these results, it is clear that ELISA is a cost-effective and sensitive technique that can be used for pesticide residue detection and quantification at below part per billion levels.

THERE has recently been increased interest in developing immunoassays for pesticides and other environmental chemicals for residue analysis (Hermann 1988; Vanderlaan et al. 1988). The use of immunoassays for the analysis of small molecules has been extensive in endocrinology, clinical chemistry and other fields. Application of immunoassay technology by environmental chemists is well behind that in other fields, primarily because the early compounds of interest to these analytical chemists were more appropriately analysed by gas–liquid chromatography.

The increasing efficacy of some new pesticides has prompted regulatory agencies to seek lower detection limits (ng/mL and pg/mL) (Plimmer and Hill 1988). Growing concern over possible adverse effects of long-term, low level exposure to agricultural chemicals has led to more comprehensive crop and environmental monitoring programs. Persistence of pesticides, especially organochlorine pesticides, has led to a serious concern about monitoring levels of contamination by their residues (Hussain et al. 1994a,b,c). Coping with the large sample loads that can be generated from such regulatory programs is often difficult with current analytical techniques, and analytical chemists are now exploring immunoassays to help overcome this problem. There are many advantages to pesticide immunoassay, such as specificity, sensitivity, precision simplicity, cost-effectiveness, speed and applicability (Jung et al. 1989). Many pesticide immunoassays have therefore been developed for a variety of pesticides such as organochlorines, triazines, cyclodienes, etc. in different environmental samples (Wittman and Hocks 1989; Brady et al. 1995; Skerritt 1995).

The DDT/DDE immunoassay is a polyclonal-antibody-based laboratory assay for the semiquantitative determination of residues of DDT (1,1,1-trichloro-2,2-bis (4-chlorophenylethane) and DDE (1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene), the latter a major and persistent metabolite of DDT in water and soil samples. The antibody also detects DDD (also known as TDE, 1,1-dichloro-2, 2-bis (4-chlorophenyl) ethane), a major metabolite. Detection of DDA (2,2-bis-(4-chlorophenyl) acetic acid), the mammalian acidic metabolite, is 20 times less sensitive than DDT (FAO/IAEA 1993).

Compounds which have been analysed by immunoassay so far include highly lipophilic organochlorine compounds such as DDT/DDE and dieldrin, and the highly insoluble s-triazine herbicides such as atra-

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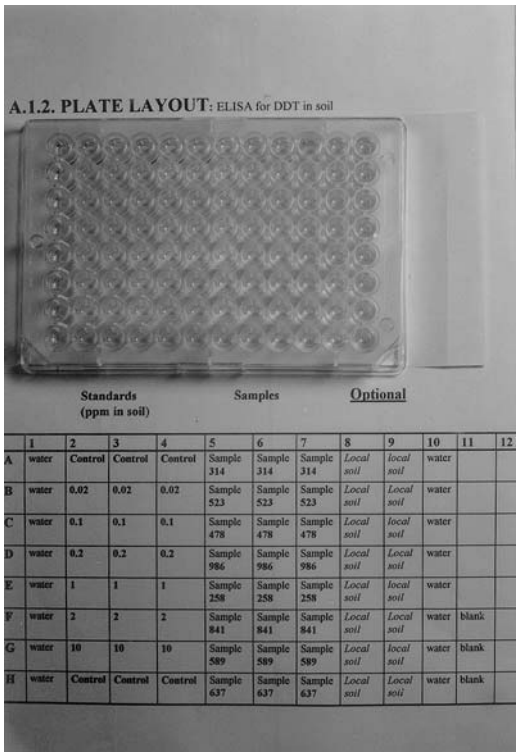
zine. Analyses have been made in various environmental matrices (Maqbool et al., these proceedings). The current paper discusses the details of research work done for residue analysis of DDT/DDE in soil.

Materials and Methods

Immunoassay for DDT/DDT residues in soil

Studies were conducted following the design shown in Figure 1 This study relates to the evaluation of the ELISA kit provided by the Coordinated Research Program of FAO/International Atomic Energy Agency for the determination of DDT/DDE residues in soil samples containing unknown concentration of residues. In addition to those samples, local soil samples (presumed to contain aged residues of DDT/DDE) were also analysed. Details follow of the reagents used for ELISA performed in this study.



	1	2	3	4	5	6	7	8	9	10	11	12
A	water	Control	Control	Control	Sample 314	Sample 314	Sample 314	*Local soil*	*local soil*	water		
B	water	0.02	0.02	0.02	Sample 523	Sample 523	Sample 523	*Local soil*	*Local soil*	water		
C	water	0.1	0.1	0.1	Sample 478	Sample 478	Sample 478	*Local soil*	*local soil*	water		
D	water	0.2	0.2	0.2	Sample 986	Sample 986	Sample 986	*Local soil*	*Local soil*	water		
E	water	1	1	1	Sample 258	Sample 258	Sample 258	*Local soil*	*local soil*	water		
F	water	2	2	2	Sample 841	Sample 841	Sample 841	*Local soil*	*Local soil*	water	blank	
G	water	10	10	10	Sample 589	Sample 589	Sample 589	*Local soil*	*local soil*	water	blank	
H	water	Control	Control	Control	Sample 637	Sample 637	Sample 637	*Local soil*	*Local soil*	water	blank	

Figure 1. Experimental design

Reagents used for DDT/DDE immunoassay

- Microwell plates coated with polyclonal rabbit anti-DDT/DDE antibody (Fig. 2).
- Enzyme-conjugate.
  Freeze-dried enzyme horseradish peroxidase–pesticide conjugate reconstituted in 100 μL nanopure water and diluted in microwell/diluent buffer solution.

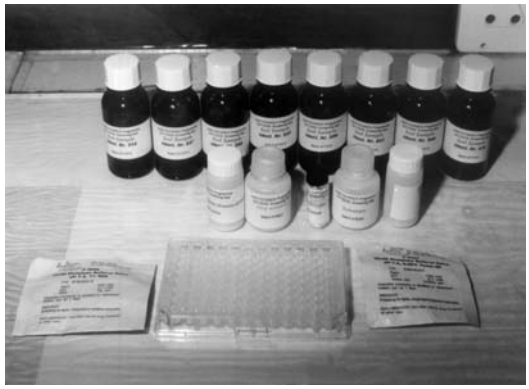


Figure 2. ELISA reagents

- Stock standard solutions.
  1 mL stock of DDT (100 ppm-100 μg/mL) in methanol diluted in a series of dilution as working standards: 0, 0.02, 0.1, 0.2, 1.0, 2.0, 10.0 ppm DDT in oil.
- Microwell diluent buffer solution
  1% (w/w) bovine serum albumin (Sigma, USA) in 10 mM phosphate-buffered saline pH 7.4 diluent power, reconstituted to 1 L with nanopure water.
- Wash buffer
  0.05% Tween 20 in 10 mM phosphate-buffered saline, pH 7.4 diluent power (Sigma, USA), reconstituted to 1 L with nanopure water.
- Chromogen solution
  600 μL TMB base in dimethyl sulfoxide
- Substrate solution
  30 mL of substrate containing 0.15% of urea hydrogen peroxide.
- Colour developer preparation.
  3 parts of chromogen to 97 parts of substrate prepared immediately before use.
- Stop solution
  1.25 M sulfuric acid.
- Methanol
  Analytical reagent-grade freshly distilled before use.

Equipment used for ELISA

- ELISA microplate reader
  Porta-Reader II, Model D 602 (Fig. 3) with an interference filter of 450 nm.

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- Plate washer
  BIO-RAD Model 1575, Immunowash ELISA plate washer.

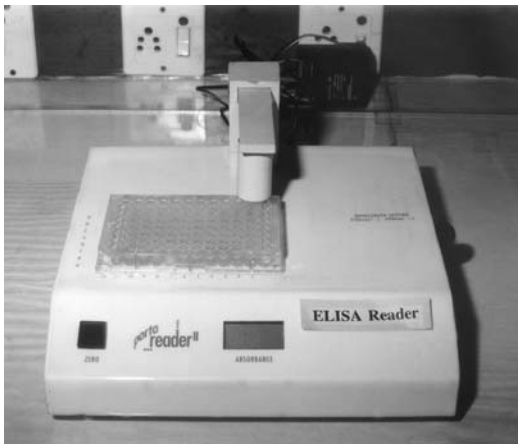

Figure 3. ELISA plate reader

- Water purification system
  Sybron Barnstead Nanopure-II.
  Water purification system (10–15 megaohm cm).
- Micropipettes
  – Single channel pipettes for measuring 20–200 μL (Fig. 4), 200–1000 μL and 1000-5000 μL (Oxford, Eppendorf, Gilson).
  – Multichannel pipette-8 channel system-range 50–200 μL (Socorex) (Fig. 5).
- Glassware
  – Centrifuge glass tubes or similar for diluting standard solution.
  – Glass vials (20 mL scintillation vials) for diluting enzyme conjugate.
  – Measuring cylinders and bottles (100 mL and 1000 mL) for preparing and storing buffer solutions.
- Shaker for soil extraction
  Wrist-action shaker
- Miscellaneous
  Vortex mixer, refrigerator, timer (preferably with an audible alarm), absorbent towels, marker pens (waterproof) and adhesive labels.

Sensitivity of immunoassay for qualitative and quantitative determination of DDT/DDE residues in soil

The following steps were involved in performing DDT/DDE immunoassay.

Reagents and sample preparation

- Soil extraction: 5 g soil samples (including IAEA soil samples and local soil samples) were extracted after drying using 25 mL 90% methanol in water for 20 hours with constant shaking by wrist-action shaker. Soil samples were allowed to settle for 30 minutes, and the supernatant phase was assayed directly.
- Preparation of standards: A series of six working standards (having 0.02, 0.1, 0.2, 1.0, 2 and 10 ppm) was prepared from standard stock solution (100 ppm) with vigorous mixing of each dilution with vortex mixer.
- Preparation of microwell/diluent buffer: The contents of one sachet containing 1% w/w bovine

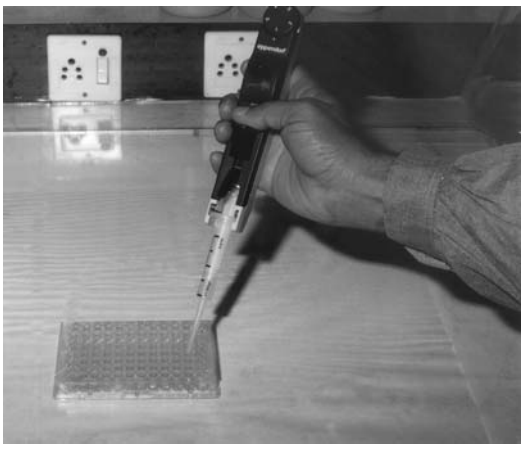

Figure 4. Single channel micropipette

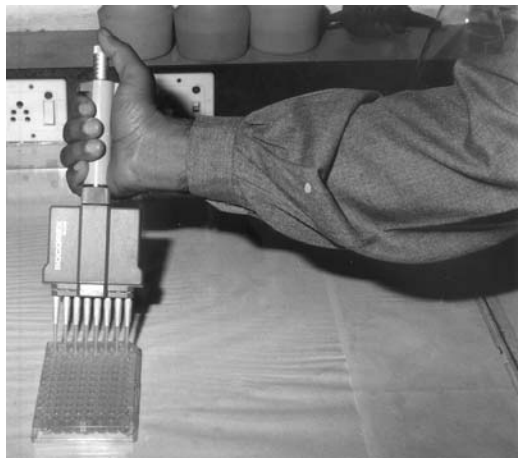

Figure 5. Multichannel micropipette

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serum albumin (Sigma, USA) in 10 mM phosphate-buffered saline, was reconstituted to 1 L with nanopure water, pH 7.4.

- Dilution of horseradish enzyme-conjugate: The freeze- dried peroxidase conjugate is reconstituted in 100 μL nanopure water and diluted with microwell diluent buffer solution in ratio of 1:280.
- Preparation of colour-developer: Colour developer was prepared by adding 3 parts of chromogen to 97 parts of substrate.

Immunoassay procedure

All the reagents and sample extracts were brought to room temperature (15– 25°C) before use.

- Hydration of the antibody plate:
  - 120 μL of microwell/diluent buffer solution was added to each well using a multi-channel pipette for the hydration of antibody on the plate.
- Addition of standards and sample extracts:
  - Microwell plate was loaded with 20 μL per well of DDT standards in methanol or sample extracts in triplicate according the planning of the strip format (Fig. 1)using single-channel pipette.
  - 20 μL of methanol to control or blank wells was added.
  - The plate was mixed gently by rotating in a circular motion for approximately 10 seconds.
- Addition of enzyme conjugate:
  - 60 μL of reconstituted and diluted enzyme conjugate was added to all wells immediately using a multichannel pipette.
  - The microwell contents were mixed gently by rotating the plate gently for approximately 10 seconds.
  - The plate was covered with parafilm and incubated at room temperature for 60 minutes.
- Washing:
  - The plate was washed with wash buffer and using a microplate washer.
  - The remaining wash buffer on the plate was shaken out and the plate tapped dry on absorbent paper.
- Addition of the substrate/chromogen:
  - 150 μL of the colour developer (that includes chromogen and substrate) was added in each well using multichannel pipette.
  - Again the plate is covered with parafilm and incubated for 30 minutes at room temperature, during which time the blue colour developed.
- Measurement of colour development:
  - The reaction was stopped by the addition of stop solution i.e. 1.25 M sulfuric acid.
  - The plate was read within 30 minutes in a plate reader with a 450 nm interference filter.

Interpretation of results

- The optical density values (O.D. values) obtained were subjected to calculate percentage maximal absorbance values (% Bo) as follows:

$$\%Bo = \frac{\text{O.D. value of the sample}}{\text{O.D. value of So}} \times 100$$

- The average O.D. values of the standards were plotted against their respective concentrations to obtain a DDT/DDE standard curve.
- The data were also subjected to regression/correlation analysis by plotting % Bo values against log concentration (Log c) to obtain a regression equation.
- The absorbance values of samples having unknown concentrations of DDT/DDE residues were plotted against the standard curve and from regression equation and the concentrations of DDT/DDE calculated.

Results and Discussion

The observed O.D. values are shown in Table 1. From these values, the average O.D. values, standard deviations (SD) and coefficients of variation (CV), and percent maximal absorbance (%Bo)values were calculated (Table 2).

The absorbance values obtained for the standards were plotted against their respective concentrations (0.02, 0.1, 0.2, 1.0, 2.0 and 10 ppm) on semilogarithmic paper to obtain the DDT/DDE standard curve which was found to be linear (Fig. 6). From the standard curve, it was observed that absorbance readings obtained from ELISA were inversely proportional to the concentration of analyte in the standard; i.e. an increase in concentration will be accompanied by a decrease in absorbance values.

The data were also subjected to regression/correlation analysis. There was a highly significant negative correlation (r = –0.970; $P \leq$ 0.01) between percent maximal absorbance values (%Bo) and concentration (log C). The regression equation (1) was obtained was calculated by plotting %Bo values against log concentration (log c) as shown below:

$$\%Bo = 37.796 \pm 30.558 \log C$$

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Table 1. Enzyme-linked immunosorbent assay for the determination of DDT/DDE residues in soil. Absorbance values taken at 450 nm

Wells	Optical density values											
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.25	1.22	1.11	1.18	0.66	0.61	0.57	1.25	0.95	0.91	–	0.16
B	1.10	1.15	1.37	0.88	0.40	0.44	0.43	1.13	1.02	0.95	–	0.19
C	1.10	0.71	0.90	0.59	1.10	0.81	0.88	0.91	1.04	0.99	–	0.27
D	1.30	0.57	0.68	0.57	0.52	0.42	0.46	1.04	0.98	1.03	–	0.20
E	1.35	0.36	0.29	0.28	0.31	0.39	0.36	0.84	0.87	0.95	–	0.21
F	1.07	0.30	0.32	0.22	0.75	0.69	0.69	0.83	0.99	0.99	0.02	0.38
G	0.98	0.16	0.18	0.15	0.56	0.50	0.42	0.85	0.91	0.97	0.01	0.39
H	0.96	1.04	1.31	1.21	1.08	0.93	1.15	0.92	0.97	1.19	0.00	0.07

Table 2. Qualitative and quantitative determination of DDT/DDE residues in soil employing ELISA

Well contents	Average O.D.	±SD	%CV	% Bo	DDT concentration	
					R.E. value	G value
Standard						
O – Top control	1.17	0.056	4.70	100.00		
O – Low control	1.19	0.136	11.40			
S1	1.13	0.245	21.70	95.76		
S2	0.73	0.156	21.30	61.86		
S3	0.61	0.063	10.30	51.69		
S4	0.31	0.043	13.90	26.27		
S5	0.28	0.053	18.90	23.73		
S6	0.16	0.015	9.40	13.55		
Soil Samples						
SS-1	0.42	0.021	5.00	35.59	1.180	0.560
SS-2	0.93	0.151	16.20	78.81	0.045	0.042
SS-3	0.71	0.035	4.90	60.17	0.185	0.115
SS-4	0.49	0.070	16.70	41.52	0.755	0.380
SS-5	0.61	0.045	7.40	51.69	0.351	0.200
SS-6	0.47	0.026	5.50	39.83	0.858	0.430
SS-7	0.35	0.040	11.40	29.66	1.846	0.830
SS-8	1.05	0.11	10.60	88.98	0.021	0.027
Local	0.96	0.11	11.60	81.35	0.037	0.039

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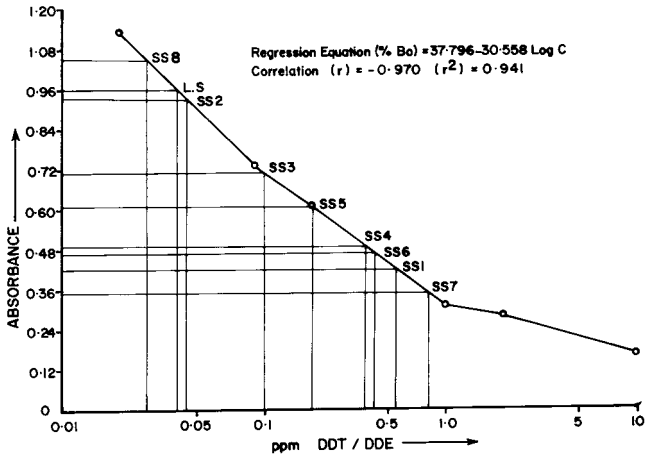

Figure 6. DDT/DDE standard curve (soil analysis)

There were slight differences in the values obtained from the regression equation and the standard curve (Fig. 6).

The regression equation gives a best-fit curve on which the sample values lie. whereas values from the curve reflect the slight perturbations in the curve. From the results (Table 2), it was observed that soil sample 7 contained the highest DDT/DDE residues (1.846 ppm from the regression equation and 0.83 ppm from the standard curve), and soil sample 8 the lowest residues (0.021 ppm and 0.027 ppm, respectively). The other six samples immunoassayed contained DDT/DDE residues in between these two extremes. The local soil sample showed very low DDT/DDE aged residues (0.037 ppm and 0.039 ppm, respectively). These results indicate the sensitivity and robustness of ELISA for qualitative and quantitative determination of DDT/DDE residue in soil.

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Analysis of the Distribution of DDT Residues in Soils of the Macintyre and Gwydir Valleys of New South Wales, Australia, Using ELISA

H.M. Shivaramaiah*, I.O. Odeh*, I.R. Kennedy* and J.H. Skerritt†

Abstract

DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl ethane)) is a persistent environmentally toxic organochlorine insecticide. It has been applied for many decades in countries around the world and its indiscriminate use and long persistence in soil have resulted in extensive soil contamination with DDT and its metabolites. Conventional gas chromatographic analytical techniques for the detection of these residues in soil are expensive and time consuming. An ELISA method was therefore developed and used to monitor DDT/DDE residues in soil samples collected from the Macintyre and Gwydir valleys of northern New South Wales, Australia. DDT was used extensively in this region particularly for protection of cotton crops, until its use was banned in 1982. The main DDT residue observed after several years in these soils when analysed by gas chromatography is DDE, in contrast to the soils at the more heavily contaminated dip sites used to control cattle tick, where DDT itself is the major residue.

Samples of soil predominantly grey-cracking clay (Vertisols) were collected from 120 sites in the Macintyre Valley and 150 sites in the Gwydir Valley. These sites were selected to cover a variety of past land uses for comparative purposes as part of a more extensive soil survey using geographical information system (GIS) techniques. Extraction with 90% methanol was found to be efficient and quantitative, using assay conditions specially developed to avoid interferences from the soil matrix. Of the samples collected in both the Macintyre and Gwydir valleys to 0–10 cm depth, more than half contained residues at a concentration in the range of 0.01–0.75 mg/kg (ppm). Soil from sites showing positive analysis in the 0–10 cm soil layer was also analysed at greater depth (10–20 cm). No positive analysis for DDE were observed with 10–20 cm samples from the Macintyre Valley whereas some contamination at 10–20cm depth was observed in samples from the Gwydir Valley where cotton was grown more extensively before the 1982 ban.

This study indicates widespread persistence of DDT residues even though DDT has not been applied for over 15 years. Analysis of the distribution of DDE residues indicates that contamination is mainly in the surface layer where there may still be some potential for impacts on surface biota, crops or grazing stock. Surprisingly, soil from cotton farms in the Macintyre Valley was found by a statistical analysis to be less contaminated with DDE than was soil from nearby native pastures. However, analysis of soils in the Gwydir Valley indicated less difference between various usage patterns.

IN many developed countries, the use of DDT has been banned for several years. Recognised as one of the more persistent organochlorines, there is nevertheless scant evidence about the extent of contamination that remains in soil more than 15 years after the previous application, although DDE in particular has been observed as a degradation product of DDT with particularly long persistence (Agarwal et al. 1994). This short paper describes a survey of the Macintyre and Gwydir valleys of New South Wales (NSW), Australia for DDE residues in a range of soil types and land uses including cotton growing with inten-

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sive DDT application in the past. Analyses for a third valley, the Namoi, are expected to be completed later. This study was conducted to determine the current extent of contamination with DDE, to examine whether there is a need for remediation and to suggest practical means of achieving it.

Materials and Methods

Soil sampling

In the river valleys studied (Fig. 1), the dominant soils are Vertisols (Soil Survey Staff1992) characterised by a large proportion of clay. These Vertisols vary from grey to black and brown clays. Red and yellow brown earth areas are intersected with Vertisols in the valleys. Sample sites were randomly located based on a stratified simple random scheme, with 120 sites sampled in the Macintyre Valley (Fig. 2) and 153 in the Gwydir Valley (Fig. 3). The scheme allowed for preferential sampling in such a way that the sites cover a variety of land use types—ranging from cultivated to stock routes, native pastures and woodland (McGarry et al. 1989; Odeh and McBratney 1994). Samples were taken with cores divided into 10 cm depth sections. For the purpose of this study on DDE, only the 0–10 and 10–20 cm sections were analysed. Soils were air-dried, ground in a mill, and sieved of large particles before storage at room temperatures. For incurred residues of a highly immobile chemical of extremely low volatility and long half-life, such routine soil preparation would not be regarded as a potential source of loss of residues. Soil samples for volatile chemicals such as endosulfan could not be treated in this fashion without losses.

Materials for DDE analysis by ELISA

Bovine serum albumin (BSA) and horseradish peroxidase (HRP) were purchased from Boehringer-Mannheim, Germany. Fish skin gelatin (FG) and Tween 20 were obtained from Sigma Chemicals, St Louis, USA. Methanol (AR grade) was obtained from Ajax Chemicals, Clyde, NSW, Australia. Maxicorp polystyrene 96-microwell plates were purchased from Nunc, Roskilde, Denmark.

Preparation of DDE standard

DDE (100 mg/L, ppm) was prepared in methanol as a stock solution. From this stock, a 1000 μg/L, ppb standard was prepared by dilution in 0.1% FG-phosphate buffered saline (PBS, 50 mM sodium phosphate/0.9% NaCl, pH 7.2) and then serially diluted to obtain 200, 40, 20, 4,2 and 0 ppb in borosilicate tubes for the standard curve. The standard curves for soil analysis were prepared in using an extract of soil diluted 1:20 in 0.1% FGPBS.

Soil spiking

The Vertisol soil sample used for spiking was established as free from pesticide residue by solvent extraction followed by gas–liquid chromatographic (GLC) analysis. Ten gram sub-samples of this soil were distributed into glass jars (with aluminum lined caps) and spiked with 0.5, 1, 2, 5 and 10 mg/kg (ppm) concentrations of DDE.

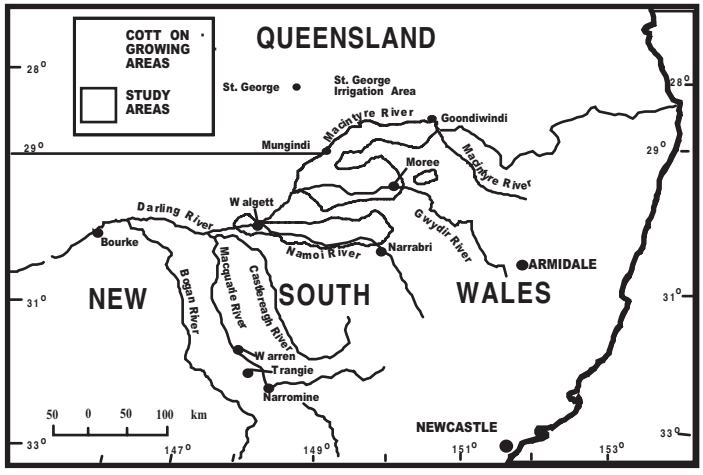

Figure 1. River systems of northern New South Wales, showing the Macintyre and Gwydir valleys

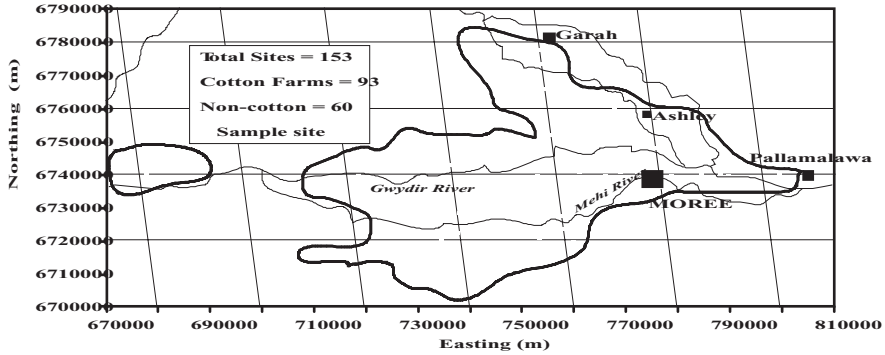

Figure 2. Gwydir Valley sampling sites. A large number of sites representing various land uses were selected and sampled to depths of 0–10 cm and 10–20 cm.

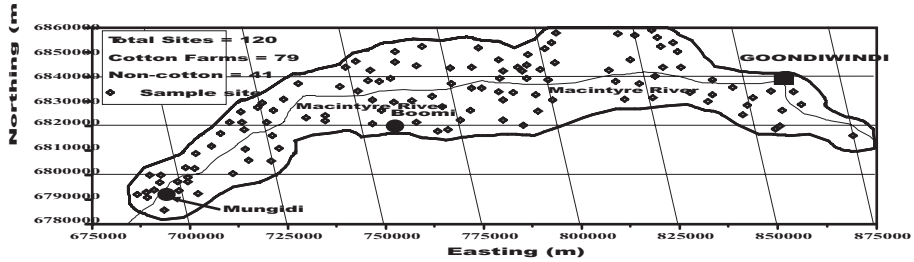

Figure 3. Macintyre Valley sampling sites. Numerous sites representing various land uses were selected and sampled to depths of 0–10 cm and 10–20 cm.

The soil was mixed thoroughly with a stainless steel spatula for 5 minutes and allowed to stand at room temperature for 3 days. The soil samples were extracted using 25 mL of 90% methanol by shaking for one hour in the original glass jars. Extraction of DDE from soil with 90% methanol was shown to be efficient by the use of spiked soil.

ELISA–laboratory microwell assays

The assay for *p,p'*-DDE was obtained (Larkin et al. 1994; Beasley et al. 1998) using an immunogen which includes all elements of the DDE structure, except that one of the *p*-chloro groups was replaced by β-alanine carboxamide for coupling to carrier proteins. The same hapten was used for coupling to horseradish peroxidase (HRP). Antibodies to protein conjugates of this hapten exhibited specificity for *p,p'*-DDE (100%) over *p,p'*-DDT (7%). Cross-reactivity (% ×) is calculated as the concentration of analyte that causes a reduction of 50% in the assay colour relative to a pesticide-free control (IC_{50}), expressed as a percentage of the IC_{50} of the cross-reaction compound. The DDE assay showed preferential recognition of the DDE and DDD compounds and were 10–80 times less sensitive for the *p,p'*-DDT parent compound. Thiobencarb, a herbicide used in rice cultivation, was detected only at very high levels (greater than 1000 µg/L) by the DDE assay (0.4% cross-reaction). Dicofol and methoxychlor were not cross-reactive (Beasley et al. 1998).

Antibodies were diluted in 50 mM carbonate buffer pH 0.6 to 10 µg/mL and were coated at 100 µL per well overnight at 20°C. The microwells were washed twice with PBS (50 mM sodium phosphate/0.9% NaCl, pH 7.2) containing 0.05% (v/v) Tween 20 (PBS/T) and then blocked with 150 µL of BSA/PBS (1% bovine serum albumin) in PBS. One hundred µL of DDE standard or sample followed by 100 µL of peroxide conjugate diluted in PBS containing 0.5% (w/v) fish skin gelatin (Sigma) was incubated for 1 hour at 20°C. Hydrogen peroxide substrate/ chromogen (3.3, 5.5 tetra methyle benzidine/hydrogen peroxide in accetate buffer, pH 5.5) 150 µL was added and incubated 30 minutes at 20°C. Colour development was stopped by adding 50 µLof 1.25 M sulfuric acid and the colour

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intensity was read at 450 nm. For control and blanks distilled water and solvent were used. The IC_{50}, minimum detectable limit, and also percent recovery were calculated from the standard graph.

Soil extraction for GLC

For instrumental analysis, 50 g of soil was weighed into a stoppered conical flask and 150 mL of 90% methanol was added. The flasks were shaken for 12 hours and the solvent was filtered through paper containing 2 g of anhydrous sodium sulfate. The filtrate was concentrated to 5 mL using a Kuderna-danish apparatus and then chromatographed on a Florisil column. The column was eluted with 150 mL of hexane: diethyl ether 3:1. The first 10 mL of elute was discarded and remainder concentrated to 5 mL. GLC analysis was performed using a Hewlett Packard 5890 Series II gas chromatograph equipped with an electron capture detector.

Immunoassay

Ten g soil of well-mixed soil was weighed into a 100 mL glass jar and 25 mL of 90% methanol was added. The jars were shaken for 1 hour and allowed to stand overnight until the particles settled. The supernatant was then collected and typically diluted 1:20 in water for ELISA.

Statistical analysis

Statistical analysis of residue data was accomplished by analysis of variance (ANOVA) to establish significant differences, using the JMP program to plot the range and mean values for different land uses.

Results and Discussion

The standard graph for DDE analysis by ELISA is shown in Figure 4. Using standard concentrations of DDE dissolved in methanol, analyses were performed in the range 2 to 200 ppb (µg/L) with an IC_{50} of 20 ppb. Since the DDE was to be detected in soil extract, a standard graph was also prepared using pesticide-free soil extract. Initial experiments indicated 1:10 dilution in 0.1% FG-PBS showed matrix interference in ELISA as indicated by elevated IC_{50} values. This could be overcome by diluting 1:20 in 0.1% FG-PBS.

Spike and recovery test

DDE after extraction with methanol and dilution in 1:20 in FG-PBS was analysed by ELISA and the recovery data were comparable with valves obtained from GLC analysis (Table 1). The data shown in Figure 5 indicate the recovery valves calculated by the ELISA method were comparable to that of GLC values. For both methods, a recovery of more than 95% was achieved compared with the amount spiked.

DDE residues in the Macintyre and Gwydir valley soil samples

When the top layer (0–10 cm) of the soil was analysed, DDE was detected in samples from 65 of 120 sites

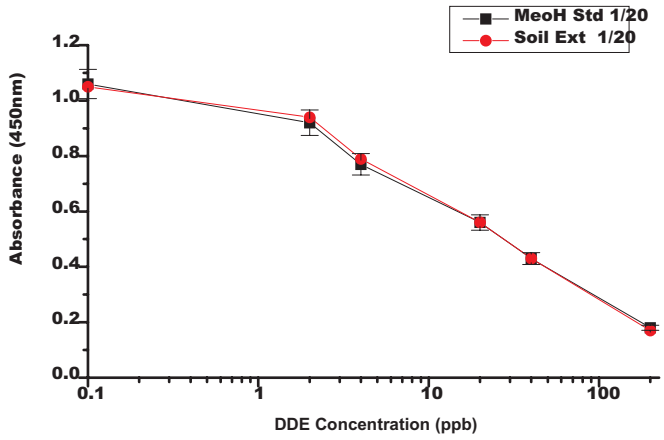

Figure 4. Standard curve for DDE in methanol and in methanol soil extract using pesticide free soil. Standard errors are shown as bars; the value indicated as 0.1 ppb was the zero level absorbance.

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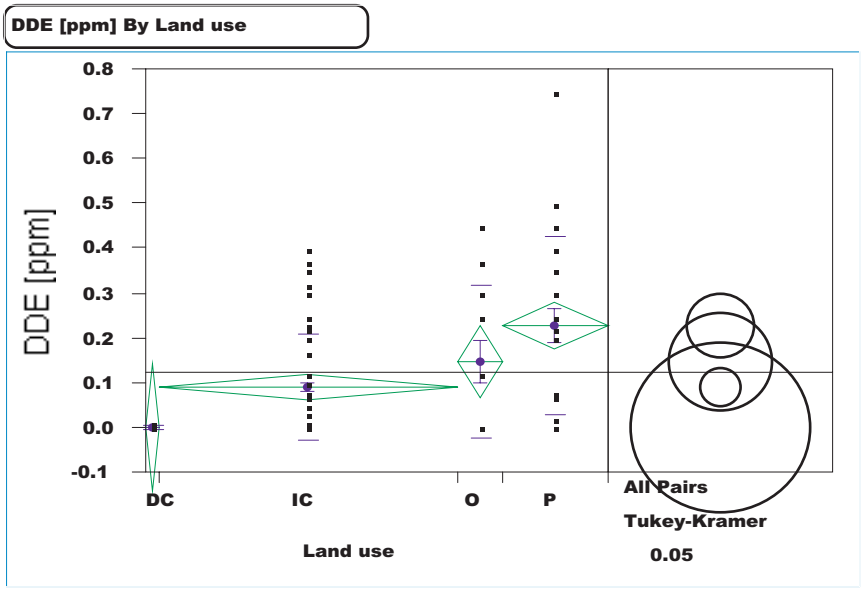
in the Macintyre Valley and samples from 97 of 151 sites from the Gwydir Valley (see Figs 2 and 3 for sampling sites). The concentration ranged from <0.01–0.40 ppm and <0.01–0.75 ppm in the Macintyre and Gwydir valleys soil samples, respectively. The soil samples analysed were classified as dryland (rainfed) cotton (DC), irrigated cotton (IC), other crops (O), native pastures (P), stock routes (SR), and woodland (WL). The relative concentration in dryland cotton soil samples, on which DDT may never have been sprayed, was very low as compared with irrigated cotton, when compared using analysis of variance in the JMP program (Tables 2 and 3).

Table 1. Comparison of ELISA versus gas–liquid chromatographic analysis data of spike and recovery of DDT from soil

Concentration spiked (ppb)	Mean ELISA recovery (ppb)	SD ELISA	Mean GC recovery (ppb)	SD GC	% ELISA recovery	%GC recovery
100	92.5	3.5	86.0	1.4	92.5	86.0
200	194.5	7.8	210.5	14.9	97.3	105.3
500	425.0	35.4	507.5	10.6	85.0	101.5
1000	992.5	10.6	1004.0	5.7	99.3	100.4
2000	1570.0	99.0	2002.5	3.5	78.5	100.1
4000	4025.0	35.4	4001.0	1.4	100.6	100.0
10000	9982.0	25.5	9795.0	106.1	99.8	98.0

Table 2. Statistical analysis of Macintyre Valley soil samples



Land use key: DC = dryland cotton O = other crops
IC = irrigated cotton P = pastures

ANOVA in the JMP program indicates a significant difference between residues in pastures and soils for cotton. Absence of overlap of circles indicates significant differences ($P < 0.05$, Tukey–Kramer method)

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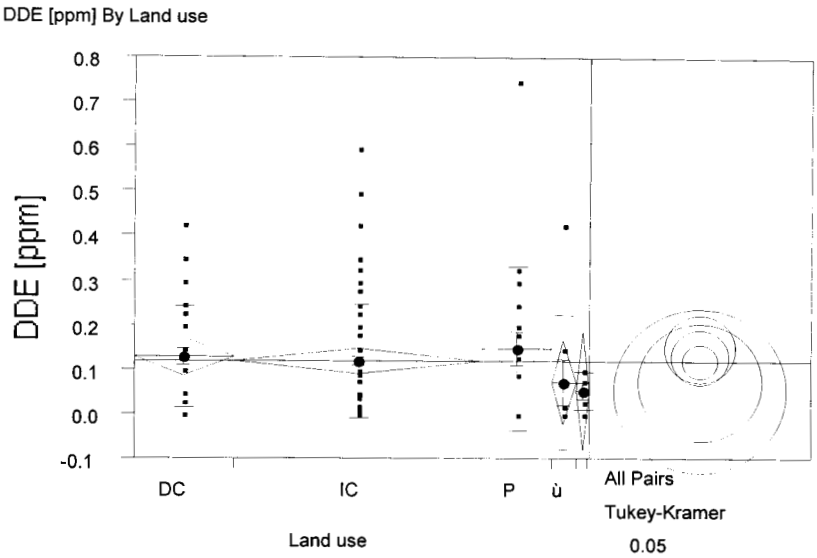
Table 3. Statistical analysis of Gwydir Valley soil samples



Land use key: DC = dryland cotton SR = stock routes
IC = irrigated cotton WL = woodland
P = pastures

ANOVA in the JMP program indicates a significant difference between residues in pastures and soils for cotton. Absence of overlap of circles indicates significant differences ($P < 0.05$, Tukey–Kramer method)

However, soils with pasture contained higher levels of DDE residues (40 ppb) in the Macintyre Valley. The highest concentration was recorded in soil of other crops such as legumes and cereals. Similarly, the relative concentration in irrigated cotton soil samples was higher than in dryland cotton soil samples but the highest concentration was recorded in native pastures near cotton farms. Possibly, more extensive cultivation and consistent watering may result in a long-term remediating affect in cotton soils. This could result from accelerated biodegradation or by erosion in run-off. The analysis of the soil samples at lower depth in all the samples revealed absence of DDE residue in the Macintyre Valley. No relationship between the crop cover on the soil and DDE residue concentrations was observed.

In the Gwydir Valley, differences between soils with different land uses were less pronounced (Table 3). Possibly, this indicates that greater usage of DDT in the Gwydir Valley, where cotton was more extensively grown when DDT was in use, may have resulted in a more even distribution and a greater amount of stored DDE. The concentration of residues ranged from 0.01–75 ppm. Whereas no soil samples in the Macintyre Valley below 10 cm depth contained residues, 12 samples of the Gwydir Valley at 10–20 cm depth were slightly contaminated.

The study indicates that the pesticide is still widely present in the soils of the study area even though DDT has not been in use since 1981, confirming the long peristence of its residues noted elsewhere (Samuel et al. 1988; Wan et al. 1989; Kausik 1991; Martijn et al. 1993; Agarwal et al. 1994; Boul 1995). Most of the residues were recovered in topsoil, indicating the absence of extensive leaching. Water movement in cotton soils is mainly lateral, during flood irrigation and in run-off from storms, these soil being regarded as having low hydraulic conductivity and low recharge of groundwater. Absence of DDT residues in the lower layers of soil indicated no downward movement of DDT in soil.

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Conclusion

The results of this study indicate widespread persistence of DDT residues as DDE in these cotton growing valleys in New South Wales even though DDT has not been applied for 15 years. Analysis of the distribution of DDE residues indicates that contamination is mainly in the surface layer where there may still be some potential for impacts on surface biota, crops or grazing stock. Management practices capable of reducing such impacts for such organochlorine residues have been discussed (McDougall et al. 1995). Surprisingly, soil from cotton farms in the Macintyre Valley was found by a statistical analysis to be less contaminated with DDE than soil from nearby native pastures. owever, analysis of soils in the Gwydir Valley indicated less difference between various usage patterns.

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Concentrations of Pesticides and the α/γ HCH Ratio in Gas and Particle Phases in Air of Alsace, Eastern France)

Astrid A. Sanusi*, Maurice Millet , Philippe Mirabel and Henri Wortham

Abstract

Eleven pesticides (HCB, α-HCH, γ-HCH, trifluraline, mecoprop, phosalone, atrazine, carbofuran, carbaryl, diuron and isoproturon) were determined in atmospheric samples which were collected in the Alsace region of eastern France. Three stations were chosen: in an urban area (Strasbourg), in a rural area (Colmar), and in a remote area (Aubure).

Pesticides samples were collected during summers of 1993 and 1994, using Hi-Vol sampler fitted with Whatman filter paper and XAD-2 resin. The particle and gas phases were collected separately during 24 hours. After extraction, samples were fractionated using normal phase high performance liquid chromatography (HPLC), and three fractions were collected. Organochlorine and trifluraline pesticides (fraction 1) were analysed by gas chromatography-electron capture detection, and others pesticides (fraction 2 and 3) were analysed by reverse phase HPLC-UV.

The concentrations of pesticides in the particle phase were lower than those in the gas phase. For some organochlorines, concentrations were higher in Aubure samples than in Colmar and Strasbourg samples.

Only the γ-HCH isomer is still used in western Europe. It could be isomerised to α-HCH by photochemical reaction in the atmosphere.

The α-HCH/γ-HCH ratio varied from 0.06 to 2.8 and only 2 samples had a α-HCH/γ-HCH ratio greater than 1. These values indicate the utilisation of pure γ-HCH, that HCH isomers generally came from countries around sampling stations and that therefore there may have been insufficient time for the γ form to isomerise to the α form.

SYNTHETIC organic pesticides have been used since World War II and the introduction of DDT which was used to control of malaria and typhus. The used of such pesticides has become more and more common, especially in agriculture for crops protection. The intensive used of these compounds has resulted in widespread contamination of the terrestrial, aquatic, and atmospheric environments. Pesticides may enter the air 'directly' during agricultural spray applications, by wind transport or by volatilisation of residues deposited on soils and plants (Spencer et al. 1973; Lewis and Lee 1976; Seiber et al. 1980). Depending on the type of formulation, physicochemical properties, and meteorological conditions, pesticides present in the atmosphere could be transported over long distances. Some authors have detected organochlorines such as polychlorinated biphenyl (PCBs), hexachlorocyclohexanes (HCHs), DDT, DDE in the air and surface water of the Arctic area, where pesticides were never been used (Pacyna and Oehme 1988 ; Hoff et al. 1992 ; Iwata et al. 1994 ; Hühnerfuss et al. 1992).

Most organochlorine pesticides have a high vapour pressure, so they could be readily transported easily

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even in light winds. The use of most organochlorine pesticides had been prohibited in North America and western Europe for some years, because of their persistence and toxicity.

γ-HCH (lindane) is an organochlorine pesticide still in used in western Europe, and which can be photochemically transformed to α-HCH by atmospheric UV-radiation(Newland et al. 1969; Steinwandter 1976). The α/γ-HCH ratio can be used to estimate the age of the air masses (Oehme 1991). Air masses are considered as old (or pesticide transported over long distance) when the α/γ-HCH ratio is greater than 3 (Lane et al. 1992) and as young air (or indicating fresh application of pesticide) when the α/γ-HCH ratio is less than 1(Hoff et al. 1992).

This paper presents the concentration levels of 11 pesticides (HCB, α-HCH, γ-HCH, trifluraline, mecoprop, phosalone, atrazine, carbofuran, carbaryl, diuron and isoproturon) measured in urban, rural and remote atmospheres (gas + particle phases). These data are used to compare the atmospheric contamination of various ecosystems. The significance of the α/γ-HCH ratio of each sampling areas is also discussed.

Methods

Sampling

Samples were collected at three sites using a Hi-Vol sampler through a 30 cm diameter glass-fibre filter (GF/A) and 20 g of XAD-2 resin. The procedure for sampling and pre-cleaning of traps has been described elsewhere (Millet et al. 1996 ; Sanusi et al. 1998). The duration of sampling was 24 hours which allowed sampling of 300–500 m^3 of air. The three sampling sites were situated in the east of France (Alsace). Their locations can be characterised as remote (Aubure), rural (Colmar), and urban (Strasbourg). For this study, three series of five sampling campaigns were performed simultaneously. During the campaigns, between spring 1993 and summer 1994, 27 atmospheric samples (gas and particles) were collected (Sanusi et al. 1998).

Extraction and analysis

XAD-2 resins and glass fibre filters were separately Soxhlet extracted for 12 hours in *n*-hexane/CH_2Cl_2 (85:15). Extracts were concentrated to about 1 mL on a rotary evaporator. Clean-up of these concentrated samples was carried out by fractionation-based HPLC using a silica column and UV detection at 254 nm (Sanusi et al. 1998). With this clean-up procedure, three fractions were obtained: 1. HCB, α-HCH, γ-HCH, trifluraline; 2. mecoprop, phosalone; and 3. atrazine, carbofuran, carbaryl, diuron and isoproturon.

Analyses of these fractions were done by several methods described in detail elsewhere (Sanusi 1996; Sanusi et al. 1998). Fraction 1 was analysed by GC-ECD (^{63}Ni electron capture detector using a J&W DB-5 (30 m, i.d. 0.32 mm, film thickness 0.25 μm) column. Quantifications were done using an internal standard: 0.05 μg/mL δ-HCH.

Fractions 2 and 3 were analysed by HPLC-UV (Waters) at 230 nm using a Nova-Pak™ C_{18} reverse phase column (30 cm ; i.d. 3.9 mm; 4 μm; 60 Å) at 1 mL/min. For fraction 2, a binary solvent (methanol:acetonitrile (80:20 v/v) mixture:water (0.17% TFA)) was used. A binary solvent was also used for the analysis of fraction 3 with methanol/acetonitrile (85:15 v/v) and water milli-Q. Quantification were done by using 2,3-dimethyl naphthalene at 0,5 μg/mL as an internal standard.

Results and Discussion

Pesticide concentration

Dates, sites, duration of sampling, volume of air collected and concentrations of the 11 pesticides in the total (gas + particles) phase for the three series of five sampling campaigns are given in Table 1.

A comparative study of the two phases shows that the concentrations of pesticides measured in the gas phase are generally higher than those in the particulate phase (Sanusi 1996). The partition between gas and particulate phases is essentially the result of the combined influence of atmospheric temperature (the gas phase increases with the temperature), the vapour pressures of the pesticides (Haragushi et al. 1994), the concentration of particles in the atmosphere (the particulate phase increase with particle concentration) (Pankow and Bidleman 1991), and the sampling method used. Indeed, because of their high flow rate, Hi-Vol samplers under-estimate particulate-phase concentrations and over-estimate gas phase concentrations (Bidleman 1988; Haragushi et al. 1994). In our results here, we compare the contamination at the three sites by considering the total concentrations (gas + particulate) of pesticides measured.

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Table 1. Date. sites, duration, volume of air sampled, and concentration (pg/m^3) of pesticides in total (gas + particle) phases for three series of five sampling campaigns

Date	Station	Duration	Volume of air (m^3)	trifluraline	α-HCH	HCB	γ-HCH	mecoprop	phosalone	carbofuran	carbaryl	atrazine	isoproturon	diuron
Series 1														
19–20/04/93	Aubure	24	162.52	5650	423	1510	290	260	500	<114	–	254	–	–
	Colmar	24	176.42	3350	191	283	1090	310	–	440	696	699	209	330
26–27/04/93	Aubure	24h30'	284.16	2520	163	572	228	5	–	<114	–	75	100	–
	Colmar	22h30'	170.29	4500	206	968	1564	99	76	4100	500	640	600	–
04–05/05/93	Aubure	24h	212.07	5740	191	1620	316	360	830	<114	130	420	900	1200
	Colmar	24h	204.05	5640	75	300	830	890	620	8100	670	970	1160	190
10–11/05/93	Aubure	25h	324.97	7980	142	900	542	–	890	<114	–	–	–	–
	Colmar	30h	266.75	3105	206	183	751	580	–	4400	240	1200	670	980
17–18/05/93	Aubure	26h	450.01	6540	214	750	1630	2470	–	<114	1800	1400	720	1600
	Colmar	24h	205.47	2450	258	208	1364	2910	–	4700	120	860	–	358
Series 2														
13–14/06/94	Aubure	21h15'	523.86	1315	76	107	80	117	460	3100	130	286	99	350
	Strasbourg	22h15'	386.15	1624	190	470	624	69300	7040	14200	505	30000	450	1250
14–15/06/94	Aubure	24h15'	620.04	2210	167	176	1050	1820	3670	1710	–	110	–	390
	Strasbourg	23h45'	379.07	3805	98	130	345	6200	11500	16330	767	30610	755	870

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Table 1. Date. sites, duration, volume of air sampled, and concentration (pg/m^3) of pesticides in total (gas + particle) phases for three series of five sampling campaigns

Series 2 (cont'd)														
22–23/06/94	Aubure	24h45'	580.39	1300	70	81	1122	1720	260	140	345	110	112	646
	Strasbourg	23h45'	371.43	3800	272	549	1738	18500	2144	12750	575	17440	620	1890
23–24/06/94	Aubure	23h15'	568.33	2780	149	101	697	2400	–	1075	110	145	49	634
	Strasbourg	24h15'	366.50	3320	307	685	1147	5200	8800	28970	580	51260	800	1680
11–12/07/94	Aubure	28h15'	742.75	300	98	126	528	1680	440	230	284	142	166	430
	Strasbourg	28h45'	458.51	940	200	508	1230	3600	1000	14300	68	1630	120	3200
Series 3														
12–13/07/94	Colmar	24h15'	609.17	465	163	145	767	3890	2533	670	166	4550	950	480
	Strasbourg	24h	367.36	743	404	688	1258	5450	1360	8150	1420	690	700	870
21–22/07/94	Colmar	23h30'	578.24	1060	279	240	807	1265	65	790	190	1700	3300	7350
	Strasbourg	24h	348.99	1510	313	268	1598	3850	1050	15760	382	20410	660	1730
25–26/07/94	Colmar	7h45'	140.64	1630	415	211	1100	2690	–	<114	130	1380	–	13800
	Strasbourg	23h45'	355.42	1950	481	675	3940	2500	–	2050	125	4340	500	5850
26–27/07/94	Strasbourg	24h45'	375.46	1891	280	370	1520	8000	950	13570	770	23890	490	1100

Results obtained for Colmar, with the exception of isoproturon, are in agreement with those obtained by Millet et al. (1997) at the same site using similar analytical and sampling methods. Generally, pesticides used locally (trifluraline, mecoprop, atrazine, isoproturon, diuron) are present in higher concentrations. The difference for isoproturon might be the result of local spraying of this compound during the sampling campaigns in previous work (Millet et al. 1997). The abundance of atrazine in Colmar samples in the present work could be the result of the type of farming practiced in the Alsatian region.

In Strasbourg, pesticides used locally are also present at higher concentrations than other pesticides, even if Strasbourg is an urban site. The presence of many crops within about 5 km around Strasbourg could explain this.

Generally, lower concentrations of pesticides were found at Aubure. For example, concentrations of atrazine measured in Aubure are 10 to 100 times lower than those measured in Colmar and Strasbourg. This phenomenon could be explained by the geography of Aubure which is at an altitude of 1100 m and is far removed from crops.

HCB concentrations measured at Aubure were generally higher than those measured in Colmar. The use of HCB is forbidden in France for crop protection, but is still permitted for wood protection. That is probably the reason for the high level of HCB in Aubure. On the other hand, organochlorine pesticides concentrations are of the same order of magnitude at the three sites, which is surprising for Aubure because of its remote location. However, these pesticides are not intensively used in Europe, so none of our sites is closer than any other to an important source of organochlorines. Moreover, these compounds have a low vapour pressure and consequently can be transported over long distances in the atmosphere.

Detailed analysis of the organochlorine pesticide concentrations shows that concentrations of α-HCH at the three stations are very similar. This result is the consequence of the use of pure lindane (γ-HCH) in western Europe. Thus, any α-HCH can come only from degradation of γ-HCH or by long range transport.

α/γ-HCH ratio

If we know the α/γ-HCH ratio, we can estimate the age of the air masses passing the sites. A α/γ-HCH ratio lower than 1 is characteristic of local emission of pesticide or of young air masses (Hoff et al. 1992). A α/γ-HCH ratio higher than 3 indicates an old air mass or long-range transport (Lane et al. 1992). By photochemical reaction in the atmosphere, γ-HCH can be isomerised to α-HCH which is more stable.

Table 2 presents the α/γ-HCH ratio of the 27 atmospheric samples collected at the three sites The ratio varied from 0.06 to 2.8. A ratio greater than 1 was obtained for Aubure samples. This result seems to show that technical HCH is not used in France and emissions of HCH are local. Technical HCH contains 50–80% α-HCH which has no pesticidal activity. Only γ-HCH is pesticidal.

The α/γ-HCH ratio greater than 1 found at the Aubure site could be explained by its geographic remoteness and high altitude. The γ-HCH arriving at Aubure comes not only from around the site but also via long-range transport.

The α/γ-HCH ratios for Colmar and Strasbourg sites are low (<0.4), so we can consider local emission is predominant and the air masses young.

Table 2. α/γ-HCH ratio for the 27 atmospheric samples analysed

Aubure	α/γ	Colmar	α/γ	Strasbourg	α/γ
19–20/04/93	1.43	19–20/04/93	0.17	13–14/06/94	0.34
26–27/04/93	0.72	26–27/04/93	0.14	14–15/06/94	0.28
04–05/05/93	0.60	04–05/05/93	0.09	22–23/06/94	0.16
10–11/05/93	2.80	10–11/05/93	0.28	23–24/06/94	027
17–18/05/93	0.13	17–18/05/93	0.18	11–12/07/94	0.15
13–14/06/94	0.99	12–13/07/94	022	12–13/07/94	0.32
14–15/06/94	0.15	21–22/07/94	0.28	21–22/07/94	0.20
22–23/06/94	0.06	25–26/07/94	0.37	25–26/07/94	0.12
23–24/06/94	0.21			26–27/07/94	0.15
11–12/07/94	0.19				

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Conclusion

Between spring 1993 and summer 1994, 27 atmospheric samples collected in remote, rural, and urban areas were analysed for 11 pesticides. The gas and particulate phases of each sample were analysed. Results shows that concentrations of pesticide in the gas phase are higher than those in the particulate matter.

Comparison of results from the three stations showed that pesticide concentrations in the remote area (Aubure) are, in general, lower than those in either the rural (Colmar) or urban area (Strasbourg) sampled.

The α/γ-HCH ratio in samples varied between 0.06 and 2.8, with at higher ratio of α/γ-HCH found at the Aubure site. These values indicated the presence of young air masses or local emission of HCH for rural and urban areas. For Aubure, air masses are sometimes older (ratio >1). This is the consequence of the geography of this station.

On the other hand, in Europe the use of pure lindane (99% γ-HCH) can explain the low α/γ-HCH ratios found at Colmar and Strasbourg.

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