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Acute Toxicity of Four Organophosphorus Pesticide Products

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Abstract Acute toxicity of phoxim, acephate, isofenphos-methyl and isocarbophos on male SD rats of clean grade was carried out by gastric lavage method at room temperature of 18°C. These rats are 4 to 5 months old with body weight of 180 to 220 kg. The results indicate that the orders of the toxicity of these four pesticides on SD rats are isofenphos-methyl, isocarbophos, acephate, phoxim. We found that the median lethal concentration of phoxim in 24 h, 48 h, 72 h and 96 h is 3.892 g/kg, 3.051 g/kg, 2.618 g/kg and 2.458 g/kg respectively; the median lethal concentration of isofenphos-methyl in 24 h, 48 h, 72 h and 96 h is 0.015 g/kg, 0.013g/kg, 0.012g/kg and 0.011 g/kg respectively; the median lethal concentration of isocarbophos in 24 h, 48 h, 72 h and 96 h is 0.049 g/kg, 0.046 g/kg, 0.043 g/kg, 0.041 g/kg respectively; and the median lethal concentration of acephate in 24 h, 48 h, 72 h and 96 h is 0.137 g/kg, 0.113 g/kg, 0.100 g/kg, 0.085 g/kg respectively. Finally, we evaluated the characteristics of toxicity effect and safe concentration of these pesticides to SD rats.

Key words Phoxim, Acephate, Isofenphos-methyl, Isocarbophos, Acute toxicity, SD rats

Most organophosphorus pesticides (OPPs) are phosphoester or thiophosphate and used in prevention and control of plant diseases and insect pests. With high effect, wide in variety, rapid degradation and low toxic residue, such pesticides are widely used in agriculture-husbandry production. In the past, most OPPs produced in China are insecticides, such as thiophosphate, demeton, malathion, Rogor, dipterex and dichlorvos. In recent years, new OPPs have been compounded, such as bactericide and raticide. However, abuse of OPPs has made such chemical compound into one type of major environmental pollutants and seriously affects food and ecological security, even threatens human health^[1–2].

Exposed to high concentration OPPs environment, plasma cholinesterase (ChE) activities will be inhibited, and acetylcholine (ACh) will accumulate at cholinergic nerve endings. Consequently, acute poisoning symptom may appear, such as muscarinic symptom, nicotinic symptom and central nervous system symptom. Severe patients may even go into a coma or die of respiratory failure^[3]. In recent years, organism injury due to exposure to OPPs environment has gradually attracted more and more social attention. Mackness B^[4] and Hernandez AF^[5] found that for gardeners exposed to OPPs environment at room temperature or herdsmen soaking wool with OPPs for a long time, the paraoxonase-1 (PON1) activity and concentration are obviously reduced. In 2008 to 2009, our research laboratory randomly selected 790 farmers who are exposed to OPPs environment for a long time. Our test indicates that the

oxidative stress (OS) and free radical level have a clear rise^[6]. Survey made by Fokina KV^[7] *et al.* indicates that people exposed to OPPs environment for a long time will have early clinical manifestation of rise of blood pressure, lipid metabolic disorder, drop of vascular tension, and cardiovascular and cerebral atherosclerosis. OPPs is one of the most commonly used pesticides, so people and animals are easily to get exposed to OPPs environment and get poisoned or even die. It is possible to test organic phosphorus content in foods and feed using phosphoantimonyl molybdenum blue spectrophotometric method^[8]. Nevertheless, the definition of safe concentration range of organic phosphorus content still depends on reference values in Instructions of pesticides, so it is easy to lead to error and consequently cause poisoning. In view of these, our laboratory conducted acute toxicity test of phoxim, acephate, isofenphos-methyl and isocarbophos on SD rats during September to November of 2010, to provide relevant data for physiological and ecological research of SD rats in adverse environment, and to provide basis for evaluation of ecological risk and treatment of pollution accidents.

1 Materials and methods

1.1 Materials

1.1.1 Experimental animals. We use 200 male SD rats provided by Henan Laboratory Animal Research Center. These rats are 4–5 months old with body weight of 180 to 220 kg. We put them at room temperature of 18 °C and raise them with conventional SD rat feeds and purified tap water.

1.1.2 Experimental pesticides. All experimental pesticides are purchased from recognized pesticide factory, and specifications and production factories of OPPs are listed in Table 1. We use distilled water to mix the experimental pesticides into mother liquor with certain mass concentration.

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Table 1 The experimental pesticides and their manufacturers

Name of pesticide	Registration number	Active constituent	Effective component	Manufacturer of pesticides
Phoxim	PD85157-2	O, O-Diethyl O-(alpha-cyanobenzylidene-amino) phosphorothioate	40%	Lianyungang Liben Agro-Chemical Co., Ltd.
Acephate	LS20041242	O,S-Dimethyl acetylphosphoramidothioate	20%	Henan Like Chemical Co., Ltd.
Isofenphos-methy	PD86165-3	N-Isopropyl-O-methy-O [2-Isopropoxycarbonyl] Phenyl] phosphoramidothioate	40%	Hubei Xianlong Chemical Industry Co., Ltd.
Isocarbophos	LS93564	O-methy-O (N-Isopropoxycarbonyl phenyl) thiophosphoramidate	17.5%	Hubei Sanonda Co., Ltd.

1.2 Methods After preliminary experiment, we determine the concentration range of experimental pesticides (the upper limit of safe concentration and lower limit of lethal concentration after 96 hours) at room temperature of 18°C. The natural status is taken as control group. We set several gradient groups of mass concentration using equally spaced method. We adopt gastric lavage method to conduct acute toxicity experiment on SD rats. Each gradient of pesticide mass concentration is provided with 10 experimental animals. We continuously observe their activities with heart stopping beating as the criteria of death. The mortality rate is recorded every 24 hours.

1.3 Data processing According to experimental results of

$$\text{MAC} = \frac{\text{Difference of median lethal concentration in certain observation time}}{\text{Difference of median lethal concentration between the first observation time and experiment time}} \times 100\% \quad (2)$$

$$\text{SC} = 0.1 \times 96 \text{ H } LC_{50}$$

where SC is the safe concentration, while is 96 h LC_{50} median lethal concentration at 96 hours after being poisoned.

2 Results and analyses

2.1 Poisoning symptom From Table 2, it is known that SD rats have different responses to different mass concentration in OPPs environment, and the difference in resistance to OPPs is also significant. Firstly, low mass concentration of isofenphos-methyl, acephate and isocarbophos groups within 24 hours

acute toxicity of pesticides on SD rats, we use SPSS13.0 to establish linear regression equation of mortality probability-mass concentration at 24 h, 48 h, 72 h and 96 h. Then, we test the reliability of the equation using r correlation coefficient ($P < 0.05$ means significant level) and F value ($P < 0.05$ means significant level), and use MAC to analyze the accumulation and degradation rules of SD rats to experimental pesticides. The safe concentration (SC) of experimental pesticides is calculated using following formulas:

$$95\% \text{ CI of } LC_{50} = (LC_{50} \pm t_{\alpha/2}, vS_x) \quad (1)$$

where CI refers to the confidence interval, and LC_{50} is median lethal concentration.

leads to death of some SD rats, while in phoxim group, no rat dies. However, most SD rats are steady in activity condition, and food taking and defecation are normal. Secondly, the high mass concentration of all four types of experimental pesticides within 24 hours leads to death of more SD rats. The survival rate is obviously lower than the low mass concentration groups. The mortality rate of SD rats in high mass concentration of isocarbophos group is 100%, 20% and 20% higher than that in phoxim, isofenphos-methyl and acephate respectively.

Table 2 The acute toxicity of four phosphate pesticides on SD rats at intervals of time at different dose

Pesticide	Experimental mass concentration // g/kg	The average cumulative mortality in different experimental time // %			
		24 h	48 h	72 h	96 h
Control group		0	0	0	0
Phoxim	1.578	0	0	0	10
	1.878	0	10	20	20
	2.178	10	10	20	30
	2.478	10	20	30	50
	2.778	30	50	70	70
Isofenphos-methy	0.011	10	20	40	50
	0.012	20	40	50	70
	0.013	30	50	60	80
	0.014	30	60	80	90
	0.015	50	70	100	100
Isocarbophos	0.038	10	20	20	30
	0.042	30	40	50	70
	0.045	40	40	50	70
	0.048	40	60	70	80
	0.051	60	70	100	100
Acephate	0.052	0	0	10	20
	0.072	10	20	30	40
	0.092	20	40	50	60
	0.112	30	50	60	80
	0.132	50	60	70	80

The experimental results indicate that the mortality rate of SD rats of all experimental pesticide groups within 24 hours is directly proportional to the mass concentration of experimental pesticides. Along with extension of experimental time, the mortality rate of each gradient group rises. The course from poisoning to death of SD rats is manifested as myosis, salivation, and amyostasia. Apart from these typical symptoms, there are also other possible symptoms, such as tearing, sweating, vomiting, diarrhoea, excited but restless, fast heart beating and breathing, even general convulsion, coma, general paralysis, and suffocation.

2.2 Acute toxicity effect of pesticides on SD rats From Table 2, we can know that along with rise of mass concentration and extension of experimental time, the acute toxicity of four types of OPPs on SD rats clearly rises and the mortality rate also obviously rises, but different experimental pesticides have different acute toxicity influence on SD rats. In the first place, within the range of experimental mass concentration, along with extension of poisoning time, the rise of mortality rate for high mass concentration gradient groups of experimental pesticides (the mortality rate after 96 hour > 50%): isocarbophos > acephate ≈ isofenphos-methy > phoxim (0 to 24 h); phoxim ≈ isofenphos-methy > isocarbophos ≈ acephate (24 to 48 h); isofenphos-methy ≈ isocarbophos > phoxim ≈ acephate (48 to 72 h); acephate > phoxim ≈ isofenphos-methy ≈ isocarbophos (72 to 96 h). In the second place, within the range of experimental mass concentration, along with extension of poisoning time, the rise of mortality rate for high mass concentration gradient groups of experimental pesticides (the mortality rate after 96 hour ≤ 50%): isofenphos-methy ≈ isocarbophos > acephate ≈ phoxim (0 to 24 h); isofenphos-methy ≈ isocarbophos > acephate ≈ phoxim (24 to 48 h); isofenphos-methy > isocarbophos ≈ acephate > phoxim (48 to 72 h); acephate ≈ phoxim ≈ isofenphos-methy ≈ isocarbophos (72 to 96 h). In the third place, according to range of experimental mass concentration and experimental results of acute toxicity on SD rats, the sequence of toxicity of 4 types of OPPs is isofenphos-methy > isocarbophos > acephate >

phoxim. The toxicity of isofenphos-methy is about 10 times that of isocarbophos, 9 times that of acephate and 246 times that of phoxim respectively.

3 Discussions

3.1 Characteristics of acute toxicity of phoxim, isofenphos-methy, isocarbophos and acephate on SD rats Different poison resistance of SD rats to 4 types of OPPs is related to OPPs molecular structure and the metabolic difference of organism to OPPs. Our research indicates that for the groups which has similar mortality rate in 96 hours after poisoning (take the mortality rate 50% as an example, shown in Table 2), the sequence of SD mortality rate is as follows: isocarbophos > isofenphos-methy > acephate > phoxim (0 to 24 h); isofenphos-methy > isocarbophos > acephate > phoxim (24 to 48 h); isocarbophos > isofenphos-methy > acephate > phoxim (48 to 72 h); isofenphos-methy > isocarbophos > acephate > phoxim (72 to 96 h). It proves that at the same level of acute toxicity, there is significant difference in distribution and accumulation speed between phoxim, isofenphos-methy, isocarbophos and acephate in SD rats. Specifically, at early state of experiment, isocarbophos has higher toxicity effect; in the middle period of experiment, isofenphos-methy has higher toxicity effect; and at later stage of experiment, the acephate and phoxim show higher toxicity effect.

3.2 Characteristics of acute toxicity effect and mortality change of phoxim, isofenphos-methy, isocarbophos and acephate Through statistical processing of the data in Table 2, we get Table 3. From Table 3, it is shown that in the same experimental condition, the difference is significant in mortality rate of different groups of experimental mass concentration. Through establishing the linear regression equation of mortality probability-mass concentration, we found that except phoxim at 0 to 72 h ($F > F_{0.05}$), other OPPs show higher degree of fitting ($r > r_{0.05}$), indicating the close correlation between mortality rate and the mass concentration in the same toxicity of OPPs.

Table 3 Analysis on acute toxicity of four phosphate pesticides on SD rats

Pesticide	Time	Regression equation	<i>r</i>	<i>df</i>	<i>p</i>	<i>F</i>	<i>LC</i> ₅₀ g/kg	LC50 95% Confidence interval//g/kg	MAC %	SC g/kg	Toxicity
Phoxim	24	<i>y</i> = 23.333 <i>x</i> - 40.820	0.904	3	0.035	13.364	3.892	2.440,5.344	- -	0.246	Low
	48	<i>y</i> = 36.667 <i>x</i> - 61.860	0.904	4	0.035	13.444	3.051	1.599,4.503	58.647		
	72	<i>y</i> = 50.000 <i>x</i> - 80.900	0.916	5	0.029	15.698	2.618	1.166,4.070	30.195		
	96	<i>y</i> = 50.000 <i>x</i> - 72.900	0.985	5	0.002	96.429	2.458	1.001,3.910	22.944		
Isofenphos-methy	24	<i>y</i> = 9000.000 <i>x</i> - 89.000	0.959	5	0.010	34.714	0.015	0.011,0.019	- -	0.001	High
	48	<i>y</i> = 12000.000 <i>x</i> - 108.000	0.986	5	0.002	108.000	0.013	0.009,0.017	50.000		
	72	<i>y</i> = 15000.000 <i>x</i> - 129.000	0.985	4	0.002	96.429	0.012	0.008,0.016	25.000		
	96	<i>y</i> = 12000.000 <i>x</i> - 78.000	0.986	4	0.002	108.000	0.011	0.007,0.017	25.000		
Isocarbophos	24	<i>y</i> = 3463.035 <i>x</i> - 119.144	0.966	5	0.007	42.434	0.049	0.041,0.057	- -	0.004	High
	48	<i>y</i> = 4416.342 <i>x</i> - 153.852	0.973	5	0.011	52.314	0.046	0.038,0.054	37.500		
	72	<i>y</i> = 5622.568 <i>x</i> - 193.891	0.966	4	0.007	42.361	0.043	0.035,0.051	37.500		
	96	<i>y</i> = 4766.537 <i>x</i> - 143.541	0.948	4	0.014	26.501	0.041	0.033,0.049	25.000		
Acephate	24	<i>y</i> = 600.000 <i>x</i> - 33.200	0.986	4	0.002	108.000	0.137	0.087,0.187	- -	0.009	Medium
	48	<i>y</i> = 750.000 <i>x</i> - 35.000	0.985	4	0.002	96.469	0.113	0.063,0.163	46.154		
	72	<i>y</i> = 750.000 <i>x</i> - 25.000	0.985	5	0.002	96.429	0.100	0.050,0.150	25.000		
	96	<i>y</i> = 800.000 <i>x</i> - 17.600	0.970	5	0.006	48.000	0.085	0.035,0.135	28.846		

Note: $F_{0.05}(1, 3) = 34.12$; $F_{0.05}(1, 4) = 21.260$; $F_{0.05}(1, 5) = 6.608$.

The accumulation and degradation of OPPs in body of SD rats are foundation for evaluating OPPs toxicity effect and analyzing poison resistance of SD rats. Table 2 shows that MAC value of phoxim, isofenphos-methy, isocarbophos and acephate are positive, indicating their accumulation is obviously higher than degradation in body of SD rats. However, the difference of OPPs molecular structure and toxicity mechanism leads to great difference in MAC values. MAC values of phoxim, isofenphos-methy, isocarbophos and acephate decrease along with extension of experimental time, indicating that these four types of OPPs have higher accumulation effect at early stage and in middle period of experiment and show the peak mortality rate, which lays a foundation for obvious rise of mortality rate. The acephate shows the second time of peak mortality rate at the period of 72 to 96 h, indicating that it possibly has secondary distribution within organism body.

3.3 Evaluation on safe concentration of phoxim, isofenphos-methy, isocarbophos and acephate to SD rats The phoxim, isofenphos-methy, isocarbophos and acephate are stomach pesticide and contact insecticide. Through inhibiting cholinesterase (ChE) activities, they cause massive accumulation of acetylcholine (Ach), consequently lead to blockage of nervous conduction and finally lead to death. Table 2 indicates that the safe mass concentration of SD rats to phoxim, isofenphos-methy, isocarbophos and acephate is 0.246 g/kg, 0.001 g/kg, 0.004 g/kg and 0.009 g/kg respectively. According to recommendation of China's standard for pesticide with acute toxicity^[9], the phoxim has low toxicity to SD rats, the acephate shows medium toxicity to SD rats, and isofenphos-methy and isocarbophos have high toxicity to SD rats.

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3.5 Prevention and control of plant diseases and insect pests The new variety 'jin yan' has high stress resistance. In general situation, it is not vulnerable to infection of plant diseases and insect pests. Nevertheless, in the *Hemerocallis* breeding test, snail seriously harms growth of *Hemerocallis* during May to August, it is required to take preventive measures in time.

4 Application of *Hemerocallis* in afforestation and landscape

Hemerocallis has jade green leaves which do not wither in the whole year. The subterranean stem can withstand temperature as low as -20 to 30 °C. It is not strict for soil environment. Besides, strong growth and convenient management are also advantages. It not only can be planted in flower border and beside roads, but also can be used as ground coverage in sparse woods; as well as in artificial hillock or near pools for interspersing. In addition, it is suitable for planting in backyard or beside wall, to set off beauty of yard landscape.

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