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UNITED STATES DEPARTMENT OF AGRICULTURE WASHINGTON, D. C.

RED-SQUILL POWDERS AS RATICIDES

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PURPOSE OF INVESTIGATION

The principal requirements for an ideal rat poison are safety, effectiveness, and economy. The rat poisons in most general use to-day (arsenic, barium carbonate, phosphorus, and strychnine) have toxic properties that seriously menace the safety not only of wild and domestic animals, but also of human beings. Red squill is relatively safer and more nearly approaches the ideal rat poison. Animals other than rats usually refuse to eat red squill in the concentrations used for rat baits. The difficulty of obtaining a uniformly toxic preparation of red squill has retarded its development for this purpose. To contribute to the information needed for the production of a potent red-squill preparation for the efficient destruction of rats, the investigation here reported was undertaken.

¹ Formerly pharmacologist of the Bureau of Chemistry.

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SOURCE AND STRUCTURE OF SQUILL

Squill, Urginea maritima (L) Baker, also called U. scilla Steinheil, is a perennial plant growing wild on the coast of southern Italy, Sicily, and Sardinia, and elsewhere along the Mediterranean Sea. The bulbs produced are pear shaped, usually from 3 to 6 inches in diameter and from 300 to 2,000 grams in weight (pl. 1), and are composed of closely overlapping fleshy scales. The outer scales are dry, brittle, and reddish brown; the inner scales vary from light yellowish white to deep cherry or mahogany; and the central ones (core) are usually white.

There are two commercial varieties of squill—white squill, which is marketed as dry, white scales and is the product official in various pharmacopœias, and red squill, which until recently was obtainable only in the fresh state. Both the viscous, mucilaginous juice and the dry scales of each variety are irritating to the skin. This stinging effect upon the skin, as well as the acrid prickly taste, is commonly attributed to calcium-oxalate raphides. White squill is used in human medicine as a heart tonic, emetic, diurotic, and nauseant expectorant. Various European investigators have recommended red squill as a rat poison.

Microscopic examination of squill was made by George L. Keenan, of the Food, Drug, and Insecticide Administration, who states:

A microscopic examination of red-squill powder shows that the anatomical structure is similar to that ζ' white squill. The powdered bulb of both varieties consists of fragments of parenchyma cells, spiral vessels, and long raphides of calcium oxalute, some of which are surrounded by dried masses of transparent refractive material, the mucilage sheath. The longest acicular crystals are approximately 1 millimeter long. Short raphides are present also. The greatest diagnostic difference is the presence of pigment cells in the red-squill scales and their absence in the white. The red pigment cells are numerous in the scales of the red squill and are readily found in the powdered material.

To facilitate its identification, the red squill was reduced to a fine powder and examined microscopically in a drop of menstruum consisting of an acidified chloral-hydrate-stycerol solution (made by dissolving 45 grams of crystals of chloral-hydrate in 25 cubic centimeters of dilute hydrochloric acid (1:8) and 10 cubic centimeters of glycerol). The slide was not warmed. The red coloring matter gradually diffused out of the colls, giving the pigmont masses a reddish appearance. Long raphides of calcium oxalate were found in the dried mucilaginous material, many in close proximity to the red masses.

When a powdered dry built containing 5 per cent of red squill was examined according to the method just described, the pigment cells were readily found.

RESULTS OF PREVIOUS INVESTIGATIONS

CHEMICAL

An exhaustive review of the chemical studies on squill since 1741 is given by George (12^2) . The active principle has not been definitely isolated and identified. Many of the products obtained by extraction with various solvents have proved to be impure mixtures. In many instances it is not plain whether red or white squill was studied. George states that the active constituents of squill are (1) scillitin, $C_{17}H_{25}O_6$, a yellow, very bitter, nitrogen-free glucoside, slightly soluble in water (0.18 per cent) and soluble in alcohol, which was isolated

¹ Reference is made by italic numbers in parentheses to "Literature cited," p. 35.





by Kopaczewski (18) as the toxic principle of squill; (2) scillidiuretin, a strong diurctic, also isolated by Kopaczewski; (3) xanthoscillide, a yellow crystalline glucoside, insoluble in chloroform, but soluble in boiling alcohol, isolated by Buschmann (2); and (4) a water-soluble toxic substance isolated, but not identified, by Ewins (8). George obtained a red glucoside from African squill, Urginea burkei, which corresponded in properties to Merck's scillitoxin. (Scillitoxin has not been chemically identified as a definite chemical entity.) Galavielle and Cristol (11) found the same chemical principle in Scilla autumnalis L. grown in the neighborhood of Montpellier, France, as in S. maritima. A patent has been granted to Stoil and Suter (27) for a process of isolating a physiologically active glucoside, "Scillaren," from white squill. The chemical composition and formula are not given in the patent.

PHYSIOLOGICAL¹

Lewin (21) reported that animals receiving squill showed a slower pulse, dyspuea, and pyrexia, and died on account of respiratory failure. Danysz and Kopaczewski (6) stressed the paralysis of the hind limbs, convulsions, and characteristic gyrations of rats following the consumption of squill. They state that the respiration is greatly depressed and that after several gasps the animal dies. Gunn and Heathcote (14) found that the subcutaneous minimum lethal dose of the glucoside prepared by Ewins's process (8) was 5 milligrams per kilo of body weight for the rabbit and 150 milligrams per kilo for the rat; also, that much stronger concentrations were required to stop the isolated rat heart than to stop the isolated rabbit heart in the same space of time. They concluded:

It is, of course, still possible that the rat may be more susceptible than other domestic animals to squill taken by the mouth; but this, if true, must be due, say, to some constituent of squill other than the glucoside or to some difference in action than the action on the heart. The possibility that this alleged hypersusceptibility of the rat may be due merely to the fact that the rat is the only domestic animal that will eat squill has not been sufficiently excluded * * *. The rat is markedly less susceptible than the rabbit.

The present experiments tended to confirm the conclusions of Gunn and Heathcote. Death following squill is apparently produced by respiratory rather than cardiac action.

TOXICITY

The toxicity of squill (whether of the red or of the white variety is not always stated) has been studied by a number of investigators since the first experiments by Orfila in 1818 (22, v. 2, pp. 99-102). The reports are summarized in Table 1.

The great apparent variation in toxicity may be attributed to differences in products, in methods of preparation, and in administration. Relatively enormous quantities of squill are apparently required to injure animals other than rats.

¹ While this bulletin was being prepared two articles by Winton (28, 59) were published. Winton concluded that "the cardine ginesside and the rat-poisoning principle in red squills are distinct subs._nees. The former occurs in about equal amounts in red and white squills. The rat-poisoning substance, however, is present in significant amounts in red squills only."

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Reference	Animals	Product	Dose	Result
Lewin (21)	Rats	Dried balb	1 gram	Death.
Kopaczewski (18)	do	Dried bulb	830 mg./kg	Do.
		Red-squill powder	260 to 1,800+mg./kg_	Do. 1
Claromont (5)	do	powder.	v,000 mg./xg	Surviyed.
		Urginea burkei powder.	2,000 mg./kg	Death,
Dulardin-Beaumetz	do	Boull	10 mg	Do.
Howarth (16, pp. 16-	ۤ	Scillitin	0.02 to 0.05 mg	Do,
Orflia (22, v. 2, pp. 90-102).	Dogs	Fresh bulb	32 mg 31⁄2 ounces	Do. Do.
Lowin (21)	Quinea pigs	Scillitoriu	0.5 mg	Do.
	Dogs, cats, and hogs_	Fresh bulb	0.25 to 2 grams	Voulting, diar-
Hortwig (10, p. 196)	da		45 grams	Death.
	2 horse and cow	do	30 grams	Diarrhea. Death
Kopaczewski (18)	Quinea pigs	Seillitin	I mg./kg	Do.
Claremont (4)	('ats Rabbits	do	2 mg./kg 216 mg./kg	Da. Da. Da.

TABLE 1.—Effect of squill preparations upon animals (reported in literature)

USE OF SQUILL AS RAT POISON

The Danish antirat law of 1907 forbade the use of strychnine, arsenic, and sulphurous preparations in rat control but permitted various squill preparations. Aumann (1) lists nine commercial squill preparations offered for sale in Germany in 1912; he also states that practice had confirmed the laboratory finding that squill preparations are twice as efficient as raticides as are the bacterial poisons. Smith (26) found that only one of five chemical extracts on the English market in 1921 killed rats. Aqueous, alcoholic, and aceticacid extracts of dried white squill failed to kill rats, although each of three alcoholic extracts of dried red squill proved fatal.

Claremont (4, 5) reported an intensive study of squill as a rat poison. The lethal doses of various red-squill powders ranged from 260 to more than 1,800 milligrams per kilo of body weight to rats and did not seem to bear any relation to the chemical characteristics of the preparations. The reducing sugars ranged from 3.32 to 10.45 per cent, and the total sugar after inversion from 6.94 to 73.73 per cent. Hydrochloric acid intensified the red color of the extract. Some powders and pastes on the British market were found to be toxic to rats. White-squill powders failed to kill in doses of 9,000 milligrams per kilo of body weight. South African squill, Urginea burkei, gave more mucilage than the ordinary varieties, and only one of three powders killed, the lethal dose of it being 2,000 milligrams per kilo of body weight. A drying temperature of 60° C. appeared to produce a satisfactory powder, although the lethal prin-ciple was not destroyed in preparing squill biscuits at temperatures around 100°. The powder preserved its texicity when stored in air-tight containers. Cold and hot water extracts were toxic, but alcohol failed to extract the toxic principle. Wild rats were rather more susceptible than tame rats. Microscopic examination showed no specific characteristic except the large number of raphides. Claremont concluded that the toxicity of squill preparations can not be inferred from analytical results but must be determined by feeding experiments on rats.

Kobert (17) quotes Kunkel's findings that red squill is more potent than white, that the outer scales act more intensely than the inner scales, and that the bulbs gathered in summer are more potent than those collected in the spring.

Lantz (19), Lereboullet (20), George (12), Howarth (16), and Schlupp (23) report the suitability of red squill as a rat poison under various conditions. Howarth states further that "it is, moreover, regarded as comparatively harmless to domestic animals." W. K. Tonkin, rat officer of Middlesex County, England, reports ' that

phosphorus pastes, barium carbonate, and one or two proprietary poisons have on occasion been used, but freshly extracted squill, both as a lethal rat poison and by reason of its being comparatively harmless to domestic animals, has been found of greater use than any other poison.

Boulenger (3) found that squill solution was the most effective rat poison, being three times as toxic as barium carbonate to rodents and also less harmful to domestic animals.

Finally, the British Ministry of Agriculture and Fisheries (13) recommended the use of squill:

Baits containing barium carbonate or red squill * * * are recommended by the ministry, and if used as directed, and with proper precautions, are less dangerous than baits containing such poisons as arsenic, strychnine, and phosphorus * * *. Red squill is the safer, and it is advisable to use it in preference to other toxic agents for application on farms and in places where special care is necessary owing to the presence of poultry, livestock, domestic animals, or stored food supplies * * *. Owing to the peculiar chemical nature of red squill in its various forms, as sold for the destruction of rats and mice, users of these preparations should satisfy themselves that they obtain guaranteed toxic red-squill raticides.

Recapitulating, the literature indicates that red squill is toxic to rats whether fed as the fresh bulb or as a powder, that it is essentially nontoxic to animals other than rats, and that white squill is nontoxic to rats (25). Apparently, the toxic principle has not been consistently isolated by chemical methods.

EXPERIMENTAL PROCEDURE

PREPARATION OF POWDER

In the preliminary experiments, 24 powders were prepared from samples taken from a 900-pound keg of squill bulbs imported from Gagliari, Sardinia, in the fall of 1923 and kept in storage at 4° to 5° C. This entire series of powders was prepared by Otis F. Black and James W. Kellcy, of the Office of Drug, Poisonous, and Oil Plants, Bureau of Plant Industry, United States Department of Agriculture.

The outer, dry, reddish-brown husks, which were found to be less toxic than the inner scales, were stripped from all bulbs. The bulbs were sliced and, in most instances, air-dried for several hours and placed in long glass tubes. A current of air was passed over the surface. The temperature was increased over a period of several days from room temperature to a given maximum, which was maintained until constancy in weight indicated that the products were thoroughly dried. They were then removed and ground in a drug mill until the powder passed through a 40-mesh sieve. These powders contained 1 to 2 per cent of moisture. After being ground, all powders were placed in tightly closed screw-top vials. Details of time and temperature are given in Table 2.

Personal communication.

		Bulbs			Prelimi- nary		Dry	ing condi	tions	:	
P. C. No.	Date made	which powder was made 1	Prevlous treatment	Condition when used	exposure to air at room tem- perature	Elec- tric oven	Vacuum oven	Cur- rent of hot air	Steam oven	Tem- perature	Remarks
Series 1: 11	1923 August	с	Just received from Sar- dinia.	Fresh	Yes	Hours 8	Hours	Hours	Days	° <i>C</i> , 60~80	
16	1924 Jan, 15	С	Stored at room tem- perature since August, 1923.	Sprouting; no decomposition.	7 days	8				60	
17. 18 19 20	do do do do		do do do do	do do dodo	do do No No	72	8	8 72		8095 6080 70 6060	Dried in current of hot air, then in
21 22 23	do	O C C	do. do. do.	do dodo	No Yes No	8 8	72			80 70 70	oven at 60° C. Husks. Core.
24 25 26	Feb, 19	C C	Stored at 5° C, since August, 1923. do	Slight decompositiondo	5 days do	- 48 - 48		16		80, 20-65 60 80	Dried in current of hot air, then in oven at 80° C.
27 28 29 20	do do Fab _ 27		do do do	do do do do	do do	24 8 8				100 110 120	
31 32 33	Mar, 1		do do do	dodo	10 days do		10 12 8 8			100 110 120	
35 36	Apr. 7	c c	do	do	45 days	12	8 	8		100 85	Dried in current of hot air, then in oven at 85° C.
37 38	Apr. 12	S	do	composition.	з days do	8		8	 	80, 20-65 80, 20-65	oven at 80° C. Do,

TABLE 2.—Method of preparation of red-squill powders

Series.	2:	1 1925	1	1	4	1 1 1 1	T	*		1	· · ·	
	[A	Dec, 7	C	Sardinian bulbs; held at room temperature	Fresh	No	68			•••••••	50	Composite scales; core: removed.
719	B	do	- C	since October, 1925.	dodo	68 hours.	4S 48			•	. <u>80</u>	Do.
	AF	Dec. 18	C	do	Alcoholicfermentation	No	74				80	cores only.
	AAF.	1926 Jan. 7	C	do	A catio acid formanta-	No	916					
		1025			tion.	110	1.10				. 00	
79	JA	Dec. 12	C	Sardinian bulbs; stored at 5° C, since October.	Slight fermentation	No	46				80	Whitish-pink bulbs.
120	lв	do	c	1925. do	đa	40 1.0						
791	jA	do	č.	do	do	No.	46				80	Do. Pink-red bulbe
	JB	do	l g	do	do	46 hours_	93				80	Do.
722	18	dō	1. 6.	do	Slight decomposition.	NO	46				0	Deep-red bulbs.
703	A	do	ĬČ	do	do	No	46				80	D0.
	γ(B	do	C	do	do	46 hours_	93				80	Do.
1.1	111	1926								1		
724	{ <u>A</u>	Jan. 8	C C	do	Fresh	No				4	70	Whitish-pink and pink-red bulbs.
	18		1 2	do	do	8 days				4	70	Do.
725	{B	do	č	do	do	NO				4	70	Nearly red and deep-red bulbs.
727	A	do	Ċ	do	do	No.	96			4	70	All colors: mixture 794 and 795 com
		Det of							1			posite.
769		1º00, 20	L C	do	do	No				12	70-80	All colors.
763		do	- č	do	Frozen	No	40				120	From composite all colors as P. C. 761.
764		Mar. 8	Č	do	do	No	41				100	Do.
- 765		Mar. 11	l Ç :	do	do	No	64				60	Do.
(00		Mar. 3	: " C	do	Fresh	No					Room.	From composite all colors as P. C.
			1									701; 14 days in desiccator over
767		Mar. 10	С	do	Acetic acid fermenta-	No				10	80	Rest of 722 and 723, combined.
768		do	S	do	do	No				10	80	Rest of 720 and 721, combined.
		mur. 4			Signt fermentation	No				23	40-50, 80	First 3 days at 40-50° C., then at
774		Mar. 17	С	do	Frozen	No	47				120	From composite P. C. 761; duplicate
775		Mar. 10	C	do	Fresh	No				10	80	Rest of P. C. 770.
182.		Mar. 30	U	do	Frozen	No	67				80	From composite P. C. 761; duplicate
		1 A 1 A 1 A 1 A 1 A 1 A 1 A 1 A 1 A 1 A					·	-	5		1.1	of P. C. 764

¹ Composite (C) or single (S).

RED-SQUILL POWDERS AS RATICIDES

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		Bulbs			Prelimi-		Dry	ing cond	itions		
P. C. No.	Date made	which which powder was made	Previous treatment	Condition when used	exposure to air at room tem- perature	Elec- tric oven	Vacuum oven	Cur- rent of hot air	Steam oven	Tem- perature	Remarks
Series 2—Con. 785	1926 Apr. 12	2 S	Sardinian bulbs; stored	Frozen	No	Hours	Hours	Nours	Days	° <i>C</i> . 80	
786 787 788 780 790 817	do do do do June 7	* S * S * S * S * S * S * S * S	at 5° C. since October, 1925. do. do. do. do. do. Stored at 5° C. since Oc- tober, 1925.	do	No No No No No	120 120 120 120 120 120 48				80 80 80 80 80 80 120	From composite P. C. 816.
818 819 820 841 842	do do Aug. 16 do	0000 0	do do do do do do	dodo do P. C. 840 used; fresh, not frozen, P. C. 840 used; frozen	No No No No	48 48 48 163 72				100 80 60 89 60	Do. Do. Do. From composite P. C. 840. Do.
843 844 845	do do do	C C C	do do do	2 days, do. do. do.	No No No	72 72 72				80 100 120	Do. Do. Do.

TABLE 2.—Method of preparation of red-squill powders—Continued

¹ Large bulbs.

I Small bulbs.

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As the results of these preliminary experiments indicated that squill is of value as a rat poison, the investigational work was continued, material from a second shipment of approximately a ton of squill bubs from Sardinia being used. Upon reaching the United States in the fall of 1925, the 200-pound bags of squill were stored at 4° to 5° C. Samples were drawn from time to time for the preparation of powders.

Essentially the same method of preparation was used as in the preliminary experiments. As previous investigation had indicated the desirability of using high temperatures in order to check fermentation or enzyme action quickly, an electric oven that could be set to within 1° was used for drying most of the powders in this series. In some instances it was desirable to duplicate commercial conditions. For such drying a commercial steam drier was used. Dried material was ground to pass through a 40-mesh sieve and packed in jars. The approximately ¼-inch-thick slices obtained in the commercial runs by using a hand-operated slicing machine proved to be too thin, as a large quantity of mucilaginous juice was exuded, facilitating fermentation and materially retarding drying. Slices half an inch thick were found to be more satisfactory.

By using the method finally adopted for the preparation of squill powders (p. 33), it has been possible to reproduce powders having the same toxicity.

METHOD OF FEEDING

Most of the toxicity determinations were made on white rats. Wherever feasible, results on wild (brown) rats were also obtained. All rats were held in cages for about a week to make the experimental animals essentially uniform. No rats weighing less than 100 grams each, and when possible only those that weighed between 150 and 250 grams each, were used. None of the rats received food for 18 hours, but all were given water freely, to insure a somewhat constant appetite and to favor the rapid consumption of the experimental diet. As previous experiments had indicated that rats readily consume 1 per cent of their body weight, efforts were made to feed approximately this quantity of total food.

Animals were weighed and placed in separate cages. The ordinary laboratory rat food—99 per cent of a mixture of whole-milk powder (one-third) and whole wheat flour (two-thirds) and 1 per cent of salt was passed through a 40-mesh screen. The sample of squill to be fed was thoroughly nixed with this food, so that approximately 1 per cent of the animal's body weight would be fed in giving the desired dose of squill. The weighed squill-rat-food mixtures were placed in individual glass sponge cups. The time when these cups were placed in the cages was noted, and frequent inspections were made to determine the time at which all food had been consumed. The dish was then removed and the rat left without food and water until the next morning.

Frequent inspections were made to determine the onset of symptoms of squill poisoning. Several times the fatal dose of fresh scales or of food containing squill powder has been readily eaten. Concentrations up to 40 per cent of squill powder in rat food have been rapidly and completely devoured, indicating that the presence of calcium-

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oxalate raphides does not deter a starved rat from eating these products. On the day following the feeding of an experimental diet, animals that were not in an obviously depressed condition or that were not gyrating viciously were offered the regular diet and water. All rats that died within five days after having been fed squill, and in which squill symptoms had been produced, were considered to have been killed by squill.

The detailed results obtained from about 3,000 white and 400 wild (brown) rats are reported in Tables 3, 4, and 5. The preparations as made were given "P. C." (pharmacology card) numbers to identify them. As these experiments were carried on over several years, the date of each series of feeding tests is given to show that there was at least a general agreement in susceptibility of test animals throughout the entire investigation. The quantities of dried squill powder fed are given in grams. The numerator of each fraction shows the number of rats dying from the indicated dose within a period of five days; the denominator shows the total number of rats fed the indicated dose. The MLD (minimum lethal dose) was selected as the smallest dose of a dried squill powder, in milligrams per kilogram body weight, that killed all, or practically all, the rats within five days.

EFFECTS OF SQUILL ON RATS

Rats that have eaten a toxic dose of squill soon become somewhat lethargic. From 4 to 14 hours later they usually exhibit characteristic tremors and sensory depression in the hind legs. This is followed by progressive paralysis of the trunk and forelegs. Respiration increases in rate and becomes labored. The animal next starts a peculiar, extremely characteristic rotation on its long axis. These gyrations have continued, at intervals, for half an hour and sometimes for 24 hours. The animal rolls over and over for some time, and then stops, apparently through fatigue. After resting, it resumes this gyration. Stimuli, such as a current of air, cause prompt resumption of gyrations. Few rats that showed this symptom recovered. Post-mortem examinations of a number of animals indicated acute cardiac dilatation, and hyperemia of the gastrointestinal tract and omentum. Although no cause for death other than acute cardiac dilatation was observed, in some instances the heart beat was detected after the cessation of respiration, indicating that respiratory paralysis played a part in producing death by squill.

RESULTS OF INVESTIGATION

During the progress of this investigation a number of individual problems were studied. For clarity of presentation, the results obtained in each separate study are reported under separate headings. The data given in Tables 6 to 13 are drawn from the results in Tables 2, 3, 4, and 5.

TABLE 3.—Toxicity of red-squill powders to rats 1

WHITE RATS

D C X75	Month of	Minimum						T	oxicit	y of 1	owder	în qu	iantiti	es of r	nillig	rams p	er kil	d lo c	ody w	eight	i i		-			
F. C. NO.	feeding	lethal dose	25	50	75	100	150	200	250	300	350	400	500	625	750	1,000	1, 250	1, 500	1, 750	2, 000	2, 500	3, 000	3, 500	4,000	5, 000	6, 000
	1924	Mg. per kgm. of body weight				- 1			0/0			1/0	n/o													
16 17	do	500 750 625										1/3	3/3 1/3 4/6	2/2	6/6 4/4	3/3		3/3								
18 19 20.	A pril February do	500 2,000+ 1,000				0/1		0/3	3/5	1/3	0/3	3/4	7/8 0/2 0/2	2/2	6/6 1/4 1/4	0/1 4/4	2/2			1/2 3/3						
21 22 23	do	1,000 3,000+						0/1				0/1	0/2 0/1	 	1/2 0/2	2/2 0/1	0/1			2/3 0/1		0/3				
24 25	April March	3,000+ 1,500							1/6				2/6		2/6 0/2	1/6 3/9	1/7	0/3 2/2		0/3		1/3	 			
26 27 28	do	1,000 2,000 2,500+						0/1			0/2		3/11		1/5 2/9	5/5 4/9 1/4	0/3	3/6 1/3		2/2 4/9	2/6		 			
29 30 31	do do	3,000+ 750 1,500+							1/1 0/3		0/2		0/1 2/7 1/5		4/4 0/6	0/3 1/1 2/11	1/3	4/6		1/7	0/5	2/3				
32 33 34	do do	750 750 2 000						0/1	 		0/2 0/2	- 1/1	2/5 2/4 0/1		3/3 3/3	3/3 2/2 0/2	 	1/4		2/2						
35 36	Aprildo	1,750											0/3			1/3 0/3		2/5 1/3	3/3 1/2	5/6	1/1					
35	May	3,000				4/13	4/5	4/6	6/6			3/3	2/3			3/3		3/3		3/3	1/3	4 4 				
714 715	1925 October September	4,000 6,000+			 		 						1/6 0/6			2/6 0/6		2/6 0/5		1/5 0/5	3/3	2/5 0/3		5/6 0/5		1/1 0/5

¹ Numerator of each fraction shows number of rats dying within five days; denominator shows total number of rats fed indicated dose.

TABLE 3.—Toxicity of red-squill powders to rats—Continued

WHITE RATS-Continued

	0.01	Month of	Minimum						т	oxicit	y of I	owder	in qu	antitio	es of r	nillig	rams p	er kil	d lo c	ody w	eight						-
	P. C. INC.	feeding	lethal dose	25	50	75	100	150	200	250	300	350	400	500	625	750	1,000	1, 250	1, 500	1, 750	2, 000	2, 500	3, 000	3, 500	4, 000	5, 000	8, 000
		1925 (December	Mg. per kgm. of body weight	{							0/2		1/3	1/3	0/2	2/3	1/1			1/1							
	[A	1926 March August	1,000	 						0/1				 	0/1 5/5	3/5 4/5	3/3 4/5		4/5		 						
		October November 1925 December				 			0/3	 1/2	 		1/2 3/6	4/5 5/5 5/7	4/5 3/3 1/1	5/5 	5/5 										
719	B	1926 August	625	{ 											-5/5	5/5	5/5									، بر ۱ 	
	C	1925 December 1926 February	1,000			 			0/2 0/1	•••••			•	0/2 0/4		1/2 0/3	1/1 3/9	0/4	8/15		1/1 1/1						
	[AAF	do 1925 December	2, 000				0/6	6/10	5/7	0/6	1/1			 1/1	0/2	0/3 1/1	0/5 1/1	3/8	7/9		5/5	ī/ī					
720	A	1926 June	} 250 }.	 			 -	2/5	12/15	4/5	••••															· · · ·	⁻
	B	1925 December 1926 June	250				0/18	11/16 0/3	5/6 13/15		2/2				÷												

										197 - La	1.1															
		1925 December			(0/1		1/6	5/9	1/1			1/1		1/1			 	 		÷	 		
(·····	1926 - June		350							5/5	4/5	11/11							 	 ••••			 		
21		November	}		l 1	****		0/1	0/2	•••••	1/4	7/8	4/5						•••••	 	 • - • • •			 	*	
	в	December		400]				0/2	0/1	2/4		6/6		5/6					 	 • • • •	•		 		
		1926 February								3/5	3/5	4/5 1/5	8/10	5/5			5/5			 	 			 		
		1925 December			[0/2		0/2	2/2	1/1			1/1		1/1	1/1		 				 	*****	
[A	1926 July	}	500+							0/5	0/5	3/10		5/10					 				 		
722		November) 1		[0/3		3/5					2/5		•••••		******	 •••••	 	••••		 		.4
		December							0/1	0/1	1/3									 	 •			 		
4	B	February		350	}					0/5	5/5 5/5	5/5		4/5 5/5						 	 			 		
		July October December								0/10	4/5	4/5	1/6 5/5					: 		 	 			 	 	
		1925 December								0/2	1/4	1/1			1/1		1/1	1/1		 	 			 		
	Δ	1926 February		000-1-									2/5				1. N. J. A.							 		
	**	March July		, ,							0/5		0/5	2/5	2/5	1/3 2/5	2/3 3/5	2/2		 	 			 		
723		November]		[0/1	2/4		 	 	 		 		
		December								0/1										 	 	 .		 		
i (B	1926 February		500	 					1/3		3/3 1/3		- 1/7-	5/5					 	 *-			 		
		July December	Į et s						1/5		1/5 0/5	0/5			5/5	4/5	5/5 5/5	5/5		 	 			 		
ſ	A	May July	}	500					 				1/3	0/0 	2/3 4/5	5/5	3/3 3/3	3/3		 	 			 		
721	в	September	} } } }	750	 {					0/3	4/6			2/3	5/5 3/3 1/5	2/5	3/3 8/10			 	 			 		

RED-SQUILL POWDERS AS RATICIDES

TABLE 3.—Toxicity of red-squill powders to rats—Continued

WHITE RATS-Continued

D C M	Month of	Minimum	· · ·			91 .		Т	oxicit	y of p	owder	in qu	antitie	s of n	ailligr	ams p	er kilo	of bo	ody w	eight		-				
F. C. 180.	feeding	lethal dose	25	50	75	100	150	200	250	300	350	400	500	625	750	1, 000	1, 250	1, 500	1, 750	2, 000	2, 500	3, 000	3, 500	4, 000	5, 000	6, 000
	1098	Mg. per kgm. of body meight							 				-					-							1	
	April May September October		 	 					2/3		0/3	1/3 4/5	3/3 2/3 5/5 4/5	2/3 5/5	2/3 3/3 5/5	3/3								 		*****
A	December	500	{		 		0/2		1/3	6/6 5/5			2/2				·						•••••	 		
725	1927 January		l					5/13	6/12																	
(_B	April September November	625	{					3/6	2/3			3/3 3/5	7/11 5/5	3/3 4/5	3/3 4/5											
727.A 748	March September March	} 500 5, 000+	{					 	0/5 0/2			0/4 2/5	3/4 11/11	2/2 5/5	5/5	2/2 		0/1		0/1		0/1	····		0/5	
749 750 751 752	do do do do	200 4,000+ 3,500 5,000+					1/3	3/3	3/3 0/3		 		3/3 0/3		1/1 	4/4 0/3 0/3	 	1/1 0/3		0/3 0/4	0/6	0/1			3/3	
753	March March Movember	5,000+ 5,000+ } 750	 {	 		 				0/3		 	2/5		5/5	0/3 3/3	 	0/3 4/4		1/1	 1/1	0/3 			0/3 1/3	
756	February April November	625							0/3 3/3	2/3		1/3 0/3	5/8 3/3 4/5	3/3 6/6	3/3	3/3			1							
759	April November	1, 500	{ 			0/6			0/3				0/3		2/9	3/3 1/3	2/3 4/5	1/1 3/3								
760 762 763 764	Marchdo	500 750 750	 	 		 		2/5	2/6 0/3 0/3	6/14	1/5 	3/3 3/3	3/3 4/9 5/9		3/3 6/6 6/6 2/5	3/3 3/3	1/1 3/3	3/3	 	2/2 3/3	 					
765	December	1,2:0	{									0/5	3/8 1/5		2/5	2/3 3/5	5/5	3/3		3/3						

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768	March		1, 250				<i>i</i> +						- 3/8	C/6		2/3	0/3	3/3	3/3		••••		.		- <u>-</u>	*****	
768	do	1	750		1				1 41		2/3	- 44	3/8	7/8		5/5		1	3/3			*****					
770	do	Į	750											0/3	2/3	4/4			2/2								
774	{do October	}	1, 250	{						0/3			0/5	0/4		2/5	1/3 5/5	5/5	2/3 5/5								
775	March	 }	625	{				•		0/3			2/5	2/3 3/5	3/3	3/3			2/3								
782	March.	ľ.	1, 250							0/3			1/2	0/6			1/3	3/3	6/6			3/3					
786	do		2,000										1/5	0/3			0/3				1/3	2/3				3/3	
788	do		250				0/3		0/3	3/3			2/12	6/6		1/i	2/2					»		•••••			
789	Mny	l	5,000+	÷			0/3		0/3	3/3			3/3	0/3			3/3 0/3				0/3		0/3			1/3	
817	June		2, 500							0/3				2/3							0/5	5/8	2/2			3/4	
	January) · ·	a sure	 	1					0/4				1/15													
	1926									1/3				0/3			2/3				0/6	7/15	4/5				
618	June	}	3,000+	{			t i								-								-1-				
	1927 Tenuery								<u> </u>	0/3				6/13		- 2710	1/5										
	February	J											*****				1/0										
	1926	h		[· ·	1		0/3		1		1/6			0/3				0/5	5/5	5/5				
819'	June	}	2, 500	1				21 C _		2/3		0/5		2/13		9/13	1/8	0/1			0/3		-,-				
	(January	J		(• • • • • •			-/0		0,0		-/		-,	-10				0,0						
820	1926 June)	2,500	{			• • • •			0/3				0/3			1/3				0/6	5/5	4/5			 	
	(December			l			•••••				([2/5		1/5	• • • • •	4/4						
641	1927		1 500				1.11		·	1				915		Die	10/16			1 ¹			· . · · ·	· · ·			
842	do		1,000											1/5		3/5 3/5	9/10	5/5	-1/0			+ 					
844	do		750 750											2/5 1/5		5/5 5/5	5/5 5/5					 					
845	do		2,000				•••••							3/5		1/5	1/5		1/5		5/5	4/5					

RED-SQUILL POWDERS AS RATICIDES

TABLE 3.-Toxicity of red-squill powders to rats-Continued

WILD RATS

	Month of	Minimum						Т	oxicit	y of I	owder	in qı	antiti	es of 1	nilligi	ams p	er kilo	o of b	ody w	eight		-				
F. C. 180,	feeding	lethal dose	25	50	75	100	150	200	250	300	350	400	500	625	750	1, 000	1, 250	1, 500	1, 750	2, 000	2, 500	3, 000	3, 500	4,000	5, 000	6, 000
		Mg. per kgm, of body							-										-							
16	March April March April	weight 250 500	{ 	 				0/1 1/1	2/2 2/3 3/3	••••• ••••	1/1	1/1 2/2			1/1	2/2 3/3		 	 							
24 38	April May	} 250 200	}		 		 	0/1 1/1	2/2			2/2	1/1	2/2			•••••	 	 							
719A F 72\\A	1926 February January	500+ 250	0/2	3/5	0/1	3/6	5/5 3/3	0/2 5/8	1/3 8/8	0/1 3/3	0/3	1/1	1/4 			 	·····							· • • • • • •		
721A 722A	dodo September	350 350 500	0/3	1/4 1/5	 	1/6 1/4	1/1	3/4 1/4	4/6 4/6	1/6	4/4 3/4 0/2	1/1	6/C		2/3											
724A 724A 724B 725A	do	500 500 500 300+			0/1	0/3	7/10 7/10	2/7 1/4	8/13 7/8	6/7 0/2 4/6	3/4	1/1	3/3 3/3				 								• • • • • • • • • • • • • •	
725B 727A	dodo do March	500 500+	{			0/1	0/3 0/1	2/5	u/2 2/3	0/1 4/5 2/4		3/5 3/4	5/S				 					 				

1		Minimum				Toxicity	in quantit	ies of milli	grams per	kilo of bod	y weight			-
190°-	Product	lethal dose	100	250	500	625	1,000	3,000	4,000	5,000	7,500	10,000	15,000	18,000
3	Calcium oralate	Mg, per kilo of body weight 1,000+	0/3	0/3	0/3	0/3	0/3							
	White squill	18,000+						0/3	0/1	0/8	1/3	1/3	0/2	

TABLE 4.—Toxicity of calcium oxalate and white squill to white rats 1

¹ Numerator of each fraction shows number of rats dying within 5 days; denominator shows total number of rats led indicated dose.

RED-SQUILL POWDERS AS RATICIDES

TABLE 5.—Toxicity of fresh squill bulbs to rats 1

WHITE RATS

P C No	Month of	Month of Minimum																	
	feeding	lethal dose	75	100	150	200	250	300	350	500	625	750	1,000	1,250	1,500	2,000	2,500	3,000	3,500
16A ³ 711 ⁴ 712 ³	1924 April 1925 October do	Mg. per kgm. of body weight 1, 250 150(?) 150+	0/2	2/3 0/2	2/2		0/6			2/6	1/1	0/3	3/5	6/6	3/3	3/3			
713 4 719 4 720 5 721 6 722 7 723 5	do December. do do do do do	150+ 1,000 1,000 500 750 350+		0/4 	0/1 	0/1		0/1	0/1	0/1 1/1 1/1 1/1 0/1	1/1 0/1	0/1 0/2 1/1 1/1	1/1 1/1 2/2						
761 • 816 10 840 10	1926 March June August	750 3,000 2,000		0/3				0/3		1/3 0/6	4/6 1/5	3/3 1/5 0/3	3/3 1/5 2/12		0/4 3/5	1/4 3/4	2/4	3/3	1/1
								WII	D RAI	r s									:
16A	1924 {March April	} 150	{	3/3 2/3	3/3	1/1	2/2	2/2											
1 All fre 2 Nume 3 Sardin 4 Sardin 5 Sardin 5 Sardin 7 Sardin 5 Sardin	sh squill res entor of each aian, red. aian, white. aian, whitish aian, pink. aian, deep re aian nearly a	ulis have bee fraction sho i pink d. red	n recalci ws num	ulated to ber of rat	dry basi s dying v	s, essum vithin 5	ling fresh days; der	bulb to cominat	contain or shows	80 per ce total nu	nt of mol mber of r	ture, ats रेंजे h	idicated	dosē.	1	<u></u>		!	

EFFECT OF TEMPERATURE ON TOXICITY

Powders made at 80° to 100° C. were more toxic than those made at other temperatures. Apparently they were more toxic than the fresh bulbs used in their manufacture, even after an 80 per cent allowance had been made for the moisture content of the bulbs. The powders made at room temperature (25° C.) and at 60° C. were less toxic than the fresh bulbs (on a dry basis), and also less toxic than powders made at 80° C. Temperatures above 100° C. seemed to decompose the active principle. Somewhat similar findings have been reported by Claremont (5). Although heating rarely increases toxicity, such a result is not unknown, as in the conversion of quinine into quinotoxin. Further investigation is necessary to determine the factors underlying this apparent increase in toxicity.

It was concluded that the most toxic powders were produced at a drying temperature of 80° to 100° C. (Table 6.)

TABLE	6Effect	of tempera	ture on toxic	ily of	′ red-squill	powder
-------	---------	------------	---------------	--------	--------------	--------

	Equiv-	Minimum lethal dose of squill dried at								
Baries No.	mini- mum lethal dose of undried fresh buib	25° C.	60° C.	80° C.	100° C.	110° C.	120° C.	140° O.		
l (hot-sir oven) 2 (hot-sir oven) 3 (vacuum oven) 4 (electric oven)	1, 250 1, 250 750	1, 250	750 1, 500 1, 250	400 1, 000 750 500	2, 000 1, 500+ 750	2, 5004 750	3,000+ 750 750	2,000		

[Mg. per kgm. of body weight]

DIRECT OVEN DRYING VERSUS PRELIMINARY AIR DRYING

One portion of each of seven pairs of samples prepared from the same composite lots of squill bulbs was weighed and placed directly in the drying oven at the desired temperature. The other portions were exposed on open watch glasses in the air for five days, after which they were dried at the same temperatures as the first set. (Table 7.)

	Minimum of squill	lothal dose powder—		Minimum lethal dose of squill powder—			
Sample No.	Direct oven dried	Prelimi- pary sir dried	Sample No.	Direct oven dried	Prelimi- nary air dried		
P. C. 710 P. C. 720 P. C. 721 P. C. 722	Mg. per kg. of body weight 1,000 250 350 500+	My, per ky, of body weight 125 200 400 350	P. C. 723 P. C. 724 P. C. 724 P. C. 725	Mg. per kg. of body weight 1,000+ 500 500	Mg. per kg. of body weight 500 750 025		

TABLE 7.-Effect of type of drying on toxicity

No difference was found in one set, but the variations in toxicity in the other sets suggested that other factors played more significant rôles in the development of toxicity. Direct oven drying is a simpler and more convenient procedure. It was concluded that direct oven drying gives as good results as preliminary air drying in the production of toxic squill powders.

EFFECT OF FERMENTATION ON TOXICITY AND YIELD OF POWDER

The 1925 experiments showed that sliced composites of fresh squill bulb fermented rapidly upon exposure to the air. Accordingly, an experiment was conducted to determine the effect of fermentation on the toxicity of squill powders, and incidentally to determine whether the fermentation was conducted upon the glucose of the toxic glucoside (or glucosides).

Portions of chopped composite taken from a fermenting lot and dried formed series 1. (Table 8.) One lot (P. C. 719A) was made by directly drying the unfermented squill in an electric oven at 80° C. Another (P. C. 719AF) was made from the same composite, which had stood in a closed fruit jar for 11 days at room temperature (25° C.). By this time the fermentaion had reached the alcoholic stage, that is, the odor of alcohol was apparent when the jar was opened. From 500,000,000 to 800,000,000 bacteria, from 5,000,000 to 18,000,000 yeasts, and 10,000 molds per gram were found in different parts of the material.⁵ After fermentation had reached the acetic-acid stage, sample P. C. 719AAF was taken.

		Yield		Toxicity				
Series No.	Unfer- mented	Alco- holic	Acolic ncid	Unfer- inented	Alco- holic	Acetic ucid		
1 2 8	Per cent 23.2	Per cent 17.8	Per cent 12.0	Mg. per kym. of baily weight 1,000 250–350 500–1,000	Mg. per kgm. of body meight 2,000	Mg. per kym. of body weight 2,000 750 750		

TABLE 8.-Effect of fermentation on yield and toxicity of red-squill powders

For series 2, a mixture of bulbs comprising the whitish-pink (P. C. 720) and the pink-red (P. C. 721), and for series 3 a mixture of the deep-red (P. C. 722) and the nearly red (P. C. 723) color groups of bulbs (p. 21) were allowed to rot to the slimy, oozy stage and were then sliced and dried in a steam-heated commercial drying oven. Samples P. C. 767 and P. C. 768 were produced.

In series 1 the yield and the toxicity decreased during formentation. The sample taken during the stage of acetic-acid fermentation gave only half as much powder as the unformented bulb, and it was only half as toxic to rats. A similar loss in activity was noted in series 2. Incomplete data were obtained in series 3, owing to lack of material.

It was concluded that chopped bulbs should be dried immediately to avoid decreases in yield and toxicity following fermentation.

EFFECT OF VARIABILITY OF SQUILL BULBS ON TOXICITY

Variations in the toxicity of squill preparations have been reported in the literature. Origin, climatic conditions during growth, and season of harvesting are reported to be factors in the toxicity of squill.

³ The e-descrimination (were made by B. A. Linden, of the Food, Drug, and Insecticide Administration,

In the 1924 experiments, the minimum lethal dose to white rats of the powders made simultaneously from two individual bulbs was found to be 250 and 3,000 milligrams per kilo of body weight. In the 1925 experiments six powders were made at the same time, and dried in the same oven at 80° C. simultaneously. Each powder was made from a single bulb, three from large bulbs (weighing around 750 to 1,000 grams each), and three from small bulbs (weighing around 200 grams each). The lethal doses to white rats were found to be 500, 500, and 2,500 milligrams per kilo of body weight for the powders from the large bulbs, and 250, 250, and over 5,000 milligrams per kilo of body weight for powders made from the small bulbs. No characteristic differences in the appearance of the bulbs or powders were apparent.

A sample of red-squill bulbs from Sardinia was found somewhat more toxic than one grown in Sicily during the same year.

Powders were prepared from composites of various portions of squill bulbs to see if material differences in toxicity could be found. The minimum lethal dose to white rats of the powder from the outer dry husks and scales was over 3,000 milligrams per kilo of body weight, that of the middle fleshy scales was 500 and 750 milligrams per kilo of body weight, and that of the central core was 1,000 and over 3,000 milligrams per kilo of body weight.

The readily available outer dry husks were stripped from all bulbs before the bulbs were sliced, in the preparation of squill powders. Although the core was less toxic than the middle fleshy scales, it constituted so small a portion of the bulb that it was not deemed necessary to remove it.

It was concluded that powders should be made from composites of a number of bulbs, and that the outer dry husks should be rejected.

EFFECT OF INTENSITY OF COLOR OF SQUILL BULBS ON TOXICITY

The color of the middle, fleshy scales of squill varies in intensity from a light whitish pink to a deep mahogany. A sackful of bulbs was arbitrarily divided into four color groups, and powders were prepared from six bulb composites of each group by two methods: (1) One portion of the chopped composite was directly oven-dried at 80° C. for 45 hours to constant weight; (2) the remainer of the chopped composite was exposed to the air for 46 hours at room temperature (25° C.) and then dried in the electric oven at 80° C. for 93 hours to constant weight. The quantities of bulbs in each color class, and the minimum lethal doses to white rats are given in Table 9.

Sample No.	Color of bulbs	Quantity	r of bulbs	Minimum lethal dose of powder made by—			
				Method 1	Method 2		
P, C, 720. P, C, 721 P, C, 721 P, C, 721 P, C, 722	Whitish pink Pink red Nearly red Deep red	Kym. 3.0 9.0 35.0 29,8	Per cent 3, 9 11, 7 45, 6 38, 8	Mg. per kgm. of body weight 250 350 1,000+ 500+	Mg. per kgm. of body weight 250 400 500 350		

TABLE 9.- Effect of intensity of color of squill bulbs on toxicity

The powders made by one method were about as toxic as those by the other in all but the nearly red group. The whitish-pink bulbs gave the most toxic powder and the nearly red ones the least, although still satisfactorily potent. As nearly red bulbs comprised almost half the shipment, it was not deemed desirable to reject them, even though they were less toxic than those of the other groups. To facilitate the application of experimental results to commercial conditions, all powders thereafter were made from run-of-shipment bulbs, without regard to color. Properly selected light-colored bulbs, however, might give more toxic powders.

TOXICITY OF WHITE SQUILL TO RATS

White squill is reported to be botanically identical with red squill and to have essentially the same microscopic appearance, except for the lack of pigment cells. The minimum lethal dose of three samples of white-squill powder fed to rats could not be determined, but it was more than 18,000 milligrams per kilo of body weight. White squill is apparently nontoxic to rats.

TOXICITY OF CALCIUM OXALATE TO RATS

The presence of calcium oxalate in squill powders has been universally recognized. Flückiger and Hanbury (9, p. 691) report the presence of 3.07 per cent of CaC₂O₄.3 H₂O in a sample of dried white squill. A dose of 1,000 milligrams per kilo of body weight of calcium oxalate (equivalent to 33,000 milligrams per kilo of squill, calculated from Flückiger's assay) was fed to rats without producing any apparent effect. Since calcium oxalate is present in white as well as in red squill, and since the squill powders that kill rats in doses around 200 milligrams per kilo would contain only 6 milligrams per kilo of calcium oxalate in the lethal dose, it was concluded that calcium oxalate itself could not be the primary cause of death in rats dying after eating red-squil powders.

PREPARATION OF RED-SQUILL POWDER ON A SEMICOMMERCIAL BASIS

From 10 to 30 kilograms of squill bulbs were dried in a 65 by 150 by 200 centimeter (25 by 60 by 80 inches, approximately) drier, holding six trays, each 27.5 by 90 centimeters (12 by 36 inches), and heated by closed steam coils. The temperature was kept constant within 4° or 5° C. during a given run. The outer scales were stripped off and the bulbs were sliced by hand or by slicing machines onto tared trays, until the trays were covered to a depth of 2 to 3 inches. The trays were placed in the oven, which had previously been heated to the desired temperature. Weights were taken from time to time until constancy of weight indicated that the bulbs had dried to equilibrium. The dried bulbs were then mixed and ground in an electric mill until the product passed through a 40-mesh sieve. The ground powder, after being again thoroughly mixed, was placed in screw-top cardboard mailing tubes for protection from the air. After six months to a year, the powder in these cardboard tubes solidified into a hard cake, but no change in toxicity could be detected. (Table 10.)

Run No.	Sample No.	Color of builts	Fermentation	Minioum lethal doso
1 2 3 4	P. C. 724 B 725 A 725 B 725 B 725 B 725 B 725 B 725 B 725 B 707 770 776 768	Deep red and nearly red	None	Mg. per kyta. of body weight 560 750 625 500 730 025 780 730

TABLE 10.- Effect of semicommercial preparation on toxicity of red-squill powders

For the first trial run, the outer scales were removed from about 150 kilograms of fresh bulbs, which were then arbitrarily divided into two groups, deep red and nearly red (P. C. 724), and whitish pink and pink red (P. C. 725). One portion of each group was sliced, weighed, placed directly in the oven, and dried, at temperatures ranging from 68° to 75° C., the average being 72°. The remainder was sliced, weighed, and left exposed to the air for four days. Slight fermentation had started when the material was loaded into the oven and dried at the same temperature as the first group.

For the second run (P. C. 760) a composite of 250 kilograms of bulbs, irrespective of color, was used. The outer husks were removed, and the bulbs were sliced into large chunks. The weighed material was dried at once at a temperature ranging from 70° to 80° C.

For the third run (P. C. 770) a composite was made from 2 sackfuls of bulbs. A machine was employed to make uniform slices approximately a quarter of an inch thick. This brought out much of the mucilaginous material from the bulbs and reduced them to a more slimy consistency before drying. Conditions were therefore unintentionally made more favorable for fermentation. The use of sharp instruments in slicing the bulbs is advisable to avoid bringing out an excess of the mucilaginous juices. During the first three days of drying the oven temperature was maintained at 40° to 50° C. On the fourth day it was brought up to 80°, where it was held during the rest of the drying period.

One portion of the fourth run (P. C. 775) consisted of the remainder of the bulbs used in the preparation of P. C. 770. They were also finely sliced. In addition, bulbs that had been stored at 4° to 5° C. for three months were used. Fermentation during storage had been marked. The bulbs were soft and spongy, with the leaves separating, and were dripping mucilaginous slime. Two composites were made. The whitish pink (P. C. 720) and pink red (P. C. 721) groups were consolidated as P. C. 768; the deep red (P. C. 722) and nearly red (P. C. 723) groups were consolidated as P. C. 767. The spongy bulbs were sliced onto a tray and placed directly in the oven to dry. During the fourth run the oven temperature was held at approximately 80° C.

Although the temperature was not held so constant as in the electric oven, the products obtained in the steam oven corresponded reasonably well in toxicity with corresponding samples from the same composite dried in the electric oven. Thus, the minimum lethal dose of P. C. 727A, dried in an electric oven, was 500 milligrams per kilo of body weight, and that of P. C. 724A and of P. C. 725A, dried in a steam oven, was the same.

Sample P. C. 760 in the second run and P. C. 775 in the fourth run were made at 80° C., and both were more toxic than P. C. 770 in the third run, which was held at 40° to 50° for three days before being heated to 80°. This suggests that a rapid rise to 80° for the fresh bulb tends to produce the most toxic squill powder.

The rate of drying was followed only until uniform weight was indicated. At 80° C. it seems that drying would be complete in about three days.

Although food containing 40 per cent of squill powder was readily caten, 10 per cent concentrations were used in most instances. With this concentration, a rat consuming 1 per cent of its body weight of food would consume 0.1 per cent of its body weight of squill, or 1,000 milligrams of squill per kilo. Accordingly, squill preparations having a lethal dose at or below 1,000 milligrams per kilo appear to be sufficiently toxic for commercial purposes. Most of the experimental powders killed in materially smaller doses.

The production under semicommercial conditions on a semicommercial scale of squill powders having similar toxicities indicates the possibility of successfully manufacturing squill powders of a satisfactory degree of toxicity on a commercial basis.

RELATIVE SUSCEPTIBILITY OF WHITE RATS AND OF WILD (BROWN) RATS TO SQUILL POWDERS

From the literature it would seem that wild (brown) rats are more susceptible to squill than white rats. The lethal doses found in the investigations here reported, when the same powder was fed to the two forms, are given in Table 11.

Sample No.	Minimu doso	m lethal for—	Patla		Minimu dose		
	White rais	Wiki rats	1110	Sample No.	White rats	Wild mts	Ratio
Р. С. 18 28 716 А.Р 7203 721 А. 722 А.	Mg, per kgm, of body xciyht 750 250 2,000 250 250 350 350 500+	M(g. per kym, of body scipht 250 500+ 250 150 350 350 500	$\begin{array}{c} 3.0\\ 1.0\\ 1.25\\ (4, 0-)\\ 1.0\\ 1.7\\ 1.0\\ (1.0+) \end{array}$	Р. С. 721 А. 724 А. 724 В. 725 А. 725 В. 725 В. 725 В. 725 В. 727 А. Хуствуе	Mg. per kgm. of hody weight 1, 000+ 500 750 500 (25 500	Mg. per kgm of body weight 500 500 500 300+ 500+	(2.0+) 1.0 1.5 (1.7-) 1.3 (1.0-) 1.5

TABLE 11.—Relative susceptibility of white rats and of wild (brown) rats to squill powders

In some series no difference in susceptibility was noted; in others the wild rats appeared to be about three times as sensitive. Lack of material and the difficulty of obtaining a fairly large supply of wild rats for feeding purposes at any definite time prevented more accurate determinations of differences in susceptibility of the two forms. Results obtained in these 14 series confirm Claremont's findings (5) that, on the average, less squill powder is required to kill wild rate than is required to kill white rats.

EFFECT OF RED-SQUILL POWDERS ON DOMESTIC AND OTHER ANIMALS

Previous investigators have stated that squill, although toxic to rats, is not toxic to cats, dogs, chickens, and other domestic animals. As no data showing the minimum lethal dose of red squill to these animals were found in the literature, a series of experiments was undertaken to determine the toxicity of squill to animals other than rats.

CATS

Squill powders were mixed with whole-milk powder, and various proportions were added to 100 grams of lean hashed meat, which was then fed to cats. In food containing 10 to 25 parts of squill powder per million the unusual flavor was detected, and the food was eaten slowly but completely. The quantities of food containing higher concentrations of squill that cats consumed within 24 hours were then determined. (Table 12.)

TABLE 12.—Relative percentages of squill-meat baits eaten by cats in a 24-hour period

Concentration of squill (parts per	Percent-	Concontration of squill (parts per	Percent-
million)	age enten	militon)	age cuten
100 200 600 1,000	100 90 84 75	2,500 5,000 10,000	8 20 7

As rat baits are customarily exposed in concentrations of 10 per cent, or 100,000 parts per million, cats probably will not eat such food.

DOGS

Dogs refused ground meat containing 1,000, 2,500, and 5,000 parts of squill powder per million of meat. When squill powder suspended in water was injected into the stomach by stomach tube, emesis and diarrhea followed in several instances after doses of 100 milligrams per kilo of body weight. As dogs refused food containing a small concentration of squill powder, such as 1,000 parts per million, it does not seem that they would be hurt by squill. Two separato instances have been noted where dogs gulped down squill rat baits containing 10 per cent of squill mixed with sausage. Emesis was the only effect noted.

CHICKENS

No effect was observed following the injection into chickens' crops of quantities less than 2,000 milligrams of squill powder per kilo of body weight. Diarrhea followed the injection of 2,000 and 3,000 milligrams per kilo. Squill powder was mixed with cracked corn and with laying mash in 10 per cent concentrations. After one or two picks, chickens refused to eat such food. Two chickens were placed on a diet containing 10 per cent of squill powder in growing mash. After refusing to eat for several days, they consumed the mash without much hesitation. This food was continued for two weeks, during which time the chickens apparently grew as well as controls fed on untreated mash. Odom Stewart, formerly of the Bureau of Biological Survey, in experiments with baby chicks at Raleigh, N. C., concluded that chicks are not likely to eat enough feed containing 10 per cent of powdered red squiil in 1, 2, or 3 feedings to cause death, even when fed it to the exclusion of other foods.

PIGEONS

After a few picks, pigeons refused to eat mash containing 10 per cent of squill powder. Injection of 2,000 milligrams of squill powder per kilo of body weight into the crop failed to produce any noticeable effect. The injection of 3,000 and 4,000 milligrams per kilo resulted in emesis, but no other untoward effect could be noted.

PIGS

A dose of 250 milligrams of squill powder per kilo was given in a gelatin capsule at 10.45 a. m. to a 4-month-old pig weighing about 16 kilograms. No evidence of squill action was noticeable until about 4 p. m., when signs of gastric distress developed. The pig vomited several times during the night. At 9 o'clock the next morning it was unable to stand without definite ataxia, and consistently refused food, but drank water freely. Some food was eaten at noon but it was vomited about 3 p. m. The pig's tail was limp and straight, although at the time of feeding it was tightly curled. On the second day after the injection the pig had practically recovered.

After a 9-day intermission, to permit complete recovery from the first dose, a mixture of cracked corn and middlings containing 10 per cent of the same squill powder was offered. The pig took only one or two bites; then grunted, refused further food, and vigorously rubbed its snout on the cage walls and floor. This reaction was probably due to the sting of the calcium-oxalate raphides. Half an hour later, and again the next morning, the same squill food was refused, but untreated food was readily consumed.

Post-mortem examination showed nothing abnormal, except some evidence of irritation in the stomach. When the body weight of a pig is considered, it is readily apparent that the quantity of squill bait required to cause toxic effects would need to be very large. For this particular animal, the quantity of squill power given would be sufficient to kill 16 kilograms (35 pounds) of rats. Under ordinary conditions of rat baiting, it hardly seems possible that a pig would consume enough poisoned bait to cause trouble.

WOODCHUCKE

Injected in aqueous suspension into the stomach of woodchucks, the minimum lethal dose of sample P. C. 18 was found to be 500 milligrams per kilo of body weight (the same as for rats). Vomiting, however, frequently followed even smaller doses. Baits containing squill were refused. Squill does not seem to be suitable for use in controlling woodchucks.

PRAIRIE DOGS AND POCKET COPHERS

Prairie dogs and pocket gophers refused to eat freshly exposed squill baits. After rains had washed off the squill, the remaining baits were readily consumed.

From these feeding and stomach-tube experiments, it seems safe to conclude that squill mixtures containing 5 to 10 per cent of squill powder either will not be eaten by animals other than rats, or will produce emesis with direct removal of the poison. Probably, therefore, squill powders deserve the reputation accorded them in the literature of being generally harmless to farm animals. Of course, failure to produce death may be due to the animal's failure to cat and retain enough squill powder, rather than to relatively high species resistance or insusceptibility.

YIELDS OF TOXIC SQUILL POWDERS ("RAT UNITS")

Generally speaking, squill bulbs lose 80 per cent of their weight during drying. The yields of powder obtained when samples were dried under stated conditions are given in Table 13. The weight of powder obtained in following a given process is not significant of the efficiency of the process unless the toxicity of the powder obtained is also considered. Two kilograms of a highly toxic powder may represent a better yield than 4 kilograms of a relatively nontoxic preparation. It is necessary to know both the yield and the toxicity in judging the efficiency of producing a squill powder under a given set of conditions.

	Bu	lb	Drying	Darria anon	Loss in weight after drying for-									
P. C. No.	Part used and color	Condition	ture	Di ying oven	18 hours	1 day	2 days	3 days	4 days	5 days	6 days	8 days	10 days	15 days
766	All	Fresh	20-25	None I	P. ct. 9.0	P. cl. 25. 2	P. cl. 60.0	P. ct. 69.5	P. ct.	P. ct. 76.0	P. ct.	P, cl. 76. 5	P. ct. 76. 5	P. ct. 76. 6
765 724A 725A	Deep red and nearly red Whitish pink and pink	Frozen. Fresh	50 72 72	Steam	01. 3	، ۱۹۹۵ 			78.5 79.4					
770 820	Alldo	do. Frazen 2 deus	1 40-80 60 60	Electric	73.3	76.8	78.0	82.1				 		76.0
719A 719B	do	Freshdo	80 1 80 1 80	do do		49.0	10.8 70.2	76.8 75.2 83.0	78.5 85.0	78.5				
719A F 719A A F	All.	Alcoholic fermentation Acetic-acid fermentation Slight fermentation	80 80 80	do		82.0	87, 6 88, 0	83.2			88.0	88.0		
720A 720B 721A	Pink red	do	3 80 80 3 80	do			76.0 87.1 74.0	89.0 87.5	89. 5 87. 6	89, 9 88, 1	89, 9 88, 1			
722A 722A 822B	Deep reddo	do	80 3 80 80	do do			78.5 55.2 75.6	79.0	79.1	79.3	79.3			
723B 727A	All 4	do Fresh	3 80 80 80	dodo			55. 0 48. 3	77.3	77.5	77.7	77.7 76.1		78.0	
764 767	do Mixture of No. 722 and Native of No. 722 and	Frozen Acetic-acid fermentation	80 80	Electric Steam	73.5	78.0	78.0						79.2	
768	Mixture of No. 720 and No. 721.	Fresh	80 80	do									79,9 79,0	
782	do	Frozendo	80 80 80	Electricdo	75. 0	75.0	79.0			72.4 79.2				
757 758 798	do do	do	80 80 80	do						74.3 76.1 73.0				
790.	dodo]do	80	do		·		.		76.7	·		· · · · · · · · · · · · · · · · · · ·	·

TABLE 13.-Rate of loss in weight of squill bulbs during drying

S10	1 411	1 Fresh		1 do	76.3	76.81	78.0	lanna can	استخدرتهم			فديد ماليا فالم		lunnanere.
541	do	da	80	dn		76.5	81.1			81.1	82.5			
- 071	do	Trann 9 dave	ŝõ	do		SI S	82.2	82.4						
- 010	do do	Frozan	100	do	70.5	76.0	77.5	77.5						
100		I Frach	100	do	75 3	78 0	78.3	1					1	
010		Engran 0 dave	100	div.	10.0	61.5	89.1	6 62	*******	*******				
811		Frozen 2 unys	100	10	67 5	80.5	50 5		********	*******	[*=====;**			
102		F RUSH	120	do	0140	20.0	77.8		*******	********		*******	1	
114	·]- ·····Q0···················	Frozen	120			-0.2	70 0	[**********		*****	********	*******		
817		Fresh.	120	{	(0. L	10.1	10-0	1			*******		*	
845		Frozen 2 days	120			80.0	84.8	83,0		*******	*******	*******		
		±						£ .			£	1		1

Dried in desiccator over calcium chloride.
 3 days at 40 to 50° C.; then at 80°.
 First at room temperature; then at 80°.
 From composite used in No. P. C. 724 and No. P. C. 725 dried in steam oven.

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In an attempt to express in a single figure the results of combining the two variables, yield and toxicity, the so-called "rat units" of powders have been calculated. A "rat unit" is the yield in grams from 1 kilogram of fresh squill bulbs divided by the minimum lethal dose in grams per kilo of body weight. It is the number of kilograms of rats that should be killed by the powder obtained from a kilogram of fresh squill bulbs treated by the method under consideration. For example, if a 20 per cent yield was obtained by a certain method, and the powder had a minimum lethal dose of 500 milligrams per kilo of body weight, the rat unit for the method would be calculated as follows:

> Yield =20 per cent×1 kilogram of fresh bulbs=200 grams Toxicity=500 milligrams per kilo=0. 5 grams Rat units= $\frac{\text{Yield}}{\text{Toxicity}} = \frac{200}{0.5} = 400.$

Another method for making a powder with 200 rat units would obviously be less valuable, being but half as efficient. The rat units determined for a number of series of samples are given in Table 14.

Sample No,	Metland of drying	Yiohi	Mini- mun lethal dose	Rnt units	Sample No.	Method of drying	Yield	Mini- mum letimi dose	Rat units
P. C. 761 719A 719A F 719A F 719A F 720A 720A 720A 721A 722A 723A 723A 724B 724B 724B 722B	Frosh bulb Electric oven do Desicentor Electric oven do do do do do do do do do do do do	Per cent 23, 2 17, 8 12, 0 21, 5 23, 2 12, 0 12, 0 21, 5 21, 4 21, 5 21, 2 21, 5 21, 7 21, 5 21, 7 21, 5 21, 7 21,	Mg. per kynn. of bady u.eiyht 4,000 1,000 2,000 2,000 1,250 3,50 1,000 4,000 4,000 4,000 4,000 4,000 4,000 5,00 1,250 3,50	250 233 30 239 239 239 450 370 450 370 450 300 300 500	P. C. 721B. 727A. 762 764 764 783 784 794 A. 725A. 725A. 725A. 775	Electric oven do do do do do Stenn oven do	Per cent 22, 3 23, 6 22, 5 22, 5 22, 5 22, 5 22, 5 22, 5 24, 6 20, 6 20, 6 20, 6 20, 6 20, 6 20, 2 21, 2 21, 2	Mg. per kgm. 500 500 500 750 1,250 1,250 1,250 1,250 1,250 1,250 500 500 500 500	450 480 200 180 110 170 190 430 410 410 340 340

TABLE	14Y	ields o	f toxic	squill	powders
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The rat unit for the fresh bulb (P. C. 761) was 250. Essentially the same value was obtained for P. C. 719A in the unfermented stage. Fermentation to the alcoholic stage reduced the rat unit to 90, and further fermentation to the acetic-acid stage caused a drop to 60, showing the loss in activity during fermentation to be progressive. Freezing caused a decrease to about 200 rat units. The rat units of powders prepared under laboratory conditions ranged from 110 to 590, while the values for powders made under semicommercial conditions ranged from 300 to 430. Attention to manufacturing details would be expected to raise the rat units for commercial powders above 500. Indeterminate values for two of the P. C. No. 719 series prevent careful comparison, but the A series averaged less than 350, whereas the B series averaged 420 rat units. This would suggest that preliminary air-drying treatment given the B series preserved the toxicity or aided in its development. The fact that many powders have rat units greater than 250, the value for fresh squill bulbs, also suggests that the toxic principle in squill bulbs may be linked to some plant ingredient in a thermolabile condition, and that the toxic principle is liberated by heating to 80° to 100° C.

STABILITY OF SQUILL POWDERS

Two red-squill powders stored in screw-top vials in the light for 15 months showed no change in lethal dose. A 10 per cent squill biscuit that had been baked was as toxic a year later as the squill content would require. A number of powders tested over a period of a year have shown no evidence of loss of activity.

ACCEPTANCE TESTS

To determine the palatability of 10 per cent squill biscuit as compared with that of commercial samples of 5 per cent arsenious-oxide biscuit and 30 per cent barium-carbonate biscuit, several dishes were placed in a cage containing a starved white rat. Each dish contained more poisoned food than would be necessary to cause death by itself. After exposure from one to five hours, the dishes were removed, and the quantities of food consumed were determined. A number of tests were run simultaneously. With the wright of squill biscuit eaten considered as unity in each case, the ratio of control biscuit to 10 per cent squill biscuit was 2.6:1.0; that of 5 per cent arsenious-oxide biscuit to 10 per cent squill was 0.17:1.0; and that of 30 per cent barium-carbonate biscuit to 10 per cent squill biscuit was 0.93 :1.0. The absolute quantities of poisons consumed would then be in the ratio of 250 squill to 750 barium carbonate to 20 arsenious oxide. These figures are roughly proportional to the minimum lethal doses of the poisons under consideration. Barium carbonate is approximately one-third as toxic as squill powder; fed in three times the concentration, about equal quantities of bait are consumed. As the toxicity of arsenious oxide depends upon its degree of fineness, closer comparisons are not available.

A field-acceptance test in comparison with phosphorus baits is reported by Tcall.⁶ At Vienna, Austria, an organized 2-day rat-killing campaign was held in January, 1927, phosphorus baits being used exclusively. Of 1,460,000 baits exposed, about 20 per cent were taken. In March, 1927, another 2-day campaign was undertaken, during which only squill baits were used. Of 1,260,000 baits exposed, more than 50 per cent were taken. It was noted that squill was taken in many houses where phosphorus bait had not been touched.

The degree of acceptance of squill baits varies with the relative palatability to rats of the foods with which the squill is mixed. Directions for preparing and distributing red-squill baits have recently been published by the Department of Agriculture (24, p. 8, 9).

COMMERCIAL SQUILL RAT POISONS

Three liquid squill extracts that had been offered for sale as rat poisons were tested. One was one-fourth and the other two were less than one-tenth as toxic as their labels would indicate. Two squill biscuits and three squill powders were found to correspond in toxicity to the labeled claims for squill content.

⁴TRALL, G. GRGANZATION AND RESULTS OF THE FIRST VIENNA RAT-RHLING CAMPAIGN. (Second supplementary report to Report No. 6025 dated May 20, 1925, in reply to department's nonumbered instruction of March 28, 1925, File No. 1027-1848.) [1927.] [Original, on file in Department of State, not seen. Copy on file in U. S. Dept, Agr., Bur. Riol. Survey.]

EXTRACTION OF TOXIC PRINCIPLES BY VARIOUS SOLVENTS

To obtain information regarding the chemical nature of the toxic principle of red squill, a mixture of two powders (P. C. 724A and P. C. 725A) was taken for intensive study. Seventy-five-gram portions in Soxhlet thimbles were extracted with water, with 95 per cent ethyl alcohol, with acetone, and with chloroform, until there was no further appearance of color. The solutions were concentrated in a current of warm air, and the resulting solid extracts were dried in an electric oven at 80° C. and finally in a vacuum desiccator over calcium chloride. The residues were removed from the Soxhlet thimbles and dried in the electric oven at 80°. Feeding tests were made with the original mixed powder and with some of the extracts and residues.

The water extraction, started on January 20, 1926, was run from 8.30 a. m. until 5 p. m. daily for 19 days. The dark-red mucilaginous extract, possessing an odor of molasses, was concentrated on a steam bath, then dried in an oven, and finally allowed to stand over calcium chloride in a desiccator until constant in weight. It was ground to pass through a 40-mesh sieve. Both the extract (P. C. 751) and the exhausted residue (P. C. 750) were very low in toxicity, indicating that the active principle is decomposed by this treatment.

The alcoholic extraction for 19 days removed a much smaller quantity of mucilage, but apparently as much color as did the water extraction. The residue caked and became very hard upon exposure to the air. The solid extract (P. C. 749) had a rather oily feel, and was the most toxic extract in this series. The lethal dose of the residue (P. C. 748) exceeded 5,000 milligrams per kilo of body weight. The residue therefore is inert (nontoxic).

Acctone extraction gave a small yield of lemon-yellow crystals (P. C. 755) and no mucilage or color. Feeding tests showed that the very fluffy residue (P. C. 754) was two-thirds as toxic as the original powder, indicating that a portion of the toxic principle is destroyed during the treatment with acetone.

Chloroform gave the same result as acetone. The solid extract (P. C. 757) was small and oily. The fluffy residue (P. C. 756) was somewhat less toxic than the original powder. The acetone and the chloroform extracts were too small to be used for feeding tests.

Distilled water was repercolated through another 75-gram charge. Channels formed, producing incomplete extraction. So much mucilage dissolved that after seven days further repercolation was found to be impossible. The thick, molasseslike percolate had a sweetish odor and reduced Fehling's solution. Evaporated to a very dark-brown, tough, solid extract (P. C. 759), it was readily eaten by rats. The iethal dose was 1,500 milligrams per kilo of body weight. The incompletely extracted residue was not fed to rats.

To study further the effect of water in extracting the toxic principle, 75 grams of squill powder were placed in a beaker with about 800 cubic centimeters of water and warmed on a steam bath. Twice a day the solvent was removed in a Buchner funnel, and a fresh charge of water was added. After 22 days the extraction appeared complete. The aqueous solution was concentrated to a solid extract (P. C. 753). The residue (P. C. 752) was a dark-brown, tough sheet, heavily laden with glittering calcium-oxalate crystals. Both the extract and the residue were nontoxic, showing that the active principle is totally destroyed by this treatment.

Table 15 shows the yield, the lethal doses, and the proportion of toxic principle from the original powder that appeared in the various extracts and in the residues.

		Yleld		Minimum lethal döse		Total toxicity	
Method of extraction	Solvent used	Solid extract	Resi- due	Solid extract	Resi- due	Solid extract	Resi- due
Original powder Percolation Exhaustion Boxhiet. Do Do Do	Waterdo dodo Acohol (05 per cent) Acetone Chloroform	Per cent 18.5 82.6 44.3 14.0 1,3 1.3	Per cent 100 81, 5 17, 4 55, 7 86, 0 98, 7 98, 7	Mg. per kg. of body weight 1, 500 5, 000- 3, 500 200	Mg. per kg. of body weight 500 5,000+ 4,000+ 5,000+ 750 625	Per cent	Per cent 100 1,7 7,0- 8,6- 60,0 80,0

TABLE 15 .- Effect of method of extraction on yield und toxicity of squill

Exhaustion with water on the steam bath, or in a Soxhlet thimble at 100° C., caused almost total destruction of the toxic principle in the extracts as well as in the residues. As dry heat at 100° C, did not appear to cause any decrease in toxicity, it seems evident that this loss in potency is related to the hydrolysis of the toxic glucoside. The alcoholic residue was nontoxic, and the acetone and choroform residues were somewhat less potent than the original powder. Even though the boiling point of each of these three solvents is less than 80°, it seems that any method of extraction tried caused some destruction of the toxic principle. The alcoholic extract possessed one-third the activity of the original powder.

Apparently the toxic principle of red squill is soluble in alcohol, but not in water, acetone, or chloroform. Water and alcohol extracted appreciable quantities of mucilage and reducing sugars. Extraction of the toxic principle suggests itself in the study of the chemistry of the active principle. It seems evident, however, that the cost of undertaking this on a commercial scale would far outweigh any possible benef. of marketing a more toxic preparation. Therefore no further experiments in this direction were conducted.

SUGGESTED METHOD OF PREPARATION OF TOXIC SQUILL POWDERS

Based upon the results obtained during a 3-year series of experiments in the manufacture of red-squill powders, the following method is offered as furnishing the most toxic product:⁷

Remove the outer dry husks from fresh red-squill bulbs obtained as soon as feasible after digging, and slice the bulbs transversely into sections one-fourth to one-half inch thick. Place the sliced composite as soon as possible in a drying oven, which has been previously heated to 80° C., and dry to constant weight at that temperature. Grind the dried material so that it will pass through a 40-mesh sieve. Pack the powder in hermetically sealed containers.

⁷ It is possible that other manufacturing conditions might yield products of equal toxicity.

Powders made by this process should kill white rats in doses approximating 250 to 500 milligrams per kilo of body weight, and should be stable for several years.

Because of the variations in toxicity of different lots of squill, the minimum lethal dose of every lot of squill powder should be determined by feeding it to rats in the laboratory before it is marketed. It should be fed in 10 per cent concentrations in ordinary rat food to white rats that have been deprived of all food for 18 hours. The minimum lethal dose is the smallest dose that kills all the rats within At least five rats should be fed with each dose tested. five days. This is necessary to insure essentially uniform toxicity in squill powders distributed commercially. As a result of feeding tests, great variations in the potency of successive lots of powders may be reduced by properly mixing powders of higher degrees of toxicity with lesspotent preparations, to produce the same standard potency. Such procedure would put a premium on the production of the most toxic squill powders, but would also permit the utilization of less toxic preparations.

As a standard of toxicity it is suggested that commercial squill powders having a minimum lethal dose of 1,000 milligrams per kilo of body weight be marketed, and that commercial squill baits having a minimum lethal dose of 10 grams per kilo be prepared. Such baits would contain 10 per cent of the standard squill powder (that is, a squill powder with a minimum lethal dose of 1,000 milligrams per kilo). If this standard bait is used, it would be necessary that a rat eat only 1 per cent of its body weight to obtain enough poison to kill it. Many of the wild rats studied during this investigation weighed between 250 and 400 grams (8 to 14 ounces). From 2.5 to 4 grams (one-twelfth to one-seventh of an ounce) of such a squill bait would be necessary to kill them. These experiments indicated that rats will readily eat much larger quantities of such baits.

CONCLUSIONS

Powdered red squill is toxic to rats; white squill is not.

Powders prepared by directly drying unfermented, sliced red squill bulbs in an oven at 80° C. are usually more toxic than those prepared under other conditions. The lethal dose of squill powders prepared by this method is usually about 250 milligrams per kilo of body weight for white rats; wild (brown) rats are killed by somewhat smaller doses.

Cats, dogs, chickens, and pigeons were not seriously harmed by squill powder. Food poisoned with squill either was not eaten or, if eaten, was promptly vonited. Consequently it has been indicated that red squill is nontoxic to these animals under normal conditions and when exposed in the concentration recommended for rat poisons.

Because of variations in toxicity, squill powders should be tested before being marketed and adjusted so that 10 grams of 10 per cent squill bait will kill a minimum of 1 kilogram of rat. (One ounce will kill 7 pounds of rats.) *

LITERATURE CITED (1) AUMANN. 1912. VERGLEICHENDE UNTERSUCHUNGEN ÜBER DIE WIRKSAMKEIT BAK-TERIELLER UND CHEMISCHER RATTENVERTILGUNGSMITTEL. Contbl. Bakt. [etc.] 63: 212-221, illus. (2) BUSCHMANN, E. 1919. UNTERSUCHUNGEN ÜBER DIE CHEMISCHEN BESTANDTEILE VON BUL-BUS SCILLAE. Arch. Pharm. 257: 79-86. (3) BOULENGER, E. G. 1920. REPORT ON METHODS OF BAT DESTRUCTION. Zool. Soc. London Proc. 1919: [227]-244. (4) CLAREMONT, C. L. 1921-22. NATIONAL RESEARCH ON BAT DESTRUCTION. Journ. Roy. Sanit. Inst. 42: 311-318. (5) ~ 1922. Notes on the analysis and use of red squill in rat poisons. Analyst 47: 60--67. (6) DANYSZ, J., and KOFACZEWSKI, W. 1914. STR LES PROPRIÉTÉS TOXIQUES DU PRINCIPE DE LA SCILLE. Compt. Rend. Soc. Biol. [Paris] 77: 59-61. (7) DUJARDIN-BEAUMETZ. 1923. LA DÉRATISATION. Journ. Pharm. et Chim. 27: 461-467; 28: 17-25. (8) Ewins, A. J. 1911-12. A NEW WATER SOLUBLE ACTIVE CONSTITUENT OF SQUILLS. Journ. Pharmacol. and Expt. Ther. 3: 155-160. (9) FLÜCKIGER, F. A., and HANBURY, D. 1879. PHARMACOGRAPHIA. A HISTORY OF THE PRINCIPAL DRUGS OF VEGETABLE ORIGIN, MET WITH IN GREAT BRITAIN AND BRITISH INDIA. Ed. 2. 803 p. London. (10) FRÖHNER, E. 1901. LEHRBUCH DER TOXIKOLOGIE FÜR THIERÄRTZE. Ed. 2, 356 p. Stuttgart. (Ref. Hertwig, p. 196.) (11) GALAVIELLE, and CRISTOL, P. 1922. LE SCILLA AUTUMNALIS L. ÉTUDE CHIMIQUE DE SES PRINCIPES ACTIFS. Bul. Sci. Pharmacol. 29: 29-31. (12) GEORGE, E. 1925. THE ACTIVE PRINCIPLES OF THE AFRICAN SQUILL, URGINEA BURKET STREET, DESCRIPTION OF OTHERWISE AS A SUBST-(SLANGKOP) AND ITS SUITABILITY OR OTHERWISE AS A SUBSTI-TUTE FOR URGINEA MARITIMA IN COMMERCE. Journ. So. African Chem, 1 ist. 8: 14-27. (13) [GHEAT BRITAIN]. MINISTRY OF AGRICULTURE AND FISHERIES. 1923. The DESTRUCTION OF RATS. [Gt. Brit.] Min. Agr. and Fisherics Leaflet 244, 8 p. (14) GUNN, J. A., and HEATHCOTE, R. ST. A.
 (1920-21. THE ALLEGED HYPERSUSCEPTIBLET: OF THE RAT TO SOULL. Journ. Physiol. (Physiol. Soc. Proc. Set. 16, 1920) 54: lxxxy-(15) HARTWICH, C. 1889. Unen die Meenzwiebel. Arch. Pharm. (3 R. 27) 227: [577]-588, illus. (16) HOWARTH, W. J. 1921. REPORT OF THE MEDICAL OFFICER OF HEALTH OF THE CITY OF LONDON FOR THE YEAR 1920. Med. Off. Health [London] Ann. Rpt. 1920, 76 p. (17) KOBERT, R. 1902-06. LEHRBUCH DER INTOXIKATIONEN. Ed. 2, 2 v., illus. Stutigart. (18) KOPACZEWSKI, W. 1914. UBER DIE PHYSIOLOGISCHEN WIRKUNGEN DES SCILLITINS UND SCILLIDIURETINS. Biochem. Ztschr. 66: [501]-508. (19) LANTZ, D. E. 1917. HOUSE RATS AND MICE. U. S. Dept. Agr. Farmers' Bul. 896, 24 p., illus.

TECHNICAL BULLETIN 134, U. S. DEPT. OF AGRICULTURE 36

- (20) LEREBOULLET, P.
 - 1916. LA LUTTE CONTRE LES RATS DES TRANCILÉES. Paris Médical 6 Année: 165.
- (21) LEWIN, L. Translated and annotated by G. 1903. TRAITÉ DE TOXICOLOGIE. Pouchet. 1120 p., illus. Paris.
- (22) ORFILA, M. P. 1818. TRAITÉ DES POISONS TIRÉS DES RÉGNES MINÉRAL, VÉGÉTAL ET ANIMAL ON TOXICOLOGIE GÉNÉRALE, CONSIDERÉE SOUS LES RAPPORTS DE LA PHYSIOLOGIE, DE LA PATHOLOGIE ET DE LA MÉDECINE LÉGALE. Ed. 2, rev., corrected, and enl. 2 v. Paris. W. F.
- (23) SCHLUPP, W. F. 1921. THE DESTRUCTION OF RODENTS BY THE USE OF POISONS. Union So. Africa Dept. Agr. Bul. 4 (1921), 34 p.
- (24) SILVER, J. 1927. RAT CONTROL. U. S. Dept. Agr. Farmers' Bul. 1533, 21 p., illus. (25) -
- 1929. RAT CONTROL AIDED BY DEVELOPMENT OF EFFECTIVE NEW POISONS. U. S. Dept. Agr. Yearbook, 1928: 518-521, illus.

(26) SMITH, W. F.

- 1921. LIQUID EXTRACT OF RED SQUILL (SCILLA MARITIMA) AS A RAT POISON. Analyst 46: 178-180.
- (27) STOLL, A., and SUTER, E. 1925. HEART-AFFECTING PURE GLUCOSIDE FROM BULBUS SCILLAE AND PRODUCING THE SAME. (U. S. Patent No. 1516552). Commr. Patents Ann. Rpt. 1924: 582.
- (28) WINTON, F. R. 1927. THE RAT-POISONING SUBSTANCE IN RED SQUILLS. JOURN. Pharmacol. and Expt. Ther. 31: 123-136, illus.
- (29) =

1927. A CONTRAST BETWEEN THE ACTIONS OF RED AND WHITE SQUILLS. Journ. Pharmacol. and Expt. Ther. 31: 137-144.



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