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Some factors affecting the toxicity of red squill.

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SOME FACTORS AFFECTING THE TOXICITY OF RED SQUILL

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DEDICATION

This thesis is hereby dedicated to my wife, Annette.

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I. INTRODUCTION

Great losses result each year from man's most destructive pest, the rat. These rodents are a serious menace to the public health, as they carry diseases of man and animals such as plague, typhus, trichinosis, rat-bite fever, infectious jaundice and others (Zinsser, 1935). The rats' economic depredations are almost unlimited. The most efficient means of destroying rats is by poisoning, and the most practical poison is red squill.

The object of this work has been to determine some of the factors affecting red squill toxicity so as to find methods of making red squill even more effective as a rat poison. To accomplish this end, experiments to determine the effect of rat sex, rat weight, heat, storage, fats, protein, carbohydrate, moisture, diet, and pectin have been carried out.

It is hoped that this thesis will yield information of value to farmers, food manufacturers, public health workers, and rodent control officers.

II. REVIEW OF LITERATURE

1. Red Squill

Red squill, Urginea maritima, called also scilla, or sea onion, is a perennial bulb belonging to the lily family. It grows wild along the coast of the Mediterranean Sea. The bulbs are pear shaped, usually from three to six inches in diameter, and weigh about five pounds. They are gathered usually during the dormant period in summer and early in the fall. The pointed, blade-like, deep-green leaves dry up before the flower blooms in spring. The small flowers which are white with green veins are borne on a tall stem. The fruit is a three celled capsule, with flat, winged seeds, having a thick black shell.

There are two commercial varieties of squill which apparently are not distinguishable botanically. The white variety of squill is employed in the official preparations of squill in the United States and Great Britain. It is used in human medicine as a heart tonic, emetic, diuretic and nauseant expectorant. Red squill is official in France and has all the properties of white squill, and in addition contains active constituents which are toxic to rats.

The toxic substance of squill is soluble in water and in concentrations of alcohol and acetone up to 90 per cent. It is destroyed by boiling with dilute acid or alkali (Winton, 1927).

Much work has been done on the constituents of squill: Rode (1929); Kopaczewski (1913); Ziegerspeck (1914); Munch, Silver and Horn (1929); Bijlsma (1936); Stoll (1934); Danysz and Kopaczewski (1914); and Buschman (1919). Buck (1936) gives a complete list of patents relating to squill in his doctoral thesis, and thoroughly covers red squill extracts and chemical and toxicological work on red squill (1934)(1936).

2. Partial Analysis of Red Squill

Claremont (1922) determined the moisture, ash, silicon dioxide, water extract, reducing sugars and total sugars of 15 red squill powders. A partial percentage analysis of a red squill powder on a dry matter basis follows:

Moisture	4.00
Ash	3.50
Fiber	5.20
Reducing sugar	10.80
Total sugar after inversion	25.60
Non-reducing sugar	14.86

3. Procedure of Assay

According to Munch, Ward, Mills, Buck, and Jarvis (1937), the methods of chemical assay have not furnished adequate indications of physiological potency so bioassays are required.

A standard technique was developed using the white rat: Munch, Silver and Horn (1929); Buck and Fellers (1935); and O'Connor, Buck and Fellers (1935). Normal male rats, not previously used for any other test, and weighing between 100 and 200 grams were starved for 18 hours, after having been fed on a stock diet for at least one week. The squill powder tested was weighed and mixed with the stock diet which was offered to the animals. The bait was consumed within fifteen minutes, and squill poisoning symptoms developed before death, which occurred within approximately five days. Munch, Silver and Horn (1930) in a number of experiments have shown that a hungry rat will consume one per cent of its body weight of food within 15 to 20 minutes.

4. Palatability of Squill Baits

General deductions from the palatability tests made by O'Connor, Buck and Fellers (1935) are that raw meat, raw fish, rolled oats, whole wheat, corn meal, bread crumbs, canned fish, canned meat, cooked cereals, cheese, meat scraps, powdered milk, fish meal, fresh vegetables, cooked vegetables, and fresh fruits rank in approximately that order. Numerous simple and complex food mixtures showed little improvement in acceptance over simple foods, though meat or fish and cereal mixtures were always readily eaten.

They also found that the use in baits of oils of caraway,

anise, catnip, cinnamon, and peppermint did not enhance palatability of the foods to rats. Peppermint oil was repulsive. O'Connor (1933) found that the addition of flavoring oils to red squill powder baits as lures has no advantage insofar as the amount of bait consumed by rats is concerned.

5. Toxicity as Affected By Sex of Rats.

In 1927 Winton found that female rats are killed by only one-half the dose of red squill required by male rats. Munch, Silver and Horn (1930), in their investigations, found no consistent difference in the susceptibility of male and female rats. LeBlanc and Lee, and Crabtree, Ward and Welch, working independently in 1939, also found that the normal male rat is at least twice as resistant as the normal female to the toxic principle of red squill.

6. Susceptibility to Red Squill Poisoning

Claremont (1922), and Munch, Silver and Horn (1929) noted that less squill is required to kill wild rats than white rats. As wild rats are not convenient for laboratory work, the results obtained by using white rats have proved satisfactory.

O'Connor (1933) found that white rats which have eaten red squill powder, but have recovered after much suffering, will not eat a lethal dose of red squill powder for some time thereafter.

Winton (1927) states that previous administration of the poison has little influence on the susceptibility of the rat to squill poisoning.

According to O'Connor, Buck and Fellers (1935) large rats on the basis of body weight require a larger dose of red squill to cause death than small rats.

7. Other Factors Affecting the Toxicity of Red Squill

A. Heat

O'Connor, Buck and Fellers (1935) found that when dry red squill powder was heated to 240° F. in a retort for 90 minutes the toxicity remained unchanged. When moistened with water or mixed with such carriers as meat, fish, or cereals, and sealed in tin cans or glass jars, no reduction in toxicity was noted.

Winton (1927) noted that the toxic substance in red squill is relatively thermostable. LeBlanc and Lee (1939) concluded that temperatures up to 100° C. are not injurious to the toxic principles of red squill. Munch, Silver and Horn (1929) reported that drying red squill bulbs at temperatures above 100° C. seemed to decompose the active principle.

B. Moisture

O'Connor (1933) found that white rats prefer dry rather than moist baits containing red squill powder.

C. Fats

Underhill (1936) reports in his book that fats hinder the absorption of drugs and poisons. Sollman (1936) states that oils, gums and other substances hinder the absorption of drugs, partly by adsorption of the drug, partly by hindering absorption, and partly by hindering its access to the absorbing surface.

D. Pectin

According to Manville, Bradway and McMinis (1936) pectin possesses the properties of a hydrophilic colloid with great adsorptive qualities. Furthermore, it is capable of giving rise to galacturonic acid upon being broken down. In work with rabbits they found that galacturonic acid from pectin can be utilized by the organism in the detoxication of menthol. Their experiment demonstrates the fact that galacturonic acid is capable of forming conjugation products with toxic materials in the same manner as glucuronic acid.

E. Fineness of Division of Red Squill Powder

The average lethal dose of a red squill powder may be 50 per cent greater if it is less finely subdivided (Winton, 1927).

F. Storage

Red squill powder shows no appreciable deterioration when stored for long periods according to Winton (1927), O'Connor (1933), and Munch, Silver and Horn (1929). The present work substantiates

this finding.

G. Diet

Winton (1927) states that abnormal diets have little influence on the susceptibility of the rat to squill poisoning. Smith (1939) in experiments made on rats showed that the toxicity of selenium in naturally occurring food is largely determined by dietary factors. He found that a level of intake of selenium which is highly toxic and tissue damaging when fed in a diet of low protein and high carbohydrate content is only slightly harmful, if at all, when fed in a diet of high protein and low carbohydrate content. The same level of selenium intake in a low protein and high fat diet causes stunted growth and extensive loss of hair, but no other demonstrable tissue damage except some fatty degeneration of the polygonal cells of the liver.

8. General

The symptoms and "modus operandi" of red squill poisoning are covered thoroughly by Winton (1927).

Hans Zinsser (1935) in his book "Rats, Lice and History" and various authors in United States Public Health Service Bulletin 30 (1910) give thorough accounts of the history of the rat and describe its destructiveness and public health importance.

The effect of red squill on other animals is covered by Silver and Munch (1931) and Munch, Silver and Horn (1929) in Leaflet 65

and Bulletin 134 of the United States Department of Agriculture. In general, there is little danger of poisoning animals other than rats and mice by the use of red squill baits. They are the safest effective baits known at the present time.

III. EXPERIMENTAL

1. Description of Samples

A standard red squill powder, called B-SS1 by Buck (1936), is known as Reference Standard Red Squill of 1934 throughout this work. This standard powder was prepared by Munch of the United States Bureau of Biological Survey to be used as a reference standard in the assay of red squill preparations. This powder was prepared by mixing together five samples of red squill which had proved to be toxic in a preliminary assay.

This Reference Standard Red Squill of 1934 was found to have the following percentage composition:

Moisture	5
Ash	5.95
Nitrogen	1.23
Ether soluble	3.17
Crude fiber	10.6
Nitrogen free extract	74.03
Reducing sugar	3.3
Starch	5.5
Total reducing sugar after inversion	9.2

Another sample of red squill powder was used in this work. This was obtained from Penick and Co., and is an ordinary commercial squill powder. It is called by the serial number of its manufacturer, Lot Sc. 3422 No. 130863, Penick and Co.

2. Procedure of Assay

Normal albino rats weighing, when possible, between 100 and 250 grams were used. These had been fed Purina Fox Chow (a balanced, complete dry feed) previous to the assay. The rats were weighed, then placed in individual cages, given water, but deprived of food for 24 hours to insure a complete clean up of the bait by the rats. They were then fed a dose of squill dependent on their body weights. This squill was mixed with enough of the desired carrier to make one per cent of the rats' body weight. Purina Fox Chow was used as the carrier unless otherwise stated. Twenty-four hours later, Fox Chow was placed in the cages as food. The animals were observed for 120 hours at frequent intervals. The number of hours after the squill feeding that death occurred was noted. This time for squill to cause death is accurate within about five hours. The results of an assay are expressed as a fraction, for example 5/10. The numerator of the fraction shows the number of rats dying from the indicated dose. The denominator shows the total number of rats fed the indicated dose. The toxicity, that is, the lethal dose, is selected as the smallest amount of red squill powder, in milligrams per kilogram of body weight that kills 90 to 100 per cent of the rats within 120 hours.

3. Presentation and Discussion of Results

A. Palatability

The possibility was considered that vitamin B_1 and its by-

products might enhance the attractiveness and the palatability of the baits. Consequently several types of baits were prepared as follows:

(1) 10 per cent brewers yeast powder, 90 per cent Fox Chow

(2) 10 per cent vitamin B_1 by-product A (39RD 388) Merck and Co., 90 per cent Fox Chow

(3) 10 per cent vitamin B_1 by-product B (39RD 389) Merck and Co., 90 per cent Fox Chow

(4) 0.5 per cent thiamin, 99.5 per cent Fox Chow

(5) 100 per cent Fox Chow as control

Ten grams of each of the five foregoing baits were placed in four individual cages, each of which contained a well-fed male albino rat. The results are shown in Table 1.

The conclusion reached was that vitamin B_1 and its by-products were of no marked value as rat lures. This experiment also gives an indication as to the variation in dietary preferences of rats.

B. Sex Differences

The differences in toxicity of two red squill powders due to sex were determined by feeding at various levels to male and female rats on the basis of body weight.

When Reference Standard Red Squill of 1934 was fed to females only, the results, as shown in Table 2, were: 0/9 at 100 mg/Kg, 1/9 at 200 mg/Kg, 5/7 at 300 mg/Kg, 10/10 at 350 mg/Kg, and 10/10 at 400 mg/Kg. These results indicate that the toxic level of this

Table 1.-PALATABILITY TESTS ON VITAMIN B_1 BY-PRODUCTS AS RAT LURES

Rat weight	Bait I	Bait II	Bait III	Bait IV	Bait V
Grams	10% Dried Brewers Yeast Powder* 90% Purina Fox Chow	10% Vitamin B_1 By-product A(39RD 388) Merck 90% Fox Chow	10% Vitamin B_1 By-product B(39RD 389) Merck 90% Fox Chow	.5% Thiamin 99.5% Fox Chow	100% Fox Chow Control
	Amt. Eaten	Amt. Eaten	Amt. Eaten	Amt. Eaten	Amt. Eaten
	Grams	Grams	Grams	Grams	Grams
270	9.5	0	9.9**	9.6	0
300	.7	0	9.9**	3	0
170	5.8	0	.7	4.6	1.4
194	1.5	0	.5	3	3.7

*Dried Brewers Yeast Powder was used because of its similarity in odor to thiamin.

**Disagreeable volatile solvent which evaporated completely from this bait causing it to be quite palatable, probably accounts for amount eaten.

Table 2.-ASSAY OF STANDARD REFERENCE SQUILL OF 1934 ON FEMALE RATS

Level	Rat Weight	Squill Fed	Time for Squill to Cause Death	Remarks
mg/Kg	Grams	Milligrams	Hours	
100	130	13	-	Normal
100	100	10	-	Normal
100	120	12	-	Normal
100	202	20	-	Normal
100	140	14	-	Normal
100	172	17	-	Normal
100	144	14	-	Normal
100	170	17	-	Normal
100	122	12	-	Normal
200	180	36	-	Normal
200	166	33	-	Normal
200	186	37	-	Normal
200	154	31	-	Very ill
200	122	24	47	
200	162	32	-	Normal
200	98	20	-	Normal
200	154	31	-	Normal
200	108	22	-	Normal
300	140	42	24	
300	148	44	-	Normal
300	142	43	72	
300	128	38	-	Normal
300	136	41	24	
300	138	41	47	
300	188	56	24	
350	220	77	20	
350	108	38	20	
350	110	39	20	
350	108	38	20	
350	138	48	20	
350	92	32	20	
350	110	39	20	
350	112	39	20	
350	124	43	20	
350	100	35	96	
400	116	46	20	
400	118	47	20	
400	124	50	20	
400	232	93	20	
400	248	99	20	
400	142	57	20	
400	128	51	20	
400	120	48	20	
400	144	58	20	
400	106	42	20	

powder was 350 mg/Kg toward female albinos. Table 3 presents data obtained by feeding this squill to male rats. The results were as follows: 1/10 at 400 mg/Kg, 9/10 at 600 mg/Kg, 7/10 at 700 mg/Kg, and 9/10 at 800 mg/Kg. From these results, Reference Standard Squill of 1934 was considered to be 800 mg/Kg in toxicity for male rats. Figure 1 shows the comparative toxicity curves of Reference Standard Squill of 1934 toward male rats and toward female rats.

Two levels were used in determining the relative toxicity of red squill powder, Lot Sc. 3422 Number 130863, Penick and Co. toward male and female rats. At 300 mg/Kg the number of male rats killed was 0/5 and the number of females 4/5 at the end of this experiment, that is, 120 hours; however, the remaining female rat died in 146 hours. At 600 mg/Kg the number of males killed was 7/10 and the number of females 5/5. This data is shown in Table 4. Accordingly, the toxicity of this red squill powder to male rats may be calculated to be between 700 mg/Kg and 800 mg/Kg. Likewise, for female rats, the toxicity may be judged to be between 300 mg/Kg and 400 mg/Kg.

The above experiments show that Reference Standard Squill of 1934 is 2.27 times more toxic for female than it is for male rats; and that squill, Lot Sc. 3422 Number 130863 of Penick and Co. may be said to be about twice as toxic for females as it is for males. This work shows the fallacy of using female rats or rats of both sexes in an assay for red squill because of the greater resistance

Table 3.-ASSAY OF STANDARD REFERENCE SQUILL OF 1934 ON MALE RATS

Level	Rat Weight	Squill Fed	Time for Squill to Cause Death	Remarks
mg/Kg	Grams	Milligrams	Hours	
400	228	91	-	Normal
400	190	76	-	Normal
400	272	109	-	Normal
400	142	57	-	Normal
400	212	85	-	Normal
400	180	72	-	Normal
400	268	107	120	
400	242	97	-	Very ill
400	196	78	-	Normal
400	142	57	-	Normal
600	134	80	20	
600	148	89	20	
600	134	80	20	
600	122	73	96	
600	130	78	20	
600	198	119	-	Ill
600	134	80	120	
600	144	86	20	
600	334	200	120	
600	184	110	120	
700	192	134	-	Ill
700	160	112	-	Normal
700	288	201	-	Ill
700	120	84	44	
700	92	64	20	
700	152	106	96	
700	90	63	20	
700	206	144	20	
700	122	85	20	
700	158	111	20	
800	162	130	96	
800	140	112	44	
800	130	104	20	
800	264	211	20	
800	150	120	20	
800	392	314	20	
800	92	74	20	
800	114	91	20	
800	70	56	20	
800	134	107	-	Normal

Figure I.-TOXICITY CURVE FOR REFERENCE STANDARD RED SQUILL OF 1934 AFTER STORAGE FOR 5 YEARS

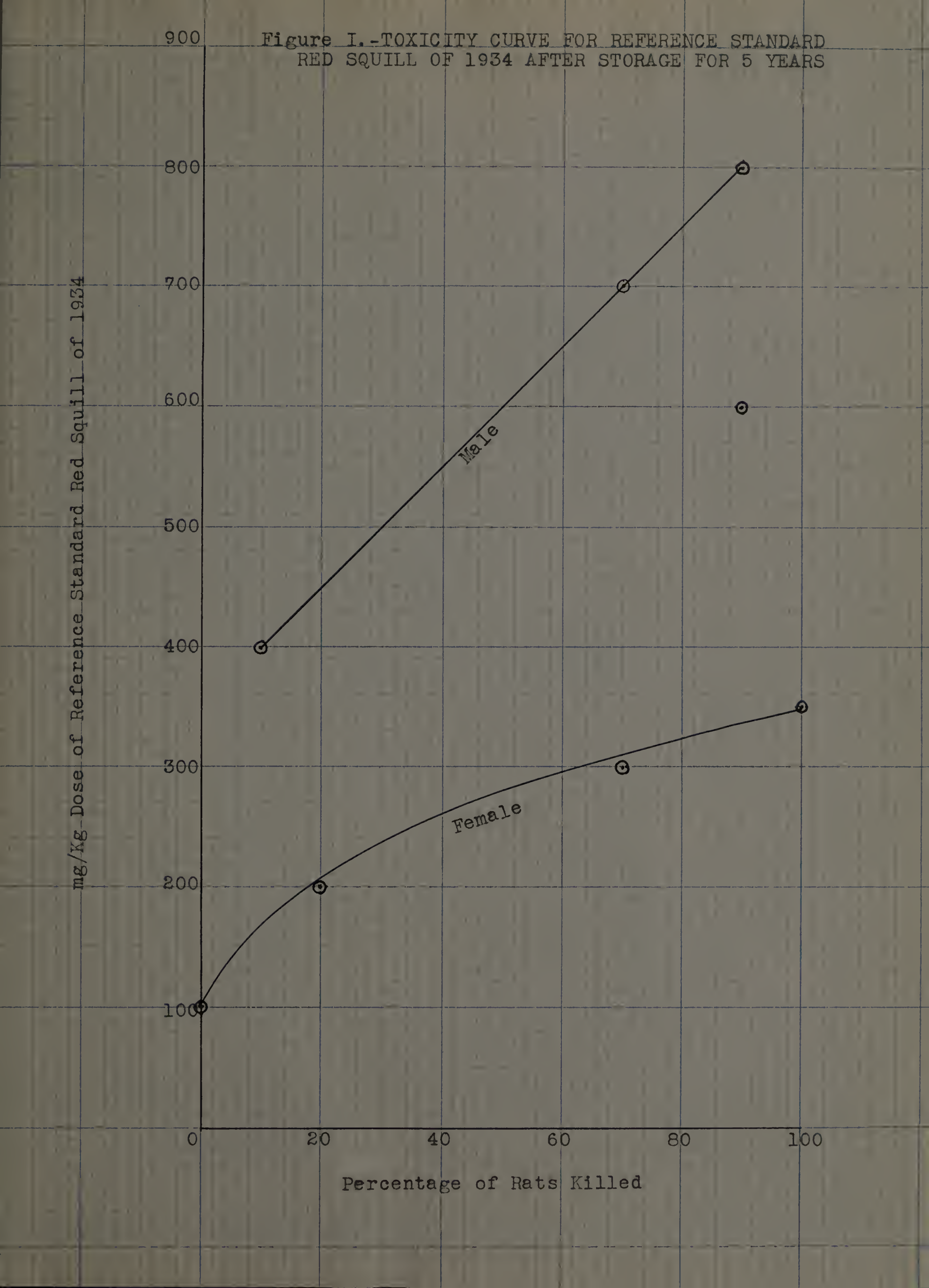

Table 4.-ASSAY OF A RED SQUILL POWDER, LOT SC. 3422, NO. 130863 OF PENICK & CO.

Level	Sex	Rat Weight	Squill Fed	Time for Squill to Cause Death	Remarks
mg/Kg		Grams	Milligrams	Hours	
300	M	94	28	-	Normal
300	M	152	46	-	Normal
300	M	142	43	-	Normal
300	M	108	32	-	Normal
300	M	120	36	-	Normal
300	F	118	35	24	
300	F	140	42	24	
300	F	235	71	9	
300	F	112	34	-	*
300	F	298	89	9	
600	M	138	83	9	
600	M	162	97	72	
600	M	128	77	-	Normal
600	M	150	90	-	Normal
600	M	92	57	24	
600	M	170	102	24	
600	M	106	64	48	
600	M	122	73	7	
600	M	200	120	-	Normal
600	M	156	94	7	
600	F	250	150	9	
600	F	140	84	9	
600	F	126	76	24	
600	F	186	112	9	
600	F	94	56	48	

*Died in 146 hours.

of male rats. For this reason a standard assay of red squill should be based on male rats.

Results of many workers in the field must now be taken cautiously as they are based on the erroneous fact that male and female rats are equally susceptible to red squill poisoning.

C. Susceptibility to Red Squill Poisoning

It is known that a tolerance is built up in many animals against continued doses of certain poisons. However, in some cases a poison is cumulative and subsequent doses of it produce dire results in animals. This question was raised in connection with red squill. If male rats are fed one-half their lethal dose of squill and then fed one-half their lethal dose again at a subsequent date about two weeks later, what will be the result? Will the rats refuse or accept a second dose of squill?

In this experiment 27 male albino rats, which had previously recovered from a 400 mg/Kg dose of Reference Standard Squill of 1934, were used. These rats were starved for 24 hours to try to insure the acceptance of the squill baits. The squill was mixed with about two grams of Fox Chow as carrier. One out of ten male albino rats which have never been fed red squill previously die when fed a 400 mg/Kg dose of Reference Standard Squill of 1934, as shown in Table 3. In this experiment 7/27 male rats died when fed a second 400 mg/Kg dose of squill, as shown in Table 5. These rats had recovered from the first 400 mg/Kg dose of Reference Standard Squill of 1934, which killed ten per cent of their brethren.

Table 5.-THE SUSCEPTIBILITY OF MALE ALBINO RATS TO A SECOND 400 MG/KG DOSE OF REFERENCE STANDARD RED SQUILL OF 1934

Rat Weight	Squill Fed	Time for Squill to Cause Death	Remarks
Grams	Milligrams	Hours	
164	66	-	Normal
158	63	-	Normal
160	64	24	
202	81	-	Normal
195	78	-	Normal
100	40	-	Normal
176	70	-	Normal
195	78	-	Very ill
82	33	8	
110	44	-	Normal
90	36	-	Normal
86	34	-	Normal
106	42	-	Normal
130	52	-	Normal
128	51	-	Normal
75	30	24	
110	44	24	
100	40	-	Normal
115	46	-	Normal
295	118	24	
228	93	-	Normal
234	94	-	Normal
90	36	-	Normal
90	36	24	
176	70	-	Normal
110	44	-	Normal
150	60	20	

This experiment indicates that male rats which have recovered from a sublethal dose of red squill are more susceptible to another sublethal dose of squill than are normal male albinos which have never before been fed squill. This may be due to the fact that the toxic principle of red squill is either cumulative or that the first dose of red squill weakened the animals. Therefore, they were more susceptible to the second sublethal dose of squill which was fed two weeks later. From this experiment it was also observed that when rats which previously had been fed red squill are properly prepared by starving for 24 hours, no trouble is encountered as far as acceptance of a bait containing a second dose of red squill is concerned.

It might also be mentioned that other investigators have found that large rats require a larger dose, that is, a larger number of milligrams per kilogram of body weight to cause death than small rats. Through a careful study of the tables in this work, this was not found to be the case, but rather that large rats require about the same dose in milligrams per kilogram as small rats to produce death.

D. Factors Affecting the Toxicity of Red Squill

a. Heat

Heat is destructive to many organic toxicologic substances. The differences in toxicity upon heating at various temperatures were determined by assaying the heat treated squill.

Reference Standard Squill of 1934, which has a toxicity of 800 mg/Kg toward male rats, was used in this work. This squill was spread in a thin layer on aluminum trays and heated in an electric oven. One hundred gram lots of this squill were used. The temperature to which the squill was heated was measured by means of a thermocouple which was placed in contact with the squill. The different portions of this red squill were heated for 30 minutes at the temperatures of 100° C. (212° F.), 125° C. (257° F.), 150° C. (302° F.), 175° C. (347° F.), 200° C. (392° F.), and 225° C. (437° F.). In every case there was a reduction in the weight of the squill caused by the heating process, due to loss of moisture accompanied in some cases by charring. To compensate for this loss of weight, a sufficient amount of powdered dextrose was added, as it is a neutral substance and has no effect on the toxicity of red squill. This was mixed with each heat treated portion of red squill to bring the weight to 100 grams. The color and relative distastefulness to a human subject are listed in Table 6. Subjection to high temperatures made the red squill less distasteful to men and darkened the color of the squill.

The toxicity determinations were performed using male rats which were starved for 24 hours, and then fed on an 800 mg/Kg level with the heat treated samples of squill in baits containing about two grams of Fox Chow as carrier.

Table 6.-COLOR AND TASTE TO HUMAN SUBJECT OF HEAT TREATED SAMPLES OF REFERENCE STANDARD RED SQUILL OF 1934

Squill Sample	Temperature of Heat Treatment °C.	°F.	Length of Heat Treatment Minutes	Color	*Relative Distastefulness
Control	-	-	-	Light Pink	6
A	100	212	30	Pink	5
B	125	257	30	Light Brown	4
C	150	302	30	Brown	3
D	175	347	30	Dark Brown	2
E	200	392	30	Black	1
F	225	437	30	Black	1

*Numbers range from least distasteful which is number 1 to most objectionable which is number 6.

This experiment indicates that the higher the temperature applied to the squill, the less toxic it becomes. When heated at 100° C. (212° F.) for one-half hour in the dry state in a hot air oven, the squill was only slightly changed in toxicity, while at 225° C. (437° F.) it was rendered non-toxic to male rats at the 800 mg/Kg feeding level. These results, given in Table 7, show that red squill is relatively heat stable. Table 8 sums up this data.

b. Moisture

The toxicity of red squill is often affected by the nature of the carrier bait. The amount of moisture in different types of baits might vary considerably. In order to determine the optimum amount of moisture in red squill baits, the following experiments were conducted.

The average amount of water ingested by normal female albino rats was determined. This was found to be 222 cc. per day per kilo of female rat, as shown in Table 9. Then the amount of water ingested by normal male albino rats was found to be 179 cc. per day per kilo of male rat as given in Table 10.

To determine the effect of moisture in red squill baits on the toxicity of the baits, male and female rats were starved and not given water for 12 hours. They then were fed a lethal dose of squill, which contained 5 per cent moisture, and enough Purina Fox Chow, which contained 8.46 per cent moisture, to make one per cent of their body weight. This squill bait was mixed with one-fourth,

Table 7.-THE EFFECT OF HEAT ON THE TOXICITY OF REFERENCE STANDARD RED SQUILL OF 1934 FED ON AN 800 MG/KG LEVEL TO MALE ALBINO RATS

Rat Weight	Temperature of Heat Treatment of Squill		Length of Heat Treatment of Squill	Squill Fed	Time for Squill to Cause Death	Remarks
Grams	°C.	°F.	Minutes	Milligrams	Hours	
164	100	212	30	131	48	
346	100	212	30	277	-	Normal
140	100	212	30	112	24	
184	100	212	30	147	68	
194	100	212	30	155	68	
292	100	212	30	234	-	Very ill
180	100	212	30	144	48	
232	100	212	30	186	-	Very ill
200	100	212	30	160	24	
170	100	212	30	136	24	
370	125	257	30	296	23	
82	125	257	30	66	-	Normal
82	125	257	30	66	48	
210	125	257	30	168	-	Normal
278	125	257	30	222	-	Ill
56	125	257	30	45	23	
104	125	257	30	83	-	Normal
60	125	257	30	48	23	
120	125	257	30	96	48	
62	125	257	30	50	23	
90	150	302	30	72	-	Normal
110	150	302	30	88	96	
364	150	302	30	291	-	Normal
86	150	302	30	69	-	Normal
180	150	302	30	144	-	Normal
80	150	302	30	64	-	Normal
86	150	302	30	69	-	Normal
254	150	302	30	203	-	Normal
188	150	302	30	150	-	Normal
100	150	302	30	80	-	Normal
86	175	347	30	69	-	Normal
276	175	347	30	221	-	Normal
90	175	347	30	72	120	
70	175	347	30	56	96	
90	175	347	30	72	-	Normal
104	175	347	30	83	-	Normal
80	175	347	30	64	-	Normal
86	175	347	30	69	96	
108	175	347	30	86	-	Normal
84	175	347	30	67	-	Normal

Table 7.-continued

Rat Weight	Temperature of Heat Treatment of Squill		Length of Heat Treatment of Squill	Squill Fed	Time for Squill to Cause Death	Remarks
Grams	°C.	°F.	Minutes	Milligrams	Hours	
70	200	392	30	56	-	Normal
72	200	392	30	58	-	Normal
86	200	392	30	69	-	Normal
68	200	392	30	54	-	Normal
72	200	392	30	58	120	
106	200	392	30	85	-	Normal
64	200	392	30	51	23	
76	200	392	30	61	-	Normal
70	200	392	30	56	96	
210	225	437	30	168	-	Normal
138	225	437	30	108	-	Normal
246	225	437	30	197	-	Normal
150	225	437	30	120	-	Normal
130	225	437	30	104	-	Normal
150	225	437	30	120	-	Normal
138	225	437	30	110	-	Normal
100	225	437	30	80	-	Normal
132	225	437	30	106	-	Normal

Table 8.-SUMMARY TABLE-THE EFFECT OF HEAT ON THE TOXICITY OF REFERENCE STANDARD RED SQUILL OF 1934 TOWARD MALE ALBINO RATS

Temperature of Heat Treatment of Squill		Length of Heat Treatment	Level	Number of Rats Killed	Remarks
°C.	°F.	Minutes	mg/Kg		
-	-	-	800	9/10	Control
100	212	30	800	7/10	Two rats very ill
125	257	30	800	6/10	One rat ill
150	302	30	800	1/10	
175	347	30	800	3/10	
200	392	30	800	3/10	
225	437	30	800	0/10	

Table 9.-DETERMINATION OF THE AMOUNT OF WATER INGESTED BY NORMAL FEMALE ALBINO RATS

Rat Weight	Time Animals Observed	Water Ingested
Grams	Hours	cc.
144	86	150
168	86	110
182	86	150
182	86	130
Sum 676		Sum 540

The average amount of water ingested per day per kilo of female rat is 222 cc.

Table 10.-DETERMINATION OF THE AMOUNT OF WATER INGESTED BY NORMAL MALE ALBINO RATS

Rat Weight	Time Animals Observed	Water Ingested
Grams	Hours	cc.
264	93	200
170	93	120
214	93	130
Sum 648		Sum 450

The average amount of water ingested per day per kilo of male rat is 179 cc.

one-half, three-fourths, all of the daily requirement of water, and no water at all. Twelve hours after this feeding, water and Fox Chow were placed in the cages, and the cups containing the squill baits were removed. All uneated squill baits were dried in a hot air dryer to remove the water present. After drying, they were allowed to remain exposed to the atmosphere for a week so that they would absorb their normal content of moisture in order to lessen a possible error due to overdrying of the baits. They were then weighed and the weight was recorded. It should be remembered that in every case in which bait was not eaten, except in cases where no water was mixed with bait, there was water present in the bait, but it could not be conveniently measured. The female rats were fed on a 350 mg/Kg level of this squill, and the male rats on an 800 mg/Kg level of this squill. The results of this experiment are shown in Table 11.

Another experiment of the same type was carried out to determine the effect of the amount of water ingested along with a dry squill bait on the toxicity of the dry red squill bait to rats. Fox Chow was used as the carrier for the red squill in the bait. In this experiment, however, instead of mixing the water with the bait, the water and the bait were placed in separate cups. Twelve hours later the feed cups were removed, the amount of water and bait remaining in them was recorded, and the rats given their regular feed and water. The results are shown in Table 12.

Table 11.-INFLUENCE OF WATER IN REFERENCE STANDARD RED SQUILL OF 1934 BAITS ON THE TOXICITY OF THE BAITS TO MALE ALBINO RATS FED AT AN 800 MG/KG LEVEL, AND FEMALES FED AT A 350 MG/KG LEVEL

Rat Wt.	Sex	Bait Fed	Squill Fed	Amount of Water Offered		Weight of Uneaten Bait	Time for Squill to Cause Death	Remarks
Gms.		Gms.	Mgs.	cc.	% of daily requirement	Gms.	Hours	
206	M	2.06	165	0	0	-	12	
136	M	1.36	109	0	0	-	12	
182	M	1.82	147	0	0	-	12	
208	M	2.08	166	0	0	-	12	
332	M	3.32	266	0	0	3.12	-	Normal
132	F	1.32	46	0	0	-	12	
122	F	1.22	43	0	0	-	12	
152	F	1.52	53	0	0	-	12	
100	F	1.00	35	0	0	-	12	
132	F	1.32	46	0	0	-	12	
254	M	2.54	203	11.8	25	-	12	
146	M	1.46	117	6.8	25	-	69	
162	M	1.62	130	7.5	25	-	12	
154	M	1.54	123	6.9	25	-	20	
180	M	1.80	144	8.1	25	-	12	
130	F	1.30	46	7.2	25	-	12	
100	F	1.00	35	5.5	25	-	12	
146	F	1.46	51	8.0	25	-	59	
160	F	1.60	56	8.8	25	-	12	
120	F	1.20	42	6.6	25	-	12	
160	M	1.60	128	14.4	50	-	12	
152	M	1.52	122	13.7	50	-	12	
184	M	1.84	147	16.6	50	-	12	
160	M	1.60	128	14.4	50	-	12	
208	M	2.08	166	18.7	50	.64	12	
170	F	1.70	60	18.7	50	1.28	12	
194	F	1.94	68	21.4	50	1.18	12	
136	F	1.36	48	15.0	50	1.14	-	Normal
124	F	1.24	43	13.6	50	-	12	
150	F	1.50	53	16.5	50	-	12	
134	M	1.34	107	18.1	75	-	12	
184	M	1.84	147	24.8	75	1.24	-	Normal
156	M	1.56	125	21.1	75	1.12	12	
126	M	1.26	101	17.0	75	-	12	
146	M	1.46	117	19.7	75	-	59	
150	F	1.50	53	24.8	75	1.22	-	Normal
140	F	1.40	49	23.2	75	1.06	12	
150	F	1.50	53	24.8	75	1.22	-	Normal
130	F	1.30	46	21.5	75	.82	69	
174	F	1.74	61	28.7	75	.89	-	Normal

Table 11.-INFLUENCE OF WATER IN REFERENCE STANDARD RED SQUILL OF 1934 BAITS ON THE TOXICITY OF THE BAITS TO MALE ALBINO RATS FED AT AN 800 MG/KG LEVEL, AND FEMALES FED AT A 350 MG/KG LEVEL (cont.)

Rat Wt.	Sex	Bait Fed	Squill Fed	Amount of Water Offered		Weight of Uneaten Bait	Time for Squill to Cause Death	Remarks
Gms.		Gms.	Mgs.	cc.	% of daily requirement	Gms.	Hours	
164	M	1.64	131	29.5	100	.80	12	
126	M	1.26	101	22.7	100	.92	69	
168	M	1.68	134	30.3	100	1.20	-	Normal
134	M	1.34	107	24.1	100	.80	12	
130	M	1.30	104	23.4	100	.62	-	Normal
134	F	1.34	47	29.5	100	1.14	-	Normal
120	F	1.20	42	26.4	100	.96	-	Normal
160	F	1.60	56	35.2	100	.78	-	Normal
112	F	1.12	39	24.6	100	.44	12	
160	F	1.60	56	35.2	100	1.34	-	Normal

The conclusions reached from the former experiment are that a dry red squill bait has greater acceptability, but the moisture in a bait has no relationship to the toxicity of the bait.

The indications garnered from the latter experiment are that rats which have been fed red squill baits drink less water than normal rats. Also, if dry red squill baits are offered to white rats, they are more readily taken; that is, are more palatable to rats, hence more toxic as more of the bait is eaten. However, the toxicity is not increased per gram of bait in dry condition. The amount of water taken along with the dry red squill bait seems to bear no relationship whatsoever to the killing power of the bait. This experiment also indicates that the popular idea that wild rats seek water and come out in the open when poisoned with red squill has no basis.

c. Fats

It is known that fats and oils have a retarding effect on the absorption of certain poisons and a lessening effect on the toxicity of these poisons to animals due to a purely physical action of the fats and oils on the poisons and the hosts. Whether or not red squill was so affected was the question raised.

Hydrogenated fat (Crisco) and petrolatum were used in experiments to determine the effect of fat on the toxicity of red squill to rats. Each rat was fed one per cent of its body weight of a

Table 12.-THE EFFECT OF WATER TAKEN WITH A DRY REFERENCE STANDARD SQUILL OF 1934 BAIT ON THE TOXICITY OF THE BAIT TO MALE RATS FED AT AN 800 MG/KG LEVEL AND FEMALES FED AT A 350 MG/KG LEVEL

Rat Wt.	Sex	Bait Fed	Squill Fed	Amount of Water Offered		Amount of Water Left	Time for Squill to Cause Death	Remarks
Gms.		Gms.	Mgs.	cc.	% of daily requirement	cc.	Hours	
224	M	2.24	179	0	0	-	-	Normal
104	M	1.04	83	0	0	-	-	Normal
350	M	3.50	243	0	0	-	-	*Normal
114	M	1.14	91	0	0	-	24	
170	M	1.70	136	0	0	-	120	
290	F	2.90	102	0	0	-	16	
170	F	1.70	60	0	0	-	12	
140	F	1.40	49	0	0	-	12	
160	F	1.60	56	0	0	-	12	
150	F	1.50	53	0	0	-	12	
94	M	.94	75	4.2	25	-	108	
270	M	2.70	216	12.1	25	-	-	Normal
260	M	2.60	208	11.5	25	-	34	
290	M	2.90	232	10.6	25	-	-	Normal
170	M	1.70	136	7.5	25	-	-	Normal
170	F	1.70	60	9.4	25	-	12	
154	F	1.54	54	8.5	25	7.5	12	
186	F	1.86	65	10.2	25	-	12	
170	F	1.70	60	9.4	25	-	12	
132	F	1.32	46	7.4	25	-	12	
88	M	.88	72	7.9	50	4	12	
160	M	1.60	108	14.4	50	7	12	
190	M	1.90	152	17.1	50	-	16	
120	M	1.20	96	10.8	50	-	12	
210	M	2.10	168	18.9	50	10	-	Normal
150	F	1.50	53	16.5	50	-	12	
136	F	1.36	48	15.0	50	4	12	
136	F	1.36	48	15.0	50	-	20	
120	F	1.20	32	13.2	50	5	-	Normal
170	F	1.70	60	18.7	50	-	20	
144	M	1.44	115	19.4	75	15	-	Normal
210	M	2.10	168	28.3	75	18	34	
190	M	1.90	152	25.6	75	19	-	Ill
174	M	1.74	139	23.5	75	15	12	
146	M	1.46	117	19.7	75	15	-	Ill
152	F	1.52	53	25.0	75	18	12	
180	F	1.80	63	29.7	75	21	12	
150	F	1.50	53	24.7	75	-	108	
124	F	1.24	43	20.5	75	10	12	
112	F	1.12	39	18.6	75	14	12	

Table 12.-THE EFFECT OF WATER TAKEN WITH A DRY REFERENCE STANDARD SQUILL OF 1934 BAIT ON THE TOXICITY OF THE BAIT TO MALE RATS FED AT AN 800 MG/KG LEVEL AND FEMALES FED AT A 350 MG/KG LEVEL (Cont.)

Rat Wt.	Sex	Bait Fed	Squill Fed	Amount of Water Offered		Amount of Water Left	Time for Squill to Cause Death	Remarks
Gms.		Gms.	Mgs.	cc.	% of daily requirement	cc.	Hours	
170	M	1.70	136	30.6	100	25	-	Very ill
160	M	1.60	128	28.8	100	21	70	
80	M	.80	64	14.4	100	6	12	
96	M	.96	77	17.3	100	-	12	
118	M	1.18	94	21.2	100	19	-	Very ill
230	F	2.30	71	50.6	100	33	12	
184	F	1.84	62	40.5	100	33	-	Normal
126	F	1.26	44	27.7	100	23	12	
184	F	1.84	62	40.5	100	30	12	
170	F	1.70	60	37.4	100	33	12	

*Weight of uneaten bait equals 3.3 grams.

bait consisting of a known amount of red squill, a known percentage of fat, and Fox Chow, when necessary, to make 100 per cent of bait. The squill used was Reference Standard Squill of 1934 which has a toxicity of 350 mg/Kg when fed to female rats and 800 mg/Kg toward male albino rats.

Hydrogenated fat combined with 350 mg/Kg dose of red squill was fed to female rats with the following results: with 10 per cent Crisco in bait 7/10 died, and with 25 per cent Crisco 8/10 died. This data is shown in Table 13.

Female rats were also fed hydrogenated fat at the 400 mg/Kg level (50 mg/Kg more than the necessary lethal dose in a non-fat bait) with the following results as shown in Table 14: 3/3 died with 10 per cent Crisco in bait, 4/4 died with 75 per cent Crisco in bait, and 3/4 with 96 per cent Crisco in bait.

Table 15 shows the results obtained when a 10 per cent hydrogenated fat bait, combined with an 800 mg/Kg dose of Reference Standard Red Squill of 1934, was fed to male albino rats. Seven out of ten male rats were killed by this bait.

In feedings of Petrolatum, U.S.P., to female rats, following the same procedure as for Crisco feedings and using a 350 mg/Kg dose of Reference Standard Red Squill of 1934, the number of females killed by a bait containing 10 per cent petrolatum was 7/10 and by a bait containing 25 per cent petrolatum was 6/10 as shown in Table 16.

Table 13.-THE EFFECT OF HYDROGENATED FAT ON THE TOXIC ACTION OF REFERENCE STANDARD RED SQUILL OF 1934 FED TO FEMALE ALBINO RATS AT A LEVEL OF 350 MG/KG

Fat in Bait	Rat Weight	Bait Fed	Squill Fed	Time for Squill to Cause Death	Remarks
Percent	Grams	Grams	Milligrams	Hours	
10	100	1.00	35	16	
10	142	1.42	50	28	
10	160	1.60	56	16	
10	114	1.14	40	-	Ill
10	142	1.42	50	16	
10	150	1.50	53	16	
10	90	.90	32	-	Ill
10	152	1.52	53	16	
10	110	1.10	39	28	
10	88	.88	30	-	Normal
25	134	1.34	47	88	
25	110	1.10	39	-	Ill
25	122	1.22	43	16	
25	110	1.10	39	16	
25	110	1.10	39	28	
25	154	1.54	54	-	Ill
25	126	1.26	44	16	
25	130	1.30	46	16	
25	118	1.18	42	16	
25	148	1.48	52	28	

Table 14.-THE EFFECT OF HYDROGENATED FAT ON THE TOXIC ACTION OF REFERENCE STANDARD SQUILL OF 1934 FED TO FEMALE RATS AT A 400 MG/KG LEVEL

Fat in Bait	Rat Weight	Bait Fed	Squill Fed	Time for Squill to Cause Death	Remarks
Percent	Grams	Grams	Milligrams	Hours	
10	222	2.22	89	20	
10	286	2.86	114	20	
10	248	2.48	99	20	
75	222	2.22	89	20	
75	242	2.42	97	20	
75	218	2.18	87	20	
75	162	1.62	65	20	
96	232	2.32	93	-	Normal
96	158	1.58	63	20	
96	214	2.14	86	20	
96	238	2.38	95	20	

Table 15.-THE EFFECT OF HYDROGENATED FAT ON THE TOXIC ACTION OF REFERENCE STANDARD SQUILL OF 1934 TO MALE RATS FED AT AN 800 MG/KG LEVEL

Fat in Bait	Rat Weight	Bait Fed	Squill Fed	Time for Squill to Cause Death	Remarks
Per cent	Grams	Grams	Milligrams	Hours	
10	180	1.80	144	67	
10	160	1.60	128	92	
10	200	2.00	160	8	
10	210	2.10	168	18	
10	174	1.74	139	18	
10	100	1.00	80	-	Normal
10	200	2.00	160	-	Normal
10	80	.80	64	18	
10	110	1.10	88	-	Normal
10	200	2.00	160	8	

Control rats were used at all percentages of Crisco and petrolatum. These were fed the required percentage of fat and enough Fox Chow to make 100 per cent of the bait. Rats were fed one per cent of their body weights of these control baits. The control rats evidenced no harmful effects from these control baits.

It is seen from the foregoing data that fat lessens the toxicity of red squill. In general, it should be noted that the concentration of fat in the bait does not seem to be of too great importance as far as the amount of decrease in the toxicity of the squill is concerned. However, in one or two cases, the indication is that the more fat present in a red squill bait the less toxic is the bait, provided, of course, that the dose of squill remains constant. In the case of Crisco baits fed to female rats, the fact that an increase in the dose of red squill from 350 mg/Kg to 400 mg/Kg overcomes any disadvantages in killing power that the amount of Crisco in the bait might exert, is interesting. Table 17 summarizes this experiment.

d. Pectin

Pectin acts as a detoxifying agent because of its physical character and because on being broken down in the organism it gives rise to galacturonic acid. This acid is capable of forming conjugation products with toxic materials in the same manner as glucuronic acid. Might pectin decrease the toxicity of red squill?

Table 16.-THE EFFECT OF PETROLATUM ON THE TOXIC ACTION OF REFERENCE STANDARD SQUILL OF 1934 TO FEMALE ALBINO RATS FED AT A 350 MG/KG LEVEL

Petrolatum Fed	Rat Weight	Bait Fed	Squill Fed	Time for Squill to Cause Death	Remarks
Percent	Grams	Grams	Milligrams	Hours	
10	120	1.20	42	88	
10	134	1.34	47	16	
10	100	1.00	35	16	
10	188	1.88	66	45	
10	110	1.10	39	-	Very ill
10	112	1.12	39	16	
10	112	1.12	39	-	Very ill
10	132	1.32	46	16	
10	132	1.32	29	40	
10	84	.84	29	-	Very ill
25	158	1.58	55	16	
25	134	1.34	47	16	
25	166	1.66	51	16	
25	120	1.20	42	28	
25	146	1.46	51	-	Very ill
25	108	1.08	38	-	Normal
25	116	1.16	41	-	Normal
25	150	1.50	53	16	
25	198	1.98	70	16	
25	144	1.44	51	-	Ill

Table 17.-SUMMARY TABLE-THE EFFECT OF FATS ON THE TOXIC ACTION OF REFERENCE STANDARD RED SQUILL OF 1934 TOWARD ALBINO RATS

Type of Fat	Fat in Bait	Red Squill Feeding Level	Rat Sex	Number of Rats Killed	Remarks
	Percent	mg/Kg			
Control (No Crisco) -		350	Female	10/10	
Crisco	10	350	Female	7/10	Two rats ill
Crisco	25	350	Female	8/10	Two rats ill
Control (No Crisco) -		400	Female	10/10	
Crisco	10	400	Female	3/3	
Crisco	75	400	Female	4/4	
Crisco	96	400	Female	3/4	
Control (No Crisco) -		800	Male	9/10	
Crisco	10	800	Male	7/10	
Control (No petrolatum) -		350	Female	10/10	
Petrolatum	10	350	Female	7/10	Three rats very ill
Petrolatum	25	350	Female	6/10	Two rats ill

Baits were prepared containing 10 per cent of pectin, 50 per cent of pectin, and 96 per cent of pectin. These baits contained 8 per cent of Reference Standard Red Squill and enough Fox Chow to make 100 per cent. They were so prepared that when fed in the proportion of one per cent of a rat's body weight, they would contain an 800 mg/Kg dose of squill along with the desired percentage of pectin and Fox Chow as carrier. Male albino rats were used.

An 800 mg/Kg dose of Reference Standard Red Squill will kill 9/10 males when fed with Fox Chow as carrier. When male rats were fed an 800 mg/Kg dose of the squill in a bait containing 10 per cent pectin, 7/10 died. When 50 per cent pectin was fed in a like bait, 3/8 died. Ten rats were used on the 50 per cent pectin bait, but as two rats refused to take the bait they were not considered in the experiment. On a 96 per cent pectin bait, combined with red squill, 4/5 rats died, but as ten rats were used on this experiment, five rats refusing bait and five rats not taking the complete amount of bait, this latter experiment will only be considered in light of the unpalatability of pectin to rats rather than as an experiment showing the toxicity lessening power of pectin. The results are shown in Table 18.

This experiment shows that pectin has a detoxifying action on red squill. The more pectin in a red squill bait, the less toxic will the bait be to rats. Also that pectin in large concentrations

Table 18.-THE EFFECT OF PECTIN ON THE TOXICITY OF REFERENCE STANDARD RED SQUILL BAITS WHEN FED TO MALE ALBINO RATS ON AN 800 MG/KG LEVEL

Rat Weight	Pectin Fed	Bait Fed	Squill Fed	Time for Squill to Cause Death	Remarks
Grams	Percent	Grams	Milligrams	Hours	
240	10	2.40	192	16	
166	10	1.66	133	-	Normal
112	10	1.12	90	16	
166	10	1.66	133	-	Normal
156	10	1.56	125	16	
236	10	2.36	189	-	Normal
134	10	1.34	107	16	
110	10	1.10	88	16	
100	10	1.00	80	16	
170	10	1.70	136	16	
160	50	1.60	128	-	Normal
210	50	2.10	168	-	*Normal
220	50	2.20	176	-	Normal
160	50	1.60	128	24	
120	50	1.20	96	24	
170	50	1.70	136	-	*Normal
190	50	1.90	152	48	
210	50	2.10	168	-	Normal
180	50	1.80	144	-	Normal
150	50	1.50	120	-	Normal
140	96	1.40	112	-	*Normal
210	96	2.10	168	-	*Normal
100	96	1.00	80	**20	
130	96	1.30	104	-	*Normal
166	96	1.66	133	-	*Normal
180	96	1.80	144	-	**Normal
120	96	1.20	96	**48	
120	96	1.20	96	-	*Normal
110	96	1.10	88	**48	
100	96	1.00	80	**48	

*Bait was refused.

**Bait was partially eaten.

is distasteful to rats.

e. Storage

Workers in the field are unanimous in their observations that red squill powders do not deteriorate to any extent on storage. This observation was also borne out by the following experiment: Reference Standard Red Squill of 1934, also known as B-SS1 by Buck (1936), was examined by him in 1934 and found to kill 7/10 rats at 400 mg/Kg level and 8/8 rats at 500 mg/Kg level. White rats of both sexes were used in his assays. This red squill powder was stored in slip-covered tin cans for five years and then reexamined to determine if there was any change in toxicity due to this long storage period. When examined in 1939, Reference Standard Red Squill of 1934 was found to have a toxicity of 350 mg/Kg toward female rats and 800 mg/Kg toward males. As Buck's finding using mixed sexes is about a mean between the results for males and females of this assay, it was concluded that there was no marked change in squill toxicity.

The conclusion reached was that Reference Standard Red Squill of 1934 shows no noticeable variation in toxicity after a five year storage period in slip-covered tin cans.

f. Diet

The action of poisons is sometimes affected by diet. The question was raised whether or not dietary factors might in some way affect the toxicity of red squill. Preliminary experiments

showed that fat in red squill baits lessens the toxicity of the baits. In feeding baits containing 92 per cent protein (casein) and 8 per cent of Reference Standard Red Squill of 1934 (800 mg/Kg dose) to male rats in amounts equal to one per cent of their body weights, there was no change in red squill toxicity (Table 19). Carbohydrate (corn starch) was fed in like manner with identical results (Table 20).

In an attempt to answer the question as to whether the type of diet has any effect on the killing power of red squill, diets were prepared as follows:

Diet I - Low protein, high carbohydrate diet, consisting of 7 per cent protein, 73 per cent carbohydrate, 11 per cent fat.

Diet II - High protein, low carbohydrate diet consisting of 45 per cent protein, 35 per cent carbohydrate, 11 per cent fat.

Diet III - Low protein, high fat diet, consisting of 7 per cent protein, 37 per cent fat, 47 per cent carbohydrate.

Diet IV - The control diet, Fox Chow, consisting of 20 per cent protein, 3 per cent fat, 46 per cent carbohydrate.

The composition of these experimental diets is shown in Table 21. The rats used were normal male albino rats which had been

Table 19.-THE EFFECT OF PROTEIN (CASEIN) ON THE TOXICITY OF REFERENCE STANDARD RED SQUILL OF 1934 TOWARD MALE ALBINO RATS FED ON AN 800 MG/KG LEVEL

Rat Weight	Bait Fed	Squill Fed	Time for Squill to Cause Death	Remarks
Grams	Grams	Milligrams	Hours	
170	1.70	136	20	
200	2.00	160	20	
164	1.64	131	20	
170	1.70	136	65	
150	1.50	120	20	
170	1.70	136	20	
160	1.60	128	20	
190	1.90	152	65	
190	1.90	152	-	Normal
170	1.70	136	20	

Baits consisted of 92 per cent Protein (Casein) and 8 per cent Squill.

Table 20.-THE EFFECT OF CARBOHYDRATE (CORN STARCH) ON THE TOXICITY OF REFERENCE STANDARD RED SQUILL OF 1934 TOWARD MALE ALBINO RATS FED ON AN 800 MG/KG LEVEL

Rat Weight	Bait Fed	Squill Fed	Time for Squill to Cause Death	Remarks
Grams	Grams	Milligrams	Hours	
222	2.22	178	20	
144	1.44	115	20	
184	1.84	147	20	
188	1.88	150	20	
180	1.80	144	20	
170	1.70	136	7	
176	1.76	141	20	
210	2.10	168	-	Normal
124	1.24	99	7	
130	1.30	104	20	

Baits consisted of 92 per cent Carbohydrate (Corn Starch) and 8 per cent Squill.

Table 21.-PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS

Diet Number	I	II	III	*IV	
Commercial casein	7	45	7	Protein (crude)	20
Corn starch	73	35	47	Nitrogen free extract	46
Crisco	9	9	35	Fat (crude)	3
Cod liver oil	2	2	2	Crude fiber	6
Dried brewers yeast	5	5	5	Also contains vitamins and minerals	
Osborne & Mendel salt mixture	4	4	4		

*Guaranteed analysis of Purina Fox Chow.

maintained on a diet of Purina Fox Chow. Rat weights ranged from 100 to 320 grams. Four groups of rats were fed the various diets over a period of one month. They then were left without food for 24 hours, after which they were fed an 800 mg/Kg dose of Reference Standard Red Squill of 1934 in enough Fox Chow to equal one per cent of the rat's body weight. This squill has a toxicity of 800 mg/Kg toward male rats. Rats were observed for 120 hours. Fox Chow was used as the carrier rather than the experimental diets in order to minimize the physical interference which the diets might have on the toxicity of the squill.

The results shown in Table 22 are described in the following paragraphs:

Diet I - Ten rats were used in this experiment. One rat died at the end of 10 days on this diet, cause unknown, and, therefore, was not considered in the results. These rats appeared normal after 30 days on this diet. When fed an 800 mg/Kg dose of Reference Standard Red Squill of 1934, 8/9 died, indicating that this diet has no influence on the toxicity of red squill.

Diet II - Ten rats were used. These rats appeared normal after 30 days of this diet. They were then fed an 800 mg/Kg dose of Reference Standard Red Squill of 1934. 2/10 rats died, indicating that this diet has a profound lessening influence on the toxicity of red squill.

Diet III - In this group there were 10 rats. These rats had a

Table 22.-THE EFFECT OF PREVIOUS DIET ON THE TOXICITY OF REFERENCE STANDARD RED SQUILL OF 1934 FED IN AN 800 MG/KG DOSE TO MALE RATS

Rat Weight	Diet Fed for 30 Days Previous to Squill Feeding	Bait Fed	Squill Fed	Time for Squill to Cause Death	Remarks
Grams		Grams	Milligrams	Hours	
	Low Protein, High Carbohydrate				
194	"	1.94	155	24	
194	"	1.94	155	24	
100	"	1.00	80	24	
180	"	1.80	144	30	
180	"	1.80	144	24	
200	"	2.00	160	-	Normal
220	"	2.20	176	30	
170	"	1.70	136	46	
130	"	1.30	104	24	
	High Protein, Low Carbohydrate				
212	"	2.12	170	-	Normal
300	"	3.00	240	-	Normal
230	"	2.30	184	46	
320	"	3.20	256	-	Normal
200	"	2.00	160	-	Normal
222	"	2.22	178	-	Normal
212	"	2.12	170	-	Normal
220	"	2.20	176	-	Normal
246	"	2.46	197	46	
252	"	2.52	202	-	Normal
	Low Protein, High Fat				
160	"	1.60	128	-	Normal
194	"	1.94	155	24	
184	"	1.84	147	24	
150	"	1.50	120	46	
140	"	1.40	112	52	
200	"	2.00	160	24	
210	"	2.10	168	24	
180	"	1.80	144	-	Normal
220	"	2.20	176	-	Normal
144	"	1.44	115	96	
	Control (Purina Fox Chow)				
216	"	2.16	173	-	Normal
250	"	2.50	200	24	
250	"	2.50	200	116	
200	"	2.00	160	24	
200	"	2.00	160	24	
184	"	1.84	147	24	
220	"	2.20	176	24	
208	"	2.08	166	24	
230	"	2.30	184	24	

greased appearance, but otherwise appeared normal. When fed an 800 mg/Kg dose of Reference Standard Red Squill of 1934, 7/10 rats died, indicating that this diet has a very slight effect on toxicity of red squill. This slight effect is due perhaps to the amount of fat remaining in the alimentary tract of the rats because of the high fat diet before being fed the red squill bait in spite of the fact that they were without food for 24 hours. In a previous experiment (page 21) it was shown that fat has a lessening effect on the toxicity of red squill.

Diet IV - Ten rats were placed on this control diet. One rat died from causes unknown at the end of 29 days, and was not considered in this experiment. All rats appeared normal at the end of 30 days. On being fed an 800 mg/Kg dose of Reference Standard Red Squill of 1934, 8/9 died, checking the fact that this squill was about 800 mg/Kg in toxicity toward male rats.

Experiments made on rats indicate that the toxicity of red squill is affected by dietary factors. It is to be noted first that one-bait feedings of 92 per cent protein and 92 per cent carbohydrate with a lethal dose of squill have no effect on the toxicity of the squill; while one-bait feedings of various percentages of fat in like manner cause a decrease in the toxicity of squill. High carbohydrate, low protein, and high fat, low protein diets when fed to rats for 30 days seem to have no effect on the toxicity of red squill fed on the 31st day. However, a high protein,

low carbohydrate diet when fed in like manner has a marked effect in lessening the toxicity of red squill.

The reason for the important role of protein in the diet on the toxicity of red squill is unknown. It may be due to raised tissue immunity toward red squill caused by the high protein diet, or to the combination of the poison with degradation products of protein present in the rat due to the diet.

V. SUMMARY AND CONCLUSIONS

1. The literature concerning red squill has been reviewed.
2. Vitamin B_1 and its by-products are of no marked value as rat lures.
3. Red squill is about twice as toxic for males as it is for female rats. It is recommended that standard assays of red squill should be based on male rats rather than female rats, or rats of mixed sexes.
4. Rats which have recovered from a sublethal dose of red squill are more susceptible to another sublethal dose of squill than are normal rats which have not been fed squill previously.
5. Rats which have been fed one dose of squill and have recovered can be induced to take another dose of squill by starving them for 24 hours.
6. Large rats require about the same lethal dose in milligrams per kilogram as small rats.
7. The higher the dry air temperature (above 100° C.) applied to dried red squill, the less toxic it becomes.
8. Dry red squill baits are more palatable than wet ones.
9. The amount of moisture taken with or in a red squill bait bears no relationship to the toxicity per gram of dry matter in the bait.
10. Rats which have been fed red squill drink less water than normal rats.
11. Considerable fat in red squill baits slightly lessens the

toxicity of the squill.

12. Foods containing large amounts of pectin are distasteful to rats. Pectin has a detoxifying action on red squill. The more pectin in a red squill bait, the less toxic will the bait be to rats.
13. The toxicity of red squill powders is not decreased noticeably by long storage.
14. Large percentages of protein or carbohydrates in squill baits do not affect the toxicity of the baits.
15. Low protein diets containing (1) high fat and (2) high carbohydrate when fed to rats for 30 days have no effect on the toxicity of red squill. However, a high protein-low carbohydrate diet when fed in like manner has a marked effect in lessening the toxicity of red squill baits.

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