Red Squill, VIII. Further Notes on Bioassay Methods*

By Justus C. Ward, D. Glen Crabtree and F. E. Garlough

For some years red squill has been used in the United States in rat-control projects. As it is a crude plant product, it is subject to the variations inherent in such a material. Many workers have recognized the fact that red squill will differ from time to time and from sample to sample in its rat-killing ability. This uncertainty in squill action has led to several attempts to introduce adequate bioassays at many separate laboratories in the country. Some years ago comparative tests were run with the same red squill powders at three laboratories and significant differences in results were obtained. These were discussed in a paper by J. C. Munch, *et al.* (1). For obvious reasons a series of studies has been carried out to determine the influence of various controllable factors on the toxicity of red squill. The first paper detailing some results of these experiments (2) showed that male rats are more than twice as resistant to this poison as are female rats and proved that this is due to the presence of the male hormone, testosterone. The second (3), which has been submitted for publication, shows the effect of altitude on the toxicity of the red squill powder, proving that there is a direct relationship between the two—the higher the altitude, the higher the toxicity.

Table I.—Influence of the Method of Preparing Bioassay Bait on Red Squill Toxicity

Test *A*. The red squill powder was weighed for each rat individually and then mixed with bait in quantity sufficient to make the total food offered each rat just 1 per cent of its body weight.

Rat No.[a]	Sex	Wt., Gm.	Dose, mg./Kg.[a]	Result	Rat No.	Sex	Wt., Gm.	Dose, mg./Kg.[a]	Result
1	M	193	1,000	Died—O. N.[b]	11	F	139	500	Died—O. N.[b]
2	M	205	750	Died—O. N.[b]	11	F	147	500	Died—O. N.[b]
3	M	212	500	Died—O. N.[b]	13	F	152	400	Died—O. N.[b]
4	M	224	500	Died—O. N.[b]	14	F	161	400	Died—O. N.[b]
5	M	224	400	Died—O. N.[b]	15	F	169	250	Died—O. N.[b]
6	M	263	400	Died—3 days	16	F	178	250	Died—O. N.[b]
7	M	269	250	Survived	17	F	178	200	Died—O. N.[b]
8	M	270	250	Survived	18	F	179	150	Died—O. N.[b]
9	M	273	200	Died—2 days	19	F	181	100	Died—4 days
10	M	279	150	Survived	20	F	184	50	Survived

Test *B*. The red squill powder and the bait were both weighed individually, but only enough bait was added to make the powder amount to 10 per cent of the total food given.

No males were used in this series.

Rat No.	Sex	Wt., Gm.	Dose, mg./Kg.[a]	Result
21	F	135	500	Died—O. N.[b]
22	F	154	500	Died—O. N.[b]
23	F	155	400	Died—O. N.[b]
24	F	161	400	Died—O. N.[b]
25	F	162	250	Died—O. N.[b]
26	F	171	250	Died—O. N.[b]
27	F	182	200	Died—O. N.[b]
28	F	183	150	Died—O. N.[b]
29	F	189	100	Died—O. N.[b]
30	F	205	50	Died—O. N.[b]

Test *C*. The red squill powder and the bait were first blended into a carefully stirred bioassay mixture containing exactly 10 per cent squill powder. This food was then weighed out in the proper proportions to give the desired dose of poison per rat.

Rat No.	Sex	Wt., Gm.	Dose, mg./Kg.	Result	Rat No.	Sex	Wt., Gm.	Dose, mg./Kg.[a]	Result
31	M	158	1,000	Died—O. N.[b]	41	F	126	500	Died—O. N.[b]
32	M	166	750	Died—O. N.[b]	42	F	152	500	Died—O. N.[b]
33	M	193	500	Died—O. N.[b]	43	F	154	400	Died—O. N.[b]
34	M	193	500	Died—O. N.[b]	44	F	155	400	Died—O. N.[b]
35	M	196	400	Died—O. N.[b]	45	F	173	250	Died—O. N.[b]
36	M	202	400	Died—O. N.[b]	46	F	174	250	Died—O. N.[b]
37	M	207	250	Died—O. N.[b]	47	F	175	200	Died—O. N.[b]
38	M	208	250	Died—2 days	48	F	176	150	Died—O. N.[b]
39	M	216	200	Survived	49	F	177	100	Died—O. N.[b]
40	M	220	100	Survived	50	F	199	50	Died—5 days

[a] Milligrams of red squill powder per kilogram of body weight.
[b] Over night.

* From the Control Methods Research Laboratory, U. S. Biological Survey, United States Department of the Interior, Denver, Colorado.

The present paper shows the effects on the toxicity of red squill of (*a*) the method of preparing the bioassay bait; (*b*) the concentration of red squill powder in the bioassay bait; and (*c*) the strain of rat used in the bioassay.

EXPERIMENTAL

In the tests listed in Table I the powder used was made from a single bulb of high potency; in those in Table II, a fortified powder having three times the toxicity of the original crude drug, the powder having been prepared in the Control Methods Research Laboratory; and in those in Table III, six red squill powders of different degrees of toxicity. The tables are self-explanatory.

In all the studies a standard food mixture was used consisting of 85 parts of dried and screened bread crumbs and 5 parts of mineral oil. The bioassay baits were prepared immediately before being fed to the rats. The rats were maintained on

Table II.—Effect of the Percentage of Red Squill Powder in the Bait on Its Toxicity

10 Per Cent Bait					8 Per Cent Bait				
Rat No.	Sex	Wt., Gm.	Dose, mg./Kg.[a]	Result	Rat No.	Sex	Wt., Gm.	Dose, mg./Kg.[a]	Result
51	M	127	500	Died—2 days	74	M	123	500	Died—3 days
52	M	101	500	Died—2 days	75	M	108	500	Died—2 days
53	M	102	500	Died—2 days	76	M	109	500	Survived
54	M	103	500	Died—2 days	77	M	109	500	Died—O. N.[b]
55	M	104	500	Died—O. N.[b]	78	M	110	500	Died—5 days
56	M	105	500	Died—3 days	79	M	115	500	Died—2 days
57	M	106	500	Died—2 days	80	M	116	500	Died—1 day
58	M	134	300	Died—6 days	81	M	128	300	Died—2 days
59	M	185	300	Died—2 days	82	M	177	300	Died—2 days
60	M	186	300	Died—4 days	83	M	178	300	Died—4 days
61	M	189	300	Died—3 days	84	M	179	300	Died—6 days
62	M	190	300	Died—O. N.	85	M	181	300	Died—O. N.
63	M	191	300	Died—4 days	86	M	181	300	Survived
64	M	195	300	Died—O. N.	87	M	184	300	Survived
65	M	137	200	Survived	88	M	132	200	Survived
66	M	138	200	Survived	89	M	133	200	Survived
67	M	140	200	Died—2 days	90	M	135	200	Survived
68	M	80	200	Died—O. N.	91	M	82	200	Died—O. N.
69	M	89	200	Died—5 days	92	M	90	200	Died—4 days
70	M	101	200	Died—3 days	93	M	98	200	Died—5 days
71	M	103	200	Died—8 days	94	M	105	200	Died—5 days
72	M	110	200	Survived	95	M	109	200	Died—5 days
73	M	113	200	Survived	96	M	113	200	Died—2 days
5 Per Cent Bait					3 Per Cent Bait				
97	M	110	500	Died—2 days	120	M	101	500	Survived
98	M	116	500	Died—2 days	121	M	124	500	Survived
99	M	117	500	Died—O. N.[b]	122	M	124	500	Died—O. N.[b]
100	M	118	500	Died—O. N.[b]	123	M	126	500	Died—1 day
101	M	120	500	Survived	124	M	126	500	Survived
102	M	121	500	Died—3 days	125	M	124	500	Died—2 days
103	M	123	500	Died—1 day	126	M	129	500	Died—O. N.
104	M	114	300	Died—2 days	127	M	104	300	Survived
105	M	169	300	Died—2 days	128	M	141	300	Died—2 days
106	M	170	300	Died—2 days	129	M	161	300	Survived
107	M	175	300	Died—2 days	130	M	166	300	Survived
108	M	176	300	Died—3 days	131	M	166	300	Died—3 days
109	M	176	300	Died—1 day	132	M	167	300	Died—2 days
110	M	177	300	Survived	133	M	168	300	Died—2 days
111	M	133	200	Survived	134	M	116	200	Survived
112	M	135	200	Survived	135	M	119	200	Survived
113	M	136	200	Survived	136	M	127	200	Survived
114	M	86	200	Died—7 days	137	M	89	200	Died—5 days
115	M	93	200	Died—5 days	138	M	95	200	Died—5 days
116	M	97	200	Died—4 days	139	M	96	200	Died—1 day
117	M	106	200	Survived	140	M	107	200	Survived
118	M	109	200	Died—7 days	141	M	108	200	Died—5 days
119	M	116	200	Died—2 days	142	M	130	200	Died—5 days

[a] Milligrams of red squill powder per kilogram of body weight.
[b] Over night.

This table shows the following percentage of kills:

Dose	10	8	5	3
500 mg./Kg.	100	85.7	85.7	57.2
300 mg./Kg.	100	71.5	85.7	42.9
200 mg./Kg.	55.5	66.7	55.5	55.5

These figures indicate that the concentration of the poison in the bioassay bait is of decided importance.

a balanced diet in the laboratory for at least 10 days before being used for the experiments. On the evening of the day before the tests were made the food was removed from the stock cages, and the following morning the test animals were removed and weighed into individual feeding cages. The proper doses were computed and the baits weighed. The rats were fed between 3 P.M. and 6 P.M., that is, after a 24-hour fasting period. Water was available in adequate quantity at all times.

Table I shows that, in so far as the two 10 per cent bait methods are concerned, there is no discernible difference, but that when the same doses are given in a bait carrying 1 per cent body weight concentration there is evidence, when the smaller doses are compared, that the larger quantity of food interfered with the action of the squill. Since the Test *C* method of bioassay bait preparation will save almost half of the weighings involved in the usual feeding test, this laboratory has adopted it as our standard system.

Table III shows that the three strains of rats studied did not exhibit any detectable difference in susceptibility to six different red squill samples of varying toxicities. The rats used were of approximately the same ages, the difference in weights being due to the more rapid growth characteristic of the Yale strain. The Baxter strain

Table III.—Influence of the Strain of Rat on Red Squill Toxicity

A. Special bulb 10 per cent bait

Baxter Strain				Denver University Strain				Yale Strain			
Rat No.	Wt., Gm.	Dose, mg./Kg.[a]	Result	Rat No.	Wt., Gm.	Dose, mg./Kg.	Result	Rat No.	Wt., Gm.	Dose, mg./Kg.[a]	Result
143	105	300	Died—O. N.[b]	155	117	300	Died—4 days	167	131	300	Died—O. N.[b]
144	142	300	Died—O. N.[b]	156	142	300	Died—3 days	168	217	300	Died—3 days
145	143	250	Died—3 days	157	153	250	Died—6 days	169	220	250	Survived
146	170	250	Died—3 days	158	161	250	Died—6 days	170	240	250	Died—O. N.
147	171	250	Survived	159	165	250	Died—O. N.[b]	171	242	250	Died—5 day[b]
148	265	250	Died—2 days	160	179	250	Died—O. N.[b]	172	293	250	Died—O. N.s
149	124	250	Died—O. N.[b]	161	135	250	Died—O. N.[b]	173	195	250	Died—O. N.[b]
150	140	250	Died—O. N.[b]	162	144	250	Died—O. N.[b]	174	215	250	Died—4 day[b]
151	144	250	Died—O. N.[b]	163	151	250	Survived	175	222	250	Died—4 days
152	169	250	Died—2 days	164	160	250	Died—O. N.[b]	176	238	250	Died—O. N.s
153	170	200	Died—3 days	165	168	200	Died—3 days	177	247	200	Died—6 day[b]
154	214	200	Died—3 days	166	176	200	Died—O. N.[b]	178	292	200	Died—6 days

Baxter Strain				Yale Strain			
Rat No.	Wt., Gm.	Dose, mg./Kg.	Result	Rat No.	Wt., Gm.	Dose, mg./Kg.	Result
			B. "Caked" squill No. 1705 10 per cent bait				
179	143	2500	Died—1 day	184	230	2500	Died—1 day
180	164	2000	Died—2 days	185	246	2000	Died—O. N.[b]
181	183	1500	Died—1 day	186	250	1500	Died—1 day
182	208	1000	Died—6 days	187	276	1000	Survived
183	216	750	Survived	188	287	750	Died—O. N.[b]
			C. Red squill powder No. 1706 10 per cent bait				
189	148	2500	Died—O. N.[b]	194	235	2500	Died—O. N.[b]
190	161	2000	Died—2 days	195	245	2000	Died—O. N.[b]
191	184	1500	Died—1 day	196	260	1500	Died—O. N.[b]
192	209	1000	Died—1 day	197	271	1000	Died—2 days
193	215	750	Died—3 days	198	284	750	Died—O. N.[b]
			D. Red squill powder No. 1699 (Fortified 2×) 10 per cent bait				
199	120	600	Died—O. N.[b]	205	215	600	Died—O. N.[b]
200	167	500	Died—1 day	206	250	500	Died—O. N.[b]
201	179	400	Died—O. N.[b]	207	257	400	Died—O. N.[b]
202	192	300	Died—O. N.[b]	208	278	300	Died—O. N.[b]
203	199	250	Died—O. N.[b]	209	281	250	Died—O. N.[b]
204	225	200	Died—3 days	210	310	200	Died—2 days
			E. Red squill powder No. 1699 (Fortified 7×) 5 per cent bait				
211	165	250	Died—O. N.[b]	217	211	250	Died—O. N.[b]
212	170	200	Died—2 days	218	251	200	Died—1 day
213	172	150	Survived	219	254	150	Died—1 day
214	196	100	Survived	220	278	100	Survived
215	197	75	Survived	221	280	75	Survived
216	236	50	Survived	222	354	50	Survived
			F. Red squill powder No. 1709 10 per cent bait.				
223	142	2500	Died—O. N.[b]	229	229	2500	Died—2 days
224	165	2000	Died—3 days	230	247	2000	Died—6 days
225	183	1500	Died—O. N.[b]	231	257	1500	Died—O. N.[b]
226	191	1250	Died—2 days	232	277	1250	Died—O. N.[b]
227	206	1000	Died—2 days	233	285	1000	Died—2 days
228	217	750	Died—6 days	234	305	750	Died—6 days

[a] Milligrams of red squill powder per kilogram of body weight.
[b] Over night.

of rats is a hybrid one, predominately Wistar. The Denver University strain is a hybrid which has been inbred at the local colony for many years. The Yale strain used was a pure line obtained from the University of Colorado School of Medicine.

CONCLUSIONS

1. Mixing weighed quantities of red squill powder with food to make 1 per cent body weight bioassay baits is not satisfactory for accurate studies, since the ratio of poison to food will vary, and a definite food interference is noted when small doses of squill are fed.
2. It is satisfactory either to weigh out both the red squill powder and the bait for the bioassay bait and mix for each individual test animal, or previously to blend a standard concentration bait and weigh the proper quantities of this food to give the doses desired.
3. The percentage of red squill powder in the bioassay bait is of importance, both as regards the ultimate toxicity and the speed of action of the poison.
4. The strain of rat used does not affect the bioassay, when the animals have been maintained on the same diet for at least a week before the beginning of the bioassay. The rats used should be of approximately the same age when bioassays on two or more strains are to be carefully compared.
5. Depending upon the quality of the squill powder being tested, from 0 to $33^1/_3$ per cent of the animals survive longer than three days, although they ultimately die with typical red squill symptoms, and accordingly must be counted in the bioassay. In many cases the tests must be allowed to continue for seven or eight days.
6. Female rats are more than twice as susceptible to red squill powder as are male rats.

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The Synthesis of Hydroxymandelonitrile Dibenzoates

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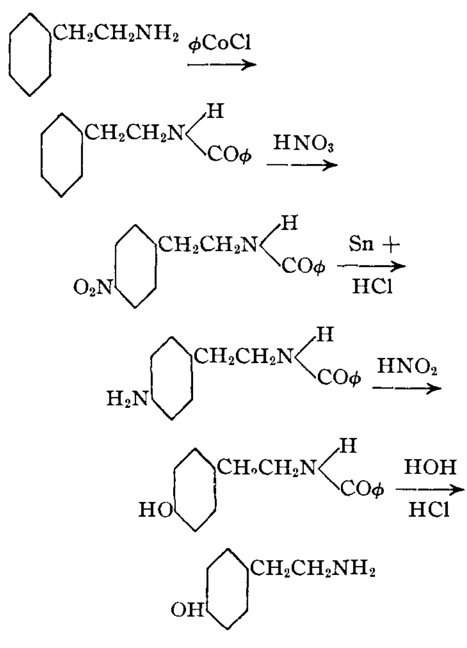
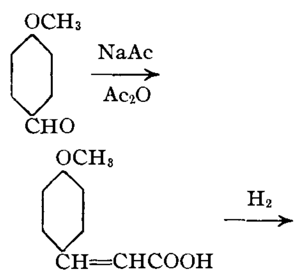
The biological importance of tyramine and the rather incompletely reported pharmacological studies of other β-hydroxyphenylethylamines led to the investigation of possible intermediates for a synthesis of tyramine and its isomers. Previous syntheses have been reported by Barger and Walpole (1) from phenylethylamine and from anisaldehyde as indicated by:



(1)



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