TOXICITY OF NICOTINE, NICOTINIUM SALTS AND RELATED COMPOUNDS BY INJECTION

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Bulletin 521 — June, 1948

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The relative toxicity of a group of nicotinium salts when applied as sprays to *Aphis rumicis* has been determined by Turner and Saunders (1947). In that study, only one of the nicotinium compounds exceeded nicotine sulfate in toxicity, and many of them were much less toxic than nicotine sulfate. It was indicated that the tertiary nitrogen atoms were very important in the toxicity of nicotine, since formation of quaternary compounds usually reduced toxicity. On the other hand, the nicotine structure had an effect on toxicity, because dodecylnicotinium thiocyanate was much more toxic than the similar isoquinolinium compound. While there were some consistent relations between chemistry and toxicity, there were also many exceptions. In general, mono-substituted nicotinium salts were more toxic than di-substituted. Mayer et al (1947) found that the di-substituted compounds were at least as toxic as the similar mono-substituted materials as tested on melonworms and the southern armyworm. While they found the dodecylnicotinium cation the most toxic, the methylnicotinium cation was second best.

In the study of Turner and Saunders, the slope of the dosage response curves for the nicotinium compounds was roughly parallel to the slope of curves for nicotine sulfate in 51 cases, flatter in 31 and steeper in 7. McCallan, Wellman and Wilcoxon (1941) state that "... it is probable that differences in slope of the toxicity curves indicate difference in mode of action ..." According to Dimond et al (1941), slope may also be affected by the type of coverage on the sprayed surface. Although the possibility of differences in coverage cannot be dismissed, there is at present no basis for believing that coverage was involved to any great extent in the curves for the nicotinium salts. In the work of Turner and Saunders (1947) no correlation was found between contact angle and toxicity.

If it is assumed that the differences in slope may indicate a change in the mode of toxic action, it follows that many of the nicotinium salts may have acted by a different mode than nicotine. For instance, dodecylnicotinium thiocyanate produced a steeper curve than nicotine sulfate. The nicotine in the molecule still had an effect on toxicity, however, because dodecylisoquinolinium thiocyanate was much less toxic than the nicotinium salt. In the many cases of parallel dosage response curves, the nicotinium salts were far less toxic than nicotine sulfate. Such a loss in toxicity might be caused by a lack of penetration. It was desirable, therefore, to study these compounds further, considering (1) the problem of penetration and (2) the possibility of changes in the mode of toxic action.

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The technique of injection of the compounds into the blood stream of the insect was selected as a means of introducing the chemicals through the integument. Hockenyos and Lilly (1932) called attention to this aspect of injection and McIndoo (1937) extended the idea to cover several species of insects. It is true that this technique does not guarantee that the chemical reaches the site of toxic action, but it does place the chemical inside the integument.

METHODS

The larger milkweed bug, *Oncopeltus fasciatus* Dal., was selected for use (Simanton and Andre, 1935). The method of rearing was adapted from the account of Worthley in Campbell and Moulton (1943). Preliminary tests showed that last-stage nymphs were at least as resistant as adults. Since the nymphs were much easier to handle, they were selected for use.

The injections were made using the device developed by Dutky (1942) for use in infecting grubs of the Japanese beetle with milky disease spores. A one cc. tuberculin syringe with a standard 27-gauge needle was used and delivered .003 ml. per stroke of the micrometer device. At first injections were made in active nymphs, but later CO_2 was used as an anesthetic. Ten and twelve nymphs were injected with each concentration, and were placed in a petri dish with a few milkweed seeds for food and a moist cellucotton plug for moisture. Mortality counts were made 48 hours after injection, with movement on prodding as the criterion for living insects.

The dosage ratio was the square root of two, and alkaloid nicotine at a top concentration of .4 per cent was the standard used in each series. All nicotinium salts were used first at .5 per cent nicotine content. The concentration used in the tests was adjusted so that the mortality from the highest concentration was less than 100 per cent. One series in each test was injected with distilled water. As a general rule mortality of the water-injected checks was very low. However, occasionally the loss would be as high as 50 per cent, although many of the low concentrations of nicotinium compounds would show no mortality in the same series. No reason has been found for this excessive loss in water-injected checks. If the mortality data appeared to be normal when compared with other replications using the same material, the data were retained without correction for the high mortality in the checks. If the data seemed inconsistent in comparison with other replications, the test was discarded.

A minimum of two replicates and a maximum of four were completed. Since the data are intended to show only general relationships, no analysis of variance has been made.

Most of the nicotinium salts were soluble in water at the concentrations required for injection. Preliminary tests using acetone as a solvent for the water-insoluble materials seemed to be promising. However, acetone proved to be too toxic to the bugs. Water-insoluble liquids were emulsified using one-fifth of the weight of nicotinium of *Atlas 1266* emulsifier, which was relatively non-toxic. At this writing no satisfactory method for handling water-insoluble solids has been developed.

RESULTS

Data for the individual compounds are given in the Appendix table. The comparison of the compound with its nicotine standard (always following the nicotinium salt in the Appendix) has been given in Table 1. All comparisons were made on the basis of the amount of nicotinium compound required, nicotine being 100 per cent. Comparison of dosage response curves was made on the basis of curves fitted by observation on logarithmic-probability paper. The toxicity of five quaternary ammonium compounds with alkaloid bases other than nicotine are included as numbers 132 to 136.

Table 2 gives a comparison of the toxicity of these compounds as sprayed on aphids by Turner and Saunders (1947) and as injected into milkweed bugs. In general, the compounds were much more toxic relative to nicotine by the injection method. Averaging the results shows that the increase is approximately ten-fold, which might be assumed to represent the improvement in toxicity with equal power of penetration through the cuticle. On the other hand, many of the injected compounds were not as toxic as nicotine itself.

For the purposes of this discussion, the compounds can be classified roughly into three groups: (1) those compounds which were relatively less toxic injected than sprayed, which would indicate superior penetrating power but inferior toxicity; (2) those compounds of which the relative toxicity was greatly improved by injection, which would indicate little difference in toxicity from nicotine but inferior penetrating power by spraying and (3) those compounds which were of relatively equal toxicity injected and sprayed, which would indicate that the penetrating power was unchanged and that any change in toxicity was inherent.

1. Only one compound, 104, 1-benzyl-3-[2-(1-methylpyrrolidyl-hydriodide)] pyridinium chloride was relatively less toxic injected than sprayed.

2. Compounds which were much more toxic by injection than by spraying were:

(*a*) *Less toxic than nicotine* (*injected*)

81 dodecylnicotinium bromide
34 hexadecylnicotinium bromide
72 benzylnicotinium bromide
31 o-chlorobenzylnicotinium bromide
106 dimethylnicotinium dibromide
92 dibutylnicotinium dibromide
93 methylnicotinium iodide
90 dimethylnicotinium diiodide
105 1-methyl-3-[2-(1-methylpyrrolidyl-hydriodide)] pyridinium iodide
137 diethylnicotinium diiodide
29 benzylnicotinium chloride
84 p-chlorobenzylnicotinium chloride
103 1-p-chlorobenzyl-3-[2-(1-methylpyrrolidyl-hydriodide)] pyridinium chloride

102 1-p-nitrobenzyl-3-[2-(1-methylpyrrolidyl-hydriodide)] pyridinium chloride
85 di-p-chlorobenzylnicotinium dichloride
96 didodecylnicotinium dichloride
121 2-methyl-2-propen-1-ylnicotinium p-toluenesulfonate
122 2-chloro-2-propen-1-ylnicotinium p-toluenesulfonate
54 methylnicotinium stearate
97 didodecylnicotinium dioleate

(*b*) *At least as toxic as nicotine* (*injected*)

141 dodecylnicotinium iodide
98 dodecylnicotinium chloride
86 2,4-dichlorobenzylnicotinium chloride
88 3,4-dichlorobenzylnicotinium chloride
87 di-2,4-dichlorobenzylnicotinium dichloride
89 di-3,4-dichlorobenzylnicotinium dichloride
77 octylnicotinium thiocyanate
35 hexadecylnicotinium thiocyanate
79 dodecylnicotinium thiocyanate
56 benzylnicotinium palmitate
83 dodecylnicotinium oleate
53 octadecylnicotinium acetate

3. Compounds of low toxicity relatively unchanged by injection:

107 methylnicotinium bromide
108 ethylnicotinium bromide
80 butylnicotinium bromide
138 ethylnicotinium iodide
139 butylnicotinium iodide
96 benzylnicotinium thiocyanate
94 didodecylnicotinium dithiocyanate
143 butylnicotinium p-toluenesulfonate
110 methylnicotinium methylsulfate

This classification shows that only ten of the compounds were relatively unimproved in toxicity by injection, while 34 were relatively more toxic. However, of these 34 only 13 were approximately as toxic as nicotine. It must be concluded, therefore, that lack of penetrating power by spraying was only partially responsible for the relatively low toxicity of the nicotinium compounds. Certainly those compounds in group 3 did not benefit by injection. Twenty-one of the compounds in group 2 were improved, but were still substantially less toxic than nicotine.

In the group of compounds which were as toxic as nicotine when injected, there is some correlation between the radical present and the toxicity. The list includes octyl, dodecyl, hexadecyl and octadecyl compounds, but no methyl, ethyl or butyl compounds. 2,4- and 3,4-dicholoro-benzyls appear, but no o- or p-chlorobenzyls. One benzyl is included, but three other benzyls were not so toxic. Dodecylnicotinium chloride was more toxic than the corresponding iodide, which was more toxic than the bromide. Three thiocyanates are in the more toxic group and one thiocyanate and one dithiocyanate in the unimproved group. Of the fatty acid compounds used, three were in the group as toxic as nicotine, and one, methylnicotinium stearate, was less toxic.

TABLE 1. SUMMARY OF THE RESULTS OF INJECTION AND COMPARISON OF TOXICITY OF NICOTINIUM COMPOUNDS WITH NICOTINE

ERL No.	Compound	Per cent Killed at Level of Comparisons	Dosage Required ERL	Dosage Required Nicotine	Toxicity of ERL[1]	Slope[2]
107	methylnicotinium bromide	20	1.0	.1	10	flat
108	ethylnicotinium bromide	60	.5	.15	30	parallel
80	butylnicotinium bromide	80	.7	.25	36	parallel
81	dodecylnicotinium bromide	80	.5	.3	60	flat
34	hexadecylnicotinium bromide	70	.45	.3	67	flat
72	benzylnicotinium bromide	80	.7	.35	50	parallel
31	o-chlorobenzylnicotinium bromide	50	.45	.25	55	parallel
106	dimethylnicotinium dibromide	30	1.0	.2	20	parallel
92	dibutylnicotinium dibromide	50	1.0	.15	15	parallel
93	methylnicotinium iodide	50	2.0[3]	.13	6	
138	ethylnicotinium iodide	60	1.0	.3	30	parallel
139	butylnicotinium iodide	60	.8	.3	37	parallel
141	dodecylnicotinium iodide	70	.5	.5	100	flat
90	dimethylnicotinium diiodide	25	1.0	.16	16	flat
105	1-methyl-3-[2(1-methylpyrrolidyl hydriodide)] pyridinium iodide	40	1.0	.25	25	parallel
137	diethylnicotinium diiodide	40	.5	.2	40	parallel
98	dodecylnicotinium chloride	70	.3	.5	167	parallel
29	benzylnicotinium chloride	70	1.0	.4	40	parallel
84	p-chlorobenzylnicotinium chloride	70	.55	.3	54	flat
86	2,4-dichlorobenzylnicotinium chloride	80	.3	.4	133	steep
88	3,4-dichlorobenzylnicotinium chloride	70	.4	.4	100	parallel
104	1-benzyl-3-[2(1-methylpyrrolidyl hydriodide)] pyridinium chloride	80	.4	.3	75	flat
103	1-p-chlorobenzyl-3-[2(1-methylpyrrolidyl hydriodide)] pyridinium chloride	80	.7	.3	43	flat
102	1-p-nitrobenzyl-3-[2(1-methylpyrrolidyl hydriodide)] pyridinium chloride	80	.9	.3	33	flat

[1]As percentage of the toxicity of nicotine.
[2]Slope of curves drawn by inspection in relation to curve for nicotine.
[3]By extrapolation.

TABLE 1. SUMMARY OF THE RESULTS OF INJECTION AND COMPARISON OF TOXICITY OF NICOTINIUM COMPOUNDS WITH NICOTINE—Concluded

ERL No.	Compound	Per cent Killed at Level of Comparisons	Dosage Required ERL	Dosage Required Nicotine	Toxicity of ERL[1]	Slope[2]
96	didodecylnicotinium dichloride	70	.75	.23	31	parallel
85	di-p-chlorobenzylnicotinium dichloride	50	2.0	.13	6	parallel
87	di-2,4-dichlorobenzylnicotinium dichloride	80	.55	.45	80	parallel
89	di-3,4-dichlorobenzylnicotinium dichloride	80	.5	.5	100	parallel
77	octylnicotinium thiocyanate	70	.3	.5	167	steep
35	hexadecylnicotinium thiocyanate	80	.5	.5	100	parallel
79	dodecylnicotinium thiocyanate	70	.3	.5	167	steep
76	benzylnicotinium thiocyanate	70	1.0	.45	45	parallel
94	didodecylnicotinium dithiocyanate	70	.9	.23	25	parallel
143	butylnicotinium p-toluenesulfonate	20	1.0	.15	15	flat
121	2-methyl-2-propen-1-yl nicotinium p-toluenesulfonate	20	.5	.1	20	flat
122	2-chloro-2-propen-1-yl nicotinium p-toluenesulfonate	70	.6	.5	85	parallel
56	benzylnicotinium palmitate	70	.45	.45	100	steep
83	dodecylnicotinium oleate	80	.4	.5	125	parallel
97	didodecylnicotinium dioleate	50	.4	.3	75	flat
54	methylnicotinium stearate	40	1.0	.3	30	parallel
53	octadecylnicotinium acetate	70	.4	.4	100	parallel
110	methylnicotinium methylsulfate	50	.6	.3	50	flat
100a	nicotine 2,4-dinitro phenolate	90	.1	.6	600	steep

TABLE 2. COMPARATIVE TOXICITY OF NICOTINIUMS SPRAYED ON APHIDS AND INJECTED INTO MILKWEED BUGS

ERL No.	Compound	Toxicity of Nicotinium as Percentage of Toxicity of Nicotine			
		Sprayed On Aphids	Slope	Injected	Slope
107	methylnicotinium bromide	8	parallel	10	flat
108	ethylnicotinium bromide	20	flat	30	parallel
80	butylnicotinium bromide	22	parallel	36	parallel
81	dodecylnicotinium bromide	10	parallel	60	flat
34	hexadecylnicotinium bromide	10	parallel	67	flat
72	benzylnicotinium bromide	16	flat	50	parallel
31	o-chlorobenzylnicotinium bromide	10	parallel	55	parallel
106	dimethylnicotinium dibromide	< 1	flat	20	parallel
92	dibutylnicotinium dibromide	2	parallel	15	parallel
93	methylnicotinium iodide	< 1	parallel	6	parallel?
138	ethylnicotinium iodide	34	steep	30	parallel
139	butylnicotinium iodide	13	parallel	37	parallel
141	dodecylnicotinium iodide	22	parallel	100	flat
90	dimethylnicotinium diiodide	< 1	flat	16	flat
105	1-methyl-3-[2-(1-methylpyrrolidyl hydriodide)] pyridinium iodide	5	parallel	25	parallel
137	diethylnicotinium diiodide	1.3	parallel	40	parallel
98	dodecylnicotinium chloride	16	parallel	167	parallel
29	benzylnicotinium chloride	4	flat	40	parallel
84	p-chlorobenzylnicotinium chloride	6	flat	54	flat
86	2,4-dichlorobenzylnicotinium chloride	6	flat	133	steep
88	3,4-dichlorobenzylnicotinium chloride	45	flat	100	parallel
104	1-benzyl-3-[2-(1-methylpyrrolidyl hydriodide)] pyridinium chloride	125	parallel	75	flat
103	1-p-chlorobenzyl-3-[2-(1-methylpyrrolidyl hydriodide)] pyridinium chloride	2	parallel	43	flat
102	1-p-nitrobenzyl-3-[2-(1-methylpyrrolidyl hydriodide)] pyridinium chloride	1.5	flat	33	flat
96	didodecylnicotinium dichloride	8	parallel	31	parallel
85	di-p-chlorobenzylnicotinium dichloride	< 1	flat	6	parallel
87	di-2,4-dichlorobenzylnicotinium dichloride	4	parallel	80	parallel
89	di-3,4-dichlorobenzylnicotinium dichloride	6	flat	100	parallel
77	octylnicotinium thiocyanate	11	parallel	167	steep
35	hexadecylnicotinium thiocyanate	10	steep	100	parallel
79	dodecylnicotinium thiocyanate	10	steep	167	steep
76	benzylnicotinium thiocyanate	43	parallel	45	parallel
94	didodecylnicotinium dithiocyanate	10	flat	25	parallel
143	butylnicotinium p-toluenesulfonate	5	flat	15	flat
121	2-methyl-2-propen-1-yl-nicotinium toluenesulfonate	4	flat	20	flat
122	2-chloro-2-propen-1-yl-nicotinium p-toluenesulfonate	52	flat	83	parallel
56	benzylnicotinium palmitate	23	parallel	100	steep
83	dodecylnicotinium oleate	7	parallel	125	parallel
97	didodecylnicotinium dioleate	12	parallel	75	flat
54	methylnicotinium stearate	5	parallel	30	parallel
53	octadecylnicotinium acetate	15	flat	100	parallel
110	methylnicotinium methyl sulfate	20	flat	50	flat
00a	nicotine 2,4-dinitrophenolate	167	steep	600	steep

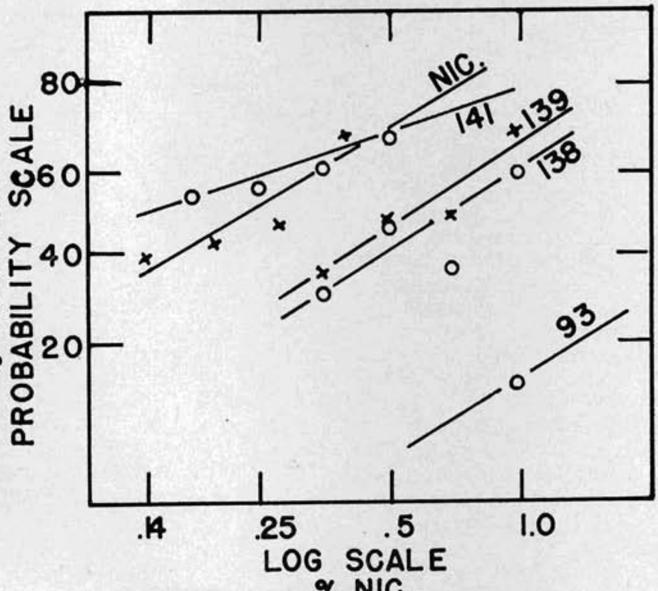

Figure 1. Dosage response curves for a series of nicotinium salts and nicotine (NIC.) 93 — methyl-, 138 — ethyl-, 139 — butyl-, 141 — dodecylnicotinium iodide.

Compound 100A, nicotine 2,4-dinitrophenolate, was again more toxic than any nicotinium salt. It was approximately six times as toxic as nicotine by the injection method. Nitro derivatives of phenols are known to be insecticidal.

SLOPE OF THE DOSAGE RESPONSE CURVES

Before discussing the results of the tests, it would be well to review briefly the meaning of slope. Dimond et al (1941) state "Statistically, a steep slope indicates that the spore population behaves more uniformly than when the dosage-response curve is flat". Slope is therefore a measure of the variability of the test insects. The original data give an idea of differences in slope, but it is much more simple to plot these data on a standard logarithmic probability grid in order to compare slopes.

Of the group of compounds tested both by spraying on aphids and by injection, the following summary of slopes of the dosage response curves has been made:

Method of testing	*Slope in relation to curve for nicotine*		
	flat	*parallel*	*steep*
Spraying	17	22	3
Injection	13	25	4

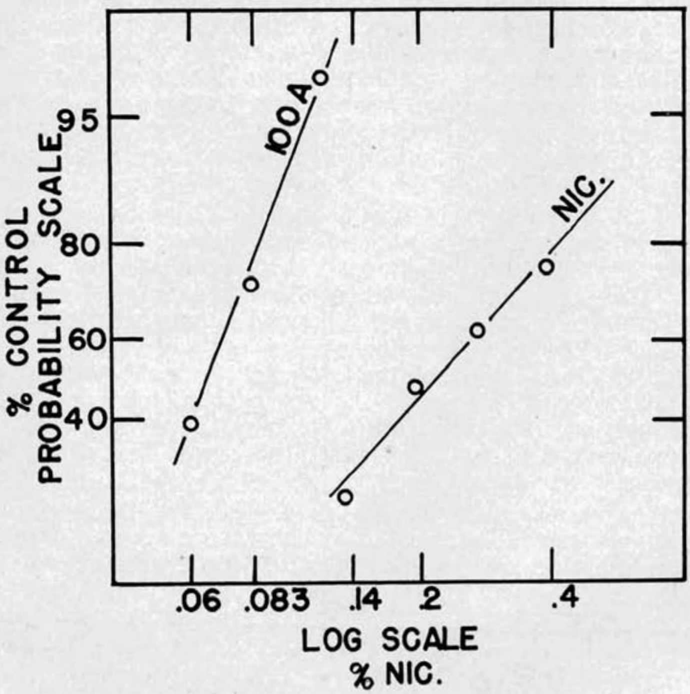

Figure 2. Dosage response curves for nicotine 2,4-dinitrophenolate (100A), and nicotine.

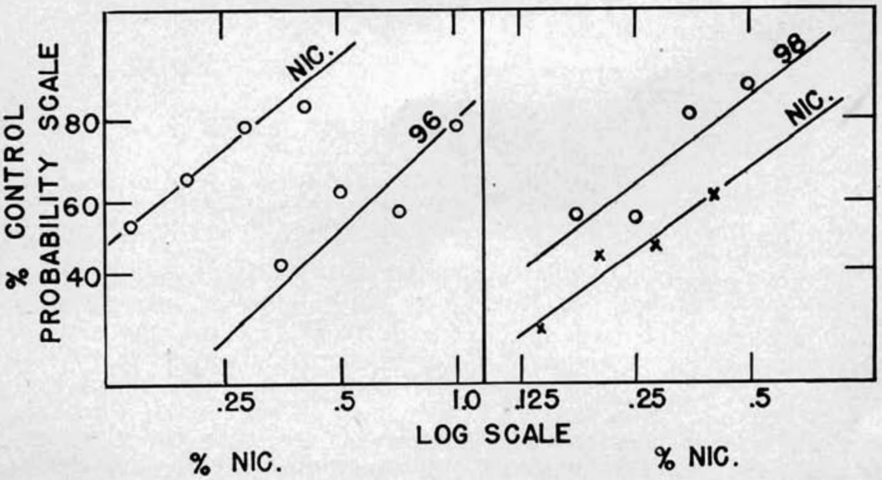

Figure 3. Dosage response curves for dodecylnicotinium chloride (98) and didodecylnicotinium dichloride (96) in comparison with nicotine.

This indicates a slight tendency towards steeper slopes in relation to the nicotine standard by injection. However, the changes in slope were not as simple as a mere tendency for steeper slopes by injection, but individual compounds were affected differently by the two methods. In nine individual cases curves were flatter in relation to curves for nicotine by injection than by spraying, in 21 cases the slopes were unchanged for the two methods, and 12 were steeper injected than sprayed.

In the 13 compounds in group 2b above, which were at least approximately as toxic as nicotine when injected, two slopes were flatter injected, four were unchanged and seven steeper relative to nicotine. While the slope was steeper in a majority of cases, there were still important exceptions. Likewise among the group three compounds which were of low toxicity by both methods, two slopes were flatter injected, two steeper, and four unchanged. It seems obvious, therefore, that slope of the dosage response curve was not directly associated with penetration through the cuticle. Moreover, relative slope seemed to be at least partially independent of the method of application.

As stated above, McCallan et al (1941) stated that changes in slope probably meant differences in mode of toxic action. In the case of the nicotinium salts, the nicotine molecule was always present. The

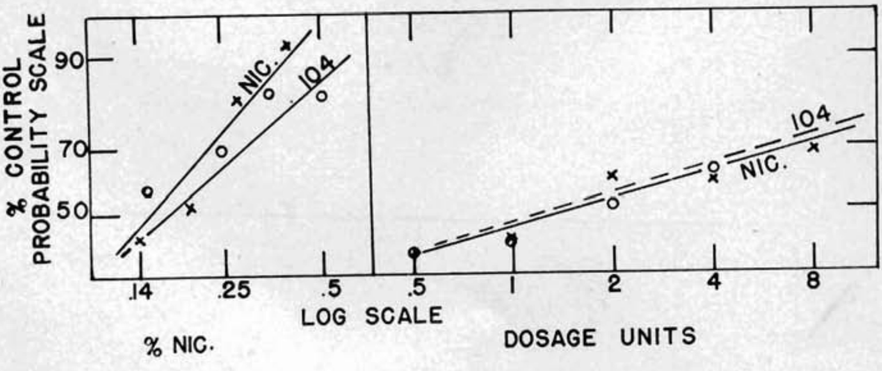

Figure 4. Dosage response curves for 1-benzyl-3-[2-(1-methyl pyrrolidyl hydriodide)] pyridinium chloride (104) by injection in milkweed bugs (at left) and by spraying on aphids (at right) (from data of Turner and Saunders, 1947).

addition of radicals to the nitrogens did not alter the basic structure. In the spray tests on aphids (Turner and Saunders, 1947), 41 compounds produced curves parallel to the curve for nicotine and 38 produced a change in slope. Of the 41 compounds injected, 24 had parallel slope and 17 were changed. If it be assumed that the parallel dosage response curve means a similar mode of toxic action, then the mode may have been changed in somewhat less than half the compounds. The data available in this experiment cannot answer the questions arising from the mode of toxic action, but do contain sufficient information to encourage speculation.

For example, Compound 85, di-p-chlorobenzylnicotinium dichloride, produced a dosage response curve parallel to that of nicotine when injected, and yet was only 6 per cent as toxic as nicotine. Since dosages were based on an equal nicotine content, the nicotine in this compound must have been almost totally inactivated. Compound 84, p-chlorobenzylnicotinium chloride, was about 50 per cent as toxic as nicotine and also had a parallel dosage response curve. Obviously, if the nicotine in this nicotinium salt was still acting as nicotine, more was available than in the di-substituted compound. Similar relationships existed in the comparable compounds of ethyl- and butylnicotinium bromide, dodecylnicotinium chloride and the dodecyl thiocyanate. Unfortunately for the theory, however, the dimethyl and diethyl

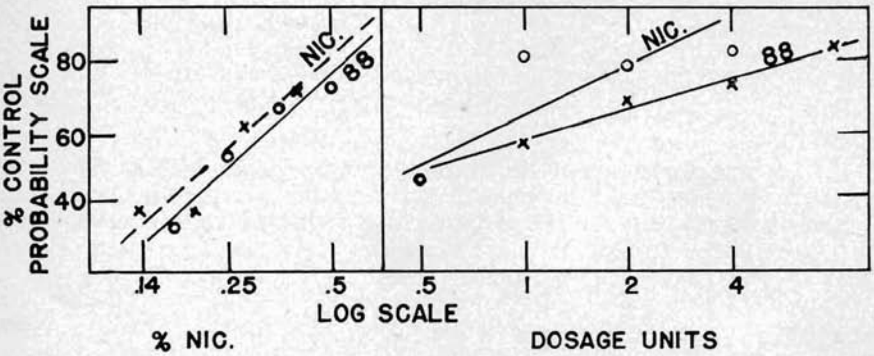

Figure 5. Dosage response curves for 3,4 dichlorobenzylnicotinium chloride (88) by injection in milkweed bugs (at left) and by spraying on aphids (at right) (from data of Turner and Saunders, 1947).

iodides reversed this pattern although there was again no change in the slope of the dosage response curves. Compound 97, didodecylnicotinium dioleate, was not only more toxic than the dodecyl oleate but also had a steeper dosage response curve. Furthermore, if the action of the added radicals was simply to inactivate the nicotine, the toxicity of the new compound would be governed by stability rather than by the chemical nature of the additives. There is, of course, no indication that this is not true, except the toxicity of the longer radicals and of the thiocyanates which is also reflected in the toxicity of most compounds containing them.

Beard (1948) has recently shown that the slope of the dosage response curve for injection steepens as the time after injection increases. In other words, slope may also be a function of the time at which the observation of mortality is made. Compounds acting slowly might therefore produce flatter curves than those acting rapidly. Whether this is true of these compounds remains to be seen.

The data appear inadequate to correlate chemical structure with slope. For instance, two thiocyanates produce slopes steeper than nicotine and two parallel. In the five dodecyl salts, two are flat, two parallel and one steeper than the curves for nicotine.

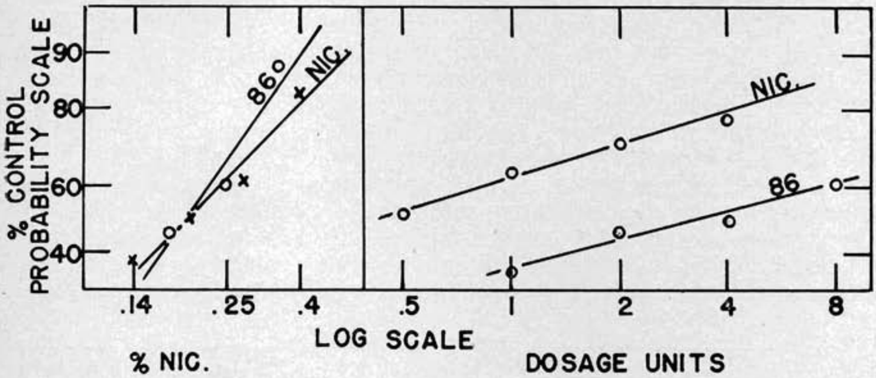

Figure 6. Dosage response curves for 2,4-dichlorobenzylnicotinium chloride (86) by injection (at left) and by spraying on aphids (at right) (data from Turner and Saunders, 1947).

The indication noted by Turner and Saunders (1947) that the two nitrogens are most important in the toxicity of nicotine seems to be borne out further by these data. The fact that many compounds produce curves parallel to that of nicotine does not necessarily mean that they act by the same means.

TOXICITY OF INTRAMOLECULAR MODIFICATIONS OF NICOTINE

Nine compounds which represented intramolecular modifications of nicotine were tested for the purpose of adding information on the relation between molecular structure and toxicity. They were used by injection, with alkaloid nicotine as the standard. The detailed results are included in the Appendix, and the summary in Table 3. *They show that dihydronicotyrine was approximately three times as toxic as nicotine.* The modification here was the removal of two hydrogens from the N-methyl pyrrolidine ring. Substitution of a hydrogen for the N-methyl group in dihydronicotyrine, to form myosmine, resulted in greatly decreased toxicity. Removal of two hydrogen atoms from

TABLE 3. TOXICITY OF INTRAMOLECULAR MODIFICATIONS OF NICOTINE BY INJECTION

ERL No.	Compound	Level of Comparison per cent	Amt. ERL	Amt. Nicotine	Toxicity ERL	Slope
70	Myosmine	70	1.8	.3	17	parallel
67	Dihydronicotyrine (N-Methyl myosmine)	80	.13	.4	300	flat
69	Nornicotine	80	.45	.4	90	flat
71	Nornicotyrine	40	2.5	.25	10	flat
68	Nicotyrine	50	2.0	.2	10	parallel
155	Metanicotine	50	1.8	.18	10	parallel
156	N-Lauryl nornicotine	50	.7	.2	29	parallel
191	N-Octyl nornicotine	50	.8	.2	25	parallel
192	N-Octadecyl nornicotine	50	2.0	.2	10	parallel

the N-methyl pyrrolidine ring of dihydronicotyrine, to form nicotyrine, reduced toxicity still further. On the other hand, nornicotine, in which hydrogen is substituted for the methyl group in nicotine, was approximately as toxic as nicotine. N-lauryl nornicotine has a lauryl radical substituted for the methyl group and the toxicity was reduced sharply. N-octyl nornicotine was slightly less toxic than N-lauryl, and N-octadecyl was still lower in toxicity.

In metanicotine the pyrrolidine ring was broken and the toxicity reduced greatly.

Richardson, Craig and Hansberry (1936) have estimated that nornicotine is about two and a half times as toxic as nicotine to aphids. It was certainly no more toxic than nicotine when injected. The substitution of the hydrogen therefore apparently increased penetration without changing inherent toxicity very much. The tests of Richardson and Shepard (1930) showed that metanicotine and nicotyrine were of a low order of toxicity. The injection method indicates that these modifications destroyed the inherent toxicity of nicotine.

It is interesting to note that the dosage response curves for nornicotine and dihydronicotyrine were definitely flatter than for nicotine.

TOXICITY OF COAL TAR BASES AND THEIR DERIVATIVES

Since the toxicity of some of the nicotinium salts was reduced by their loss in power to penetrate the insect cuticle, it was of interest to determine whether any of the coal tar bases and their derivatives that were relatively much less toxic than nicotine as determined by Tattersfield and Gimingham (1927) would be toxic when injected. Results of tests of such a group of compounds are summarized in Table 4.

TABLE 4. TOXICITY OF COAL TAR BASES AND THEIR DERIVATIVES

ERL No.	Compound	Level of Comparison per cent	Amt. ERL	Amt. Nicotine	Toxicity ERL	Slope
157	Pyridine	30	2.0	.12	6	parallel
158	2-picoline	7	2.0	.08	4	?
159	3-picoline	30	2.0	.12	6	flat
160	4-picoline	40	2.0	.25	12	parallel
161	2-hexylpyridine	50	2.0	.35	17	flat
162	2-vinylpyridine	30	2.0	.12	6	flat
163	2,4-lutidine (no toxicity at 2%)					
164	2,6-lutidine	15	2.0	.16	8	?
168	Piperidine	30	2.0	.18	9	parallel
165	Quinoline	7	2.0	.13	6	?
167	Isoquinoline	70	2.0	.3	15	parallel
166	Quinaldine	30	2.0	.12	6	flat

None of these compounds approached nicotine in toxicity even when injected. It is obviously not lack of penetration which is responsible for their low toxicity. It is of interest to note that the toxicity of these compounds by injection is relatively the same as by spraying as determined by Tattersfield and Gimingham.

TOXICITY OF QUATERNARY COMPOUNDS OF COAL TAR BASES AND THEIR DERIVATIVES

A series of five quaternary compounds was injected, and the results are given in Table 5. 2-methylisoquinolinium iodide was as toxic as nicotine, and much more toxic than methylnicotinium iodide. In fact the similar pyridinium, quinolinium and picolinium and 2,6-lutidinium iodides were all more toxic than the corresponding nicotinium. Since the corresponding coal tar bases and their derivatives were much less toxic than nicotine, it is obvious that these quaternaries are acting in a different way. It may also be true that the effect of radicals in a pyridinium differs from a nicotinium salt.

TABLE 5. TOXICITY OF QUATERNARY COMPOUNDS OF COAL TAR BASES

ERL No.	Compound	Level of Comparison	Amt. ERL	Amt. Nicotine	Toxicity ERL	Slope
132	2-methylisoquinolinium iodide	80	.5	.6	120	parallel
133	1-methylpyridinium iodide	50	.9	.5	55	flat
134	1-methylquinolinium iodide	50	.6	.3	50	parallel
135	1-methyl-2-picolinium iodide	40	.6	.22	37	parallel
136	1-methyl-2,6-lutidinium iodide	60	.6	.35	58	parallel

DISCUSSION

The determination of toxicity by the method of injection produced different results than by spraying. On the whole, the relation between molecular constituents and toxicity was more logical by injection than by spraying. A comparison of the two methods, moreover, serves to distinguish lack of toxicity caused by lack of penetration and by low inherent toxicity. The implication that the tertiary nitrogens and the basic structure of nicotine are responsible for the toxicity of the molecule was confirmed by injection, and further strengthened by the fact that related coal tar bases and their derivatives were of very low toxicity even when injected.

There was nothing in the data obtained to prove that the nicotinium salts acted in a different way than nicotine.

SUMMARY

The relative toxicity of a group of nicotinium compounds was determined by the method of injection into milkweed bugs.

In general the compounds were more toxic in relation to nicotine injected than when sprayed on aphids by Turner and Saunders (1947).

The individual compounds could be grouped as follows: (1) those which were relatively less toxic injected than sprayed, which indicated superior penetrating power but inferior toxicity; (2) those whose relative toxicity was greatly improved by injection, which indicated inferior penetrating power and about the same toxicity as nicotine, and

(3) those compounds of low toxicity by either method, which indicated low inherent toxicity but fair penetrating power. Lack of penetrating power by the spraying method was responsible for low toxicity of a relatively few nicotinium compounds.

The relation between chemical constitution and toxicity seemed to be more logical by the injection method than by spraying. Dodecylnicotinium chloride was more toxic than the bromide or iodide. Octyl, dodecyl, hexadecyl and octadecyl compounds were more toxic than the corresponding methyl, ethyl or butyl derivatives. The benzyl compounds were not highly toxic. 2,4- and 3,4-dichlorobenzylnicotinium salts were more toxic than the corresponding o- or p-chlorobenzylnicotinium salts. Compounds containing fatty acid and thiocyanate radicals were relatively toxic.

In general, slopes of injected compounds were steeper in relation to nicotine than by spraying, but curves for individual compounds were in some cases flatter in relation to nicotine by injection than by spraying.

The data on slopes of dosage response curves could not be correlated with changes in mode of toxic action.

Toxicity of nornicotine by injection was approximately equal to nicotine. The substitution of the hydrogen therefore apparently increased the penetrating power of nornicotine, since Richardson, Craig and Hansberry (1936) found it to be two and a half times as toxic as nicotine. Dihydronicotyrine was much more toxic than nicotine when injected.

The toxicity of coal tar bases and their derivatives when injected was about the same as determined by spraying by Tattersfield and Gimingham (1927). Penetrating power was not involved in their lack of toxicity.

Quaternary compounds of the coal tar bases and their derivatives were relatively much more toxic than the parent compounds.

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APPENDIX

DETAILED RESULTS OF INJECTION OF MILKWEED BUGS

Material	Per cent Nicotine	Mortality in Replicate 1	2	3	4	Average Mortality
ERL 29	1.0	83	50	67	83	71
	.7	58	17	42	17	34
	.5	33	17	8	75	33
	.35	8	8	8	17	10
ERL 31	.5	75	17	25	67	46
	.35	0	17	58	58	33
	.25	0	25	7	33	16
	.175	33	17	17	42	27
Nicotine	.4	100	42	58	83	68
	.28	42	75	25	67	52
	.2	42	67	33	42	46
	.14	33	8	17	25	21
ERL 34	.5	67	75			71
	.35	58	67			63
	.25	42	42			42
	.175	42	33			37
Nicotine	.4	75	83			79
	.28	58	58			58
	.2	58	58			58
	.14	17	0			9
ERL 35	.5	100	92	58		83
	.35	83	75	50		69
	.25	83	83	58		75
	.175	42	58	58		53
Nicotine	.4	50	92	83		75
	.25	50	75	50		58
	.2	58	75	42		58
	.14	67	42	25		45
ERL 53	.5	83	83	50		72
	.35	83	50	75		69
	.25	83	54	42		61
	.175	100	8	0		36
ERL 56	.5	75	83	67		75
	.35	67	42	42		50
	.25	33	33	25		30
	.175	8	25	25		19
Nicotine	.4	92	83	33		69
	.28	42	50	33		42
	.2	83	42	25		50
	.14	33	42	25		33
ERL 54	1.0	33	42			38
	.7	42	0			21
	.5	33	0			17
	.35	0	0			0
Nicotine	.4	83	67			75
	.28	8	42			25
	.2	25	33			29
	.14	0	33			18

Detailed Results of Injection of Milkweed Bugs

Material	Per cent Nicotine	Mortality in Replicate 1	2	3	4	Average Mortality
ERL 72	1.0	92	92	83		89
	.7	83	58	83		75
	.5	58	75	67		67
	.35	67	58	67		64
Nicotine	.4	67	75	83		75
	.28	58	92	58		69
	.2	75	83	67		75
	.14	67	83	42		64
ERL 76	.5	42	58			50
	.35	17	25			21
	.25	8	17			13
	.175	8	17			13
Nicotine	.4	83	67			75
	.28	8	42			25
	.2	25	33			29
	.14	0	33			17
ERL 77	.5	100	92			96
	.35	83	75			77
	.25	83	33			57
	.175	42	25			34
ERL 79	.5	100	92			96
	.35	75	75			75
	.25	58	50			54
	.175	33	25			29
Nicotine	.4	50	67			59
	.28	42	42			42
	.2	25	33			29
	.14	33	0			17
ERL 80	.7	83	83			83
	.5	67	50			59
	.35	58	58			58
	.25	25	50			38
Nicotine	.8	100	83			92
	.56	92	92			92
	.4	92	83			88
	.28	83	50			67
ERL 81	.5	75	83	83		80
	.35	100	75	58		78
	.25	92	83	50		75
	.175	92	67	67		75
Nicotine	.4	83	83	83		83
	.28	75	92	75		81
	.2	50	75	75		67
	.14	42	83	58		61
ERL 83	.5	92	67	67		76
	.35	92	83	75		83
	.25	83	83	50		72
	.175	75	75	42		64

DETAILED RESULTS OF INJECTION OF MILKWEED BUGS

Material	Per cent Nicotine	Mortality in Replicate 1	2	3	4	Average Mortality
Nicotine	.4	50	92	83		75
	.28	50	75	50		58
	.2	58	75	42		58
	.14	67	42	25		45
ERL 84	.5	100	70	42	58[1]	66
	.35	90	50	25	62	58
	.25	70	40	17	25	35
	.175	70	50	8	26	34
Nicotine	.4	90	70	92	85	80
	.28	90	60	75	58	68
	.2	80	50	25	29	43
	.14	70	20	8	20	28
ERL 85	1.0	25	25			25
	.7	17	17			17
	.5	8	8			8
	.35	0	0			0
Nicotine	.4	83	83			83
	.28	75	83			79
	.2	58	75			67
	.14	58	50			54
ERL 86	.5	100	100	100		100
	.35	80	100	83		88
	.25	60	90	33		61
	.175	20	90	33		46
Nicotine	.4	70	90	92		84
	.28	40	90	58		63
	.2	30	80	42		51
	.14	20	70	25		38
ERL 87	.5	80	90	67		79
	.35	60	100	83		81
	.25	40	20	17		26
	.175	20	70	17		36
Nicotine	.4	70	80	92		81
	.28	40	70	58		56
	.2	30	40	42		37
	.14	20	60	25		35
ERL 88	.5	60	100	70	67	74
	.35	60	90	70	50	68
	.25	30	90	50	42	53
	.175	10	30	40	42	31
Nicotine	.4	70	80	70		73
	.28	40	70	60		63
	.2	30	40	40		37
	.14	20	60	30		37
ERL 89	.5	90	70	62		74
	.35	90	30	50		57
	.25	20	50	33		34
	.175	10	40	42		31

[1]Mean of 2 replicates.

DETAILED RESULTS OF INJECTION OF MILKWEED BUGS

Material	Per cent Nicotine	Mortality in Replicate 1	2	3	4	Average Mortality
Nicotine	.4	80	70	75		75
	.28	70	60	58		63
	.2	40	50	25		38
	.14	60	20	17		32
ERL 90	1.0	0	33			17
	.7	8	17			13
	.5	17	17			17
	.35	0	8			4
Nicotine	.4	75	83			79
	.28	58	58			58
	.2	58	58			58
	.14	17	0			9
ERL 92	1.0	20	60	92	33	51
	.7	20	20	92	33	41
	.5	10	10	50	17	22
	.35	10	0	67	50	32
Nicotine	.8	40	60	100	83	71
	.56	30	50	92	92	66
	.4	30	40	92	83	61
	.28	20	50	83	50	51
ERL 93	1.0	8	17			13
	.7	0	0			0
	.5	0	0			0
	.35	0	0			0
ERL 94	1.0	67	75			71
	.7	67	58			63
	.5	42	50			46
	.35	33	25			29
ERL 96	1.0	83	75			79
	.7	58	58			58
	.5	75	50			63
	.35	50	33			42
Nicotine	.4	83	83			83
	.28	75	83			79
	.2	58	75			67
	.14	58	50			54
ERL 97	.5	17	58	100		37
	.35	50	58	83		54
	.25	17	50	17		33
	.175	25	50	33		37
Nicotine	.4	83	42	83		63
	.28	33	42	50		37
	.2	42	42	42		42
	.14	33	0	25		17
ERL 98	.5	83	92	83		86
	.35	75	92	75		81
	.25	25	83	58		55
	.175	50	75	50		56

DETAILED RESULTS OF INJECTION OF MILKWEED BUGS

Material	Per cent Nicotine	Mortality in Replicate 1	2	3	4	Average Mortality
Nicotine	.4	50	50	83		61
	.28	42	33	67		47
	.2	42	42	50		44
	.14	33	8	33		25
ERL 100	.175	92	100	100		97
	.125	83	92	100		92
	.0875	58	83	33		58
	.0625	42	50	25		39
ERL 100a	.175	100	100	100		100
	.125	100	92	100		97
	.0875	75	83	58		72
	.0625	33	17	33		28
Nicotine	.4	92	92	42		75
	.28	50	83	50		61
	.2	33	83	25		47
	.14	25	8	33		22
ERL 102	1.0	92	67	92		84
	.7	67	67	92		75
	.5	42	8	67		39
	.35	75	42	92		70
ERL 103	.5	42	67	92		67
	.35	42	58	42		47
	.25	33	50	33		35
	.175	25	42	42		36
ERL 104	.5	83	83	83		83
	.35	67	92	92		84
	.25	67	75	67		70
	.175	50	58	67		58
Nicotine	.4	83	92	100		92
	.28	83	75	92		83
	.2	50	75	33		53
	.14	42	25	58		42
ERL 105	1.0	33	42			38
	.7	28	25			27
	.5	33	17			25
	.35	25	8			17
ERL 106	1.0	17	42			29
	.7	17	33			25
	.5	17	0			8
	.35	17	0			8
ERL 107	1.0	17	17			17
	.7	8	17			13
	.5	17	25			21
	.35	0	25			13
Nicotine	.4	42	42			42
	.28	58	33			45
	.2	42	42			42
	.14	17	33			25

DETAILED RESULTS OF INJECTION OF MILKWEED BUGS

Material	Per cent Nicotine	Mortality in Replicate 1	2	3	4	Average Mortality
ERL 108	.5	50	50	50	100	63
	.35	10	33	58	83	46
	.25	20	25	25	75	36
	.175	70	8	33	17	32
Nicotine	.4	83	92	75		83
	.28	75	75	67		72
	.2	67	67	56		61
	.14	67	67	42		59
ERL 110	.5	33	50			42
	.35	42	33			38
	.25	33	17			25
	.175	17	25			21
Nicotine	.4	83	67			75
	.28	8	42			25
	.2	25	33			29
	.14	0	33			18
ERL 121	.5	17	33	0	25	19
	.35	17	8	0	25	12
	.25	17	8	33	0	14
	.175	0	0	75	75	37
Nicotine	.4	58	42	42	58	50
	.28	50	33	83	58	56
	.2	33	75	33	42	45
	.14	8	25	25	25	21
ERL 122	.5	58	75			66
	.35	17	67			42
	.25	17	67			42
	.75	8	42			25
ERL 132	.5	92	67			80
	.35	58	50			54
	.25	92	58			75
	.175	92	50			71
Nicotine	.4	42	58			50
	.28	83	58			70
	.2	33	42			38
	.14	25	25			25
ERL 133	.5	67	25			46
	.35	25	25			25
	.25	42	0			21
	.175	50	8			29
ERL 134	.5	58	33			46
	.35	42	25			34
	.25	25	8			17
	.175	8	17			13
ERL 135	.5	58	58			58
	.28	42	50			46
	.2	25	42			34
	.14	8	42			25

DETAILED RESULTS OF INJECTION OF MILKWEED BUGS

Material	Per cent Nicotine	Mortality in Replicate 1	2	3	4	Average Mortality
ERL 136	.5	25	83	58	54[1]	55
	.35	50	50	58	38	46
	.25	17	8	67	17	24
	.75	0	17	58	13	19
Nicotine	.4	67	58	58	50[1]	56
	.28	67	17	50	42	44
	.2	67	75	33	54	56
	.14	33	17	33	17	23
ERL 137	1.0	17	42	75		45
	.7	8	8	25		14
	.5	0	25	42		22
	.35	8	8	33		16
Nicotine	.4	33	67	83		61
	.28	25	50	67		47
	.2	33	33	58		41
	.14	33	42	50		42
ERL 138	1.0	67	33	83		61
	.7	50	33	25		36
	.5	33	58	50		47
	.35	50	8	33		30
ERL 139	1.0	67	67	75		70
	.7	58	58	33		50
	.35	33	33	75		47
	.25	50	8	42		33
ERL 141	.5	67	50	83		69
	.35	67	42	75		61
	.25	58	42	67		56
	.175	50	50	58		53
Nicotine	.4	50	83	75		69
	.28	42	42	58		47
	.2	33	42	50		42
	.14	25	50	42		39
ERL 143	.5	17	17			17
	.35	25	0			13
	.25	17	17			17
	.175	17	8			13
Nicotine	.4	83	67			75
	.28	8	42			25
	.2	25	33			29
	.14	0	33			18
ERL 67	.14	92	75	75	92	83.5
	.1	75	75	67	75	73
	.07	58	67	75	58	64.5
	.05	75	50	75	42	60.5
Nicotine	.4	67	75	83	83	77
	.28	67	58	58	75	64.5
	.2	58	58	58	67	60
	.14	17	17	0	58	30

[1]Mean of two tests.

DETAILED RESULTS OF INJECTION OF MILKWEED BUGS

Material	Per cent Nicotine	Mortality in Replicate 1	2	3	4	Average Mortality
ERL 68	2.0	33	42	58	67	50
	1.4	17	17	33	42	27
	1.0	17	25	0	33	18
	.7	25	25	0	0	12.5
Nicotine	.4	67	83	83	83	79
	.28	50	67	83	75	71
	.2	17	50	67	58	48
	.14	8	42	50	42	35.5
ERL 69	.4	75	75			75
	.28	67	75			71
	.2	50	50			50
	.14	42	42			42
ERL 70	1.0	50	17			34
	.7	33	33			33
	.5	17	8			13
	.35	33	17			25
Nicotine	.4	92	67			80
	.28	25	67			46
	.2	50	58			54
	.14	8	17			13
ERL 155	1.0	55	17			36
	.7	8	0			4
	.5	8	17			13
	.35	0	0			0
Nicotine	.4	92	67			80
	.28	25	67			46
	.2	50	58			54
	.14	8	17			13
ERL 156	1.0	50	67	25	58	50
	.7	50	75	25	58	52
	.5	50	50	42	50	48
	.35	25	42	8	33	27
Nicotine	.4	67	83	83	83	79
	.28	50	67	83	75	71
	.2	17	50	67	58	48
	.14	8	42	50	42	35.5
ERL 157	2.0	25	17			21
	1.4	0	0			0
	1.0	0	33			16
	.7	8	8			8
ERL 159	2.0	33	25			29
	1.4	25	25			25
	1.0	17	33			25
	.7	0	33			16
ERL 160	2.0	25	50			38
	1.4	8	25			17
	1.0	0	17			8
	.7	8	25			17

DETAILED RESULTS OF INJECTION OF MILKWEED BUGS

Material	Per cent Nicotine	Mortality in Replicate 1	2	3	4	Average Mortality
Nicotine	.4	83	83			83
	.28	75	75			75
	.2	58	67			63
	.14	42	17			29
ERL 158	2.0	17				
	1.4	0				
	1.0	0				
	.7	8				
ERL 164	2.0	17				
	1.4	0				
	1.0	0				
	.7	0				
ERL 165	2.0	8				
	1.4	0				
	1.0	0				
	.7	0				
Nicotine	.4	83				
	.28	75				
	.2	58				
	.14	42				
ERL 161	2.0	50	42			46
	1.4	8	25			17
	1.0	25	25			25
	.7	17	50			33
ERL 167	2.0	67	75			71
	1.4	17	33			25
	1.0	25	25			25
	.7	8	17			13
ERL 168	2.0	33	25			29
	1.4	0	25			12
	1.0	0	0			0
	.7	0	0			0
Nicotine	.4	83	83			83
	.28	58	75			67
	.2	8	67			37
	.14	0	17			8
ERL 162	2.0	25				
	1.4	25				
	1.0	17				
	.7	0				
Nicotine	.4	75				
	.28	58				
	.2	50				
	.14	58				
ERL 166	2.0	33				
	1.4	25				
	1.0	25				
	.7	17				

DETAILED RESULTS OF INJECTION OF MILKWEED BUGS

Material	Per cent Nicotine	Mortality in Replicate 1	2	3	4	Average Mortality
ERL 163	2.0	0				
	1.4	0				
	1.0	0				
	.7	0				
Nicotine	.4	83				
	.28	58				
	.2	7				
	.14	0				
ERL 71	2.0	17	75	17		36
	1.4	8	33	17		19
	1.0	8	42	17		22
	.7	8	33	42		28
ERL 191	2.0	100	92			96
	1.4	33	58			45
	1.0	58	50			54
	.7	50	42			46
ERL 192	2.0	58	67			62
	1.4	50	50			50
	1.0	17	25			21
	.7	8	50			29
Nicotine	.4	75	75	67		72
	.28	67	67	58		64
	.2	50	33	33		39
	.14	33	58	25		39