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PESTICIDE ACTIVITY AND STRUCTURE

Structure and Nematocidal Activity of Allylic and Acetylenic Halides

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Special techniques for estimating the dosage-response curves of some allylic and acetylenic halides using larvae of the citrus nematode, *Tylenchulus semipenetrans* Cobb, have been developed. The toxicities of these halides, as measured by the concentrations required to produce 50% inhibition of mobility, were found to be related to their reactivities in the S_N2 reaction with potassium iodide in acetone. A possible mode of action for these halides is suggested.

ORGANIC HALIDES are currently being used extensively as soil fumigants for the control of plant parasitic nematodes and other pathogenic soil organisms (*22*). The two most common types are the saturated aliphatic halides such as methyl bromide, ethylene dibromide, and 1,2-dibromo-3-chloropropane, and the 2,3-unsaturated alkyl halides such as 1,3-dichloropropene.

Although some of these have been utilized as nematocides for almost 15 years, very little is known concerning their mode of action. It has been suggested that these compounds act as narcotics and that physical properties such as vapor pressure, water solubility, and ability to dissolve wax are most important (*5, 21*). However, toxicological data were recently obtained which suggested that reactivity was involved (*29*). In a series of halides RX, where R was constant, the order of toxicity was RI>RBr>RCl. *cis*-1,3-Dichloropropene was more toxic than *trans*-1,3-dichloropropene and 2,3-unsaturated alkyl halides were more toxic than the corresponding saturated derivatives. In each case, nematocidal activity parallels the rate of reactivity of the halide in bimolecular nucleophilic displacement—S_N2—reactions. The present investigation was undertaken to study this relationship in more detail.

This report summarizes the techniques that were developed for the estimation of dosage-mortality curves using citrus nematode larvae and compounds which are toxicants at concentrations less than their solubilities in water. Results are also indicated for a number of allylic and acetylenic halides, and the relationship between the toxicities of these halides and their reactivities in the S_N2 reaction with potassium iodide in acetone.

Apparatus

VIALS. 25-ml. capacity with plastic snap-on caps (Merck, Sharpe and Dohme, Nos. 3352 and 6301). Drill a 27/32 inch diameter hole in half the caps.

FILTER CLOTHS. $1^1/_{32}$ inches in diameter. Drill from plastic cloth (Style PM-2711-C Polymax), 176 × 73 filaments per inch and a nominal diameter of 0.006 inch.

VIAL HOLDERS. Cut $^3/_4 \times 3 \times 31^1/_2$ inch hardwood lumber lengthwise, drill twelve $1^1/_4$-inch diameter holes on $2^1/_8$-inch centers $4^1/_{16}$ inches from each end, and line each hole with $^1/_{16}$-inch felt. Combine the two halves and through the $^3/_4$-inch side, drill three $^1/_4$-inch diameter holes, in the center and $1^1/_2$ inches from each end. Secure the two halves with $^3/_{16}$-inch stove bolts and wing nuts.

Petri dish holder and counting grid (Figure 1). Drill a $2^1/_8$-inch diameter hole in the center and four $^1/_8$-inch diam-

eter holes, 1/2 inch from the four corners of two 1/4 × 4 × 4 inch Lucite plates. Prepare a circular grid pattern 2 1/32 inches in diameter on a 2 1/2 × 2 1/2 inch film plate and protect the emulsion side with a spray application of Krylon No. 1303. The diameter of the grid is such that a single segment is in view under 30× magnification. Center the grid between the Lucite plates with the emulsion side down and secure with four 3/32-inch bolts. Mount a steel wire between two of the bolts to prevent movement of variable-sized Petri dishes.

Wooden blocks, 3/4 × 3 × 2 inches.

Petri dishes, 60 × 15 mm.

The room should be maintained at a temperature of 25° ± 1° C.

Microscope, 30× magnification.

Blood cell counter, single unit, hand tally.

Sieves, 8 inches in diameter, 60-, 80-, 100-, and 400-mesh.

Materials

The allylic and acetylenic halides were purified from commercial samples by washing with water and aqueous sodium bicarbonate, drying over calcium chloride, and fractionating through an efficient column. Allyl chloride, allyl bromide, 3-chloro-2-methyl-1-propene, 2,3-dichloro-1-propene, propargyl chloride, and propargyl bromide, after purification had the following physical properties: boiling point 44.2° C. at 733 mm. of mercury, n_D^{27} 1.4105; boiling point 69.5° C. at 732 mm. of mercury, n_D^{22} 1.4679; boiling point 70.5–0.8° C. at 733 mm. of mercury, $n_D^{26.2}$ 1.4240; boiling point 92.5° C. at 734 mm. of mercury, $n_D^{27.2}$ 1.4562; boiling point 56.3° C. at 740 mm. of mercury, n_D^{25} 1.4315; boiling point 56.5° C. at 300 mm. of mercury, $n_D^{20.1}$ 1.4930. *cis*- and *trans*-1,3-dichloropropene were separated by the fractionation of D-D, a dichloropropene-dichloropropane mixture. The fractions distilling at 100–5° C. and 110–10.5° C. at 731 mm. were collected separately and purified by the above procedure. The cis and trans isomers had the following physical properties respectively: boiling point 102.5 to 102.8° C. at 733 mm. of mercury, n_D^{22} 1.4682 and boiling point 110.5 to 111° C. at 734 mm. of mercury, n_D^{23} 1.4730.

Acetone was purified by distillation through an efficient column.

Colloidal sticker spreader (nonionic, X-77 Colloidal Products Corp.) was used.

A stock suspension of citrus nematode larvae, *T. semipenetrans* Cobb was prepared as follows: Approximately 2 pounds of citrus feeder roots (<0.25 inch in diameter) were collected from infested lemon trees, shaken to remove adhering soil particles, placed in 2 gallons of water, and aerated for 24 hours. The aqueous suspension, after removing the roots, was passed through successively smaller mesh sieves to eliminate soil, sand, and root particles and then was slowly poured through a 400-mesh sieve held at about a 45° angle. The larvae which collected on the surface of the sieve were washed with water and separated from a small amount of silt and organic matter using Polymax plastic cloth as a filtering medium in a modified Baerman funnel technique (*27*). After 24 hours the larvae were transferred to a 50-ml. volumetric flask and diluted with water. This stock suspension was stored at 0° C. until needed.

About 75 to 85% of the larvae in this suspension were second-stage females, while the remainder consisted mainly of the four stages in the life cycle of the male plus some mature males (*36*).

Procedure

In each experiment three compounds were usually tested at the same time, so that comparative toxicities could be calculated when different nematode populations were used. This eliminated any variation in susceptibility. Six concentrations, in sextuplicate, of each compound were used plus twelve untreated controls.

A 500-ml. standard aqueous suspension, containing approximately 200 mobile larvae per ml., was prepared from the stock suspension and 3 ml. were added to 120 of the 25-ml. vials. The suspension was shaken gently just before removing each 3-ml. aliquot. The vials were treated with cleaning solution and rinsed with water prior to the addition of the nematodes to prevent the loss in water droplets which would form on the walls of the vials when they are inverted later in the procedure.

The compounds were weighed into three 100-ml. volumetric flasks and diluted with acetone. Six dilutions of each were made with acetone such that the final concentrations would be about equally spaced on a log concentration-probit scale. These were then diluted 1 to 100 with water and six 2-ml. aliquots were added to 108 of the vials containing the larvae suspensions. The remaining 12 vials were diluted with 2 ml. of 1% acetone–water. The vials were then capped and allowed to stand at 25° C. for 96 hours.

The lower halves of 120 Petri dishes containing 5 ml. of water were placed in 10 rows of 12 each. Prior to the addition of the water, the bottom of each dish was wet with a 0.07% aqueous solution of the sticker spreader. This provides a better distribution of larvae on the bottom of the dishes by reducing the surface tension of the water.

After 96 hours the vials were recapped with those containing the filter cloths, placed in the racks, and inverted over the Petri dishes as shown in Figure 2. The vials are positioned in the racks before inversion so that the filter cloths will be below the surface of the water

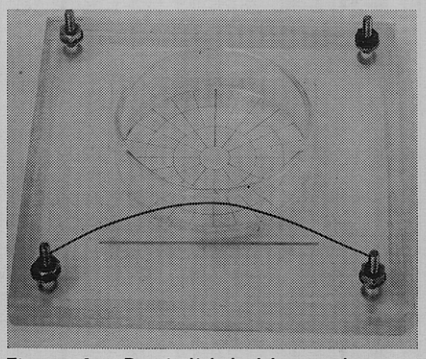

Figure 1. Petri dish holder and counting grid

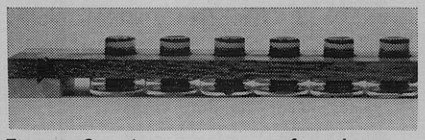

Figure 2. Arrangement of racks over Petri dishes

but above the bottom of the dishes. After 42 hours, the racks were removed and the number of larvae in each dish were counted under 30× magnification using the combination holder-counting grid (Figure 1) and the hand tally blood cell counter. As the time required for one operator to count all of these samples takes more than 8 hours, the vials can be capped and stored at 0° C. until needed.

The proportion of killed or immobile larvae at each of the six concentrations, for each compound, was calculated from the average number of survivors at each of the concentrations used and the average number in the control group. The values for each compound were then plotted as log molar concentration *vs.* the probit of inhibition of mobility. The probit regression lines for each set of three compounds were subjected to the constraint of parallelism and the estimation of the various parameters such as the molar concentrations required to produce 50% inhibition of mobility, ED_{50}, and log relative dosage values, M, was carried out by the method of Finney (*14*). In each experiment, M values were determined using one halide as a standard and were set up so that toxicities relative to *cis*-1,3-dichloropropene could be calculated using just one intermediate halide. This compound was selected as the standard and it was tested in all but two experiments.

Results and Discussion

Estimation of Percentage Mortality. Before the halides were tested, preliminary experiments were conducted to find an accurate method for differentiating mobile from immobile larvae. The usual visual technique was tried, but was unsatisfactory because of the time required for differentiating large numbers and the operator variability. Large numbers were used, because accuracy is a

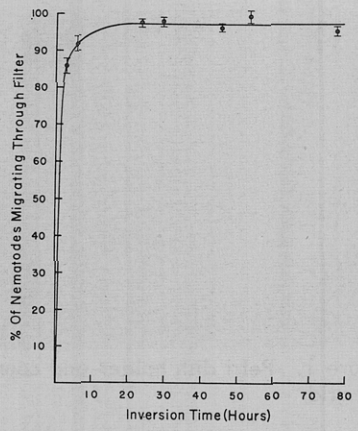

Figure 3. Efficiency of plastic filter cloth as a function of inversion time

Lines indicate standard errors

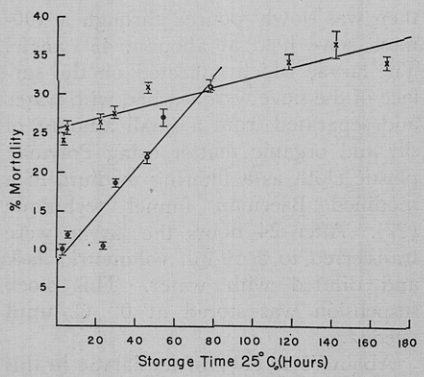

Figure 4. Effect of storage time at 25° C. on per cent mortality

Prior storage at 0° C. for 6 days (o) and 36 days (x)

Lines indicate standard errors

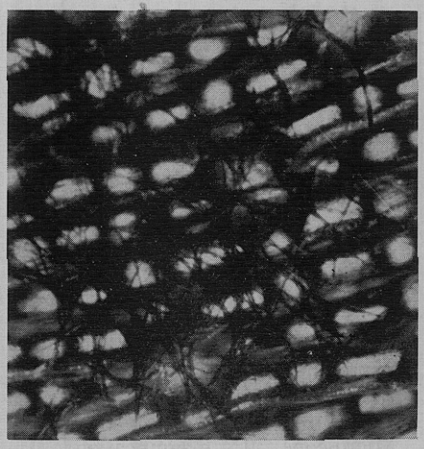

Figure 5. Photomicrograph of plastic filter cloth and citrus nematode larvae, 95× magnification

function of the total number counted (*25*).

Various membranes were tested to determine their efficiency in separating mobile from immobile larvae. The membranes tested were sharkskin and Whatman No. 1 filter paper, one and two layers of toilet tissue, 400-mesh phosphor-bronze wire cloth, standard mesh bolting silk, and Polymax filter cloth. Tests were conducted with suspensions containing different ratios of mobile and immobile larvae. These were prepared either by a 4-hour treatment with 8 to 14% (by volume) acetone, or by allowing the nematode stock suspension to stand at 0° C. The plastic filter cloth was the most effective. The rate of separation was rapid, and the number of larvae which came through approximately equaled the number visually estimated as mobile. The results of one experiment using this filter, with a stock suspension which had been stored at 0° C. for 6 days prior to use, are shown in Figure 3. The percentages are based on the number of visually estimated mobile larvae. This was obtained from the total number of larvae and the per cent mortality in uninverted samples at time, $t = 0$ (Figure 4). Twelve replicates, containing 678 ± 4 larvae, were used at each time interval. The maximum number of larvae which pass through the filter is reached somewhere between 10 and 24 hours. For a safety margin, an inversion time of 42 hours was selected for use in subsequent chemical tests.

When this experiment was repeated 30 days later using the same suspension, a similar curve was obtained, but the immobile larvae had increased from 8.41 to 25.6% and the maximum percentage of larvae passing through had decreased from 97 to 87%. This would indicate that after storage at 0° C., a certain proportion of the population, which appear mobile, can no longer penetrate the membrane. A plot of estimated mortality *vs.* storage time at 25° C. for the 6- and 36-day-old stock suspensions is shown in Figure 4. The younger population, as might be expected, has a higher mortality rate, as it presumably contains a larger proportion of more susceptible larvae.

A photograph of a number of larvae on the surface of the plastic cloth, which illustrates their length and diameter in relation to the thread size, is shown in Figure 5. Most of them are second-stage females with an average length of 323 microns and a diameter of 12 microns (*36*).

Effect of Acetone. In the above filter-efficiency experiments, acetone was found to be relatively nontoxic. Samples of a standard aqueous suspension were treated with acetone at four concentrations and allowed to stand at 25° C. for 4 hours. These and an untreated sample were centrifuged and the larvae were rinsed and resuspended in water. After 48 hours at 25° C., four replicates of each were inverted and the number of survivors counted at different time intervals. With the plastic filter and a 16-hour inversion time, the ED_{50} and the slope of the probit regression equation were $1.53M$ and 18.2, respectively. This would indicate that 1% ($0.136M$) acetone used in the chemical tests has very little effect on the mobility.

Effect of Sample Size. An investigation was made of the efficiency of the filter as a function of the total number of larvae. Dilutions of a standard aqueous suspension were made so that the total number of mobile larvae varied from 100 to 500. Replicate samples were then inverted for 42 hours, and those which came through at each nematode level were counted and compared with uninverted samples. Data from one experiment are presented graphically in Figure 6. It is apparent that the number of larvae on the surface of the filter has no influence on the number which migrate through.

Effect of Storage and Exposure Time. Citrus nematode larvae in water have a relatively long shelf life if kept at 0° C. When the same stock suspension was used over a period of time in tests with the allylic and acetylenic halides, the standard statistical analysis gave revised estimates of the number of survivors in the control groups (*14*). From these estimates, and the total number of larvae in each experiment, the mortalities in the control groups were calculated and plotted *vs.* the storage time at 0° C. This is shown in Figure 7 for two different suspensions. Over a 2-month period the percentage of larvae which are able to migrate through the filter is fairly constant. However, after this time, the mortality increased rapidly. In contrast to this initially low mortality rate at 0° C., the rate of 25° C. was fairly high (Figure 4).

The effect of temperature on mortality has also been noted with other species. When 10 larvae of the burrowing nematode, *Radopholus similis* (Cobb) Thorne, were used in water at room temperature, only a few were alive at the end of 49 days and these died in an additional 17 days (*4*). Studies have shown that the stages in the life cycle of a nematode differ in their shelf life. Second-stage female larvae of *Hemicycliophora arenaria* Raski died within 1 week, at room temperature while third-stage larvae were active for 2 to 3 weeks and adults for 4 to 5 weeks (*37*). An interesting mortality-temperature relationship has been noted for *Trichodorous Christie* (*34*). Larvae of this species die overnight if kept in water at 0° C., but can be kept for almost 1 week at 25° C.

One of the important variables in toxicity studies is the exposure or contact time. This was evaluated by testing allyl chloride and *cis*-1,3-dichloropropene, using exposure times of 96, 120, and 240 hours. There was very little, if any, change in mortalities as a function of time. For convenience, the lowest exposure time of 96 hours was chosen

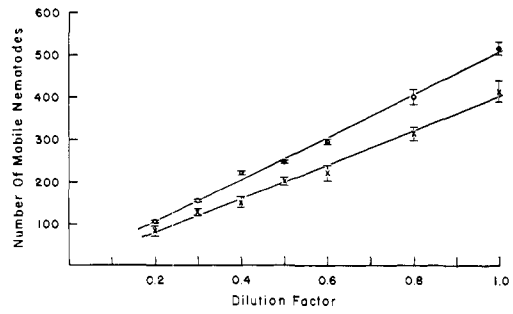

Figure 6. Efficiency of filter as a function of the total number of mobile nematodes

o. Estimated number mobile
x. Number through filter after 42 hours' inversion
Lines indicate standard errors

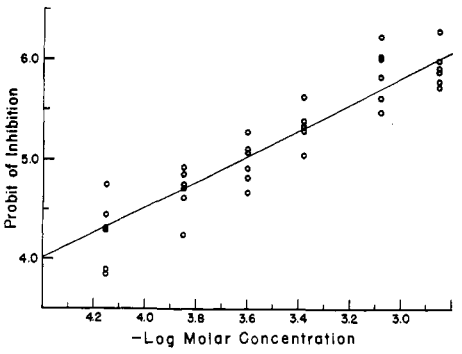

Figure 9. Dosage-response curve for propargyl chloride

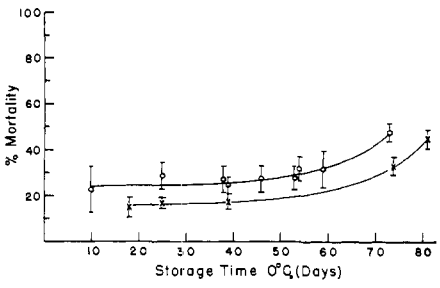

Figure 7. Effect of storage time at 0° C. on subsequent per cent mortality of two nematode suspensions after 96 hours at 25° C.

Lines indicate standard errors

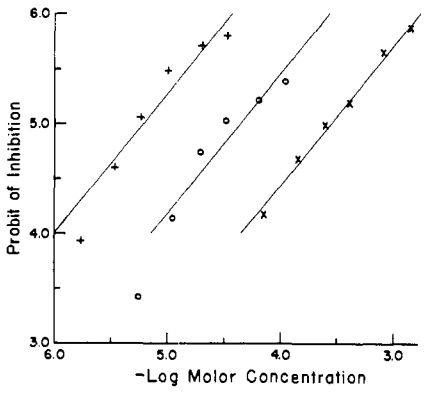

Figure 8. Dosage-response curves

o. Propargyl chloride
x. *cis*-1,3-Dichloropropene
+. Propargyl bromide

in subsequent tests with the halides.

Allylic and Acetylenic Halides. Eight halides were tested in eight separate experiments. The results, using propargyl chloride, propargyl bromide, and *cis*-1,3-dichloropropene are presented in Figure 8.

Although the variation between replicates was fairly large, as illustrated with propargyl chloride in Figure 9, it is much less than that previously noted with D-D in a 48-hour exposure at 20° C. using a mixed population of *Rhabditis* sp. and *Panagrellus* sp. in moist sand (*33*). The greater variability in the latter case may be due to the use of a mixed population or to the relatively low numbers of larvae. Both the LD_{50} (31.2 p.p.m.) and slope (5.18 ± 1.29) calculated, using the method of Finney (*14*) and assuming a total number of 200 larvae, are somewhat different from those found in the present investigation. The ED_{50} for *cis*- and *trans*-1,3-dichloropropene are 3.62 and 8.58 p.p.m., respectively (Table I). The slopes in the eight experiments ranged from 0.97 ± 0.13 to 1.35 ± 0.22.

The toxicities of the halides to citrus nematode larvae are summarized in Table I and were based on a weighted mean for the standard, *cis*-1,3-dichloropropene (*15*). The other halides were spaced at distances from it based on their M values. When possible, weighted means were also used to obtain improved estimates of these log relative toxicities.

The relative reaction rates of the halides with potassium iodide in anhydrous acetone, and in 50% ethyl alcohol–50% dioxane, and water are also shown in Table I. The toxicities of the halides are related to their reactivities in the S_N2 reaction with iodide ion—as the reactivity increases, the toxicity also increases. The most toxic compound is propargyl bromide, and the least toxic is allyl chloride. Their reactivities differ by about 900 and their toxicities by 300. This relationship is presented graphically in Figure 10, which shows a plot of the log of the relative toxicities, M, against the log of the relative rate constants for the reaction of the halides with iodide ion in acetone.

This relationship between toxicity and reactivity suggests that the mode of action of these halides is, perhaps, a displacement reaction with some required nucleophilic center in the nematode, such as a sulfhydryl or amino group present in one or more of the essential enzyme systems or in some protein or peptide of biological importance:

$$\left.\begin{matrix}\text{P}\\\text{R}\\\text{O}\\\text{T or Z}\\\text{E}\\\text{I}\\\text{N}\end{matrix}\right.\!\!\left.\begin{matrix}\\\text{E}\\\text{N}\\\text{Y}\\\text{M}\\\text{E}\end{matrix}\right\}\!: + \text{R:X} \rightarrow \left.\begin{matrix}\text{P}\\\text{R}\\\text{O}\\\text{T or Z}\\\text{E}\\\text{I}\\\text{N}\end{matrix}\right.\!\!\left.\begin{matrix}\\\text{E}\\\text{N}\\\text{Y}\\\text{M}\\\text{E}\end{matrix}\right\}\!:\text{R} + :\text{X} \qquad (1)$$

The pronounced toxicity of other compounds such as α-haloketones, α-haloesters, isothiocyanates, and 1,2-epoxides adds additional support to this mode of action, because they are also reactive in S_N2 reactions.

Two compounds that do not fit the curvilinear relationship are allyl bromide and 2,3-dichloro-1-propene. Based on their reactivities, the former is much less toxic than expected, while the latter is somewhat more toxic. This would indicate that either the mode of action on nucleophilic centers in the nematode is inadequately represented by the iodide ion displacement reaction, or that other factors besides S_N2 reactivity, such as the rate of solvolysis of the halide in water and the toxicity of the alcohol resulting from solvolysis, are also involved.

The allylic and acetylenic halides tested differ markedly in their relative reactivities toward water (Table I). Some, like allyl bromide, solvolyze rapidly, while others, such as propargyl bromide, are relatively inert. Therefore, the larvae will be subjected either to a rapidly decreasing or to a relatively steady-state concentration of halide. Allyl bromide has a specific rate constant in water at 25° C. of 1.66×10^{-5} sec.$^{-1}$ (*10*). This corresponds to a half life of only 11.6 hours. As the exposure time was 96 hours, 99.7% of the halide had disappeared at the conclusion of the test because of reaction with water alone. Propargyl bromide, on the other hand, has a very low rate of solvolysis: 1.59×10^{-7} sec.$^{-1}$ (*10*). After 96 hours, 94.7% of the halide is still left. Propargyl chloride has an even lower rate of solvolysis, as bromides react much faster than chlorides in both unimolecular and bimolecular reactions (*32*). This is illustrated in Table I by its low rate of solvolysis in 50% ethyl alcohol. The remaining allylic halides solvolyze at rates intermediate between those of allyl bromide and propargyl chloride. If their relative solvolysis rates in 50% ethyl alcohol and in 50% dioxane (Table I) can be used as an index of their rates in water, the most reactive would be 3-chloro-2-methyl-1-propene. About 57% of this compound is still left after 96 hours at 25° C. Allyl chloride solvolyzes less rapidly and about 68% is still left at the end of this exposure time.

The other factor which will influence the nematocidal activity of a 2,3-unsaturated alkyl halide is the toxicity of the alcohol resulting from the solvolysis. Allylic and acetylenic alcohols were previously shown to be very effective nematocides, with some of them possessing toxicities in the same range as the halides (*29*). Allyl and propargyl alcohols, *cis*- and *trans*-3-chloropropen-

Table I. Relative Reaction Rates and Relative Toxicities of Some Allylic and Acetylenic Halides for Larvae of the Citrus Nematode

Compound	ED_{50}, Molar Concn.	KI-Acetone, 20° C.	50% Ethyl Alcohol, 44.6° C.[a]	50% Dioxane, 30° C.[b]	Water, 25° C.
$CH_2{=}CHCH_2Cl$	1.52×10^{-3}	1.00[c,d]	1.00	1.00	1.00[e]
$CH_2{=}C(Cl)CH_2Cl$	2.59×10^{-4}	0.72[c]	...	0.83	...
$CH_2{=}C(CH_3)CH_2Cl$	2.84×10^{-4}	1.58[c]	1.52	1.33	1.46[f]
$CH{\equiv}CCH_2Cl$	2.05×10^{-4}	1.78[d]	0.049	...	...
trans-$ClCH{=}CHCH_2Cl$	7.73×10^{-5}	2.9[c]	0.98	...	...
cis-$ClCH{=}CHCH_2Cl$	3.27×10^{-5}	8.58[c]	0.98	0.71	...
$CH_2{=}CHCH_2Br$	7.49×10^{-5}	506[d]	...	21	15.0[g]
$CH{\equiv}CCH_2Br$	4.53×10^{-6}	909[d]	...	...	0.143[g]

[a] Vernon (*38*). [b] Pourrat *et al.* (*31*). [c] Hatch *et al.* (*20*). [d] Fierens *et al.* (*12*); extrapolated from 25° C. using Arrhenius energy of activation and frequency factor. [e] Fierens *et al.* (*11*). [f] Extrapolated from 45° C. (*8*) assuming E^* equal to that of allyl chloride and $k_{50\%C_2H_5OH}/k_{H_2O} = 5.3$ (*7*). [g] Fierens *et al.* (*10*).

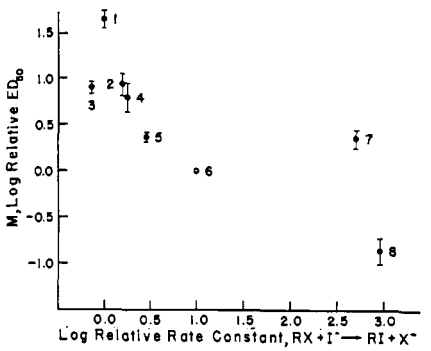

Figure 10. Relation between log molar dosage value, relative to *cis*-1,3-dichloropropene for larvae of citrus nematode and log bimolecular rate constant for reaction RX + I^- → RI + X^- relative to allyl chloride

1. $CH_2{=}CHCH_2Cl$
2. $CH_2{=}C(CH_3)CH_2Cl$
3. $CH_2{=}C(Cl)CH_2Cl$
4. $CH{\equiv}CCH_2Cl$
5. *trans*-$ClCH{=}CHCH_2Cl$
6. *cis*-$ClCH{=}CHCH_2Cl$
7. $CH_2{=}CHCH_2Br$
8. $CH{\equiv}CCH_2Br$

Lines indicate standard errors

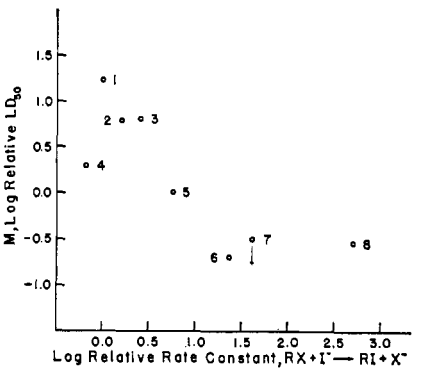

Figure 11. Relation between log molar dosage value, relative to *cis*- and *trans*-1,3-dichloropropene for larvae of oriental fruit fly and log bimolecular rate constant for reaction RX + I^- → RI + X^-, relative to allyl chloride

1. $CH_2{=}CHCH_2Cl$
2. $CH_2{=}C(CH_3)CH_2Cl$
3. $CH_3CH_2CH_2CH_2Br$
4. $CH_2{=}C(Cl)CH_2Cl$
5. $ClCH{=}CHCH_2Cl$
6. $ClCH_2COOCH_2CH_3$
7. $ClCH_2CN$
8. $CH_2{=}CHCH_2Br$

2-ol-1, the solvolysis products of *cis*- and *trans*-1,3-dichloropropene, all gave 95 to 100% control of the citrus nematode in soil tests at 25 p.p.m. The exceptional nematocidal activity of these alcohols is yet to be explained.

The toxicity of a 2,3-unsaturated alkyl halide is probably due to an integrated effect: its reactivity in S_N2 reactions (potassium iodide in acetone being used as an index), its rate of solvolysis, and the toxicity of the resulting alcohol. The relative importance of these three factors will determine the toxic dose.

Application of Structure-Toxicity Relationship to Other Halides. This relationship can be used for predicting the toxicites of substituted allylic and acetylenic halides, because the effect of substituents, in these systems, on their reactivities in S_N1 and S_N2 reactions is well established (*9*, *32*). 3,3-Dimethylallyl chloride reacts 28 times faster than allyl chloride with iodide ion in acetone (*19*) but would probably be a poor nematocide. This compound has a half life of approximately 3.2 sec. in 50% ethyl alcohol at 44.6° C. (*39*). If used in moist soil, most of it would have disappeared before coming in contact with the nematodes.

Substituted allylic halides with electron-withdrawing substituents such as halogen in the γ-position, would be expected to be active nematocides. They are very reactive in S_N2 reactions and possess relatively low rates of solvolysis (*38*). 3,3-Dichloro-2-methylallyl chloride reacts more than 33 times faster than allyl chloride with potassium iodide in acetone (*20*), and reactive allylic chlorides when tested in a cuprous chloride-catalyzed hydrolysis (*17*). The bromo analog of this compound, 3,3-dibromo-2-methylallyl bromide, was only recently patented for use as a nematocide and fungicide (*26*).

Although the acetylenic halides are usually only slightly more reactive in S_N2 reactions than the corresponding allylic halides (*3*, *12*, *13*, *16*, *18*, *23*, *30*, *39*),

Table II. Relative Displacement Rates and Toxicities of Some Halides for Larvae of Oriental Fruit Fly

Compound	LD_{50}, Molar Concn.[a]	KI-Acetone, 20° C.
$CH_2{=}CHCH_2Cl$	9.15×10^{-4}	1.00[b]
$CH_2{=}C(Cl)CH_2Cl$	1.04×10^{-4}	0.78[b]
$CH_2{=}C(CH_3)CH_2Cl$	3.20×10^{-4}	1.58[b]
$CH_3CH_2CH_2CH_2Br$	3.43×10^{-4}	2.57[c]
$ClCH{=}CHCH_2Cl$	5.41×10^{-5}	5.74[b,d]
$ClCH_2COOCH_2CH_3$	1.14×10^{-5}	23.6[e]
$ClCH_2CN$	1.72×10^{-5}	41.4[f]
$CH_2{=}CHCH_2Br$	1.49×10^{-5}	506[b]

[a] Balock *et al.* (*1*).
[b] From Table I.
[c] Extrapolated from 25° C., using E^* = 16.55 kcal./mole (*35*) and k for allyl chloride from (*12*).
[d] Average of cis and trans isomers.
[e] McBee *et al.* (*28*).
[f] Conant *et al.* (*6*). Calculated from extrapolated value of allyl chloride to 20° C., using A = 4000 in the equation $\log_{10}(k_1/k_2) = A(1/T_2 - 1/T_1)$.

their greater toxicity, as found in these experiments, is best explained by their exceptional stability in water. Allyl bromide reacts with water 104 times faster than propargyl bromide at 25° C. Even the corresponding saturated halide, *n*-propyl bromide, reacts almost twice as fast as propargyl bromide (*10*). Two acetylenic halides, propargyl bromide and 3 - chloro - 1 - iodo - 1-propyne, were recently patented as nematocides (*2*, *24*).

Application of Structure-Toxicity Relationship to Other Organisms. If the mode of action of 2,3-unsaturated alkyl halides, with citrus nematode larvae, involves a displacement reaction with essential nucleophilic centers, Equation 1, then the toxicities of these compounds to other organisms which are dependent upon intact nucleophilic centers should also be related to their reactivities, although the amount and types of these centers may vary in the different organisms. The toxicities—LD_{50} and LD_{95}—of a number of allylic and other type halides have been reported using both the eggs and larvae of the oriental fruit fly, *Dacus dorsalis* Hendel (*1*). A summary of the LD_{50} of some of the halides tested and their relative reactivities in the iodide ion displacement reaction are shown in Table II. As the reactivity increases there is a corresponding increase in toxicity (Figure 11). The coordinates are the same as those in Figure 10, except that the relative toxicities are based on both *cis*- and *trans*-1,3-dichloropropene, instead of only on the cis isomer. Not

only are the toxicities of the allylic halides related to their reactivities, but also those of the saturated halide, *n*-butyl bromide, and the two α-substituted halides, chloroacetonitrile and ethyl chloroacetate.

A similar plot can be obtained with the eggs of this insect, which suggests that the mode of action of these halides is the same with the two different stages in the life cycle of the oriental fruit fly and also with the two different organisms.

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INSECTICIDE RESIDUES IN MILK

Effects of Feeding Low Levels of Insecticide Residues on Hay to Dairy Cattle on Flavor and Residues in Milk

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EXCELLENT CONTROL OF FORAGE insects is possible with insecticide treatments which leave no more than 1 or 2 p.p.m. of residue at harvest (*2, 10, 12, 13, 21*). While these low residues were believed not to affect the health of the dairy cattle or to produce detectable off-flavors and odors or residues in milk, the lack of exact data had prevented the recommendation of many of the new organic insecticides for use on forages. In 1950, a 3-year study was initiated on the various effects of feeding insecticide-treated hay to dairy cattle. Two early progress reports of very limited distribution were issued (*11, 15*). This is the final, complete report of that work.

[1] Deceased.

Methods and Materials in 1950–51 Tests

Four insecticides, DDT, lindane, parathion, and aldrin were selected for forage insect control. These materials were formulated as dusts and applied to a second-cutting stand of alfalfa of the variety Ontario Variegated at the rate of 1 pound of actual toxicant per acre. Thirty-six days after treatment, the plots were harvested, mow-dried, and stored. Samples of hay were taken for residue analysis at the time of treatment, harvest, start of feeding, 3 weeks after feeding, and at the end of the feeding period (Table I).

Breeds of Cattle and Experimental Design in 1950 Tests

Five Holstein and five Brown Swiss cows were chosen from the regular dairy herd and one cow of each breed was assigned to each insecticide and the untreated check. The cows were then assigned in a completely randomized order to the stalls they were to occupy during the experiment. Each stall was so built that no cow could steal feed from her neighbor. This arrangement made it possible