Arch. Environm. Contam. Toxicol. 6, 325–335 (1977)

Archives of Environmental Contamination and Toxicology

Partition Distribution of Insecticides as a Critical Factor Affecting Their Rates of Absorption from Water and Relative Toxicities to Fish[1,2]

Chia-Fang Yang[3] and Yun-Pei Sun[4]

Department of Plant Pathology and Entomology, National Taiwan University, Taipei; Plant Protection Center, Taiwan, Taichung Hsien, Taiwan, Republic of China

Abstract. Loaches, *Misgurnus anguillicaudatus* (Cantor), a common fish in Taiwan, were treated with DDT, dieldrin, and monocrotophos by continuous exposure in aqueous solutions (or suspensions) and by injection. DDT and dieldrin were 150 and 220 times more toxic, respectively, than monocrotophos, to the fish exposed in aqueous solutions (24-hr LC_{50}), but only 1/9 and 1/4 as toxic as monocrotophos by injection (24-hr LD_{50}). Results of GLC analyses indicate that, at the end of 24-hr exposure, 96.5% of DDT, 92.7% of dieldrin, and 14.3% of monocrotophos were absorbed by loaches from aqueous solutions. The initial rates of absorption for DDT and dieldrin were about 10 to 20 times faster than that for monocrotophos. The large differences in relative toxicity may be due to partition distribution which in turn caused differences in absorption, as DDT and dieldrin are lipophilic and monocrotophos is hydrophilic. Statistical analysis of the relationship between fish toxicities and partition coefficients supports the present finding. The coefficient of correlation is 0.70 between partition coefficients (benzene/water) and toxicities to fish (rainbow trout) of 12 organophosphorus insecticides, 0.74 between coefficients and corrected fish toxicities, and 0.96 between partition coefficients and corrected fish toxicities for organophosphates only. Results of analyses are significant at <1% probability level. Similar correlation was also obtained between partition coefficients for hexane/water and toxicities of 8 organophosphorus and 5 organochlorine insecticides to rainbow trout.

The toxicity of an insecticide varies with the species tested, and any species may have different degrees of susceptibility to various toxicants. In order to understand the basic factors responsible for such differences in activity, we may assume that, in addition to the difference in inherent toxicities of compounds,

[1] This investigation was supported by grants from the Joint Commission on Rural Reconstruction, Republic of China.

[2] For reprints, please write to the Plant Protection Center, Taiwan, Taichung Hsien, Taiwan 431, Republic of China.

[3] This paper constitutes partial fulfillment of requirements for the M.S. degree of the senior author at the National Taiwan University.

[4] Present address, 1918 La Villa Rose Ct., Modesto, Calif. 95350, U.S.A.

other factors, which may be physical, chemical, or biological in nature, may contribute to their complex toxicology.

To simplify the understanding of complex interactions between insects and insecticides, Sun (1968) summarized the factors affecting the activities of toxicants into two main categories: (1) penetration, which represents a combination of the effects of various physical factors, such as permeation, adsorption, partition, excretion, and transportation; and (2) detoxication, which represents various chemical and enzymatic factors, such as decomposition, metabolism, and conjugation. These relationships were expressed by simple mathematical equations and by graphical evaluation. Generally, both penetration and detoxication are involved in the toxicity of insecticides. Unfortunately, in many reports, there is more speculation than experimental data in the discussion of the structure-activity relationship. To understand and evaluate the toxicology of a compound, it is necessary to prove experimentally the presence of the critical factor(s) in each hypothesis.

In comparing the toxicities of organochlorine (OCl) and organophosphorus (OP) insecticides, it was found that the toxicities of OCl compounds were generally much higher than those of OP compounds to fish, but much lower to rats. For example, the acute oral toxicities of endrin (OCl) and monocrotophos (OP) to rats are about the same, but endrin is about 17,000 times more toxic to rainbow trout than monocrotophos (Anon. 1968). From these comparisons, it appears that the distribution of toxicants between the aqueous and lipid phases could be responsible for large differences in their toxicities to fish and rats (Anon. 1968).

This paper reports the toxicities of DDT, dieldrin, and monocrotophos to fish by injection and by continuous exposure in aqueous solutions (or suspensions), the rate of absorption of insecticides by fish, and the correlation between partition coefficients of insecticides and their toxicities to fish.

Materials and Methods

Materials

Purified DDT, dieldrin, and monocrotophos were used for biological tests and for analysis. All solvents used were analytical grade. Acetone, *n*-hexane, chloroform, and ethyl acetate were redistilled to assure better quality for GLC analysis. To remove organic materials, Florisil was treated at 650°C and then treated again at 100°C for 24 hr before use.

Loach, *Misgurnus anguillicaudatus* (Cantor), was selected as a test fish, because it is small and readily obtainable, and can be reared in a small amount of water without aeration. The latter property is important for tests in water to minimize losses of toxicants during aeration. Young loaches were obtained in the market and reared in the laboratory to a proper size before testing. Uniform sized fish were selected for tests; the average weight was 2.7 g per fish.

Bioassay methods

For determining the toxicity of each insecticide by continuous exposure of fish in aqueous solutions (or suspensions), 1 ml of 4 or 5 concentrations of acetone solution of each insecticide was dispersed in 400 ml of water to give a series of concentrations of test solutions. After thorough mixing, 5 loaches were added to each of 8 (or more) replicated tests, and kept at 21 ± 1°C, without food.

Injection toxicity was obtained by injecting 5 μl of an acetone solution with a microsyringe into each of 5 loaches between two pelvic fins on the ventral side. Three replicates were made for each of 4 concentrations. Treated fish were kept in 400 ml of water at 20 ± 1°C, without food.

For both types of tests, mortality was observed at 24- and 48-hr intervals, and the criterion of death was based on the cessation of gill movement. The LC_{50} or LD_{50} value of each toxicant was obtained from the dosage-mortality curve.

The rates of absorption of insecticides by fish were determined by comparing the amounts of toxicants recovered from water with and without fish. Five loaches were added to 400 ml of aqueous solutions, containing 10 μg of DDT, 10 μg of dieldrin, or 200 μg of monocrotophos; these concentrations were not toxic to the fish. Two 50- or 100-ml samples of each test solution were taken from a different container at 0.5-, 1-, 2-, 4-, 8-, 24-, and 48-hr intervals, and analyzed for each toxicant.

Since young loaches were obtained from the market, some of them were slightly contaminated with DDT. In addition, due to incomplete extraction and separation of toxicants from fish extracts, recoveries were generally poor. Therefore, in the partition tests, the amount of toxicant in fish was calculated from the difference between the toxicant found in water with fish at the end of each exposure time and the amount found in the water without fish at the same time interval.

Although 48-hr results were obtained for both toxicity and absorption of insecticides, only 24-hr data are reported, because 48-hr data did not give additional useful information.

GLC Methods of Analysis

OP and OCl insecticides were analyzed by the methods described by Lau (1966) and Shell Development Co. (MMS-R-355-1), respectively, with some modifications.

DDT in 100 ml of water was extracted twice with a mixture of 30 ml of isopropyl alcohol, 70 ml of *n*-hexane, and 5 g of sodium chloride. Dieldrin was extracted by the same method, with an additional 30 ml of *n*-hexane. After shaking and separation, the *n*-hexane fraction was concentrated in a Kuderna-Danish concentrator, cleaned up with a 3-g Florisil chromatography column, pre-rinsed with *n*-hexane, and eluted with 125 ml of 10% ethyl ether in *n*-hexane. Each sample was then concentrated and analyzed with a gas-liquid chromatograph (Varian Aerograph Model 200), equipped with a tritium electron capture detector. The glass column was 5′ × ¼″ id, packed with 4% SE-30 and 6% QF-1 on Chromosorb W 80/100 mesh. The injection port temperature was 190°C, column temperature 175°C, and detector temperature 185–190°C. The carrier gas was nitrogen, at 40 ml/min. The average recoveries of 3 replicates were 93% and 89% for DDT and dieldrin respectively.

For analyzing for monocrotophos, 50 ml of aqueous solution was extracted with 50 ml of chloroform. The chloroform fraction was concentrated to about 1 ml with a rotary evaporator, 10 ml of ethyl acetate was added, and the solution was concentrated again until all the chloroform was substituted by ethyl acetate. Each sample was analyzed with a gas-liquid chromatograph (Varian Aerograph Model 1700) with an alkali flame ionization detector. The glass column was 5′ × ¼″ id, packed with 3% diethylene glycol succinate on Aeropack-30. The injection port temperature was 230°C, column temperature 220°C, and detector temperature 220°C. Gas flow rates were: Nitrogen (carrier), 20 ml/min; hydrogen, 42.6 ml/min; and air, 600 ml/min. The average recovery of three replicates was 96% for monocrotophos. Due to the absence of interfering materials in water, the recoveries were also high (87 to 100%) for control tests (no fish) of all 3 insecticides up to a 4-hr interval.

Results and Discussion

Toxicity of DDT, Dieldrin, and Monocrotophos to Loaches

LC_{50} and LD_{50} values of DDT, dieldrin, and monocrotophos for loaches are in Table 1. Differences in toxicity of DDT and dieldrin are minor (up to about 2 times) for both test methods. However, monocrotophos is much less toxic than

Table 1. Toxicity of DDT, dieldrin, and monocrotophos to loaches

Toxicant	Exposure in aqueous solution, 24-hr LC_{50} (ppm)	Injection, 24-hr LD_{50} (μg/g)
DDT	0.35	25.0
Dieldrin	0.24	11.2
Monocrotophos	53.5	2.7

either DDT (1/150) or dieldrin (1/220) by exposure in solutions, but more toxic than either DDT (9 times) or dieldrin (4 times) by injection. The differences are so large that a critical factor appears to be responsible for these results. Dicrotophos was also tested and gave results similar to monocrotophos, but it was discontinued from further evaluation, because the sample contained both *cis* and *trans* isomers and the results would have been difficult to analyze and to evaluate.

Absorption of DDT, Dieldrin, and Monocrotophos by Loaches.

Since the toxicities of OCl insecticides to fish exposed in solutions are generally much higher than those of OP insecticides (Anon. 1968), and since the toxicity of monocrotophos to loaches by injection indicates a reversal of relative activity (*i.e.*, more toxic than OCl insecticides) (Table 1), it is reasonable to suspect that hydrophilic compounds, such as monocrotophos, would be absorbed less rapidly from water into fish than lipophilic compounds, such as DDT and dieldrin. To prove this point, the rates of absorption of DDT, dieldrin, and monocrotophos in fish in aqueous solutions were determined.

Data in Table 2 show the amounts recovered from 10 μg of DDT, 10 μg of dieldrin, and 200 μg of monocrotophos dispersed in 400 ml of water with or without fish after 0.5- to 24-hr exposures. The percentage of recovery of each insecticide was obtained by the difference between values and calculated by the following equation:

$$\% \text{ of toxicant absorbed} = \frac{\begin{array}{c}\mu\text{g toxicant in} \\ \text{control (no fish)}\end{array} - \begin{array}{c}\mu\text{g toxicant in treated} \\ \text{container (with fish)}\end{array}}{\mu\text{g toxicant in control (no fish)}} \times 100$$

The data clearly indicate that DDT and dieldrin were readily absorbed from water by fish, while monocrotophos was absorbed slowly. At the end of 24 hr, 96.5% of the DDT, 92.7% of the dieldrin, and only 14.3% of the monocrotophos were absorbed. The recoveries of toxicants from aqueous solutions without fish decreased slightly with time. This loss may have been due to adsorption on glass and/or codistillation with water. When the amounts absorbed were converted to the rates of absorption by the method described by Sun (1968), and plotted (Figure 1), there was a positive relationship between partition coefficients (benzene/water) (Table 3) and the degree of absorption. The maximum initial rates of absorption for DDT and dieldrin were 10 to 20 times that for monocrotophos.

Table 2. Absorption of DDT, dieldrin, and monocrotophos by loaches from aqueous solutions

Time (hr)	Recovery of toxicant from control without fish (μg)			Recovery of toxicant from treatments with fish (μg)			Absorption of toxicant by fish (%)		
	DDT	Dieldrin	Monocrotophos	DDT	Dieldrin	Monocrotophos	DDT	Dieldrin	Monocrotophos
0.5	9.996	10.000	—	8.315	7.990	—	16.8	20.1	—
1	9.126	9.800	200	6.564	7.055	195.0	28.1	28.0	2.5
2	8.716	9.288	196.0	5.912	3.659	188.9	32.2	60.6	3.6
4	8.736	9.156	182.5	4.635	1.270	164.3	46.9	86.1	10.0
8	8.128	8.388	175.1	3.088	0.921	155.2	62.0	89.0	11.4
24	8.472	8.806	170.6	0.296	0.640	146.2	96.5	92.7	14.3

The orders of activity of insecticides in aqueous solutions, partition coefficients, and rates of absorption of toxicants by fish were similar, *i.e.*, dieldrin > DDT > monocrotophos. When toxicities are expressed as toxicity ratios, with DDT as a standard, it shows that monocrotophos is much less toxic than DDT or dieldrin to loaches by exposure in aqueous solutions. However, by injection, the toxicities of all three insecticides to fish are different and of about the same order of activity as the acute oral toxicities to rats (Table 3). From these comparisons, it appears that the difference in the toxicities of insecticides to fish is greatly affected by their partition coefficients, although the number of insecticides used for this study is not large enough to permit conclusions for insecticides in general.

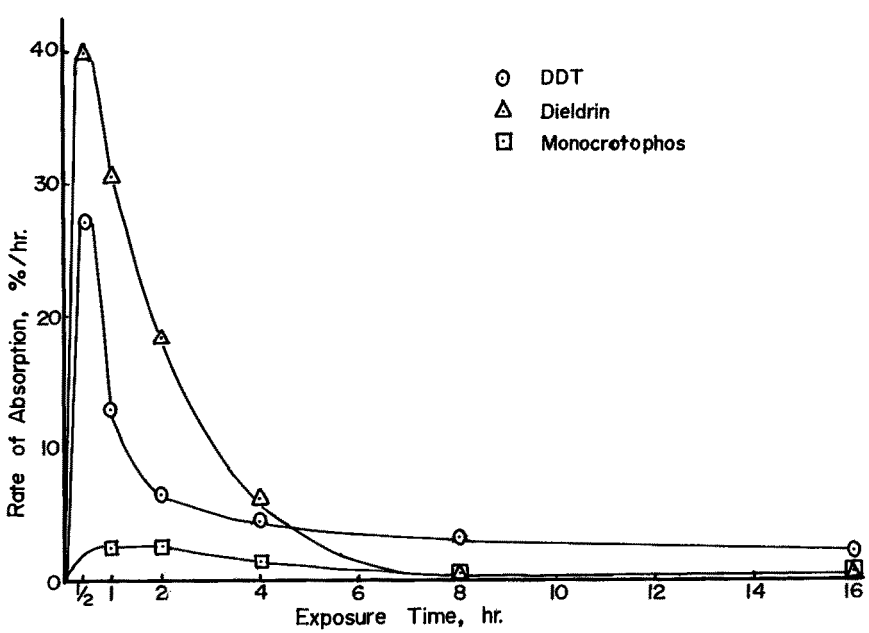

Fig. 1. Rate of absorption of insecticides from aqueous solutions by loaches

Table 3. Relationship between partition coefficient, route of administration, absorption of toxicant, and relative toxicity of DDT, dieldrin, and monocrotophos

Toxicant	Partition coefficient, benzene/water	Absorption by loaches, 24 hr (%)	Toxicity ratio		
			For loaches		For rats, acute oral
			In water	Injection	
DDT	116	96.5	1	1	1
Dieldrin	408	92.7	1.5	2.2	2.5
Monocrotophos	0.61	14.3	0.0065	9.3	6.5

Correlation Between Fish Toxicity and Partition Coefficients of Insecticides

Partition coefficients have successfully been used for the correlation of biological activities of closely related compounds (Boyce and Milborrow 1965, Fuller *et al.* 1968, Hansch and Fujita 1964, McGowan 1963, Metcalf and Fukuto 1962, and Quintana 1965). The success of such correlations is mainly due to the fact that the properties of compounds, including toxicity, usually change gradually and continuously in the direction of the change in activity. However, in more diversified structures, properties may be quite different and their changes may be unpredictable. Therefore, it would be difficult to correlate the activity with one of the properties, unless that particular property was a critical factor overshadowing the effects of the others. From the evaluation of the present data, partition distribution of toxicants between fish and water appears to be the critical factor. Therefore, we have calculated the correlation between partition coefficients and toxicities to fish of a number of insecticides.

Several solvents have been reported in the literature for determining partition coefficients. The reasons for selecting the benzene/water coefficients of OP insecticides and their toxicities to rainbow trout are the availability of more published data, better correlation, and a wider range of coefficients. Figure 2 shows the relationship between partition coefficients (benzene/water) (Sun and Johnson 1965) and toxicities to rainbow trout (Anon. 1968) of 12 OP insecticides. The following equation for the regression line was calculated from these data.

$$\log Y_1 = 0.867 - 0.657 \log X_1$$

where X_1 is the partition coefficient for OP insecticides and Y_1 is the toxicity (in ppm) to rainbow trout. The coefficient of correlation is 0.70 which is significant at $<1\%$ probability level.

In Figure 2, mevinphos and phorate (points 7 and 9) are far from the regression line. After reviewing the properties of these two insecticides, it was found that their dermal toxicities (Anon. 1968) are very high, indicating high rates of penetration. To reduce the effect of this factor, corrected fish toxicity (Table 4, footnote d) and partition coefficients were used for calculating the following equation:

$$\log Y_2 = 1.900 - 0.787 \log X_2$$

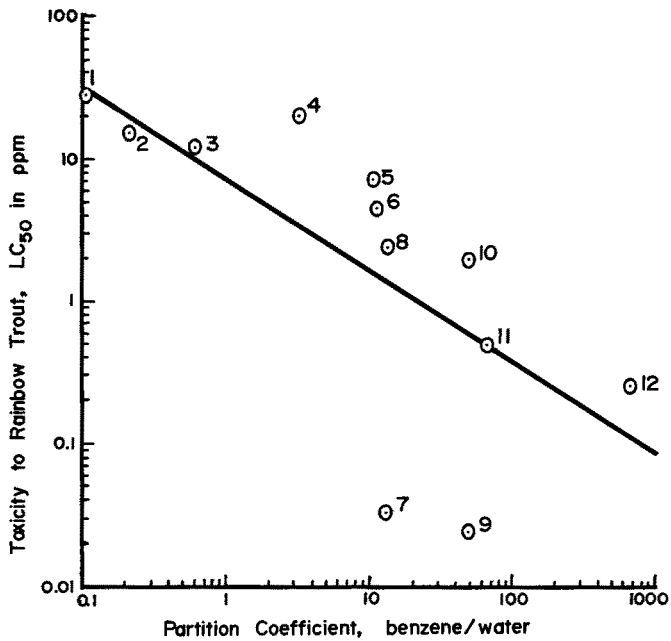

Fig. 2. Correlation between partition coefficient (benzene/water) and toxicity to rainbow trout of organophosphorus insecticides: 1 = trichlorfon, 2 = dicrotophos, 3 = monocrotophos, 4 = dimethoate, 5 = methyl parathion, 6 = phosphamidon, 7 = mevinphos, 8 = disulfoton, 9 = phorate, 10 = parathion, 11 = dichlorvos, and 12 = naled

where X_2 is the partition coefficient for OP insecticides and Y_2 is the corrected toxicity to rainbow trout. The coefficient of correlation is 0.74 which is significant at <1% probability level. Although there is only a slight improvement in the coefficient of correlation, points 7 and 9 have moved much closer to the regression line in Figure 3, as compared to their positions in Figure 2. A slight improvement in the coefficient of correlation may indicate the effects of other factors.

To further reduce the effects of other possible factors, only organophosphates (*i.e.*, points 3, 6, 7, 11, and 12 with double circles in Figure 3) were used to calculate the following equation:

$$\log Y_3 = 1.571 - 0.806 \log X_3$$

where X_3 is the partition coefficient for organophosphates and Y_3 is the corrected toxicity (Table 4) of organophosphates to rainbow trout. The dotted line, representing the regression line for organophosphates only, is parallel to the solid line, representing 10 organophosphorus insecticides. The coefficient of correlation for organophosphates is 0.96 which is significant at <1% probability level. The significant improvement in the coefficient of correlation appears to be due to the use of organophosphates only; these do not require bio-activation to be toxic.

Other partition coefficients (Sun and Johnson 1965) and toxicities to either rainbow trout or blue gill (Anon. 1968) would give similar correlations. Data in

Table 4. Relationship between partition coefficient and toxicity to fish (rainbow trout) of organophosphorus insecticides

No.	Toxicant	Partition coefficient, benzene/water[a]	Toxicity to rainbow trout LC_{50} (ppm)[b]	Dermal toxicity ratio,[c] rat	Corrected fish toxicity[d]
1	trichlorfon	0.098	27.5	—	—
2	dicrotophos	0.21	15	—	—
3	monocrotophos	0.61	12	3.2	38.4[f]
4	dimethoate	3.17	20	1.0	20
5	methyl parathion	10.7[e]	7	6.0	42
6	phosphamidon	11.5	4.5	2.8	12.6[f]
7	mevinphos	13	0.034	85	2.9[f]
8	disulfoton	13.3[e]	2.45	26.7	65.5
9	phorate	49	0.025	64.5	1.6
10	parathion	49[e]	2.0	19.1	38.2
11	dichlorvos	65.7	0.5	3.7	1.9[f]
12	naled	666	0.25	0.5	0.13[f]

[a] Sun and Johnson 1965
[b] Anon. 1968
[c] Anon. 1968
[d] Corrected fish toxicity = LC_{50} for rainbow trout × dermal toxicity ratio
[e] Original data
[f] Organophosphates

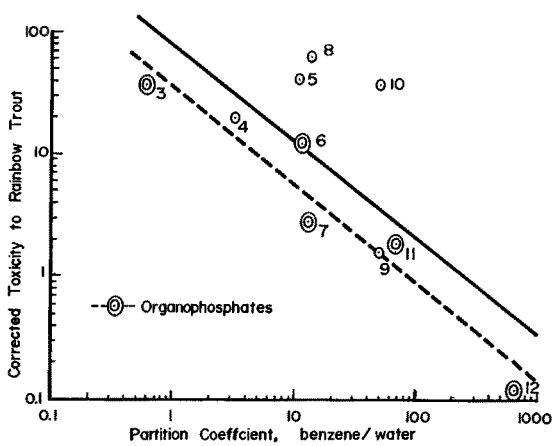

Fig. 3. Correlation between partition coefficient (benzene/water) and corrected toxicity to rainbow trout of organophosphorus insecticides: —⊙— = regression line for all OP insecticides, —◎— = for organophosphates only.

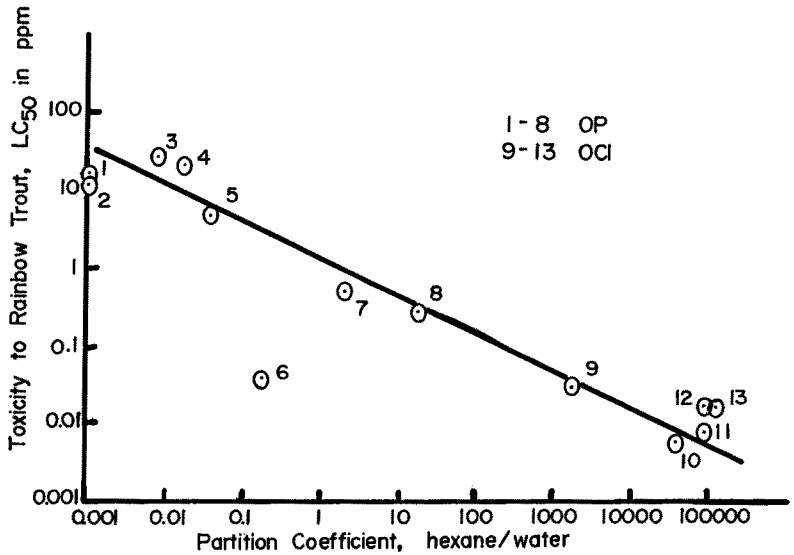

Fig. 4. Correlation between partition coefficient and toxicity to rainbow trout of OP and Ocl insecticides: 1 = dicrotophos, 2 = monocrotophos, 3 = trichlorfon, 4 = dimethoate, 5 = phosphamidon, 6 = mevinphos, 7 = dichlorvos, 8 = naled, 9 = lindane, 10 = dieldrin, 11 = DDT, 12 = aldrin, and 13 = heptachlor

Figure 4 show the relationship between the hexane/water coefficients for OP (Sun and Johnson 1965) and OCl (Voerman 1969) insecticides and their toxicities to rainbow trout (Anon. 1968). There is no particular reason that both OP and OCl insecticides should correlate with partition coefficients in the same curve. Their apparent good relationship may be explained by either coincidence or by the predominant effect of partition distribution which minimizes the differences in other properties and inherent toxicities of these insecticides. The relative importance of partition coefficients may be illustrated by the following examples. Comparing the difference in toxicity to rainbow trout and rats (Anon. 1968), DDT is 1500 times more toxic to rainbow trout than monocrotophos, but monocrotophos is 6.5 times more toxic to rats than DDT. A reversal in toxicity to fish may be explained by the large difference in partition coefficients between hexane and water (Sun and Johnson 1965 and Voerman 1969); the difference in relative ratio is 91,000,000 times higher for DDT than monocrotophos (Figure 4).

Based on an eye-fitted curve, one can see a similar relationship between Figures 2 and 4 where mevinphos (point 7 in Figure 2 and point 6 in Figure 4) is far from the regression lines. It appears that the use of different solvents to obtain partition coefficients does not substantially affect the correlation, but changes the log-log levels in the graph.

In addition to rainbow trout and blue gill, OCl insecticides were also reported to be much more toxic than OP insecticides to brook trout, brown trout, fathead, bass (Sunshine 1969), seven estuarine fish (Eisler 1970), and three fresh water fish (Nishiuchi and Hashimoto 1967). These data further support our conclusion

that partition distribution is a critical factor affecting the toxicities of insecticides to fish.

Although experimental data indicate the importance of the partition distribution of insecticides in their toxicities to fish, the same degrees of difference in toxicity do not hold true for aquatic organisms in general. The factor(s) causing large differences in toxicity between OP and OCl insecticides may be partly due to the respiratory mechanism of fish.

The chief respiratory organs of all fishes are the gills. Water is drawn in through the mouth, forced back into the pharynx, and then forced out through the gills. In the process of circulating water through the gills, blood is brought into close contact with water in the thin-walled gills, and gas exchange takes place through the thin intervening membrane. During the process, the toxicant in water could be partitioned through the gills into the blood of fish, and the rate of absorption could depend upon the partition coefficient of each insecticide.

From the results of this study, the proof that partition distribution is a critical factor affecting toxicities of insecticides to fish may have both theoretical and practical implications in the study of toxicology of insecticides to insects and animals. If the activity of a group of insecticides is predominantly affected by a single critical factor, such as partition coefficient *vs.* fish toxicity, this factor can readily be determined and used for further evaluation of other structures. However, in many cases, two or more factors may be responsible for the difference in activity and the correlation would be difficult to find with a simple graphic relationship. With the assistance of a computer, however, critical factors should readily be identified if enough data are available. In practical applications, if fish toxicity is the major concern when controlling aquatic insects with insecticides, partition coefficients would be an important factor for selecting commercial insecticides or for preparing new toxicants. Water solubility of insecticides could be a simple means for a preliminary selection.

Reference

Anon.: Suggested guide for the use of insecticides to control insects affecting crops, livestocks, households, stored products, forests, and forest products. *U.S. Department of Agriculture,* Agriculture Handbook No. 331 (1968).

Boyce, C. B. C., and B. V. Milborrow: A simple assessment of partition data for correlating structure and biological activity using thinlayer chromatography. Nature **208,** 537 (1965).

Eisler, R.: Acute toxicities of organochlorine and organophosphorus insecticides to estuarine fishes. *U.S. Bur. Spórt Fish. Wildlife,* Tech. Paper 46, pp 1–12 (1970).

Fuller, R. W., M. M. March, and J. Mills: Inhibition of monoamine oxidase by *N*-(phenoxyethyl) cyclopropylamines. Correlation with Hammett constants and partition coefficients. J. Med. Chem. **11,** 397 (1968).

Hansch, C., and T. Fujita: Rho-sigma-pi analysis. A method for the correlation of biological activity and chemical structure. J. Amer. Chem. Soc. **86,** 1616 (1964).

Lau, S. C.: Separation and measurement of 3-hydroxy-*N,N*-dimethyl-*cis*-crotonamide dimethyl phosphate (Bidrin insecticide) and 3-hydroxy-*N*-methyl-*cis*-crotonamide dimethyl phosphate (Azodrin insecticide) residues in crops by selective cleanup (partition) procedures. J. Agr. Food Chem. **14,** 145 (1966).

McGowan, J. C.: Partition coefficients and biological activities. Nature **200,** 1317 (1963).

Metcalf, R. L., and T. R. Fukuto: *Meta*-sulfurpentafluorophenyl diethyl phosphate and *meta*-sulfurpentafluorophenyl *N*-methylcarbamate as insecticides and anticholinesterases. J. Econ. Entomol. **55,** 340 (1962).

Nichiuchi, Y., and Y. Hashimoto: Toxicity of pesticides ingredients to some fresh water organisms. Botyu-Kagaka. **32,** 5 (1967).

Quintana, R. P.: Relationship between partition coefficients and cholinesterase inhibition of carbamoyl-piperinoalkanes. J. Pharm. Sci. **54,** 462 (1965).

Sun, Y. P.: Dynamics of insect toxicology—A mathematical and graphical evaluation of the relationship between insect toxicity and rates of penetration and detoxification of insecticides. J. Econ. Entomol. **61,** 949 (1968).

Sun, Y. P., and E. R. Johnson: Integration of physico-chemical and biological techniques in specific bioassay, with special reference to Bidrin insecticide. J. Econ. Entomol. **58,** 838 (1965).

Sunshine, I.: Toxicity to fish. *In* Handbook of analytical toxicology. P. 582. *Chemical Rubber Co.,* Cleveland, Ohio (1969).

Voerman, S.: Distribution ratio of some chlorinated hydrocarbon insecticides between hexane and water. Bull. Environ. Contam. Toxicol. **4,** 64 (1969).

Manuscript received October 10, 1976; accepted February 4, 1977