Penetration of Insecticides through Rat Skin

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The penetration through rat skin of DDT, famphur, carbaryl, malathion, and dieldrin was examined using radioactive compounds. The penetration rates varied markedly, increasing in the above order, and the rate was not related to the olive oil–water partition coefficient. Compounds also varied in the proportion which could be recovered from skin by acetone washing. The vehicle in which the compounds were applied also influenced penetration rate, which increased in the order corn oil, benzene, acetone.

It is probable that a factor of importance in dictating toxicity of dermally applied toxicants is the rate of penetration through the skin. One might hope that one or a few physical factors would determine the rate, and consequently that one could make appropriate predictions of rates. Polarity, particularly as measured by partition coefficients between water and a lipid phase, would seem to be the parameter of major interest. Such a situation was recently shown by Olson and O'Brien (*4*) for the case of penetration of nonelectrolytes into cockroach cuticle. However, the literature on factors affecting penetration into mammalian skin is limited and contradictory.

The present study was carried out in accordance with the beliefs that only intact skin on a living animal should be used; one should minimize alterations of the skin which could occur by exposure to water, salves, etc.; and measurement of penetration should be direct and quantitative. These goals were achieved by applying radioactive compounds to the shaved skin of intact rats in a small volume (1 μl.) of organic solvent (which evaporated promptly), removing the skin at the desired time, and measuring its radioactivity after digestion.

Methods

Insecticides. The H^3 carbaryl (1-naphthyl *N*-methylcarbamate) was ring-labeled by B. D. Hilton by the method of Hilton and O'Brien (*2*) with a specific activity of 3.7 mc. per mole. The H^3 famphur (dimethyl *p*-*N*-dimethylsulfamoylphenyl phosphorothionate) was a gift from the American Cyanamid Co. with labeling in the ring, specific activity 78.4 mc. per mmole. The C^{14}-DDT in benzene solution was purchased from Nuclear-Chicago Corp., labeled in the alkane carbons, at 4.93 mc. per mmole. The World Health Organization kindly donated benzene solutions of C^{14}-ethane–labeled malathion [*O*,*O*-dimethyl *S*-1,2-bis(ethoxycarbonyl) ethyl phosphorodithioate] at 3.9 mc. per mmole and C^{14}-ring-labeled dieldrin at 20.5 mc. per mmole.

The rats were 200-gram albino females from Holtzman Co., Madison, Wis. They were anesthetized with 0.6 ml. of 50% urethane (average dose 1500 mg. per kg.) administered in two intraperitoneal injections of 0.3 ml. each at 8 minutes apart (if the dose was given in one injection, the animals sometimes died within 3 minutes). Each rat's belly, from the front legs to the back legs, was clipped, lathered, and shaved with a safety razor. Extreme caution was taken to prevent abrading or nicking the skin. The belly was then marked with a ballpoint pen in approximately 12-mm.-diameter circles and numbered.

One microgram of the compound was applied in 1 μl. of the indicated solvent to each circle of skin by a micropipet. Samples were usually taken at 3 minutes and at 1, 3, 4, 6, 18, and 24 hours. At the given time the skin sample was removed by holding the skin with a hemostat and cutting around the circle with scissors. The skin was then digested and counted by the method of O'Brien (*3*), which involves nitric digestion, 10-fold dilution, and counting with buffer in a dioxane scintillation solution. Recovery ("zero time") experiments were performed by first removing the disk of skin and then applying the compound. An untreated skin sample was taken from each animal to correct for background and endogenous counts from the skin. Standards were made by adding the labeled compound directly to a counting vial containing 18 ml. of dioxane counting solution, 1 ml. of the above buffer, and 1 ml. of 10% clear nitric acid. The samples were counted in the Packard Tri-Carb scintillation counter for three rounds of 3-minute counts.

With this procedure, recoveries of compounds at zero time approximated 100%, except in the case of DDT, when erratic recoveries averaging about 50% were encountered. Experiments ruled out the possibility that volatile radioactivity—e.g., $C^{14}O_2$—was being produced and lost, and showed that instead a large proportion (about 50%) of the radioactivity adhered to the digestion tube. This occurred only if skin was present prior to digestion. Consequently it was necessary to digest samples of DDT-treated skin in a counting vial with 1 ml. of nitric acid, dilute as usual with 9 ml. of water, then remove 1 ml. for usual counting. Meanwhile, 8 ml. of the residual solution was discarded and the remaining 1 ml. was counted in the original vial. From the counts on the two vials, one could calculate the total radioactivity.

Diffusion of solvent along the skin surface from the point of application was very small in the case of acetone and benzene. However, corn oil spread rapidly, as observed visibly and as measured by first removing the 12-mm. treated area and then a circle of skin, diameter 20 mm., surrounding it, and digesting and counting both disk and circle. About 50% of the radioactivity was found in the ring. Consequently the disk and ring were routinely counted in all work with corn oil.

Partition coefficients were determined for olive oil–water at room temperature. Five microliters of a solution of the compound in organic solvent were added to 10 ml. of olive oil and shaken, and 8 ml. were added to 400 ml. of distilled water in a separatory funnel, shaken 1 minute, and allowed to stand for 10 minutes. The water was run off, a fresh 400-ml. portion of water added, and the procedure repeated. The remaining oil was centrifuged to remove water, and a 0.5-ml. sample of it and of original oil solution were counted in dioxane counting solution. Pipets were rinsed with counting solution to assure quantitative transfer. This procedure was necessary because of the very low polarity of the compounds. If P is the partition coefficient (oil-water), R is the ratio of water to oil volumes, L is the count in the original oil, and Q that in the oil after the two partitionings, one can show that

$$P = \frac{R\left(1 + \sqrt{\frac{L}{Q}}\right)}{\frac{L}{Q} - 1}$$

Results

Table I shows the oil-water partition coefficients, demonstrating a considerable spread in polarity among these five apolar compounds.

Table I. Relation between Polarity and Penetration

	Olive Oil–Water Partition Coefficient	Time for 50% Penetration, Hours
Sevin	64.5	14.5
Famphur	174	19
Malathion	413	5.5
DDT	932	26
Dieldrin	1805	3.5

The compounds were applied in benzene solution, except in certain experiments detailed below. The results were plotted semilogarithmically (Figures 1 through 5), so that a linear relation would imply conformity with first-order kinetics. Each point shown is the mean from the number of experiments shown below the vertical line, which indicates the range. Each experiment involved two samples from one rat. It is apparent that first-order kinetics are not usually followed throughout the 24-hour period.

Radioactivity is being measured, and expressed as original compound. Consequently if metabolism occurs in the skin, a much more complex situation than a simple one-compound diffusion would take place. Although one would prefer to know the amount of parent compound, it proved to be impossible (see below) to extract a large percentage of the radioactivity out of the skin, and in fact the rather severe nitric digestion was the only technique by which all of the radioactivity could be solubilized for counting.

In an attempt to establish whether a fraction of a compound was bound in some way, whereas another fraction was "free" (or at least extractable), experiments were carried out in which the skin disks were thoroughly rinsed with 2 ml. of acetone, which was collected in a counting vial and counted with 10 ml. of a toluene scintillation solution made up of 5% of PPO (2,5-diphenyloxazole) and 0.03% of dimethyl-POPOP [1,4-bis-2(4 - methyl - 5 - phenyloxazolyl)benzene] in toluene.

The quantities of DDT recovered in this way were at first substantially equal to those recovered by digestion of parallel skin sample. Thus at 1 hour, 71% of the applied dose could be washed out, whereas 83% of the dose was found with total digestion. At later times, a smaller fraction could be washed out: at 16 hours 37% of the dose (compared with 77% in the digest) and at 24 hours 23% (compared with 53% in the digest). With famphur and dieldrin a somewhat more controlled approach was used: At certain times, one skin sample was digested and counted ("whole digest"); and a second sample was washed, the wash was counted, and the washed skin was digested and counted. Excellent agreement was found between the sum of the washings and washed-skin values as compared with the whole digest. As Figure 6 shows, with dieldrin, the extractable fraction declined steadily, so that at 16 hours only 18% of the skin radioactivity could be washed out. By contrast, about 75% of famphur could be extracted at all times.

Application in a very small volume of solvent was the nearest available approximation to the ideal of placing compound—in the required very small

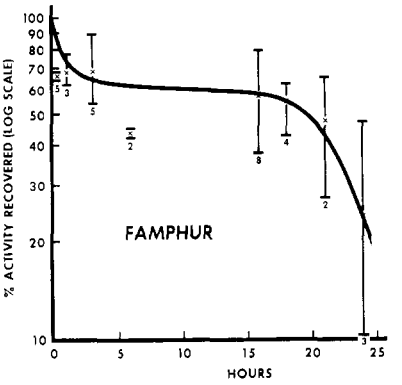

Figure 1. Penetration of famphur

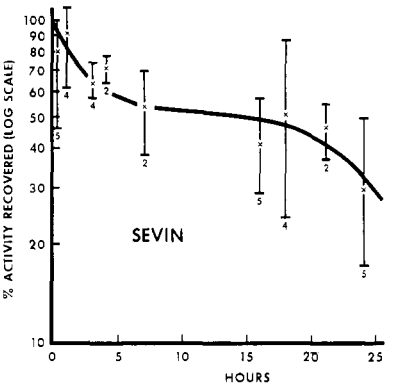

Figure 2. Penetration of carbaryl (Sevin)

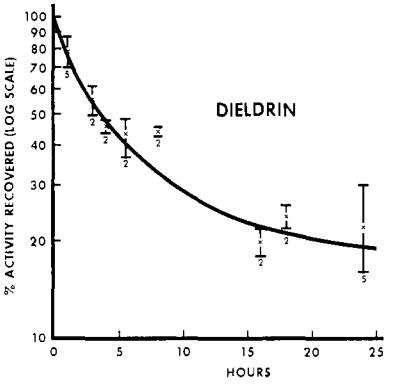

Figure 3. Penetration of dieldrin

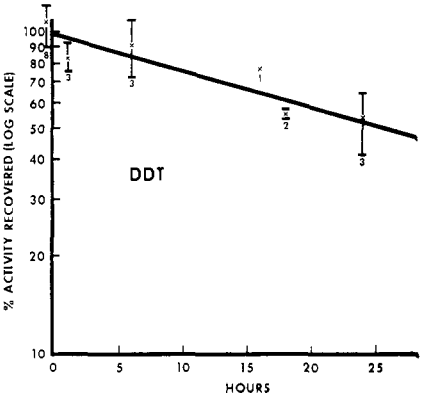

Figure 4. Penetration of DDT

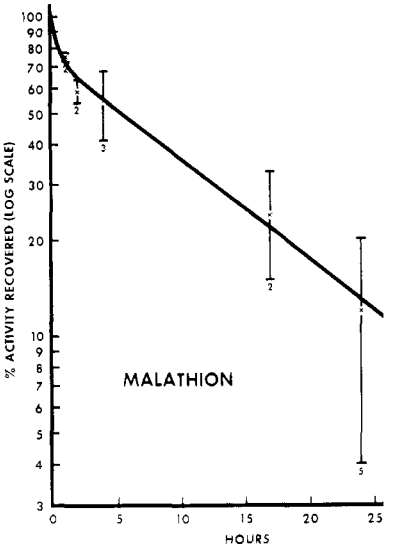

Figure 5. Penetration of malathion

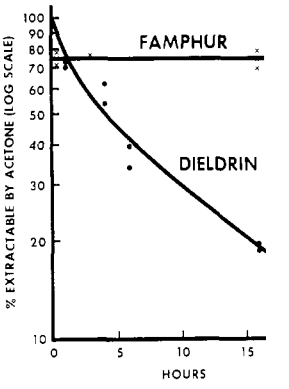

Figure 6. Proportion of compound extractable by acetone

Each point from one experiment involving 2 samples. Data calculated as per cent of radioactivity present at indicated time which was extractable by acetone

Table II. Effect of Solvent upon Penetration

	% Remaining in Skin at 3 Hours	
	Sevin	Famphur
Benzene	62.8 (4)	66.8 (5)
Corn oil	87.8 (4)	66.5 (4)
Acetone	42.4 (5)	38.5 (4)

Values in parentheses show number of replicates.
Famphur. Benzene value not significantly different (S.D.) from corn oil; acetone value S.D. from others at $P = 0.01$.
Sevin. Corn oil value S.D. from acetone at $P = 0.01$; benzene S.D. from others at $P = 0.05$. Significance evaluated by t test.

amounts—directly onto skin. Inevitably the solvent must have some effect upon the skin, although the rapid volatilization of the 1 μl. gave grounds to hope the effect would be slight. In an attempt to evaluate the solvent effect, two of the compounds were studied with three different solvents, and penetration at 3 hours was examined. Table II shows that in fact the solvent effect was rather large. For both compounds, acetone in particular hastened penetration.

Discussion

Examination of the form of the penetration curves suggests that for dieldrin and DDT, for which relatively little metabolic alteration in skin is anticipated, there is roughly first-order penetration—although with dieldrin, departure from such kinetics is pronounced at 16 hours. Organophosphates and carbamates are more metabolically labile. One might speculate that for carbaryl and famphur, the early rapid penetration is of parent compound and that a primary degradation product (such as 1-naphthol and *p*-dimethylsulfamoylphenol, respectively) persists within and is lost by slow diffusion, perhaps subsequent to further metabolism.

In a study with cockroaches, Olson and O'Brien (*4*) found that the rate of penetration from the surface grease was directly related to the polarity of the compound; thus half times of penetration of 16 minutes for phosphoric acid and 1584 minutes for DDT were observed. Buerger (*1*) used a technique identical with that of the present study, and found for three compounds [DDT, famphur, and dimethoate or *O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl)-phosphorodithioate] that the relative penetration rates increased with increasing polarity of the compound for the toad, chameleon, Mexican horned toad, mealworm adult (*Tenebrio molitor*), and American cockroach, but not for the cricket (*Gryllus domesticus*).

Table III. Partition Coefficients[a] in Various Solvents

	Carbon Tetrachloride	Benzene	Chloroform	Hexane	Triacetin
Parathion	..	493	447	213	815
Paraoxon	28	54	435	4.2	37
Malathion	34	36	37	27	39

[a] For organic solvent–water, determined by single partitioning followed by counting phases.

It appears from Table I that no simple relationship between polarity and penetration exists for the case of rat skin. However, there are two possible interfering factors. First, the data here are based primarily on benzene as the application solvent, and clearly different solvents give different values. It may be that for some solvent which interacts minimally with skin, a simpler relation between solute polarity and penetration could be found. Second, the solvent selected for determining partition coefficient can be of importance. Although approximately the same order of polarities for any series of solutes should be given by any set of partition coefficients (which must necessarily be between two solvents differing widely in polarity), yet gross departures can be expected whenever some special solute-solvent interaction occurs. Olive oil was used here because it is the classic solvent for correlation with cell permeabilities, and because it probably gives a better approximation to the lipid components of skin than would common organic solvents. Unfortunately, there are very few data in the literature to enable one to estimate how much variation in partitioning one should expect from different solvents. Table III presents previously unpublished data obtained by us in 1960 for three organophosphates, which demonstrate such variation. Paraoxon appears far more polar than parathion except when chloroform is the solvent, and far more polar than malathion when hexane is the solvent, but far less when chloroform is the solvent. With these exceptions, the five very different solvents all show that parathion is much more apolar than paraoxon or malathion, which have comparable polarity.

It was not possible to distinguish parent compound from metabolites because of the extraction problem, although for the cases of DDT and dieldrin, little such metabolism in skin is expected. The results also show substantial scatter in the data, compensated in part by extensive replication. The scatter might be lessened by using male rats (to avoid periodic fluctuations in hormonal levels) and closely controlled ambient temperature.

Literature Cited

(1) Buerger, A. A., "Penetration of Nonelectrolytes through Animal Integuments," M.S. thesis, Cornell University, 1964; *J. Cellular Comp. Physiol.*, in press.
(2) Hilton, B. D., O'Brien, R. D., J. AGR. FOOD CHEM. **12,** 236 (1964).
(3) O'Brien, R. D., *Anal. Biochem.* **7,** 251 (1964).
(4) Olson, W. P., O'Brien, R. D., *J. Insect. Physiol.* **9,** 777 (1963).

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