Proceedings of the Iowa Academy of Science

The Absorption of Chlorinated Hydrocarbon Insecticides by Frog Skin

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Recommended Citation

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SYNOPSIS: The absorption of lindane, heptachlor, aldrin, dieldrin and p, p' DDT by frog skin has been studied. Various experiments indicate that water-soluble fractions of these insecticides are selectively absorbed by the skin. The relative quantities of insecti-

cides absorbed appear to be partially related to their water solubilities. Selected studies using isolated frog skin indicate that the passage of insecticides across the skin does not alter the electrical potential difference across the skin. The observed results are discussed with respect to the accumulation of these chemicals in the tissues of the frogs.

Considerable interest has been generated in recent years as to the use, effect, and distribution of large quantities of organochlorine pesticides in our environment. The relative value of the use of large amounts of these chemicals is now in question. Governmental agencies are taking action with respect to the use of some of these substances (Gillette, 1971).

The exact location of these insecticides and their residues in the environment has been under intensive study. For example, a study of the location and concentration of DDT is given by Woodwell, Craig, and Johnson (1971). Their article presents data on the location and amounts of DDT in the air, soils, water, and the biota. It also points out some of the difficulties in assessing the effect of DDT on the biota and its circulation in living cycles.

A more specific example is shown in the studies of the toxicity of certain halogenated hydrocarbon insecticides to frogs in a study by Kaplan and Overpeck (1964). Their studies indicate that even small concentrations of pesticides have a detrimental effect on frogs. Pathologic conditions such as lethargy, depression of blood cell count and digestive upsets appear to occur above certain insecticide concentrations. Convulsions and death may also result from exposure to higher concentrations of the chemicals.

The studies reported here show the absorption of five selected organochlorine insecticides by frog skin and, in one case, the passage of insecticide through the skin. The concentrations of the insecticides in solutions that contacted frog skins or whole frogs were of the order of magnitude that would be expected in saturated water solutions.

MATERIALS AND METHODS

The ventral abdominal skin of a frog, *Rana pipiens*, was removed and placed between two symmetrical chambers. The chambers and associated connectors are similar to the apparatus used by Ussing and Zerahn (1951). The surface of the skin exposed to the solutions in the two chambers was approximately 3 cm². The amount of the bathing solutions

in each chamber was 300 ml. Contact with the solutions was made to calomel electrodes through 3 M KCl-agar bridges. The potential difference across the frog skin was recorded continuously with a potentiometric recorder. These millivolt potentiometric values of the skin were compared for accuracy with a calibrated oscilloscope. In some experiments the skin was soaked in Ringer's fluid for periods of 30 minutes to an hour before it was placed between the chambers. Equilibration of the skin bathed by normal Ringer's solution on the apparatus was 30 minutes or longer. The equilibration time was dependent on the establishment of a relatively steady potential difference. A magnetic stirrer was employed to provide uniform oxygenation and mixing of the bathing fluids.

The Ringer's solution contained NaHCO₃ 2.5 mM, NaCl 112 mM, KCl 2.0 mM, and CaCl₂ 1.0 mM per liter of solution. All experiments were run at room temperatures, 23° to 26° C. A 5-ml control sample was removed from the chamber. A 2-ml sample of a lindane-acetone solution (7.5 micrograms per milliliter) was then added to the Ringer's solution which made contact with the outside of the skin. This experimental solution was rapidly mixed by the magnetic stirrer. One 5-ml sample was taken every hour for up to 8 hours. The next day the same procedure was used on the other half of the skin which was then dead. Chemicals such as KCN were not used to kill the skin since the change in osmotic pressure might have destroyed individual cells and caused complications in the analysis of the results. Each sample was placed in a clean 10-ml capped, silanized culture tube with 1 ml of hexane. The tubes were shaken for approximately 10 minutes to allow the partitioning of the lindane into the hexane layer.

Each sample was then individually injected into the gas chromatograph, which was equipped with an electron capture detector. The chromatographic columns that were used were 4 mm x 6 ft in length and were filled with Anakrom 70/80 supporting material, coated with DC 200. Periodic chromatograph injections of standard lindane solutions (0.1 ng/4μl) were made. Both the live skin samples and the dead skin samples were run the same day in order to be certain of having identical conditions for all samples.

The average of the peak heights of the standard runs was determined. This made possible the calculation of the concentration of the insecticide sample. The peak heights of all samples were determined to the nearest 1/3 of 0.1 inch. This procedure was followed for all the chromatographic work in these experiments. These experiments were repeated using the insecticide heptachlor.

Beakers containing aproximately 500 ml of water or Ringer's solution were saturated with pesticide. The solutions were prepared using excess pure pesticide standards in water or Ringer's solution over a magnetic stirrer. These solutions were allowed to equilibrate for 48 hours. The saturated, filtered solutions contained approximately 10 ppm of lindane, 11 ppb of aldrin, 110 ppb of dieldrin, 10 ppb of p, p' DDT, and 6 ppb heptachlor. The figures given for the first four pesticides are taken from the *Pesticide Reference Standards* of the Food and Drug Administration received in 1971 and Woodwell *et al.* (1971). Heptachlor is listed as being virtually insoluble in water. Our determinations indicate that it is soluble to the extent of about 6 ppb in water and in Ringer's solution. The method of detection is by partitioning between water and hexane, although this method is not completely efficient (Konrad *et al.*, 1969). Recovery of pesticides from distilled water ranges from 92 to 102 percent.

Frogs were pithed and suspended in the solutions. They were removed at appropriate times. A control frog was submerged for one minute and then removed. Each frog, when removed, was washed in running distilled water for 1 minute. A large portion of the ventral skin was then removed and placed in a vial. This skin was then washed in hexane for about 1/2 to 1 minute to remove the insecticide from the surface and the adhering water. Additional control animals were placed in water and in Ringer's solution and their skins treated in a similar manner.

The skins were spread between two sheets of powder paper and allowed to dry. The skin outline was determined using carbon paper, the outline was cut-out and weighed. From a calibration curve the area of the skin was determined. The skins were then cut into small pieces about 2 mm², placed in vials and the insecticide extracted by partitioning with hexane. This method proved as reliable as methods of grinding the skin in a glass homogenizing apparatus. Ten control determinations using each type of preparation were within a 2 percent variation. Samples were then injected into the gas chromatograph and the concentrations determined by comparison to a standard solution of the insecticide.

RESULTS

It was found that individual skins (half skins) displayed a high degree of consistency of rate of movement of lindane. However, the variation between skins was great enough that it was impossible to distinguish between live and dead skins. Figure 1 shows an initial rate of movement of 0.680 ppb/hour for both live and dead skins. At eight hours, lindane was found to go through live skin at a rate of 0.691 ppb/hour and through dead skin at a rate of 0.666 ppb/hour. The difference, which is 0.025 ppb/hour, cannot be shown to be significant as shown by the variation indicated by the standard error of the mean (see Figure 1). It is possible that this is a real difference, but our limited data prevented our making this statement conclusively. Live skins showed a potential difference which ranged from 8 mV to 16 mV. Skins were considered dead when they showed a 0.0 mV potential difference.

The results of this experimental procedure using heptachlor were entirely negative. No movement of heptachlor with either live or dead skins was detected. The control samples
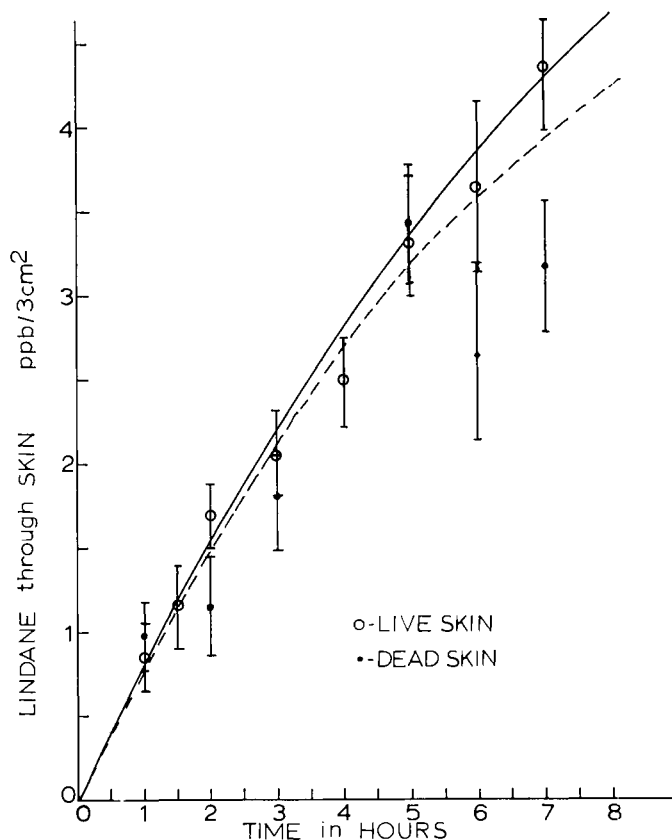

Figure 1. Rate of passage of lindane through live and dead isolated frog skin. The points are average values (7-13 skins). The bars on points represent ± 1 S.E.M.
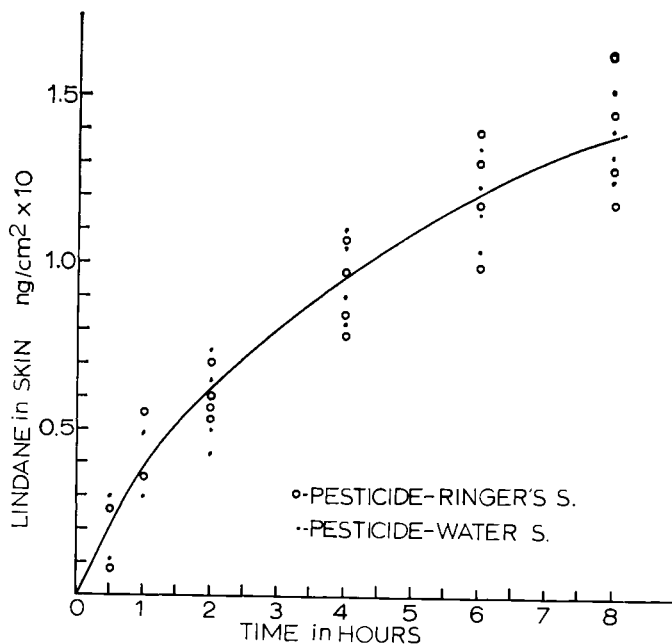

Figure 2. Rate of absorption of lindane by frog skin. Each point represents the value for the skin of a frog.

for lindane and heptachlor showed negative results. Because of the negative results found for the movement of heptachlor across the skin, we became interested in the amount of pesticide accumulated by the skin itself.
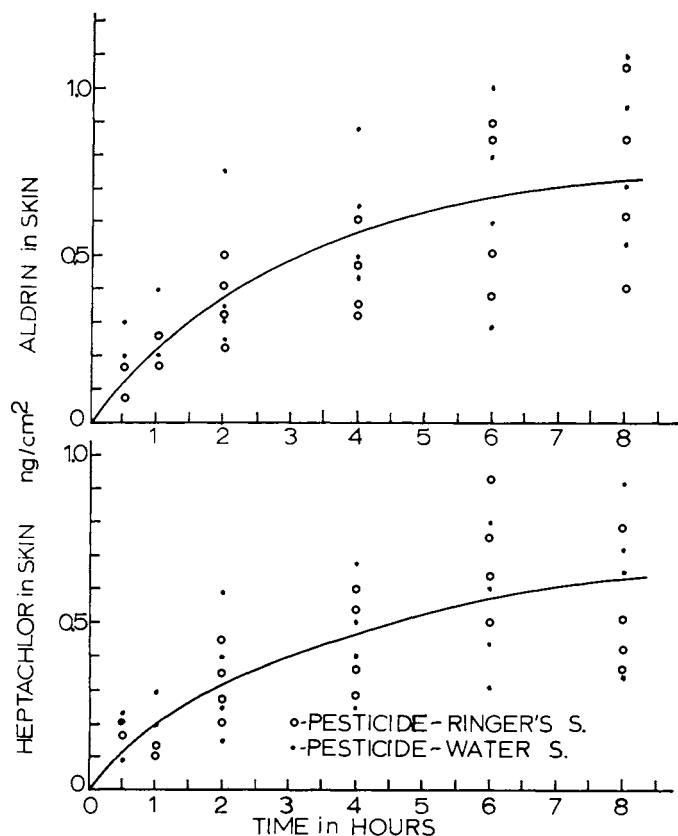

Figure 3. Rate of absorption of aldrin and heptachlor by the frog skin. Each point represents the value for the skin of a frog.

Figures 2, 3, 4, and 5 show the rates of accumulation in the skin of lindane, heptachlor, aldrin, dieldrin, and p, p' DDT, respectively, from both distilled water saturated with pesticide and Ringer's solution saturated with pesticide. Each graph shows a decreasing rate of accumulation of pesticide vs time. Lindane is taken up by the skin in greatest amount followed by dieldrin, aldrin, heptachlor, and p, p' DDT. The results of the analysis of control skins showed no detectable amount of any pesticide to be present.
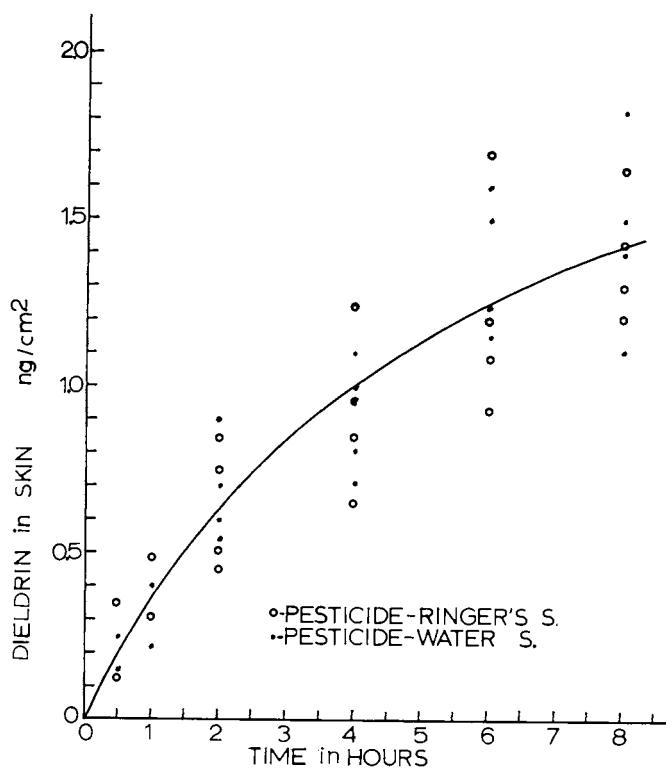

Figure 4. Rate of absorption of dieldrin by frog skin. Each point represents the value for the skin of a frog.

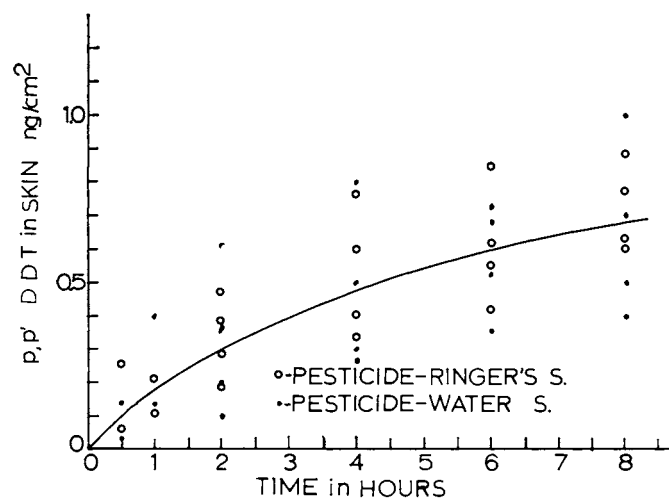Figure 5. Rate of absorption of p, p' DDT by frog skin. Each point represents the value for the skin of a frog.

CONCLUSION

Lindane is the most water soluble insecticide studied in these experiments. Due to its higher concentration in the bathing solution one might expect it to pass through the skin and to be accumulated in the skin at a faster rate than the other pesticides. This may be true if the movement is a simple water diffusion process. The movement through the skin may be a diffusion based on concentration and the relative partitioning of the chemical with the lipids of the frog's skin. This conclusion is possible since no differences are shown between the live and dead skin. The negative results for passage of heptachlor through the skin may simply

be due to the length of time and/or the detection limits of the methods used. The trend toward a differential rate of passage of the lindane through the dead and live skin may, in fact, be a reality but the limits of these experiments preclude such a conclusion.

The absorption of the insecticides by the skin may be explained on the basis of their relative solubility in water solutions and in lipid material (*i.e.*, the more concentrated they are in the bathing solution, the more goes in or through the skin). It is well known that the cell membranes and other components of cells are partially composed of lipid material. One might expect the pesticides to dissolve in this lipid material in higher concentrations than would remain in water. The skin did not concentrate the pesticides during the experimental period from the bathing solution although this might be postulated over a longer period of time.

The article by Woodwell *et al.* (1971) indicates that the reason for the biota's failure to absorb larger quantities of pesticide is unclear. It is possible that even though the total quantities of pesticides in the environment may be relatively large their concentrations in natural waters are relatively low as shown by their low water solubilities. The rate of pesticide accumulation may be slow enough for certain biota (*i.e.*, frogs) to metabolize, excrete, or deposit the pesticides in the lipid layers of the skin.

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