DETECTION AND MEASUREMENT OF C^{14}-LABELLED INSECTICIDE METABOLITES IN FRESHWATER OSTRACODS USING THIN LAYER CHROMATOGRAPHY AND LIQUID SCINTILLATION SPECTROPHOTOMETRY

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One protective mechanism by which many animals cope with synthetic insecticides, insidiously present in their environment and their foods, is through biological detoxification of the chemicals. The insecticides thus metabolized might be stored innocuously within the body or subsequently eliminated by the animal (1). Chemical detection and measurement of many insecticide residues in biological samples is laborious and tedious due to the presence of interfering biological contaminants, and the final results of such analyses often are viewed with some skepticism. Detection and measurement of possible insecticide metabolites in trace amounts is still more precarious.

This paper presents a method which has been used successfully to rapidly detect and measure trace amounts of C^{14}-aldrin, C^{14}-dieldrin, and possible C^{14}-metabolites of these insecticides extracted from water and small freshwater crustaceans, ostracods.

EXPERIMENTAL METHOD

Preparation of Samples; Different amounts of radiologically pure C^{14}-aldrin or C^{14}-dieldrin[1] were dispersed via acetone in 500 ml of water containing varying numbers of insecticide-free laboratory-reared ostracods *(Chlamydotheca arcuata* (Sars)). Samples of water and animal tissues were prepared as follows to monitor changes which occurred in insecticide residues.

Removal of insecticide residues from water samples was accomplished by two successive extractions with petroleum ether (one volume of ether to 10 volumes of water). The water and ether were shaken vigorously for two minutes in a separatory funnel of appropriate volume, the system was allowed to equilibrate, and the water was drawn off. If emulsions formed, they were destroyed by the addition of 5-10 ml of a saturated aqueous solution of sodium sulfate or anhydrous sodium sulfate granules. The ether extracts were combined and evaporated to a small volume for spotting on thin layer plates.

[1] C^{14}-labelled aldrin and dieldrin (Nuclear Chicago Corporation) possessed specific activities of 222 uc/mg and 184 uc/mg, respectively. The terms "aldrin" and "dieldrin" as used here are synonymous with HHDN and HEOD, the respective principal active ingredients in the commercial insecticides aldrin and dieldrin.

Intact ostracods, dissected shells, and dissected bodies were transferred to tared grinding vessels (16 x 150 mm, round-bottom test tubes) and dried at 60°C for 90 minutes. After cooling in a desiccator to room temperature, the grinding vessels were weighed with a Mettler model M5 microbalance. One to five ml of acetone were added to each vessel; then the tissues were homogenized using a motor-driven Teflon pestle. The volume of each homogenate was reduced by evaporation preparatory to spotting on thin layer plates.

Thin Layer Chromatography; The thin layer chromatographic (TLC) procedure used was basically that of Kovacs (2). However, prepared commercial TLC plates (Eastman 6062 Alumina) were substituted for the conventional glass-backed plates. The commercial plates consist of an aluminum oxide adsorbent layer tightly bound to an inert polyethylene terephthalate backing.

Plates were prepared for spotting by removing the lower 1 cm of adsorbent at the base of each plate. Standards and samples were spotted 2 cm from the lower edge of adsorbent. Non-radioactive standards were generally spotted on top of radioactive samples to facilitate visualization since the radioactive samples contained considerably less insecticide than the amount which was detectable by TLC. Since the subsequent quantitation of separated residues was entirely radiological (liquid scintillation counting), the presence of non-radioactive standards caused no interference.

Plates were developed at room temperature (23-25°C) in the chromatographic chamber which was presaturated (at least 30 minutes) with the appropriate solvent -- heptane or 2% acetone in heptane (v/v). The solvent was allowed to ascend to a mark 10 cm above the spotting line, after which the plates were removed from the chamber and dried for 5 minutes prior to spraying with the chromogenic agent. After spraying, the plates were dried, exposed to ultraviolet light, and examined for residues.

Radioactive insecticides or metabolites not revealed by this method were detected by autoradiography and/or liquid scintillation counting. No-screen x-ray film was securely superimposed on the developed TLC plate, the plate was placed in a light-tight box, and the film was exposed for an appropriate length of time. After development of the autoradiogram, the pattern of radioactive residue spots on the TLC plate was visible on the x-ray film. The spots on the TLC plate were then carefully cut out with a scissors and placed into scintillation vials for counting. Alternately, the area above an eluted sample on a TLC plate was divided into several regions based on previous experience gained through the visual observation of non-radioactive aldrin and dieldrin standards. Each region was cut out, and its radioactivity was measured by the liquid scintillation technique. This procedure permitted the omission of the autoradiographic step and thus reduced the chance of residue loss by volatilization. The sum of all

radioactivity found above any one spotting site was considered to represent 100% of the C^{14}-labelled material in that particular sample. Consequently, the concentration of any radioactive metabolite found was expressed as a percentage of the total in terms of the parent compound.

Liquid Scintillation Counting; The scintillation fluid used was toluene which contained the organic scintillators PPO (4 g/1) and POPOP (0.1 g/1). Measurement of radioactivity was accomplished by using a Packard Tri-Carb liquid scintillation spectrometer equipped with model 574 automatic control. All samples were counted for 10 minutes using the C-D window setting, 7.5% gain, and a temperature of 0°C. Counts due to background radiation were automatically subtracted. All sample vials were theoretically identical. Consequently, recorded counts per minute (CPM) were converted directly to nanograms (ng) of insecticide by use of a standard curve which was constructed after counting standard C^{14}-labelled residue solutions. Within the range of CPM used (ca. 100 to 30,000), the relationship between CPM and ng of insecticide was linear (Table 1). Significant variation between individual samples due to counting efficiency was detected and adjusted by use of an external standard.

RESULTS AND DISCUSSION

When either insecticide was dispersed in water, the concentration of insecticide decreased dramatically with time (Table 2). This decrease represented removal of insecticide from solution primarily by adsorption to the walls of the containers due to the hydrophobic nature of the compounds. In ostracod-free water, an appreciable amount of aldrin was converted to its epoxide, dieldrin, within five days; dieldrin was not degraded. In sterilized aqueous solutions, the epoxidation of aldrin did not occur; and therefore, the conversion with five days was presumably due to the activities of microorganisms. Aldrin was more rapidly absorbed from water by ostracods than was dieldrin (Table 3). Aldrin was converted to dieldrin by ostracods, but dieldrin was not metabolized.

Table 1. Relation between c p m and ng insecticide

ng Aldrin	CPM	ng Dieldrin	CPM
0.456	232	0.512	250
1.368	711	1.536	775
2.280	1,224	2.560	1,315
4.560	2,403	5.120	2,579
22.800	11,175	25.600	12,786
45.600	23,328	51.200	25,288

This procedure provided a means of determining with relative ease, whether metabolism or alteration of insecticide had occurred. Furthermore, it allowed a rapid quantitation of separated residues with a sensitivity far in excess of that achieved by conventional TLC and considerably greater than that of gas-liquid chromatography. As little as 0.2 ng of C^{14}-aldrin, C^{14}-dieldrin, or equivalent C^{14}-metabolite per ostracod (ca. 0.5 mg) could be detected and measured. If the number of ostracods per sample were increased, the sensitivity of detection (per ostracod) was accordingly increased. Minute concentrations of insecticide in the water were likewise detected, the precision varying with the size of the sample taken.

Table 2. Fate of C^{14}-aldrin and C^{14}-dieldrin in ostracod-free solutions at room temperature

Time (hours)	Aldrin (ppb*)	Dieldrin (ppb)
0	6.41	9.06
1	5.42	9.06
3	4.90	9.06
5	4.90	8.72
8	4.51	8.77
24	2.89	8.01
48	1.44	7.01
72	0.75**	6.23
120	0.29***	4.96****

* parts per billion
** 100% aldrin
*** 65% aldrin, 35% dieldrin
**** 100% dieldrin

The use of prepared commercial thin layer plates permitted rapid removal of the residue contained in the spots. Since the presence of the polyethylene terephthalate backing in the scintillation vials did not appreciably affect the counting efficiency, transfer and subsequent measurement of all C^{14}-insecticide present in the spots was assured by placing the entire spot (backing included) into the counting vial. Such is not the case when the spots (adsorbent) are scraped from the conventional glass plates.

Care should be exercised to avoid over-loading the thin layer plates with insecticide. If plates are over-loaded, distinct separations are generally not accomplished, and often streaks are produced. This method is restricted to samples having relatively little lipid contamination, since lipid contaminants will also produce streaks and unclear separation of residues. If fatty contaminants are encountered, preliminary cleanup procedures are recommended prior to the qualitative TLC.

Table 3. Uptake and metabolism of C^{14}-aldrin and C^{14}-dieldrin by live ostracods during 48 hours of continuous exposure to C^{14}-aldrin or C^{14}-dieldrin in water

Hour	Residue in water (ppb)	Qualitative analysis of water	ng per gram of tissue (ppb)	Qualitative analysis of tissue residue
0	11.2 a*	100% a	---	---
24	5.5	91% a, 9% d**	55,773	17% a, 83% d
36	4.0	86% a, 14% d	79,867	10% a, 90% d
48	2.76	79% a, 21% d	112,667	8% a, 92% d
0	14.1 d	100% d	---	---
24	12.9	100% d	33,038	100% d
36	11.3	100% d	35,244	100% d
48	10.5	100% d	42,051	100% d

* a= aldrin
** d= dieldrin

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