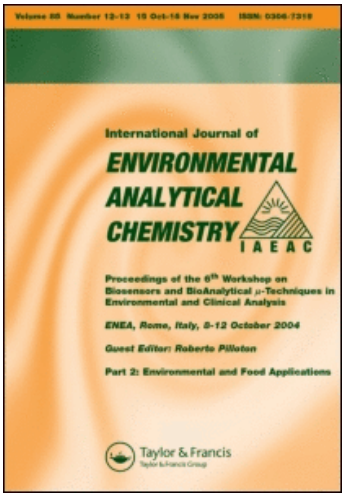

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Uptake and Elimination of ^{14}C-Aldrin and ^{14}C-Dieldrin by the Ostracod *Chlamydotheca Arcuata* (Sars)†

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Laboratory-reared, insecticide-free fresh-water ostracods, *Chlamydotheca arcuata* (Sars), were exposed to various concentrations of ^{14}C-aldrin and ^{14}C-dieldrin. Aldrin and dieldrin were accumulated in or by ostracod tissue both with and against concentration gradients from water. The initial rate of aldrin accumulation was approximately twice that of dieldrin, and accumulation of either insecticide was dependent upon the insecticide concentration in water, the duration of exposure, and the activity of the animals. Aldrin was readily metabolized to dieldrin in living ostracods, and small amounts of dieldrin were eliminated. The rate of elimination was in part dependent upon ostracod activity, although some elimination occurred with dead animal tissues. Dieldrin was eliminated more rapidly by dieldrin-exposed ostracods than by aldrin-exposed ostracods.

INTRODUCTION

Because ostracods are both primary consumers and scavengers in fresh-water systems, they potentially have appreciable capacity to concentrate persistent insecticide residues from the substrate and to transfer or recycle the residues

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to organisms at higher trophic levels. The capacity of ostracods to accumulate insecticide residues, to metabolize these compounds into more or less toxic forms, and to eliminate the residues is important not only to the survival of ostracods but to other animals in the ecosystem.

This paper presents information on the accumulation and elimination of the chlorinated hydrocarbons aldrin and dieldrin† in the fresh-water ostracod *Chlamydotheca arcuata* (Sars).

MATERIALS AND METHODS

Insecticide-free rearing and exposure water was prepared by adding appropriate salts to de-ionized water as described by Sanders and Cope.[1] The pesticide-free food used to sustain ostracods was reconstituted from a chloroform-extracted chicken food base employing the method of Stober and Payne.[2] Examination by routine gas-liquid chromatography revealed that all components of the rearing system were insecticide-free to the limits of detection of aldrin and dieldrin (0.001 ppb for food, 0.002 ppb for water, 3 ppb for algal growth, and 10 ppb for ostracods).

Chemically pure ^{14}C-aldrin and ^{14}C-dieldrin‡ were dispersed via acetone in 500 ml of insecticide-free water containing the ostracods. The concentration of acetone in exposure water never exceeded 1 ml per liter. The ^{14}C-insecticides were purified prior to experimentation by autoradiographic thin-layer chromatography.[3] Food was withheld during the exposures to ^{14}C-insecticides, and the temperature was 21.7 ± 1.1°C.

Because of the procedural limitations in analyzing for small amounts of ^{14}C, it was necessary to subject the animals to the ^{14}C-insecticides at concentrations near the 24-hr EC_{50} (immobility) dosages. These concentrations of insecticides were generally well below those which were immediately lethal to ostracods. The 24-hr immobility EC_{50} values for aldrin and dieldrin are 1.15 and 2.45 ppb, respectively. The estimated EC_{50} values for fatal exposure to either insecticide are 1000 ± 500 ppb.[4] The ^{14}C-insecticide concentrations of the several exposures ranged between 1.40 and 44.9 ppb. Although the numbers of ostracods present in exposure vessels varied with time as animals were removed for tissue examination, the maximum number of animals in any one vessel never exceeded a mass/volume ratio of 0.35 g live tissue/500 ml water. Insofar as determinable, the amount of ^{14}C-insecticides

† 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.

‡ ^{14}C-labeled aldrin and dieldrin (Nuclear Chicago Corp.) possessed specific activities of 222 mc Ci/mg and 184 mc Ci/mg, respectively.

removed from the exposure water by ostracods was insignificant compared to the total insecticide present in the systems.

Residue levels were monitored from the following components of the test systems during different exposures: water, suspended materials, container walls, and ostracods. During some exposures, intact ostracod bodies were taken for analysis; at other times, the bodies and shells were dissected prior to analysis for ^{14}C. For some experiments, dead intact animals or separate dead shells were subjected to ^{14}C-insecticides. The dead shells were remnants of ostracods obtained from the substratum of rearing aquaria. As necessary, ostracods were killed prior to or during exposures by submerging them into hot (ca. 50°C) tap water for a few seconds.

Insecticide residues were doubly extracted from water samples either with petroleum ether or directly with the scintillation counting fluid (toluene containing PPO and POPOP). Ostracod tissues were homogenized in glass vessels with a motor-driven Teflon pestle together with a small volume of acetone. These extracts were then either evaporated to incipient dryness prior to adding scintillation fluid or concentrated for analysis by thin-layer or gas-liquid chromatography. Adsorbed insecticide residues were removed from the walls of exposure vessels by an acetone rinse. Suspended materials were removed by filtering aliquots of exposure water through Whatman No. 1 paper; insecticides present were extracted with acetone. These extraction procedures, the qualitative thin-layer chromatographic separation of residues, and the liquid scintillation counting of the ^{14}C are described in greater detail elsewhere.[3]

RESULTS

When ostracods were exposed to either ^{14}C-aldrin or ^{14}C-dieldrin, the majority of insecticide generally was retained in solution. However, during exposures in which the initial aqueous insecticide concentrations were relatively high (10–50 ppb), a correspondingly greater percentage of the total insecticide was removed from the water by suspended materials, beaker walls, and ostracods. Conversely, when the initial exposure concentrations were low (1–10 ppb), a greater percentage of the insecticide remained in solution. This observation is consistent with and reflects the low water solubilities of aldrin and dieldrin (ca. 30 and 180 ppb, respectively).

The accumulation of aldrin and dieldrin by intact living ostracods occurred rather quickly after initial exposure. After exposure to aldrin or dieldrin for 24 hr at initial aqueous concentrations of 6.6 and 8.4 ppb, respectively, all ostracods were immobilized, but none were dead; even after 96 hr of continuous exposure this was true. Although aldrin was accumulated about twice

as rapidly as dieldrin during the first 24 hr of exposure, after 48 hr there was essentially no difference between the amounts of aldrin and dieldrin found in the ostracods (Table I). This observation is consonant with the observed toxicity of the two insecticides, i.e., initially aldrin is more toxic than dieldrin, but the latent toxicities of aldrin and dieldrin (48 to 96 hr) are similar.[4] After

TABLE I

Uptake of ^{14}C-aldrin and ^{14}C-dieldrin by intact live ostracods during 96 hr of continuous exposure to the insecticides, and retention of insecticide during a post-exposure period in insecticide-free water.

	Aldrin		Dieldrin	
Hour	Water	Ostracods	Water	Ostracods
0	6.64[a]	0.	8.42	0.
1	5.45	809[a].(20)[b]	8.46	214.(20)
4	4.36	1,680.(15)	7.97	816.(12)
8	3.57	2,689.(14)	7.77	1,399.(12)
12	3.18	3,677.(18)	7.35	1,258.(16)
24	2.19	6,057.(10)	6.37	3,465.(14)
48	1.45	4,227.(20)	5.54	3,604.(17)
72	1.22	7,735.(15)	4.66	7,840.(14)
96	0.87	6,656.(10)	3.92	8,857.(8)
97	—	7,128.(10)	—	8,870.(10)
100	—	8,833.(8)	—	10,619.(9)
102	—	9,061.(7)	—	6,609.(12)
106	—	8,896.(6)	—	6,212.(9)
120	—	7,350.(4)	—	7,905.(9)
144	—	—	—	8,972.(6)

[a] ^{14}C-accumulation is expressed as ppb insecticide in terms of the parent compound (mcg/l water or ng/g dry tissue).

[b] Figures in parentheses are numbers of ostracods per composite sample.

96 hr of exposure, aldrin was concentrated in the tissues 7,600 times over the concentration in the exposure water, and dieldrin was concentrated 2,300 times. During 24 and 48 hr of post-exposure in insecticide-free water, no changes in insecticide concentrations occurred within the tissues of immobilized moribund ostracods.

When ostracods were exposed to higher concentrations of aldrin or dieldrin (27.5 and 44.9 ppb, respectively), the uptake of insecticide was greater in concert with the greater aqueous exposure concentrations (Figures 1 and 2). Again, the concentration ratio (ppb in tissue/ppb in water) for aldrin-exposed ostracods was consistently greater than for dieldrin-exposed ostracods. The

body tissues, exclusive of the shell, accumulated 7–28 times more aldrin and 5–26 times more dieldrin than shells alone when exposed to the respective insecticides for the same periods of time. However, since shell tissue constitutes a much greater percentage of the total body weight, the absolute

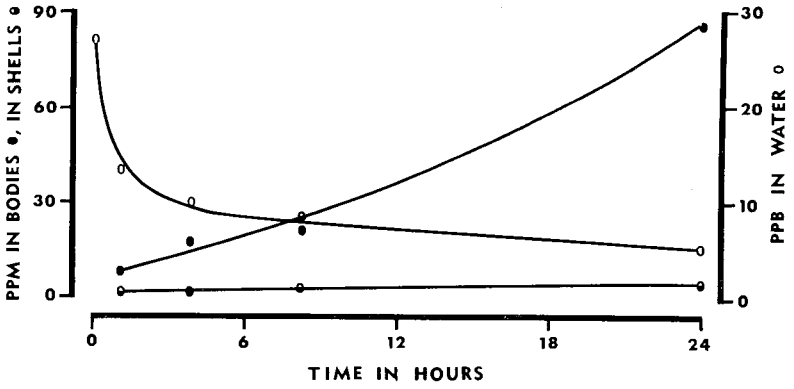

FIGURE 1 Uptake of ^{14}C-aldrin by live ostracods during 24 hr of continuous exposure to the insecticide. Shells and bodies were dissected after exposure. Curves are drawn by inspection.

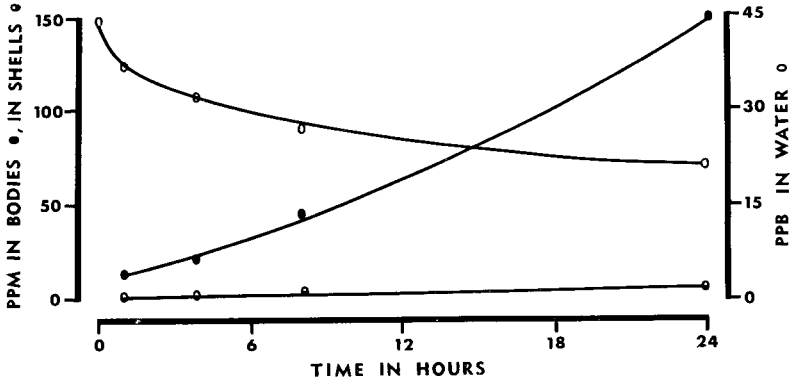

FIGURE 2 Uptake of ^{14}C-dieldrin by live ostracods during 24 hr of continuous exposure to the insecticide. Shells and bodies were dissected after exposure. Curves are drawn by inspection.

accumulation (insecticide per ostracod) in body tissues was only about two times the absolute accumulation in the shell tissues. The insecticide found in shell tissue, in this instance, did not include that which may have been adsorbed externally since the shells were rinsed briefly with 50% acetone prior to analysis. Qualitative analyses of the insecticide residue in ostracod

bodies sampled during these experiments (Figures 1 and 2) revealed that aldrin was metabolized to its epoxide, dieldrin; dieldrin was not further altered.

The uptake of aldrin and dieldrin by live ostracods was compared with the insecticide accumulation by dead shells, i.e., shells containing only the carbonate skeletal materials (Table II). Per unit weight and in absolute terms, dead shells accumulated appreciably less insecticide than live tissues. Because these were weathered shells which were not rinsed with acetone prior to analysis, the insecticide measured had been accumulated entirely by adsorption. Again, there was a more rapid accumulation of aldrin than dieldrin

TABLE II

Uptake of ^{14}C-aldrin and ^{14}C-dieldrin by live intact ostracods and dead shells.

	Aldrin			Dieldrin		
Hour	Water	Live ostracods	Dead shells	Water	Live ostracods	Dead shells
0	1.40[a]	0.	0.	2.91	0.	0.
24	0.75	2,470[a].(12)	182.(21)	2.61	3,823.(13)	632.(10)
48	0.45	12,886.(15)	677.(17)	2.30	10,426.(16)	1,126.(10)

[a] ^{14}C-accumulation is expressed as ppb insecticide in terms of the parent compound (mcg/l water or ng/g dry tissue).

[b] Figures in parentheses are numbers of ostracods or whole shells per composite sample.

by live ostracods even though initially the exposure concentration of dieldrin was twice that of the aldrin concentration. However, 2 to 3 times more dieldrin than aldrin was adsorbed to the shells which is consistent with the behavior of these insecticides with various chromatographic adsorbents[5] and in soil.[6] Aldrin and dieldrin accumulation in whole dead ostracods was compared with that of whole live ostracods after simultaneous nine-hour exposure to the insecticides in common exposure vessels. The uptake of aldrin by dead animals was about one-sixth that of live animals. Similarly, the uptake of dieldrin by dead ostracods was slight.

In another series of exposures (Figures 3 and 4) after 24 hr of exposure to 5.5–11.2 ppb of aldrin, live animals accumulated 56 ppm of residue, of which 83% was converted to dieldrin. Dieldrin-exposed ostracods, under similar conditions, accumulated 33 ppm of dieldrin which was not metabolized. At the time of the 24-hr sampling, one half of the exposed ostracods from each exposure vessel (aldrin and dieldrin) were killed and transferred to post-exposure chambers containing insecticide-free water. Further conversion of aldrin to dieldrin in dead ostracods during post-exposure did not occur, and their rates of residue elimination were less than those of live ostracods.

DISCUSSION

When ostracods were exposed to insecticides for long test periods, i.e., 48 hr exposures or longer, the insecticide uptake followed somewhat of a biphasic curve. The insecticides were rapidly accumulated initially, but the uptake leveled off in later hours of exposure. This leveling-off may have reflected equilibration of the concentration gradients, reduced insecticide transport within tissues and away from sites of uptake, and/or concurrent active

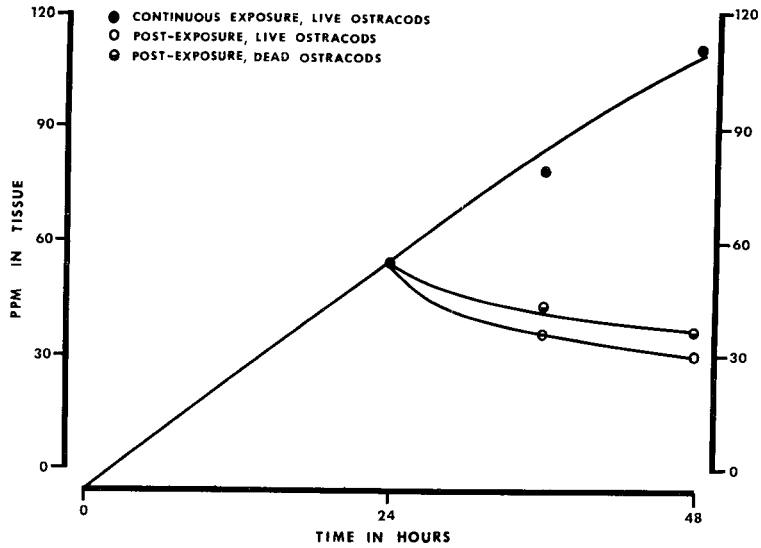

FIGURE 3 Uptake of ^{14}C-aldrin by live ostracods during 48 hr of continuous exposure to the insecticide and elimination of insecticide by live and dead ostracods after 24 hr of exposure when placed in insecticide-free water. The initial exposure concentration of aldrin was 11.2 ppb. Curves are drawn by inspection.

elimination of insecticide by ostracods. Variations in tissue insecticide concentrations at the ends of predetermined exposure periods might also be explained by these phenomena. Although tissue concentrations varied during exposures, for any one continuous exposure the concentration ratio increased consistently with time. In addition, the magnitude of the insecticide accumulation during the initial phase of any exposure was generally related directly to the initial aqueous insecticide concentration.

The rates of aldrin and dieldrin accumulation from water are probably governed by the differences between lipid and water solubilities of the insecticides as well as by ostracod activity. Since aldrin is less water-soluble than

dieldrin, its initial rate of uptake was greater than that of dieldrin. Consequently, aldrin is probably more toxic to ostracods than dieldrin simply because a greater total body burden of the insecticide is accumulated more rapidly. Presumably the two insecticides have the same mode of toxic action.

The elimination of insecticide by ostracods may have been partly due to passive phenomena since dead ostracods did eliminate insecticide. However, since dead animals eliminated insecticide less rapidly than did live ones, the elimination is at least partly due to active mechanisms. This conclusion is

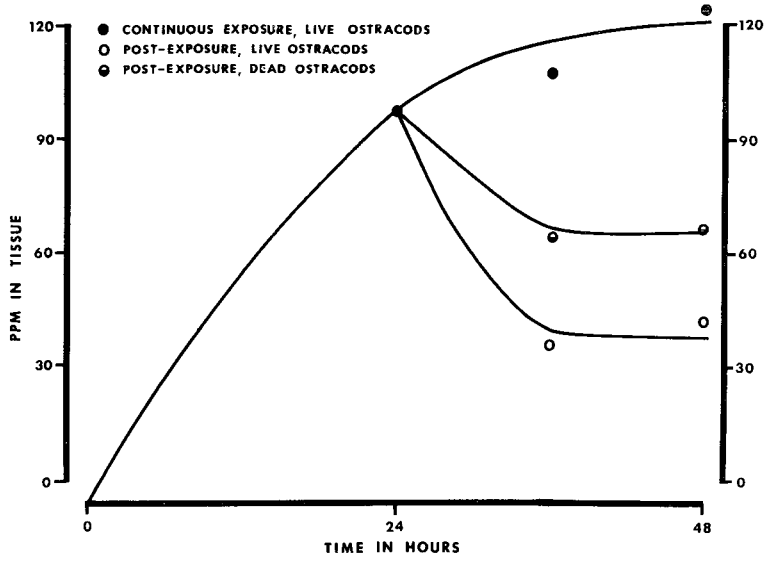

FIGURE 4 Uptake of ^{14}C-dieldrin by live ostracods during 48 hr of continuous exposure to the insecticide and elimination of insecticide by live and dead ostracods after 24 hr of exposure when placed in insecticide-free water. The initial exposure concentration of dieldrin was 14.1 ppb. Curves are drawn by inspection.

further reinforced by the observation that those ostracods exposed to substantially greater insecticide concentrations and/or for longer periods of time eliminated less insecticide than ostracods exposed to lesser concentrations and/or for shorter periods of time.

Dieldrin-exposed ostracods eliminated insecticide more rapidly than aldrin-exposed animals. Gakstatter and Weiss[7] demonstrated that the rate of elimination of three chlorinated hydrocarbons (dieldrin included) from fish corresponded to the water solubilities of the insecticides, i.e., insecticides which were more water-soluble were eliminated more rapidly than those which were less water-soluble. In the case of ostracods, virtually all aldrin was

quickly converted to dieldrin, and the insecticide was eliminated as dieldrin. Consequently, the differences in water solubility do not adequately explain the variation in insecticide elimination between aldrin-exposed and dieldrin-exposed ostracods because both eliminated the same compound. A more plausible explanation would be that aldrin-exposed animals quickly accumulated more insecticide and hence were affected to a greater degree than dieldrin-exposed animals. The aldrin-exposed individuals were, therefore, less able to effect elimination of insecticides. This same phenomenon may have occurred when ostracods were exposed to higher concentrations of either insecticide and/or for longer periods of time.

The elimination of insecticide by ostracods did not occur as rapidly as it does in higher animals. Perhaps in ostracods the process occurs primarily at a general cellular level, whereas higher animals may utilize specialized tissues or organs for collection and removal of insecticides. For example, the elimination of aldrin and its metabolites in rats and rabbits occurs primarily by excretion with urine and feces via the kidney and bile.[8] The fact that the rate of insecticide elimination in ostracods is relatively low augments their capacity to concentrate and store these compounds. In addition, the apparent inability of ostracods to metabolize or detoxify these inimical hydrocarbons could result in the production of a dangerous food supply at a low trophic level in fresh-water ecosystems.

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