Biological Magnification and Degradation of DDT and Aldrin by Freshwater Invertebrates

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JOHNSON, B. T., C. R. SAUNDERS, H. O. SANDERS, AND R. S. CAMPBELL. 1971. Biological magnification and degradation of DDT and aldrin by freshwater invertebrates. J. Fish. Res. Bd. Canada 28: 705–709.

Magnification of persistent pesticides by invertebrates occurred rapidly in fresh water. Invertebrates accumulated residues many thousands of times that of surrounding water after exposure to ^{14}C-labeled aldrin or DDT at levels <100 pptr (ng/liter). In addition, evidence of marked degradation of these pesticides within specific trophic levels was found. Our studies suggest that aquatic invertebrates influence both the quantity and quality of insecticide residue passed via the fish food chain.

Received December 29, 1970

THE magnification of organochlorine insecticides by biological components of terrestrial and aquatic ecosystems is well documented in numerous field studies (Hunt and Bischoff 1960; Hickey et al. 1966; Woodwell et al. 1967; Meeks 1968). Biological magnification implies the increased accumulation of a pesticide at each succeeding trophic level. The ultimate result is the accumulation of residue levels in predators that may be 1,000,000 times the concentration in water. In Lake Michigan water, concentrations of DDT remain essentially constant at the level of 1–5 parts per trillion (pptr) (Reinert 1970) as a result of terrestrial runoff (Nicholson 1970). Yet, DDT residues of 5–10 ppm have been reported (Reinert 1970) in predaceous coho salmon (*Oncorhynchus kisutch*).

We were concerned with biological magnification of persistent insecticides in freshwater invertebrates over a short interval under controlled laboratory conditions, and with the nature of residue changes that accompany degradation while within these specific trophic levels. Aquatic Crustacea and immature insects were exposed in a continuous-flow apparatus to ^{14}C-labeled aldrin and DDT.[1] We measured both the magnification from water and the degradation of these compounds by invertebrates.

Methods

Experimental animals were obtained from local ponds with the exception of a strain of *Daphnia magna* Strauss, which has been maintained in our laboratory for a number of years. All organisms were acclimated to laboratory well water.

Pesticide exposure was made through a continuous-flow apparatus at 21 ± 1 C employing 1-liter glass chambers fed from a glass reservoir through a metering pump at approximately 2.5 ml/min. Through this system, we were able to maintain a constant water concentration of pesticide less than 100 pptr (100 ng/liter). One chamber receiving untreated water served as a control. Stock solutions of ^{14}C-labeled DDT or aldrin were introduced directly into the reservoir and the apparatus was run for 24 hr prior to the introduction of experimental animals. Water residue values were obtained from the experimental chambers, not the reservoir.

[1]Both ^{14}C-labeled Aldrin (81.0 mc/mM, 0.05 mc) and DDT (19.1 mc/mM, 0.05 mc) were purchased from Nuclear-Chicago. Chemical names of compounds used in this paper are as follows: aldrin, 1,2,3,4,10,10-hexachloro-1,4,4*a*,5,8,8*a*-hexahydro-1,4-*endo-exo*-5,-8-dimethanonaphthalene; dieldrin, 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4*a*,5,6,7,8,8*a*-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene; DDT, 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane; DDD (TDE), 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethene; DTMC (Kelthane®, dicofol), 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethanol; DBP, 4,4′-dichlorobenzophenone.

Printed in Canada (J2000)

Because the animals were not fed during the experiment, most exposures were terminated at 3 days to preclude potential nutritional stresses. All samples were taken in triplicate and expressed as mean values ± standard error. Data were not used when mortality in experimental groups exceeded that of control by 10%.

After exposure of the invertebrates in the continuous-flow apparatus, they were removed, washed several times with water to separate any extraneous adhering debris, and dried to a constant weight at 50 C. Samples were homogenized with a teflon-coated tissue grinder, for both total body residue and degradation studies. Organisms used for degradation studies were not dried.

Total body residues were obtained at 24-, 48-, and 72-hr intervals. The homogenate was obtained by adding 6 ml of a Triton X-100®: toluene (2:3 v/v) emulsifier to the sample during grinding. The homogenate was quantitatively transferred to a glass scintillation vial, brought to 15 ml with a toluene-fluor mixture (Rodgers and Stalling 1970), and placed in a liquid scintillation counter. Pesticide residues are expressed as a pesticide/whole body dry weight ratio.

Separation, identification, and quantitation of the ^{14}C-labeled insecticides and degradation products was accomplished by the following procedures. First, the organisms were homogenized in a tissue grinder with several milliliters of redistilled petroleum ether. Then, the homogenate was transferred to a separatory funnel, extracted with petroleum ether, dried over anhydrous Na_2SO_4, and carefully evaporated to 1 ml. Extraction efficiency of spiked DDT and aldrin control samples always exceeded 95%.

Separations with thin-layer chromatography were done by spotting the sample extracts on Eastman precoated silica gel sheets and developing them in hexane: diethyl ether:acetic acid (200:2:2 v/v/v) (Siewierski and Helrich 1967). Authentic standards of the insecticide and known degradation products were co-chromatographed with the experimental extract and located with an ammoniacal $AgNO_3$ spray. An autoradiogram was made by exposing the chromatograms to Kodak no-screen X-ray film for 7 days. Identification of ^{14}C-labeled degradation products was made by comparing the autoradiogram with the authentic standards on the chromatogram. The degradation products were quantified by use of liquid scintillation assay. Radioactive areas of the silica gel plate were scraped into scintillation vials containing a fluor and counted.

Triplicate, composite, 100-ml water samples of the flow chambers were taken at 1-, 2-, and 3-day intervals. They were extracted with redistilled hexane and counted by the previously described liquid scintillation method. Autoradiograms of water samples and stock insecticides were made routinely.

Results

We found rapid direct uptake from water and magnification of the two insecticides by all freshwater invertebrates (Table 1). There was no correlation between residue levels and either surface/volume ratios or taxonomic group. The greatest degree of magnification was in the cladoceran *D. magna* and the mosquito larva *Culex pipiens* Linnaeus with residue levels over 100,000× that of the water concentration. In all instances, we observed no plateau effect on the uptake or retention of DDT and aldrin during the 3-day exposure. In addition, continued pesticide magnification by the glass shrimp *Palaemonetes kadiakensis* Rathbun was observed even through the 7th day of exposure. A linear regression of DDT magnification to time was significant at $P = 0.0005$ (Fig. 1). This suggests a biological magnification factor of 1396 per 24-hr period. Clearly, this linear relation cannot continue ad infinitum.

Autoradiograms of 3-day samples indicated some degree of aldrin and DDT degradation by all organisms (Table 2). Aquatic insects converted DDT primarily to the ethene derivative DDE, a process that was 85% complete in the mayfly nymph (*Hexagenia bilineata* (Say)) over the exposure period. The degradation of DDT by some Crustacea appeared more complex with the recovery of additional compounds other than DDE (Table 2). For instance, the glass shrimp *Palaemonetes* produced three other compounds: DDD, DTMC, and DBP. *Hexagenia*, *Daphnia*, and *Chironomus* converted aldrin to dieldrin via the well-known epoxidation reaction. For the 3-day exposure period, this conversion rate was less than 25% in all instances. No other degradation products were detected.

Discussion

This is an investigation of one segment of an aquatic ecosystem, the lower level consumer component. The results suggest that biological magnification of insecticides at lower trophic levels provides a method of introduction of these substances to higher trophic levels even though those organisms may or may not be directly exposed to a pesticide (Macek and Korn 1970).

Emphasis in the past has been on the parent pollutant with minimum concern for their degradation products. We found not only magnification of a micropollutant but also an increase in the number of pollutants. These products of DDT and aldrin were recovered repeatedly from whole body residues of all invertebrates. We feel that the presence of these degradation products and their biological magnification in organisms may pose a long-range threat to the aquatic community. Recent investigations of DDE, a degradation product of DDT, strongly suggest the need for concern. Peakall (1970) and Bitman et

TABLE 1. Biological magnification of ^{14}C-labeled *p,p'*-DDT and aldrin by freshwater invertebrates.

Pesticide	Organism	Stage of development	No./sample	Pesticide residue (mean value ± SE[a])				Biological magnification factor		
				Water (*ng/liter*)	Total body (*ng/mg*)					
					1 day	2 days	3 days	1 day	2 days	3 days
DDT	Cladocera									
	Daphnia magna	Mature adult	60	80.3±13.7	2.04±0.04	5.55±0.31	9.17±0.17	25400	69100	114100
	Amphipoda									
	Gammarus fasciatus	Mature adult	1	81.3±13.0	0.38±0.04	0.99±0.15	1.68±0.15	4600	12100	20600
	Decapoda									
	Orconectes nais	Mature adult	1	80.3±13.7	0.071[b]	0.171	0.233	880	2100	2900
	Palaemonetes kadiakensis	Mature adult	1	100.0±0.07	0.152±0.01	0.375±0.02	0.503±0.06	1500	3700	5000
	Ephemeroptera									
	Hexagenia bilineata	Nymph	1	52.1±10.0	0.49±0.04	0.87±0.02	1.68±0.06	9400	16700	32600
	Siphlonurus sp.	Nymph	10	47.0±5.1	0.48[b]	0.94	1.08	10200	20000	22900
	Odonata									
	Ischnura verticalis	Naiad	1	101.3±5.8		0.375±0.02			3500	
	Libellula sp.	Naiad	1	79.3±4.3		0.072±0.005			910	
	Diptera									
	Chironomus sp.	Larvae	10	46.3±3.5	0.36±0.07	1.13±0.20	2.2±0.21	7800	24500	47800
	Culex pipiens	Larvae	10	104.6±8.8		13.9±0.78			133600	
Aldrin	Cladocera									
	Daphnia magna	Mature adult	60	16.7±0.37	1.0[b]	1.7	2.4	58000	100000	141000
	Ephemeroptera									
	Hexagenia bilineata	Nymph	1	21.3±2.4	0.29±0.04	0.44±0.04	0.66±0.08	13800	20900	31400
	Diptera									
	Chironomus sp.	Larvae	10	21.3±2.4	0.26±0.01	0.35±0.04	0.48±0.06	12300	16600	22800

[a]Data represent the mean value of at least triplicate samples.
[b]Data represent the mean value of duplicate samples.

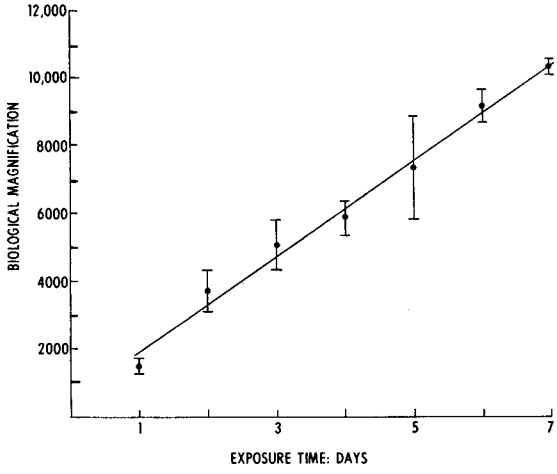

FIG. 1. Biological magnification by *Palaemonetes kadiakensis* of ^{14}C-labeled *p,p'*-DDT from water during 7 days' exposure. Water concentration was 100 $\pm$ $s_{\bar{x}}$ 0.07 ng/liter DDT. Biological magnification = total body residue/water concentration. Points are means and vertical bars represent standard errors. N = 3 or greater. r_{xy} = 0.92 $\pm$ 0.03, P = 0.005.

al. (1970) have shown DDE inhibits shell gland carbonic anhydrase of piscivorous birds, which results in production of thin egg shells.

We propose that the role of invertebrates in biological magnification of pesticides at higher trophic levels is threefold: (1) they can contribute to the rapid accumulation under situations in which the pollution exposure is of limited duration. For example, Meeks (1968) reported that the level of DDT in a freshwater marsh declined in 1 month. The invertebrates, however, remained as a source of pesticide for predators entering the treated area. (2) In many large bodies of water such as Lake Michigan, the concentration of the organochlorine insecticides remain at relatively constant low levels. For example, DDT concentrations in Lake Michigan remain approximately at 1–5 pptr as a result of constant runoff (Reinert 1970; Nicholson 1970). It is not difficult to envision the role of important forage organisms in contributing through biological magnification to the 3–10 ppm residues found in predaceous coho salmon. (3) They may be a source of biological magnification of insecticide degradation products.

TABLE 2. Degradation of ^{14}C-labeled *p,p'*-DDT by freshwater invertebrates during 3-day exposure in a continuous-flow apparatus.

Organism	Stage of development	No./sample	DDT & degradation products expressed as % of total body residue[a]	
Cladocera				
Daphnia magna	Mature adult	60	DDE	19.7
			DDT	73.4
			DDD	6.6
Amphipoda				
Gammarus fasciatus	Mature adult	1	DDE	20.9
			DDT	79.1
Decapoda				
Palaemonetes kadiakensis	Mature adult	1	DDE	13.2
			DDT	50.9
			DDD	7.2
			DTMC	13.1
			DBP	15.5
Ephemeroptera				
Hexagenia bilineata	Nymph	1	DDE	85.0
			DDT	14.9
Odonata				
Ischnura verticalis	Naiad	1	DDE	60.2
			DDT	39.2
Libellula sp.	Naiad	1	DDE	28.4
			DDT	56.3
			DTMC	15.0
Diptera				
Chironomus sp.	Larvae	10	DDE	19.1
			DDT	80.8

[a]Data represent the mean value of triplicate samples.

Although we have studied only one facet of an aquatic community, such background information may contribute to the development of a model system that in the future may aid in predicting the interaction of a pesticide and the biological components.

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