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Received for publication October 18, 1971.

DIELDRIN UPTAKE IN THE THREE-RIDGE NAIAD[1]

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Abstract: Specimens of the three-ridge naiad (*Amblema plicata*) were placed in a continuous flow system containing two concentrations of dieldrin (HEOD)—20 parts per billion (ppb) and 20 parts per trillion (ppt). For 10 weeks the gills of naiads were monitored for uptake and retention of HEOD by means of liquid gas chromatography. HEOD concentrations in the gills stabilized at approximately 1 ppm and 55 ppb, respectively, in the two systems after 2 weeks of exposure. During the next 12 weeks HEOD was not added to the test aquaria. Release rate of HEOD by naiads held at 20 ppb was one-sixth that of the uptake rate, and those exposed to 20 ppt released HEOD at one-fourth the uptake rate. The experiment was terminated when concentrations in the gills reached 700 ppb and 13 ppb (the background level in gills of freshly caught naiads was 5.7 ppb). The gills of the three-ridge naiad appear to be acceptable monitors of HEOD.

The use of naiad mollusks (Bivalvia) as biological indicators of pollution has been practiced for many years. Ingram (1957) reviewed the literature for the first half of this century. However, the use of naiads as monitors of pollution is a recent development. Bedford et al. (1968) collected naiads from an upstream location, placed them at six stations downstream, and analyzed them for pesticide content after 2, 6, and 10 weeks. Statistical analysis showed that the naiads concentrated DDT, TDE, DDE, methoxychlor, and aldrin significantly above their background levels and the concentrations detected in the stream. It was also noted that the concentration of pesticides in the naiads seemed to reach an equilibrium associated with the amount of pesticide in the stream. Studies with oysters have shown that they can concentrate pesticides to levels 70,000 times greater than those in their environment (Butler 1966).

If naiads are to be used as pollution monitors, more specific details on the kinetics of the metabolism of specific pollutants are necessary. We subjected the three-ridge naiad to a pesticide under controlled conditions and analyzed for pesticide content. *A. plicata* was chosen because it is one of the most abundant species in the Midwest, and it is found in both lakes and streams (Bates 1970).

We thank A. Hegedus of the Columbus Zoological Gardens for the use of their facilities and Drs. D. H. Stansbery and W. Collins for reviewing the manuscript.

[1] This study was supported by the Ohio Cooperative Fishery Unit and the Ohio Division of Wildlife.

MATERIALS AND METHODS

Specimens of the three-ridge naiad were collected from a local stream and placed in a continuous flow system containing two concentrations of the chlorinated hydrocarbon pesticide, dieldrin [not less than 85 percent of 1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-endo-exo-5, 8-dimethanonaphtahlene (HEOD)]. For 10 weeks the naiads were subjected to this treatment, and during the following 13 weeks untreated water (containing a background level of less than 1 ppb of dieldrin) ran through the system. An investigation of the uptake, establishment of an equilibrium, retention, and release of dieldrin was conducted using gas chromatography for dieldrin analysis.

A modification of Hille's (1969) continuous flow system was used because dieldrin is rapidly lost from a static system (Hille 1969). The system was assembled at the Arthur C. Johnson Aquarium at the Columbus Zoological Gardens (Fig. 1). Water entered the 380-liter head tank (A) through a 16-mm hose connected to a float-actuated control valve that maintained the tank near capacity. Water flowed by gravity from the base of the head tank through 13-mm Tygon tubing that was attached to a *T* joint (B). A 13-mm Tygon tube (C), open to the air, was fitted to the upper section of the *T* joint; this facilitated the flow of water by allowing trapped air to escape. The lower section of the *T* joint was connected to a 13-mm Tygon tube 30 cm long (D). A plastic connector, secured by a screw clamp (E), was inserted into the end of this tube. A 7-mm Tygon tube (F), which delivered the water to the chambers where water and a concentrated dieldrin solution were mixed (G), was attached to this connector. Four 5-mm glass siphons (H) ran from the mixing chambers to the

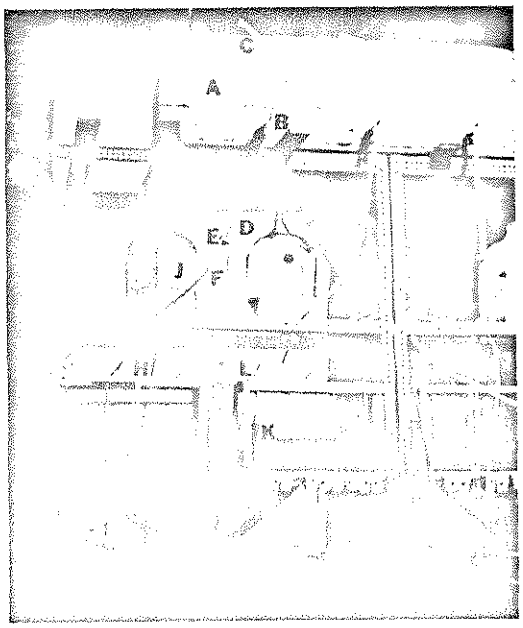

Fig. 1. A continuous flow system—(A) head tank, (B) *T* joint, (C) Tygon tube open to air, (D) Tygon tube, (E) screw clamp, (F) Tygon tube, (G) mixing chambers, (H) glass siphons, (I) test chambers, (J) Mariotte bottle, (K) aerator connector, (L) siphon tube, and (M) Tygon tube.

test chambers (I). From here the water overflowed into a galvanized trough equipped with a drain.

Three glass carboys (J), each with a 20-liter capacity, were adapted as Mariotte bottles according to Hille's (1969) procedure and placed below the head tank and above the mixing chambers. These bottles were constructed to deliver a concentrated dieldrin solution, at a constant rate, to the mixing chambers. Each of two bottles contained concentrations of dieldrin sufficient to provide, when mixed with water, test concentrations of 20 ppb and 20 ppt of dieldrin. The dieldrin was dissolved in 100 percent ethanol. The third bottle served as the control.

The mixing chambers were 42-liter aquaria, each equipped with an overflow consisting of an aerator connector (K),

siphon tube (L), and Tygon tube (M). The function of the overflow device was to keep the flow system in balance. The test chambers were 127-, 63-, and 42-liter aquaria with one aquarium of each size for the controls and one aquarium of each size for each of the dieldrin concentrations. One air stone in each of the 42- and 63-liter aquaria and two in each of the 127-liter aquaria provided aeration and circulation. Two siphons from the mixing aquaria fed each of the 127-liter aquaria, and one siphon fed each of the other aquaria.

Average flow rates from the head tank to the mixing aquaria were 2.6, 3.2, and 2.6 liters per minute in the dieldrin concentrations of 20 ppb and 20 ppt and in the control, respectively. Flow rates were regulated by adjusting the length of the Tygon tube from the *T* joint to the mixing aquaria and by inserting short segments of glass tubing into the end of the delivery tubes. The average delivery rate from all the Mariotte bottles was adjusted to 1.6 ml per minute. With the above flow rate, complete turnover of the dieldrin solution in the testing aquaria was accomplished at least every 1.5 hours.

On October 20, 1969, 298 specimens of naiads were collected at Little Darby Creek at Rosedale–Plain City road bridge, 2.8 miles east of Rosedale, Pike Township, Madison County, Ohio. Twenty-six naiads were sacrificed to obtain background levels of dieldrin. The remaining 272 were taken to the Zoological Gardens, scrubbed lightly with a toothbrush to remove dirt and attached algae, and placed in the test aquaria. The naiads soon began siphoning, and no trouble was encountered maintaining them in this system.

The head tank, delivery tubes, mixing aquaria, and siphons were cleaned biweekly with brushes and a chromic acid cleaning solution. The naiads were scrubbed weekly with a soft brush attached to a power drill, and the interiors of the testing aquaria were cleaned weekly with steel wool. Frequent cleaning was necessary because of the accumulation of ferric oxide in the water lines and on the naiads. Also, during the first 10 weeks, when ethanol was added to the system, an aerobic bacterium (*Sphaerotilus*) grew throughout the system, clogging the water lines, coating the surfaces of the aquaria, and completely covering the naiads.

Beginning on the 26th day, and every 5 to 6 days thereafter, small amounts of algae (*Colenkinia* sp.) were added to the testing aquaria. We had observed earlier that the three-ridge naiad could survive utilizing *Colenkinia* as a food source.

Dead naiads were removed daily and frozen. Dieldrin and ethanol were added to the Mariotte bottles as needed, and flow rates from the head tank and Mariotte bottles were checked periodically and adjusted when necessary.

The gas chromatograph used was a Model 7620 equipped with a Ni-63 electron capture detector and a Westronics strip chart recorder. The column was a 1.83 m × 4.0 mm U-shaped glass tube packed with 4 percent SE-30 on Anacrom ABS 70–80 mesh. Nitrogen gas was used as the carrier gas at a flow rate of approximately 80 ml per minute. The temperature readings remained quite constant and were as follows: injection port 230 C, column oven 198 C, and detector 270 C. The standard solution was 0.0197 ng/μl of 99 percent plus purified HEOD. This standard solution was injected at the beginning of each series of injections and after every six sample injections, to obtain a standard curve for calculating the data by using heights of peaks (Gaul 1966). Confirmation of HEOD in samples was obtained by injecting a few samples on a Barber–Colman Gas Chromatograph Model 5052 equipped with a tritium elec-

tron capture detector and a column packed with a 1:1 mixture of 15.2 percent QF-1 and 10.5 percent DC 200 on Gas Chrom Q 100/120 mesh. Similar results were obtained on both columns. Due to the sensitivity of the gas chromatograph, all solvents used in dieldrin extractions were glass-distilled before use.

Two water sources were used in the continuous flow system. The primary water source was the well at the Arthur C. Johnson Aquarium, but on December 18 the pump broke. Thus, from December 19 to January 6, the well that supplies the remainder of the Zoological Gardens was used. Two water analyses were made of each well (Table 1). The water temperature was checked daily; it ranged from 12.2 to 16.6 C over the 23-week period.

Water samples were collected every 4 days from the siphons leading from the mixing aquaria and analyzed for HEOD by a modification of Teasley and Cox's (1963) procedure. Water samples of 100 ml from the 20-ppb aquaria and samples of 225 ml from the 20-ppt aquaria, and from the control aquaria, were extracted with three fractions of hexane (redistilled Skelly Solve B) totaling 50 ml. The fractions were passed through 1.2 cm of anhydrous sodium sulfate and combined in a 100-ml beaker. The 20-ppt and control samples were concentrated to approximately 1 ml on a warm hot plate under a filtered airstream before injection into the gas chromatograph, whereas the 20-ppb samples were injected directly. Calculations of the data were based on the 50 ml of hexane used in the extractions. Recovery studies using this technique and aquarium well water showed 96.9 percent recovery of HEOD. The time-lapse between collection of the water samples and analysis varied from 3 days to 2 months, but no trend in the data indicated that no variation in the background levels of dieldrin could be attributed to this time differential.

Table 1. Average of two analyses[a] of the well water of the Arthur C. Johnson Aquarium and of the Columbus Zoological Gardens. Concentrations are expressed in mg per liter.

Test	Aquarium Well	Zoological Gardens Well
pH	7.73	7.35
Alkalinity	233	292
Chloride	30.0	76.3
Chromate	0.12	0.09
Copper	0.25	0.22
Fluoride	0.53	0.63
Total hardness	327	451
Iron	0.17	0.30
Manganese	0.41	0.25
Nitrate	10.6	7.0
Nitrite	0.019	0.011
Phosphate	0.16	0.15
Silica	15.7	17.6
Sulfate	88	108

[a] The analyses were conducted with a Hach Water Analysis Kit. The average values are within 10 percent of the actual values except in those cases underlined, which are 22.2, 20.0, and 47.1 percent, respectively, for chromate, manganese, and nitrate.

Each week three naiads of different sizes were removed from the two test concentrations. Different sizes were selected to obtain average uptake, retention, and release rates of HEOD over different ages. However, no attempt was made to determine the age of the specimens. The weekly samples included naiads from the 127-, 63-, and 42-liter test aquaria. Only two naiads were sampled each week from the 127- and 63-liter control aquaria because of contamination of the 42-liter control aquarium.

After a naiad was removed from the shell, the gills and foot were removed. Each portion was then blotted on a paper towel, wrapped in aluminum foil, and placed in a freezer. Dead naiads, and those sacrificed immediately after collection from Little Darby Creek, were also treated in this manner. Six naiads were removed and weighed with a Mettler balance prior to

freezing for a comparison of wet weight and weight after thawing. After thawing, the gills of these six naiads averaged 59.1 ± 4.2 percent of the wet weight.

After careful consideration, it was decided to analyze the gills for HEOD every 14 days after reaching equilibrium levels. Accordingly, the gills of the frozen naiads from weeks 1, 2, 4, 7, 10, 11, 13, 16, 19, and 22 were used in the analysis. Each pair of gills was thawed, blotted on Whatman No. 1 filter paper, weighed to the nearest 0.01 gram with a Mettler balance, cut into small (3 mm^2) pieces, and placed in a grinding vial with a small amount of anhydrous sodium sulfate and 3 ml of a 1:1 solution of acetone and acetonitrile. The samples were extracted three times during grinding with a Virtis "45" homogenizer using the microshaft attachment at approximately 16,500 to 20,500 rpm. Fractions of acetone–acetonitrile were collected from the vial using a pipette and combined in a 60-ml separatory funnel. Distilled water was added to make a ratio of 4:1 acetone–acetonitrile and water. The extracted pesticide was partitioned three times into petroleum ether, and these petroleum ether fractions were combined in a 60-ml separatory funnel. Clean-up procedures followed those of the Pesticide Residue Analytical Laboratory of the Ohio Cooperative Extension Service (A. C. Waldron, personal communication). An equal volume of 2 percent sodium bicarbonate was added to the petroleum ether and shaken. The clean-up columns, 19 × 300 mm, were packed with 6.3 cm florisil, 60/100 mesh preactivated at 650 C, then with 6.3 cm silica gel, and topped with 2.5 cm anhydrous sodium sulfate. The column was rinsed with 50 ml of benzene and 50 ml of 4:1 hexane and benzene before the petroleum ether extract was placed on the column. After this, the column was eluted with approximately 150 ml of 4:1 hexane and benzene and then with approximately 300 ml of benzene (depending upon the standardization of each batch of florisil). To prevent the formation of air canals, the column was never allowed to run dry. The benzene fraction was collected and either concentrated with a Buchi rotary vacuum evaporator prior to injection into the gas chromatograph or was injected directly. Calculations were based on the amounts of HEOD detected. Periodic recovery studies using this technique showed a 92.4 percent recovery of HEOD. The time-lapse between sampling of the naiads and analysis varied from 5 to 12 months, but no trend in the data could be correlated with this variation.

A laboratory accident contaminated samples taken during the 9th week of exposure and the control sample taken during the 10th week.

RESULTS AND DISCUSSION

Dieldrin concentrations in the water of the aquaria exposed to 20 ppb were variable. During the entire 10-week exposure period, October 20 through November and December, the HEOD concentrations, averaged over 17-day periods, were 17.8 ± 6.1, 14.8 ± 9.6, 15.8 ± 6.8, and 20.3 ± 2.1 ppb, respectively (all ± values are standard deviations). This variability was probably due to the fluctuations in the flow rates caused by the accumulations of ferric oxide and bacteria in the water lines and by small air leaks in the Mariotte bottles. The average dieldrin concentration increased and stabilized during December, probably because all the tubing and glassware finally became saturated with dieldrin. Analyses of the control water showed no evidence of dieldrin, but the sensitivity of the gas chromatograph was not great enough to detect dieldrin in the 20-ppt water samples.

Table 2. Average uptake retention and release rates of HEOD in the gills of *A. plicata*. Ranges are in parentheses.

WEEK	HEOD EXPOSURE CONCENTRATIONS		
	20 ppb ($n = 3$)	20 ppt ($n = 3$)	Control ($n = 2$)
0	5.7 (4.3– 8.2)	5.7 (4.3– 8.2)	5.7 (4.3–8.2)
1	10.3 (9.1–12.2)[a]	38.2 (26.5–51.7)	18.2 ± 0.6
2	16.2 (13.5–17.5)[a]	36.7 (26.1–44.6)	10.5 ± 0.4
4	18.1 (11.0–22.5)[a]	54.2 (54.5–58.0)	11.5 ± 0.5
7	16.5 (15.9–16.9)[a]	53.9 (32.1–83.8)	11.8 ± 1.2
9	—[b]	—[b]	—[b]
10[c]	16.8 (15.0–18.9)[a]	56.1 (41.1–69.3)	—[b]
11	12.1 (8.1–15.8)[a]	42.9 (25.9–59.4)	11.8 ± 2.6
13	8.3 (4.7–10.5)[a]	32.2 (17.2–57.8)	5.0 ± 0.4
16	3.6 (0.4– 7.2)[a]	13.0 (8.2–14.9)	6.0 ± 0.9
19	1.1 (0.7– 1.8)[a]	13.3 (11.8–15.4)	4.1 ± 0.1
22	0.7 (0.6– 0.8)[a]	8.9 (6.7–11.8)	5.0 ± 0.5

[a] Ppm; all other concentrations are ppb.
[b] Samples contaminated during analyses.
[c] End of the exposure to HEOD.

We assume that the general trend found in the 20-ppb exposure chambers occurred in the 20-ppt exposure chambers.

The background level of HEOD in the gills of the 26 naiads sacrificed immediately after collection from Little Darby Creek was 5.7 ± 1.0 ppb, with a range from 4.3 to 8.2 ppb. A background level was expected because Little Darby Creek flows through agricultural land. The average HEOD level in the gills of the control naiads during the first 10 weeks was 12.4 ± 3.0 ppb. This indicated some minor dieldrin contamination between the control aquaria and those with higher dieldrin concentrations. During the last 13 weeks, the HEOD concentration in the control naiads decreased gradually to 1.5 ppb (average of weeks 19 and 22), a value below the average background level. Unexplainedly, all control naiads held in the 42-liter aquarium were grossly contaminated with dieldrin, and their data were not included.

HEOD levels in the gills of the test organisms held in 20-ppb and 20-ppt dieldrin concentrations are shown in Table 2. Even though concentrations of HEOD in the gills for most of the weeks showed much variability, the uptake, retention, and release were similar for both concentrations of dieldrin in the water. There were three periods at both concentrations: (1) rapid accumulation of dieldrin in the gills, (2) stabilized period of HEOD retention, and (3) rapid release of dieldrin from the gills. The period of rapid accumulation began immediately after introduction into the test aquaria. This rapid accumulation was not anticipated at the low experimental concentrations, and thus the first sample was not taken for analysis until after a week's exposure to dieldrin. The rapid rate of uptake indicated that the rate of accumulation resembled a logarithmic curve. At approximately 2 weeks, the rapid accumulation ceased and the concentration of dieldrin in the gills remained relatively constant. With 14 days as the period of rapid accumulation, calculations showed that the naiads held in 20 ppb of HEOD concentrated dieldrin in their gills at a rate of 1.2 ppm per day, or 60 times the HEOD concentration in the water. Corresponding uptake for naiads held in 20 ppt were 3.6 ppb per day or 180 times the concentration in the water.

In both test concentrations, the HEOD in the gills of the naiads seemed to stabilize or come to an equilibrium with the dieldrin in the surrounding water. Thus, this equilibrium concentration was much greater in the gills of the naiads held in the higher dieldrin concentration (17 ppm versus 55 ppb). This is consistent with investigations of different organisms held at various concentrations of a given pesticide (Chadwick and Brocksen 1969, Mount and Boyle 1969).

The equilibrium concentration was retained during the remainder of the dieldrin exposure time, but dieldrin concentrations decreased logarithmically after removal of dieldrin from the water. During the 12 weeks after the end of treatment, the gills of naiads that had been held in 20 ppb dieldrin lost approximately 200 ppb per day. The rate of dieldrin release was one-sixth as fast as the rate of uptake. During the 6 weeks after the end of treatment, the gills of naiads that had been held in 20 ppt dieldrin lost approximately 925 ppt per day, or a release rate approximately one-fourth at fast as the rate of uptake. These limited data indicated that active uptake and release of dieldrin from the gills of naiads occurred more rapidly in the lower dieldrin concentration. The gills in both dieldrin concentrations did not return to the background level of 5.7 ppb but seemed to be asymptotic at concentrations above the background level (700 ppb and 13 ppb). It appeared that much time was needed for these naiads to reduce dieldrin concentrations to the background level. Thus, the low background level indicates that Little Darby Creek had not recently been highly contaminated with dieldrin. The rather rapid initial release of dieldrin, followed by a much slower release, is similar to the release of DDT from oysters (Butler 1966).

The rapidity of uptake and of release of dieldrin from the gills of the three-ridge naiad and their sensitivity to the 20 level strongly indicate that the gills of naiad are an excellent monitor for diel Other investigations have also shown different species of naiads could con trate other pesticides (Miller et al. Bedford et al. 1968). Thus, this study sh ing the interaction between a pollutant the gills of a naiad should serve as a mo for the use of naiads as monitors for pe cides and other detectable chemicals. major difficulty is that most naiads can live in highly polluted waters and th would have to be used primarily for d tecting low levels of pollutants or su pended in heavily polluted streams for only brief periods.

The feet and the visceral masses of naiads will be analyzed at a later date to determine how they compare with gills as monitoring tissues. This study and others, such as changing the pollutant concentration and having algae present throughout the exposure period, are necessary before correlations between laboratory studies and surveys of dieldrin in stream populations of naiads can be meaningful.

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Received for publication July 2, 1971.

ALLIGATOR DIETS ON THE SABINE NATIONAL WILDLIFE REFUGE, LOUISIANA

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Abstract: A total of 413 alligator (*Alligator mississippiensis*) stomachs collected in 1961, 1962, and 1964 from Sabine National Wildlife Refuge, Louisiana, were examined for food contents. Crustaceans and fishes were important food for alligators of all sizes. Reptiles and birds ranked fairly high in the diets. Fluctuations in mammal populations were reflected in the alligator food habits. Muskrats (*Ondatra zibethicus*) rated high as food in 1946, and nutria (*Myocaster coypus*) rated high in 1961.

The first comprehensive food habits study of the North American alligator was made by Kellogg (1929). Most of the 157 stomachs examined by him came from alligators taken in Vermilion and Cameron parishes, Louisiana. McIlhenny (1935) listed the contents of 24 alligator stomachs from Avery Island, Iberia Parish. O'Neil (1949:115–136) examined 96 stomachs in 1941 and 28 in 1942 from Iberia, Vermilion, and Cameron parishes. Giles and Childs (1949) analyzed 318 stomachs from alligators killed during the harvest of 1946 on the Sabine Refuge. Foods and feeding habits of 20 alligators collected from two habitats on the Rockefeller Refuge, Cameron Parish, were compared by Chabreck (In press). Our study reports on three series from 1961, 1962, and 1964 and compares them with the 1946 series (Giles and Childs 1949).

AREA DESCRIPTION

The Sabine National Wildlife Refuge, an area of 142,000 acres in the southwest corner of Cameron Parish, Louisiana, lies between two large estuarine lakes, Calcasieu and Sabine. Water from these lakes is connected to the refuge drainage system by canals. Much of the refuge is prairie marsh with salt-meadow cordgrass (*Spartina patens*) the dominant plant. Prior to 1958,