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Biomonitoring of Organochlorine Insecticides Using the Clam, Amblema plicata

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BIOMONITORING OF ORGANOCHLORINE INSECTICIDES USING THE CLAM, AMBLEMA PLICATA

(TITLE)

BY

Terry M. Hogan

THESIS

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I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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The undersigned, appointed by the Head of the Department of Zoology,

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BIOMONITORING OF ORGANOCHLORINE INSECTICIDES

USING THE CLAM, *AMBLEMA PLICATA*

Presented by

Terry M. Hogan

a candidate for the degree of Master of Science

and hereby certify that in their opinion it is acceptable.

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INTRODUCTION

The application of organochlorine insecticides over large agricultural areas has recently been cause for concern. Studies have shown insecticides and their metabolites are easily transported to the aquatic ecosystem of silt-laden runoff (Chesters and Konrad, 1971). Monitoring of major agricultural river basins has revealed insecticides in water (Breidenbach and Lichtenberg, 1963, Lichtenberg, Eichelberger, Dressman, and Longbottom, 1970), suspended particulate matter (Pfister, Dugan, and Frea, 1969), and sediment (Sparr, et al., 1966). Higher levels have been found in aquatic organisms, including phytoplankton (Butler, 1966c), mollusks (Butler, 1966c, Godsil and Johnson, 1968), and fish (Morris and Johnson, 1971).

In addition to being potentially lethal to nontarget aquatic organisms (Harrington and Bidlingmayer, 1958, Feltz, Sayers, and Nicholson, 1971, Hogan and Roelofs, 1971), insecticides have been associated with severe sublethal effects. The sublethal effects include reproduction failure in fish (Macek, 1968) and clams and oysters (Davis and Hindu, 1969), depression of growth rates in oysters (Butler, 1966b), behavior changes in fish (Warner, Peterson, and Borgman, 1966), and decreased photosynthesis in phytoplankton (Menzel, Anderson, and Randtke, 1970).

Because of frequent use, hazards, and degree of persistence, aldrin, dieldrin, heptachlor, heptachlor epoxide, and DDT and its metabolites have been listed as chemicals to be monitored in the National Pesticide Monitoring Program (Schechter, 1971).

Freshwater and marine lamellibranch mollusks are efficient biomonitoring organisms, reflecting ambient insecticide level (Bedford, Roelofs, and Zabik, 1968, Casper, et al., 1969, Bedford, 1970). Despite this fact, freshwater clams, unlike their marine relatives, have not been widely used as monitoring organisms. Mollusk biomonitoring studies have been generally limited to estuaries and bays and major river basins, with little study being devoted to the small tributaries receiving the inital influx of insecticides.

Thus, the purpose of this study is to monitor the organochlorine pesticides in Polecat Creek, Coles County, Illinois, a stream draining approximately 28 square miles of primarily agricultural land and flowing into the Embarrass River (a tributary of the Wabash River). The clam Amblema plicata (Say, 1817) was used as the biomonitoring organism.

LITERATURE REVIEW

With the discovery of the insecticidal properties of DDT in 1939, its use by the military in the early 1940's, and its commercial application in the mid-1940's, a major industry evolved. The potential for chemical control or eradication of agricultural pests encouraged studies on other organochlorine insecticides, such as chlordane, dieldrin, heptachlor, aldrin, endrin, and toxaphene.

The U.S. Department of Agriculture reports (HEW, 1969) that agriculture applies 85 percent of the utilized insecticides, 67 percent of which are applied to cotton, corn and apples. Corn receives about 10 percent of the total agriculturally-applied insecticides, including 96 percent of aldrin and 84 percent of the heptachlor.

Organochlorides are classified as "persistent" or "hard" insecticides, requireing 2 to 5 years to lose from 5 to 100 percent of their insecticidal activity (HEW, 1969). For this reason they pose a potentially serious threat to the environment by widespread contamination and accumulation in biota (Carson, 1962).

Numerous studies have been undertaken to determine how organochlorine insecticides can enter the aquatic ecosystem. Inasmuch as organochlorides are highly insoluble in water (Park and Bruce, 1968, Gunther, Westlake, and Jaglan, 1968), studies have been conducted to determine the role of silt and other suspended particulate matter in transporting insecticides in surface runoff. Pfister (1969) found

that microparticulates suspended in Lake Erie contained organochlorine pesticides. He further noted that lindane was associated with the heavier inorganic suspended matter whereas aldrin and endrin were associated with the less dense material comprised of organics, detritus and microorganisms.

Johnson and Morris (1971) noted, while monitoring Iowa rivers for chlorinated hydrocarbon pesticides, that a significant portion of the pesticides were carried in suspended matter. Similar observations were reported by Morris and Johnson (1971), who concluded that pesticides are apparently adsorbed on soil particles and move into streams during rain and snow-melt runoff as erosion silt, much of which settles as sediment.

Chesters and Konrad (1971) conclude that pesticides enter the aquatic system primarily by direct application to surface waters and by particulate erosion from pesticide-contaminated soil.

Once in the aquatic environment, the pesticides are able to enter the aquatic food web by various routes, thus accumulating in aquatic organisms. Leshniowsky, et al. (1970) indicated that flocculent bacteria isolated from Lake Erie can absorb and concentrate aldrin from colloidal dispersion, removing it from the water phase. Numerous authors have noted that organochlorine insecticides can be accumulated by algae and protozoa (Butler, 1966a, Gregory, Reed, and Priester, 1969, Vance and Drummond, 1969, Matsumura, Patil and Boush, 1970, Wheeler, 1970, and deKoning and Mortimer, 1971).

Upon entering the lower trophic levels of the aquatic food web, insecticides may undergo biological concentration. Woodwell, Wurster and Issacson (1967) present a well-documented example of trophic DDT

magnification in the estuarine environment, tracing increasing DDT levels from plankton to vertebrates such as fish and birds.

The U.S. Department of Interior (1966) reported the magnification of DDD applied to Clear Lake, California. Ambient level was 0.02 ppm, plankton - 5 ppm, fish - 2000 ppm, and grebes, which were found dead, had 1600 ppm DDD.

Under experimental conditions, Johnson, Saunders, Sanders, and Campbell (1971) noted that aquatic invertebrates not only magnify organochlorine pesticides, but also metabolize them. They concluded that aquatic invertebrates aid in biomagnification of pesticides and their metabolites. Additional examples of biomagnification can be found in the literature search by Fox (1970).

Direct absorption of pesticides via gills has also been reported as a bioaccumulation mechanism (Fromm and Hunter, 1969, Chadwick and Brocksen, 1969). Recently additional importance has been attributed to the role of gills in pesticide exchange. Hamelink, Waybrant, and Ball (1971) have suggested that the levels of pesticides in lentic organisms are dependent upon the absorption and solubility differences of insecticides in lipids and water and not upon the food web. They propose that residue levels in aquatic organisms reflect the retained levels which are the result of ". . . absorption and solubility differences acting through systems of exchange equilibria."

Gaufin (1967) has suggested that the ratio of a pesticide to its metabolite may reflect the degree to which direct absorption and food web accumulation have contributed to the total body burden.

Lichtenstein, et al. (1966) reports that under experimental conditions aldrin precipitates in water within a 7-day periods. Further

investigation revealed that lake mud enhances precipitation and has limited degradation ability due to unidentified microbes.

Relatively large amounts of insecticides are being concentrated in benthic and benthic-feeding organisms. Odum, Woodwell, and Wurster (1969) reported finding very high levels of DDT and metabolites in detritus particles from estuaries. They concluded that detritus with its associated microorganisms act as a reservoir for DDT and metabolites.

Bottom-feeding fish such as catfish have higher levels of dieldrin, than do pan and game fish such as bass and crappie. This is attributed to the deposition of dieldrin-contaminated silt as sediment and differences in feeding habits (Morris and Johnson, 1971).

Summarizing, Chester and Konrad (1971) noted that ". . . most investigations of the fate of pesticides added directly to aquatic systems have shown that decrease in pesticide concentrations in the water with time are accompanied by concomitant increases in the concentration of pesticides found in bottom sediments and/or aquatic organisms."

Aquatic organisms frequently have been used for pesticide monitoring. Moore (1966) clearly outlines the criteria for choice of a monitoring species: (1) abundant and easy to collect, (2) large enough to provide an adequate tissue sample, (3) easy to age, (4) sedentary, if conducting a local change study, and (5) high pesticide accumulation ability. Evaluating lamellibranch mollusks, Moore (1966) concluded that they are not suitable monitoring organisms in Great Britain because they fail to accumulate high contaminant levels.

Numerous studies in North America indicate that clams and oysters are suitable monitoring organisms for pesticides. Butler (1969b) reports oysters are able to concentrate DDT up to 70,000 times ambient level.

West (1966) reports similar concentrating abilities for oysters, finding that after 7 days in 10 ppb DDT ambient, oysters contained 151 ppm.

Butler (1966a) further notes that oysters' metabolic activity is relatively uninfluenced by age, sex or size. He further notes that oysters remain physiologically active, pumping in excess of 20 hours a day, until water temperature approaches the freezing point.

Oysters are also attributed with the ability to cleanse themselves of pesticides. Butler (1966b) reports that oysters having a body burden of 150 ppm DDT may lose 67 percent within 50 days and may reach a level of 6 ppm in another 40 days, after being placed in pesticide-free water. Butler (1966c) concludes that this ability contributes to the oysters overall suitability as a bioassay organism.

In 1965, the first federal oyster monitoring program for chlorinated pesticides in the estuarine environment was initiated. Evaluating the results, Casper, Hammerstrom, and Robertson (1969) report that shellfish monitoring in the estuarine environment is important not only for detecting low level contaminants, but also for detecting "slugs" of pesticides resulting from agricultural misuse or industrial accidents. They conclude ". . . in many situations the most reliable and economical pesticide monitoring program would be the suspension of oysters, clams, or mussels at a convenient sampling station, together with twice-a-month sampling and analysis of the suspended shellfish."

Bedford, Roelofs, and Zabik (1968) found that although lacking the tremendous concentrating abilities exhibited by oysters, freshwater clams were suitable as pesticide monitoring organisms. Their study revealed that freshwater clams were able to reflect low level contaminants as well as a "slug" of aldrin which passed through the monitoring stations.

In a later study, Bedford (1970), examined the organochlorine insecticide concentrating and cleansing abilities of freshwater clams. He found that clams in lake water were able to concentrate DDT 2400 fold and aldrin 1200 fold. By varying conditions, he also determined that water quality and temperature affected pesticide uptake and release.

Fikes and Tubb (1971) reported that the freshwater clam Amblema plicata concentrated dieldrin about 1000 fold at 20 ppb ambient level and 2500 fold at 0.02 ppb ambient level. A logarithmic decline in dieldrin levels was noted in the tissues when ambient levels declined.

In a non-monitoring study, Starrett (1971) reported that clams collected from the Illinois River and analyzed contained DDE, heptachlor epoxide, dieldrin, and DDT.

Presumably due to the attributes of shellfish as monitoring organisms, several subsequent monitoring programs have been conducted. Oysters were one of several types of organisms used by Novak and Rae (1965) in monitoring endrin levels in the Mississippi River.

Butler (1966a) studied fixation of DDT in estuaries by monitoring plankton and oysters and concluded that plankton plays a major role in introducing pesticides into the estuarine food web. The pesticides are concentrated by filter-feeding animals which later release the pesticides, making them available to detritus-feeders. Butler and Springer (1963), noting the ability of oysters to concentrate and fix pesticides in their fecal deposits, expressed concern about its possible role in the food web.

A 2-year study of the pesticide levels in shellfish of the Louisiana estuarine areas revealed median levels of 0.04 ppm of DDT and metabolites and lesser levels of dieldrin, endrin, heptachlor, heptachlor

epoxide and methoxychlor (Hammerstrom et al., 1967). Rowe, Canter, Synder and Mason (1971) conducted a subsequent study and reported dieldrin and endrin levels of similar magnitude.

Other studies conducted in the estuarine and bay environments include pesticide levels in the Eastern Oyster, Crassostrea virginica, from the Gulf of Mexico and South Atlantic (Bugg, Higgins, and Robertson, 1967); dieldrin, endrin and DDT levels in oysters, mussels and clams in California bays and estuaries (Modin, 1969); DDT levels in Canadian Atlantic shellfish and fishes (Sprague and Duffy, 1971); and dieldrin, DDT and metabolites in oysters, mussels, and clams in Long Island estuaries (Foehrenbach, Mahmood, and Sullivan, 1971).

Casper (1967), using oysters, and Smith and Isom (1967) and Fredeen and Duffy (1970), using clams, have monitored the application and uptake of pesticides. The work of Smith and Isom indicated that mussels were able to concentrate significant levels of 2,4-D, a herbicide, within 96 hours after its application to the ecosystem to control watermill.

Godsil and Johnson (1968) reported on an extensive 2-year monitoring program conducted by the Federal Water Pollution Control Administration at Tule Lake National Wildlife Refuge in California. Using samples of water, aquatic plants, clams, and fish, it was established that the occurrence of endrin was directly associated with contaminated irrigation water entering the lakes.

Butler (1969a) summarizes the results of several studies in which shellfish were utilized. He reports that monitoring river basins which drain agricultural areas reveal seasonal spring and fall peak pesticide levels. Referring to another study, he notes that oysters, properly placed and periodically monitored, have been used to locate a particular pesticide pollution source.

With the exception of the research of Bedford, Roelofs, and Zabik (1968), mentioned earlier, those studies utilizing shellfish as monitoring organisms have dealt largely with estuarine and bay environments or with major river basins. Knowledge of insecticide levels in small streams draining agricultural land have been either inferred by monitoring the major river basin or by monitoring stream water and sediment samples. It is hoped that the following study may provide some insight into the residual levels of pesticides in these streams.

MATERIALS AND METHODS

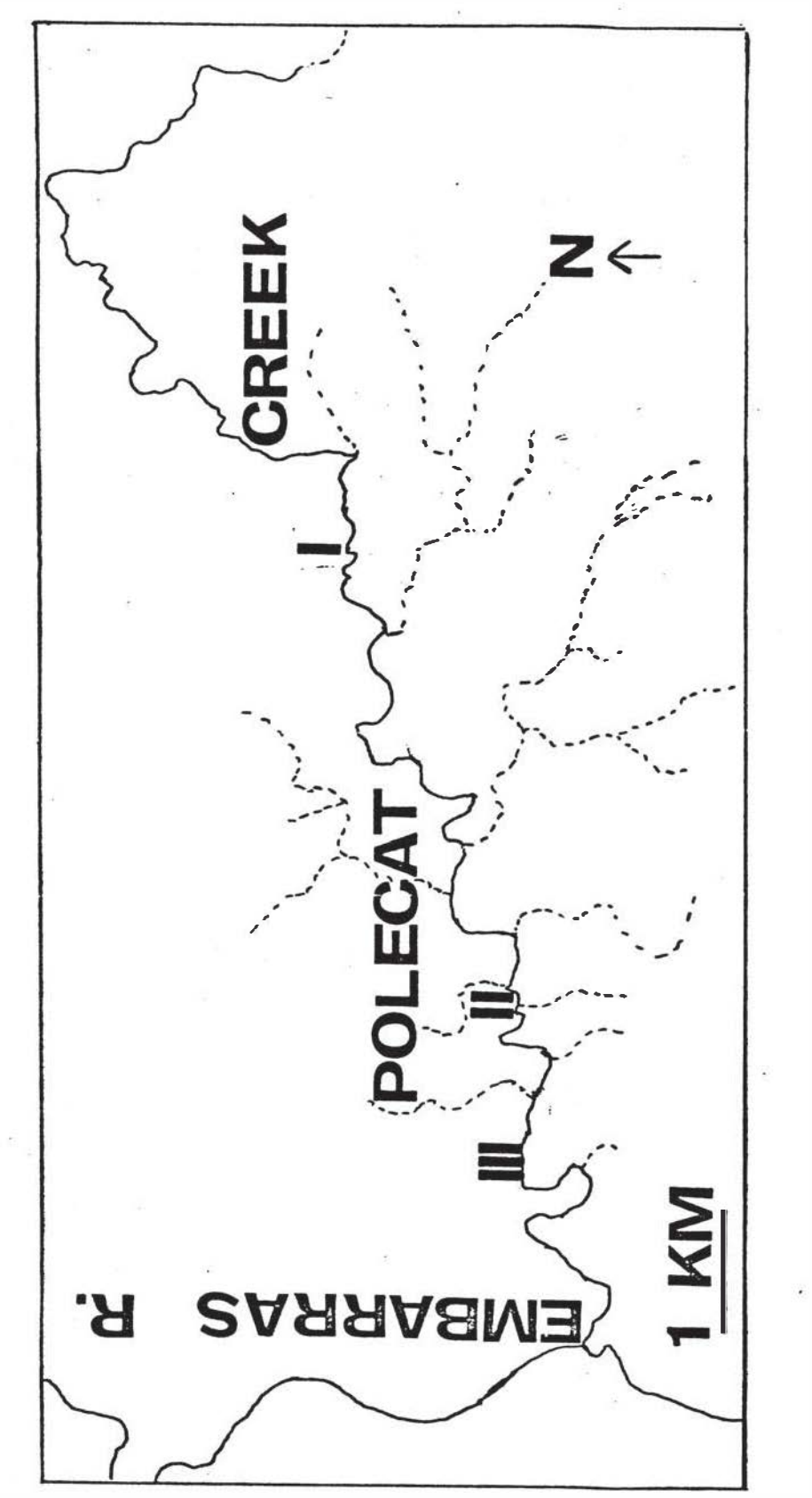
On July 28, 1972, clam retaining pens were established at 3 locations along Polecat Creek, Coles County Illinois (Figure 1). Stations I, II, and III were located in the northeast quadrant of Section 1, the southwest quadrant of Section 3, and the northeast quadrant of Section 9, respectively, in Range 10E of Township T12N. Each pen consisted of 6 meters of 20 cm. wide polyethylene plastic strips formed into a triangle. The bottom edge was buried into the sand and gravel substrate to an approximate depth of 5 cm. At Stations I and III the pens were held in place by 3 stakes driven into the stream bottom. The pen at Station II was held by stacked rocks inasmuch as a layer of sandstone beneath the substrate surface prevented the use of stakes.

On July 29th, 90 Amblema plicata were collected from Polecat Creek, in the immediate vicinity of Station I, and 25 were placed in each of the pens. The remaining 15 clams were "controls" for establishing initial insecticide and lipid levels.

Biweekly collections of 3 clams from each of the pens were conducted until October 22, 1971, when it was observed that the clams at Station III had burrowed into the substrate and ceased to feed. All collected clams were transported in stream water to the laboratory. The clam tissue was immediately removed from the shell, drained, weighed, double-wrapped in aluminum foil, placed in a plastic bag, and frozen.

Turbidity, pH, temperature, dissolved oxygen (D.O.) and biochemical oxygen demand (B.O.D.) were determined weekly at each of the

FIG. 1--Map of Polecat Creek, Coles County Illinois, with pesticide monitoring station locations.


CREEK
I
N
POLECAT
II
III
EMBARRAS R.
1 KM

stations during the monitoring period. Turbidity was determined by using a Hach D.R. Colorimeter, and pH by using a Sargent pH Meter. Two water samples per station per week were collected in a 1000 ml aspirator bottle and transferred to dissolved oxygen bottles. Dissolved oxygen was determined in the first bottle by using the Winkler method (Welch, 1948). The second water sample was incubated in the dark at 20 ± 1°C for 5 days and analyzed by the Winkler method; this second D.O. value, subtracted from the first D.O. value, provided the B.O.D. level.

In order to evaluate the feasibility of using a one-step pesticide extraction and clean-up technique, 4 clams were analyzed for lipid levels. A 10-gram collection of randomized tissue from a clam was ground with anhydrous sodium sulphate in a mortar until a free-flowing powder resulted. This powder was transferred to 10, 10 ml centrifuge tubes, and made to volume with petroleum ether (BP 30-60°C). The tubes were shaken for 1 minute and centrifuged for 5 minutes at 1500 rpm. The solvent layer was decanted into a tared 250 ml Erlenmeyer flask. The remaining sediment was re-extracted twice, with solvent layers being decanted into the tared flask. The solvent (approximately 150 ml) was evaporated to dryness in a hot water bath and the weight of lipid residue was established.

A modification of a one-step extraction and clean-up procedure reported by Langlois, Stemp, and Liska (1964), was adoped for use. "Nanograde" petroleum ether, dichloromethane (methylene chloride), and benzene, manufactured by Mallinckrodt, were used. Activated 60/100 mesh "Florisil", manufactured by Floridin and used as an absorbent for water and lipids, was reactivated at 140°C for 12 to 14 hours. Upon

cooling, 5 percent water (volume/weight) was added. The water-Florisil mixture was stored at room temperature for 48 hours prior to use.

Kontes Chromaflex columns (size 224, with teflon stopcocks and fritted plates) were used. A glass wool plug (1 cm. high) was firmly tapped against the fritted plate, 25 grams of Florisil were added to the column and washed with 50 ml of 50/50 (volume/volume) dichloromethane/petroleum ether.

The wet weight of Amblema plicata used ranged from 28.58 to 99.44 grams (mean 62.0 ± 17.0 grams). The frozen tissue was finely chopped and mixed before thawing. A 10-gram portion (containing less than 1 gram of fat) was ground to a powder with approximately 25 grams of Florisil in a mortar. The powder was added to the prepared column and a separatory funnel containing 600 ml of 20/80 (volume/volume) dichloromethane/petroleum ether was connected to the top of the column (Figure 2). The flow rate of the solvent leaving the column was adjusted to approximate 20 drops per 10 seconds. The flow entering the column was adjusted accordingly to provide a solvent reservoir of 1 to 2 cm. over the surface of the sample. The eluant passing out of the column was collected in a 1000 ml Erlenmeyer flask.

The eluant was concentrated in a 1000 ml Kuderna-Danish evaporative concentrator containing several boiling chips in the graduated receiver. The 3-ball Synder column of the concentrator was pre-wet with several ml of petroleum ether and wrapped in asbestos and aluminum foil to enhance evaporation (Figure 3).

When the solvent was reduced to 10 ml, the collecting tube was removed and the volume was further reduced to less than 5 ml by application of dry nitrogen. It was then transferred to a 5 ml "Mini Vial"

FIG. 2--Prepared Chromaflex columns for pesticide extractions (in progress), containing Florisil and Florisil/sample mixtures with attached separatory funnels containing 20/80 dichloromethane/petroleum ether. Erlenmeyer flasks receive eluants containing pesticide residues.

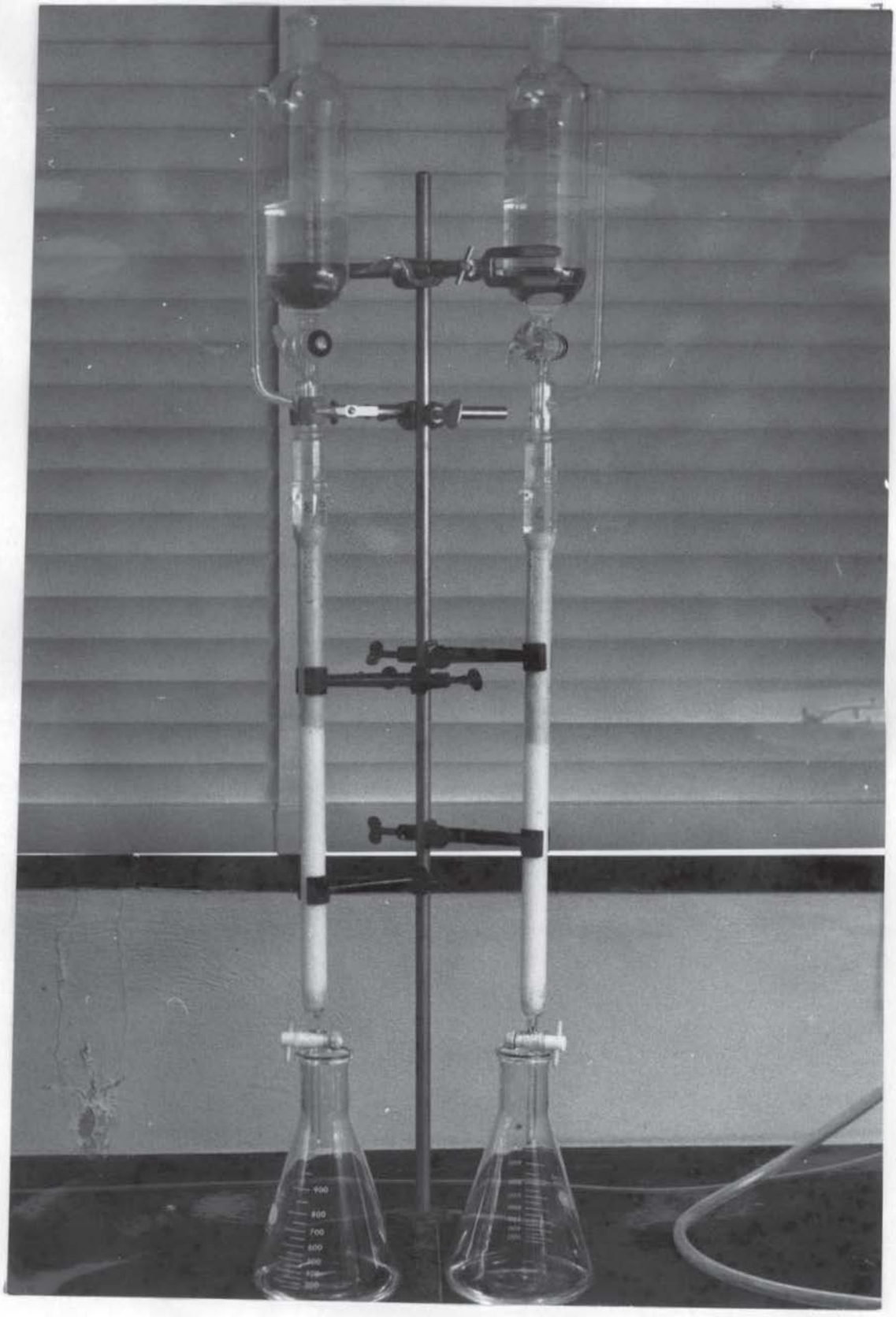


FIG. 3--Kuderna-Danish evaporative concentrators (in a ventilation hood). Note that the 3-ball Synder columns are wrapped in asbestos and aluminum foil to enhance evaporation.

(distributed by Altech Associates) and evaporated to dryness using dry nitrogen. The residue was re-dissolved in 1 ml of nanograde benzene, and frozen until chromatographic analysis.

All glassware was washed in "Chromowash" (a saturated solution of potassium dichromate in concentrated sulphuric acid) and rinsed repeatedly in distilled water. Immediately prior to use, the glassware was rinsed in nanograde 50/50 dichloromethane/petroleum ether. Teflon stopcocks and mini vial lids were not washed in Chromowash.

Forty-four samples (2 samples per station per biweekly sample and 8 controls) were analyzed with a Varian Aerograph 200 dual column gas chromatograph equipped with an electron capture detector. All samples were analyzed on a 3 percent QF-1 column with 23 confirmatory analyses conducted on a 5 percent DC-11 column (Table 1).

In addition to the above analysis, the first series of samples analyzed on October 28, 1971, were also examined on a Varian Aerograph 1200 gas chromatograph equipped with an electron capture detector and a 5 percent SE-30 column (Table 1). This was done to insure adequate tissue clean-up prior to analysis using the more sensitive QF-1 and DC-11 columns.

Quantitative/qualitative standards for aldrin, dieldrin, and p,p'DDE (Appendix 1) were injected before each sample series, after each fifth sample, and at the conclusion of each series. The standards were made by adding 50 microliters of 1000 ± 5 ppm (parts per million) aldrin to each of 2, 50 ml volumetric flasks, and 100 microliters of 1000 ± 5 ppm of dieldrin to 1 flask and an equal amount and concentration of p,p'DDE to the other. Contents of each flask were raised to volume with nanograde benzene, resulting in 2 standards--the first

TABLE 1.--Operating conditions of electron capture gas chromatographs used in the analysis of organochlorine pesticides in Amblema plicata.

Chromatograph	Varian Aerograph 1200	Varian Aerograph 200	Varian Aerograph 200
Column size	6 ft. long 1/8 in. I.D.	6 ft. long 1/8 in. I.D.	6 ft. long 1/8 in. I.D.
Liquid phase	5% SE-30	3% QF-1	5% DC-11
Solid support	80/100 mesh Chromosorb W AW-DMCS*	80/100 mesh Chromosorb W AW-DMCS	80/100 mesh Chromosorb W AW-DMCS
Oven temperature	185°C	185°C	185°C
Detector temperature	214°C	225°C	195°C
Injector temperature	-----	195°C	195°C
Carrier gas	N_2	N_2	N_2

*AW-DMCS is acid-washed and treated with dimethyldichlorosilane.

containing 1 ppm aldrin and 2 ppm dieldrin and the second containing 1 ppm aldrin and 2 ppm p,p'DDE. In addition, 3 sets of qualitative standards, provided by Argonne National Laboratory, were also injected during each series of analyses (Table 2).

Qualitative analyses were conducted by comparing the retention times of the standards to the unknown samples. Relative retention times were also compared using aldrin as the internal standard.

Confirmation of the presence of aldrin, dieldrin and p,p'DDE was made periodically by adding 0.5 microliters of the appropriate standard to 1.5 microliters of the unknown sample in a microsyringe. These "spiked" samples were then injected into the chromatograph and the results were compared with "unspiked" samples (Figure 4).

Quantitative analysis was determined by the peak area response of the chromatograph, using the QF-1 column. Quantitation was based upon the relative response of the detector to the sample of unknown concentration and to the standard. The peak area was determined by a disc integrator which recorded the response.

These data were converted to concentration (ppm) with the following formula, modified from Warnick and Gaufin (1965):

$$PPM = \frac{Vwd_2}{Wvd_1} \quad \text{when,}$$

W = Sample weight in grams.

V = Volume of extract in milliliters.

w = Weight of pesticide in sample standard injected (in nanograms).

v = Volume of extract injected in microliters.

d_1 = Recorder response multiplied by attenuation factor for "w".

d_2 = Recorder response multiplied by attenuation factor for "v".

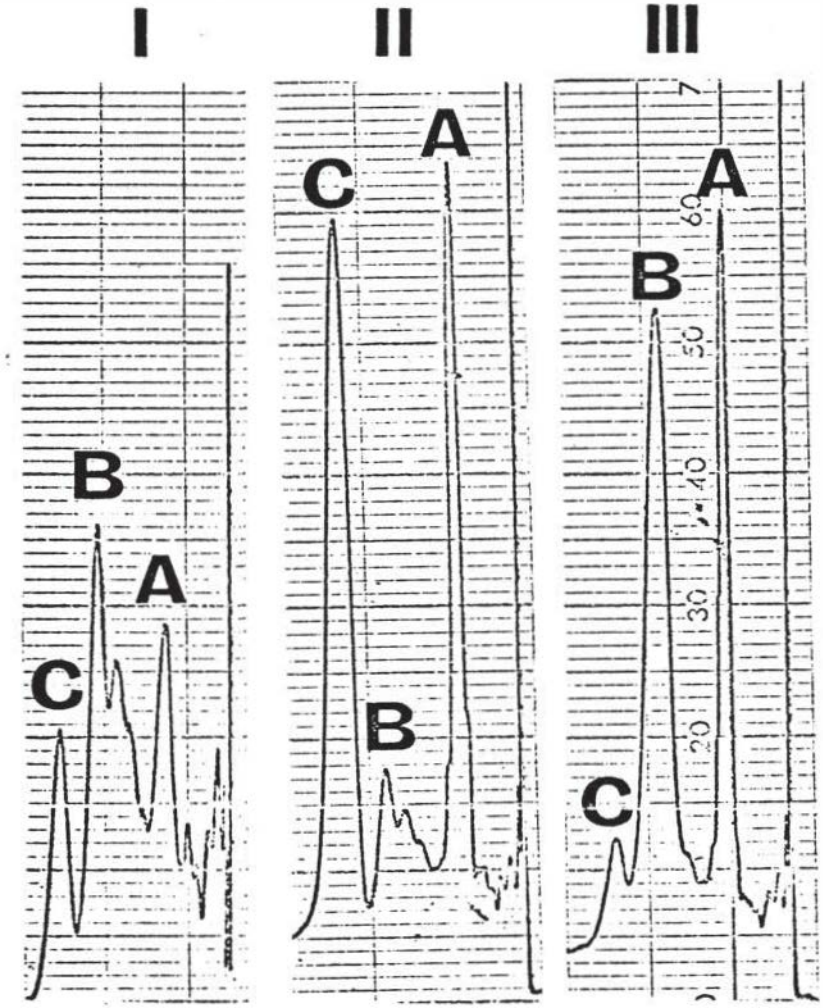
TABLE 2.--Composition of qualitative organochlorine pesticide standards utilized for identification of samples from Amblema plicata.

Standard mix 1	Standard mix 2	Standard mix 3
1[a], BHC[b]	1, Lindane	1, Heptachlor
1, Heptachlor	1, Aldrin	1, Aldrin
1, Aldrin	2, p,p'DDE	1, Heptachlor epoxide
1, Heptachlor epoxide	2, Dieldrin	2, p,p'DDT
2, Dieldrin	2, p,p'DDT	
2, p,p'DDT		

[a]Concentration in ppm.

[b]Appendix 1 for chemical names.

FIG. 4--Verification of the presence of aldrin (A), p,p'DDE (B), and dieldrin (C) in a sample from Amblema plicata (I) analyzed by electron capture gas chromatography. The addition of aldrin and dieldrin standards (II) and aldrin and p,p'DDE standards (III) to the original sample confirm the presence of aldrin, p,p'DDE, and dieldrin by increasing the respective peak heights and area.


I
II
III
A
B
C
A
B
C
A
B
C
60
50
40
30
20

Percent recovery of aldrin, dieldrin and p,p'DDE was determined by analyzing 2, 10-gram tissue samples each of 3 clams. One sample from each clam was extracted and analyzed in normal manner. The second sample from each clam was "spiked" with 1 ml of benzene containing 1 ppm each of aldrin, dieldrin, and p,p'DDE, prior to being ground with Florisil. The percent recovery was calculated by subtracting the insecticide levels of the "unspiked" samples from the corresponding "spiked" samples. The remaining values were then calculated as a percentage of the initial "spiked" levels. Control and monitoring sample levels were not adjusted for percent recovery.

RESULTS

The lipid levels analysis of the clam tissue revealed a mean value of 0.31 ± 0.18 percent with minimal differences between replicate values (Table 3). This was sufficiently low, allowing the use of the Langlois, Stemp, and Liska (1964) extraction method with an adequate tissue sample size.

Qualitative analysis of the chromatograms revealed the presence of aldrin, dieldrin and p,p'DDE in all samples analyzed. The presence of heptachlor was indicated by using the DC-11 column, and heptachlor epoxide by using the QF-1 column, but confirmation could not be established using the reciprocal columns. The SE-30 column indicated the presence of both in the initial 5 controls examined.

Analysis of aldrin, dieldrin, and p,p'DDE in the controls revealed mean levels of 0.0489, 0.0592, and 0.0464 ppm, respectively (Table 4). Percent recovery was highest for dieldrin and lowest for aldrin (Table 5). The biweekly mean levels of aldrin, dieldrin and p,p'DDE encountered in the monitored samples are given in Tables 6 through 8.

A two-factor analysis of variance was conducted for aldrin, dieldrin and p,p'DDE levels to determine the significance of the observed differences. There was no significant difference in the aldrin levels (Table 9). The dieldrin levels were significantly different ($p = 0.05$) both as a factor of time and location (Table 10). The observed differences of p,p'DDE were not significant in regards to location but were significant as a function of time (Table 11).

Duncan's multiple range test showed that most of the difference in the dieldrin levels between stations was attributable to the higher levels at Station II. It also revealed that the sampling periods in August were responsible for most of the observed differences in dieldrin (Table 10) and p,p'DDE (Table 11).

Determinations using a correlation coefficient indicate that there is no significant correlation ($p = 0.05$) between the wet weight of the controls and their levels of insecticides (Table 12).

The results of the environmental analysis at the stations are given in Table 13. The stations appear to be quite similar except for higher B.O.D. and turbidity levels encountered at Station I.

TABLE 3.--Lipid levels (percent) of 10 gram tissue samples of Amblema plicata.

Wet weight of clam (grams)	Replicate A	Replicate B
40.50	0.15%	0.15%
82.92	0.40%	0.58%
64.16	0.33%	0.44%
87.26	0.23%	0.20%
Mean	0.31 ± 0.18%	

TABLE 4.--Levels (ppm) of organochlorine insecticides in the tissues of 8 Amblema plicata controls from Polecat Creek.

Insecticide	Mean	Standard error	Confidence interval
Aldrin	0.0489 ± 0.0365	0.0129	0.0307
Dieldrin	0.0592 ± 0.0231	0.0082	0.0194
p,p'DDE	0.0464 ± 0.0186	0.0066	0.0156

TABLE 5.--Percent recoveries of aldrin, dieldrin and p,p'DDE from Amblema plicata, using a 1-step extraction and clean-up procedure of Langlois, Stemp, and Liska (1964).

Insecticide	Sample A	Sample B	Sample C
Aldrin	60%	61%	100%
Dieldrin	103%	139%	133%
p,p'DDE	63%	77%	128%

TABLE 6.--Mean (n= 2) aldrin levels (ppm) in Amblema plicata collected from Polecat Creek.

Collection Date	Station I	Station II	Station III
08/13/71	0.0826	0.0523	0.0358
08/27/71	0.0488	0.0272	0.0452
09/10/71	0.0195	0.0287	0.0312
09/24/71	0.0295	0.0309	0.0281
10/08/71	0.0390	0.0303	0.0299
10/22/71	0.0394	0.0225	0.0233

TABLE 7.--Mean (n= 2) dieldrin levels (ppm) in Amblema plicata collected from Polecat Creek.

Collection Date	Station I	Station II	Station III
08/13/71	0.0607	0.0862	0.0526
08/27/71	0.0707	0.0944	0.0727
09/10/71	0.0250	0.0377	0.0334
09/24/71	0.0428	0.0407	0.0334
10/08/71	0.0285	0.0348	0.0376
10/22/71	0.0301	0.0359	0.0364

TABLE 8.--Mean (n= 2) p,p'DDE levels (ppm) in Amblema plicata collected from Polecat Creek.

Collection Date	Station I	Station II	Station III
08/13/71	0.0479	0.0577	0.0527
08/27/71	0.0508	0.0800	0.0524
09/10/71	0.0380	0.0402	0.0416
09/24/71	0.0362	0.0405	0.0341
10/08/71	0.0345	0.0359	0.0390
10/22/71	0.0305	0.0324	0.0330

TABLE 9.--Results of an analysis of variance test for the significance of observed differences of aldrin in Amblema plicata with respect to length of time and location in Polecat Creek.

Source	Sum of Squares	Degrees Freedom	Mean Squares	F Exp.	F 0.95
Location	0.0006	2	0.00030	2.1	4.1
Time	0.0020	5	0.00040	2.9	3.3
Residual	0.0014	10	0.00014		
Total	0.0040	17			

Note:

$$F \text{ (variance ratio)} = \frac{\text{between-treatment mean square}}{\text{residual mean square}}$$

TABLE 10.--Results of an analysis of variance and Duncan's multiple range tests for the significance of observed differences of dieldrin in *Amblema plicata* with respect to length of time and location in Polecat Creek.

Source	Sum of Squares	Degrees Freedom	Mean Squares	F Exp.	F 0.95	F 0.99
Location	0.0006	2	0.00030	7.5	4.1	7.6
Time	0.0063	5	0.00126	31.5	3.3	5.6
Residual	0.0004	10	0.00004			
Total	0.0073	17				

Note:

Station	I	III	II
Means*	0.0430	0.0444	0.0550

Weeks	09/10	10/08	10/22	09/24	08/13	08/27
Means*	0.0321	0.0336	0.0341	0.0390	0.0665	0.0793

*Means not underlined by common double line are significantly different ($p = 0.05$).

$$F \text{ (variance ratio)} = \frac{\text{between-treatment mean square}}{\text{residual mean square}}$$

TABLE 11.--Results of an analysis of variance and Duncan's multiple range tests for the significance of observed differences of p,p'DDE in Amblema plicata with respect to length of time and location in Polecat Creek.

Source	Sum of Squares	Degrees Freedom	Mean Squares	F Exp.	F 0.95	F 0.99
Location	0.0003	2	0.00015	3.8	4.1	----
Time	0.0019	5	0.00038	9.5	3.3	5.6
Residual	0.0004	10	0.00004			
Total	0.0026	17				

Note:

Time	10/22	10/08	09/24	09/10	08/13	08/27
Means*	0.0320	0.0365	0.0369	0.0399	0.0528	0.0611

*Means not underlined by common double line are significantly different (p= 0.05).

$$F \text{ (variance ratio)} = \frac{\text{between-treatment mean square}}{\text{residual mean square}}$$

TABLE 12.--Coefficient of correlation analysis results of wet weight and insecticide levels of 8 Amblema plicata controls from Polecat Creek.

Insecticide	Correlation Coefficient Exp.	Correlation Coefficient p= 0.05
Aldrin	-0.498	±0.666
Dieldrin	-0.460	±0.666
p,p'DDE	-0.476	±0.666

TABLE 13.--Mean values of weekly monitored environmental conditions at three pesticide monitoring stations on Polecat Creek, Coles County Illinois.

Time	07/21/71 - 08/27/71 (N= 5)			09/03/71 - 09/24/71 (N= 4)			10/01/71 - 10/22/71 (N= 4)		
Analysis Station	I	II	III	I	II	III	I	II	III
Temperature (C.)	22.0±4.0	21.0±3.0	21.0±3.0	21.0±5.0	18.0±5.0	19.0± 6.0	17.0±3.0	16.0±4.0	16.0±4.0
Dissolved Oxygen (ppm)	6.8±1.3	7.6±0.8	7.7±0.8	5.9±1.0	6.5±1.5	7.0± 0.3	5.2±0.4	5.9±1.2	6.1±1.1
Biochemical Oxygen Demand (ppm)	3.7±1.5	1.4±1.1	1.2±0.6	3.5±1.9	0.9±0.6	0.6± 0.2	3.0±1.3	1.3±0.8	0.8±0.5
Turbidity (J.T.U.)	25.0±4.0	15.0±5.0	12.0±5.0	26.0± 3.0	18.0±8.0	19.0±11.0	13.0±6.0	9.0±5.0	9.0±5.0
pH	7.4±0.2	7.7±0.1	7.8±0.1	7.5±0.1	7.7±0.2	7.7± 0.1	7.5±0.1	7.6±0.1	7.6±0.1

DISCUSSION AND CONCLUSIONS

The low fat levels in Amblema plicata facilitated the analysis of a large (10 gram) tissue sample without over-loading the column. The mean value of 0.31 percent fat (wet weight) is somewhat lower than reported by Bedford (1970) for 3 other genera of freshwater clams.

Aldrin and dieldrin in the tissues probably reflect the use of the former as a corn insecticide. Aldrin is used to control rootworm, wireworm and other soil insects (Sparr, et al., 1966). Decker, Bruce, and Bigger (1965) report that Illinois farmers treat 3 to 4 million acres (1/3 to 1/2 of the total corn acreage) with aldrin each year.

After application, the insecticide can be transported in runoff water, tightly bound to particulate matter, to streams (Chesters and Konrad, 1971). Here or while on the soil, aldrin can undergo epoxidation to form dieldrin (Figure 5) (Decker, Bruce, and Bigger, 1965, Johnson et al., 1971). Morris and Johnson (1971), monitoring Iowa streams, found dieldrin to be the primary insecticide in fish and attributed its presence to the agricultural use of aldrin. Dieldrin is the most common insecticide contaminant in U.S. surface waters (Lichtenberg, et al., 1970).

The presence of p,p'DDE, a primary metabolite of DDT (Figure 6), without the presence of DDT in traceable amounts, may indicate that the observed levels reflect long-term residues. DDT was widely used as a corn insecticide for approximately 15 years but it has been largely discontinued in the last 5 years (Johnson and Morris, 1971).

The persistence of DDT's metabolites in soil has been shown by Terriere, Kiigemargi, Zwick, and Westigard (1966), who found that 40 percent of the applied DDT to orchards since 1946 remained as analogs and metabolites in the top 12 inches of soil, 20 years later. Therefore, the presence of p,p'DDE may reflect residues from DDT application several years previously.

Another interpretation is possible. The ratio of a parent insecticide to its metabolite in an organism may reflect the biological pathway by which the insecticide was incorporated. A large amount of the parent compound, e.g. DDT, may suggest direct absorption by the organism and therefore a rather quick accumulation (Gaufin, 1967, Butler, 1969). A relatively high level of its metabolite, e.g. p,p'DDE, may suggest accumulation by way of the food web (Butler, 1969).

Recently, Johnson, et al. (1971) found that aquatic invertebrates can rapidly metabolize significant amounts of DDT and aldrin to DDE and dieldrin, respectively, and pass both parent and metabolite on via the food web. The food web is therefore important in concentrating both the parent compound and its metabolites.

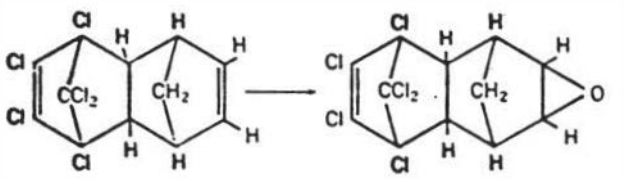
However, this alternative is compatible with the earlier suggestion that the major influx of DDT into Polecat Creek was not recent. Accumulation by either method, following a recent application of DDT would result in at least trace amounts of DDT.

The tentative identification of heptachlor and its metabolite, heptachlor epoxide (Figure 7) may have an agricultural source since heptachlor still receives limited use as a corn insecticide (Johnson and Morris, 1971).

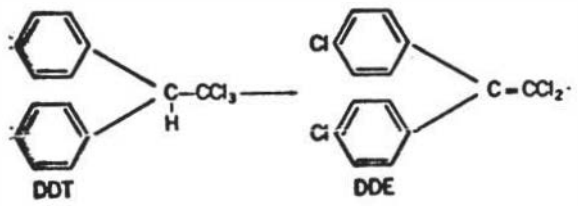
FIG. 5--Epoxidation of aldrin to dieldrin (from Van Middelem, 1966).

FIG. 6--Dehydrochlorination of DDT to DDE (modified from Van Middelem, 1966).

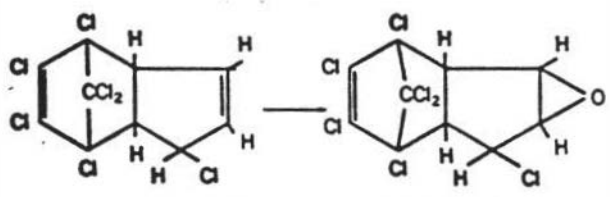
FIG. 7--Epoxidation of heptachlor to heptachlor epoxide (from Van Middelem, 1966).



Aldrin Dieldrin



DDT DDE



Heptachlor Heptachlor Epoxide

There were no significant differences in the levels of aldrin or p,p'DDE between the 3 monitoring stations. Dieldrin varied significantly ($p = 0.05$) only at Station II (mean 0.0550 ppm) which had considerably higher levels than Stations I (0.0430 ppm) and III (0.0444 ppm). This indicates that Polecat Creek may not exhibit a gradual increase in insecticide level in a downstream direction, as has been reported in larger rivers receiving agricultural runoff from tributaries (Morris and Johnson, 1971).

Both dieldrin and p,p'DDE exhibited significantly higher ($p = 0.05$) concentrations in the 2 August samples (Tables 10 and 11). The control mean values of dieldrin (0.0592 ppm) and p,p'DDE (0.0464 ppm), collected on July 29th (Table 4), were lower than either of the August levels, but higher than the September and October levels (Tables 10 and 11). This suggests an increase in insecticide levels was in process in July which peaked in August and declined in September. Dieldrin in Iowa rivers was found to peak in July and decline in August (Johnson and Morris, 1971). This seasonal peak, along with the increased toxicity of insecticides in warmer waters (Johnson, 1968), probably accounts for the highest incidence of pesticide-caused fish kills occurring in July and August, as reported by Feltz, Sayers, and Nicholson (1971).

The variation of p,p'DDE levels suggests that residues do not remain constant and passive in the tissues. Perhaps the fluctuation of levels in the tissues reflects an influx of the metabolite from the soil into the stream or a redistribution of that already present in the stream. Bottom sediments as well as living organisms can act as insecticide reservoirs (Cope, 1966).

It is surprising that aldrin levels failed to exhibit a significant difference ($p = 0.05$) in response to time and location in Polecat Creek. Since it is easily converted to dieldrin on soil (Decker, Bruce, and Bigger, 1965, Sparr, et al., 1966), as well as water (Johnson, et al., 1971), one would expect aldrin to exhibit a variety of levels attributable to time and location. Instead, levels fluctuated greatly but without any recognizable trend. The extent of variation is evident in the controls (Table 4).

The relative level of aldrin to dieldrin is considerably higher than has been generally reported. Morris and Johnson (1971) reported that the ratio of dieldrin to aldrin was about 10 to 1 in fish, even though the primary source of contaminant was the agricultural use of aldrin. Sparr, et al. (1966) concluded that the soil application of aldrin results in essentially dieldrin residues in water runoff.

Decker, Bruce, and Bigger (1965) found that in a variety of Illinois soils, the epoxidation of aldrin to dieldrin begins soon after its application and in 90 days, approximately 1/2 of the soil residue is dieldrin, reaching its peak level within 60 to 120 days after application. After 1 year, the aldrin soil level was 0.015 ppm and dieldrin levels were from 0.074 to 0.106 ppm, with the total residue representing 10 to 15 percent of the amount applied. Finally they noted that aldrin required 4 years to be eliminated from the soil.

Assuming this to approximate the fate of aldrin applications in the Polecat drainage area, it is not surprising that significant aldrin levels were detected. This is especially true in that annual application of aldrin in the recommended dosage for a period of 10 years may lead to a residual level of 91 percent of the annual application rate

(Decker, Bruce, and Bigger, 1965). The Polecat drainage area consists primarily of Fincastle-Xenia and Strawn-Lawson soil associations (Hallbick, 1968).

In a study of the clams in the Illinois River, Starrett (1971) analyzed organochlorine insecticide levels in 14 clams, comprising 7 species including Amblema plicata. He found that 9 had measurable amounts of dieldrin (mean 0.0072 ppm), all had DDE (mean 0.0132 ppm), 13 had heptachlor epoxide (mean 0.0126 ppm), 2 had DDT (mean 0.0253 ppm), but none had detectable levels of aldrin. These insecticide levels in the Illinois River were very low, much lower than the respective levels detected in the present study. Starrett (1971) suggested that the low levels might have been due to the pesticides having been absorbed in the bottom muds of the tributaries. This may in fact be the case. Bedford, Roelofs, and Zabik (1968) observed that the bottom muds contained higher levels of DDT and its metabolites than did clams in the same area.

Although sufficient data are lacking,it is possible that the most severe insecticide contamination occurs in the small tributaries receiving the immediate runoff, where much of the contaminant is rapidly fixed by biotic and abiotic material. The larger river basins would receive only a portion of the total contaminant.

The extent to which insecticides are held by tributaries appears to be dependent upon several factors. The initial dosage, the manner by which it enters the stream, and its water solubility are important. The flow rate of the stream, not only as a matter of time exposure to the absorptive factors of the stream, but also as a factor of the ability of the stream to transport suspended silt, is important. In a rapid-

flowing stream, both abiotic and biotic matter have less exposure to a particular volume of contaminated water. Also a rapid flow tends to keep contaminated erosion silt in suspension, until it reaches the larger, slower river where it may settle out.

Morris and Johnson (1971) noted that dieldrin content in catfish increased downstream in larger rivers and attributed it to accumulation of rowcrop silt from smaller tributaries. They further noted that the impounding of streams promotes a buildup of dieldrin in bottom-feeding organisms due to contaminated silt deposition.

Polecat Creek transports relatively little silt in the monitoring areas, as reflected by the low turbidity readings (Table 13) and the only infrequent silty bottom sediments encountered.

Since pesticides can be accumulated through the food web and by direct absorption through gills (Fromm and Hill, 1969, Chadwick and Brocksen, 1969) and other body surfaces (Johnson, et al., 1971), it is logical to question which, or to what extent each, is reflected in the observed levels. The ratio of parent compound to metabolite has been suggested as an indicator, as discussed earlier.

However, Hamelink, Waybrant, and Ball (1971) have suggested that the observed insecticide body burden is the result of an exchange equilibrium between body lipids and ambient insecticide levels. Whether the compound is ingested or absorbed, the retained level is dependent upon absorption and solubility differences. Presumably in clams, the level is controlled by exchange of insecticide between ambient water and cellular lipids by way of the gills and blood, dependent upon the relative solubility of the insecticide in water and in lipids.

Support for this exchange equilibrium mechanism in freshwater clams can be found in the work of Bedford (1970), concerning the uptake and elimination of DDT and dieldrin by Elliptio dilatatus, Anodonta grandis and Lampsilis siliquoidea. He noted that clams concentrated DDT and dieldrin in proportion to ambient insecticide levels. He further noted that when transferred from contaminated water to pesticide-free water, the clams cleansed themselves at a uniform rate dependent upon the tested pesticide. The cleansing half-life of DDT in lake water was 13.6 days whereas dieldrin was 4.7 days. The clams concentrated DDT in lake water 2400 fold and dieldrin 1200 fold when exposed to levels between 0.05 and 1.0 ppb. Since the water solubility of DDT is 1.2 ppb (Bowman, Acree, and Corbett, 1960) and dieldrin is 186 ppb (Park and Bruce, 1968), the results are compatible with an exchange equilibrium mechanism based upon solubility differences.

Fikes and Tubb (1971) found that Amblema plicata also concentrates dieldrin in response to ambient concentrations, but noted different concentration factors at different ambient levels. At 0.02 ppb ambient, dieldrin was concentrated 2500 fold, but at 20 ppb, dieldrin was concentrated only 1000 fold.

The exchange equilibrium mechanism is compatible with the observations that clams rapidly reflect ambient pesticide levels in their tissues at a predictable level (Bedford, 1970, Fikes and Tubb, 1971); that clams may rapidly cleanse themselves of pesticides (Bedford, 1970); that, as some authors contend (Reinert, 1969), a correlation between lipid level and pesticide levels exists; and that pesticide retention in tissues is inversely related to its water solubility (Bedford, 1970, Hamelink, Waybrant, and Ball, 1970).

Polychlorinated biphenyls (PCB's) are a source of possible contamination that was not considered in this study. PCB's are industrial plasticizers that have been used in a variety of products including paints, inks, varnishes, dust inhibitors, waxes, adhesives, fungicidal insulations and in pesticide applications (Veith and Lee, 1970, Veith and Lee, 1971). They have retention times similar to those of some organochlorine pesticides and are not separated from insecticides by normal extraction and clean-up procedures (Bagley, Reichel, and Cromartie, 1970).

Despite their wide application, PCB's have generally been found in aquatic organisms exposed to industrial and urban wastes (Veith and Lee, 1971). The presence of PCB's in mussel and fish samples have been reported in the U.S. (Bagley, Reichel, and Cromartie, 1970) and in Europe (Koeman, Ten Noever De Brauw, and De Vos, 1969). However p,p'DDT and its metabolites, most severly affected by PCB's, are present in much higher levels than PCB's, thereby minimizing the problem of quantitative error (Richardson, Robinson, Crabtree, and Baldwin, 1971).

There are several conditions that could affect the results of clam monitoring studies. The work of Bedford (1970) suggests that water temperature and amount of plankton may affect the uptake rate by clams. He found that, in dechlorinated tapwater, increasing the temperature increased the rate of uptake and elimination of pesticides. He further reported that clams concentrate lower levels of DDT in water lacking plankton (1000 fold) than in water with plankton (2400 fold).

Variation in filtering rates as a result of size differences may also affect pesticide accumulation. Rice and Smith (1958), working with the marine clam Venus mercenaria, found that more water was filtered per

gram body weight in smaller clams. In this study, the control mussels exhibited a decrease in pesticide levels with an increase in body weight, although not significant at p= 0.05 (Table 12).

Amblema, as noted by Baker (1926), varies in response to its environment, having compressed forms in streams and more inflated forms in larger rivers. Starrett (1971) has used this phenomenon (Ortmann's "law") as an indication that there is only 1 species--Amblema plicata--and the other "species" are forms. This makes Amblema plicata particularly well-suited for pesticide monitoring in that the same species can be used in monitoring the small tributaries (Amblema plicata form costata) as in the larger river (Amblema plicata form plicata). It is found throughout the Mississippi River drainage (Parmalee, 1967). Amblema plicata is also one of the more organic-pollution-tolerent species of mussels (Starrett, 1971), perhaps because of its ability to survive extended periods in nearly anaerobic conditions (Imlay, 1971). In fact, Starrett (1971) found that 62 percent of the collected clams from the highly polluted Illinois River were Amblema plicata.

Amblema plicata is adaptable to many different types of substrates. Murray and Leonard (1962) noted that Crenodonta p. peruviana and Crenodonta peruviana costata (both synonyms for Amblema plicata) inhabit most types of stream bottoms in Kansas, except shifting sands. Parmalee (1967) noted that Amblema costata (Amblema plicata form costata) is found most frequently on sand and gravel, but that it is one of the species that can adjust to a mud bottom.

Amblema plicata's most serious weakness is shared with other mollusks--elimination due to severe siltation (Ellis, 1936) and/or severe organic pollution (Starrett, 1971).

SUMMARY

Aldrin, dieldrin, and p,p'DDE were found in all Amblema plicata analyzed from Polecat Creek. Heptachlor and heptachlor epoxide were tentatively indentified. Dieldrin and p,p'DDE were found to vary significantly as a function of time, exhibiting a significant increase in the August samples suggesting a seasonal peak. Dieldrin levels were noted to be significantly higher at Station II (mean 0.0550 ppm). Aldrin varied greatly throughout the study, without apparent predictability. The aldrin to dieldrin ratio was much higher than has been generally reported.

Evidence was presented suggesting that aldrin, dieldrin, and p,p'DDE and heptachlor and heptachlor epoxide (if present) represent residues from the agricultural application of aldrin, DDT, and heptachlor. The lack of detectable levels of DDT indicated that p,p'DDE may be a long-term residue.

It was suggested that clams may reflect ambient insecticide levels by an equilibrium exchange mechanism based upon relative solubility differences of the insecticide in body lipids and ambient water.

Amblema plicata was recommended as an excellent species for mussel biomonitoring studies due to its ability to adapt to a wide range of environmental conditions and its presence throughout the Mississippi River drainage.

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APPENDIX

Chemical Names of Pesticides Discussed.

Aldrin	Not less than 95% of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene.
BHC	1,2,3,4,5,6-hexachlorocyclohexane, mixed isomers.
DDE	1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene.
DDT (including its isomers and dehydrochlorination products)	1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane; technical DDT is a mixture of p,p'-isomer and o,p'-isomer in a ratio of 3 or 4 to 1).
Dieldrin	Not less than 85% 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-dimethano=naphathalene.
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene.
Heptachlor Epoxide	1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene.
Lindane	1,2,3,4,5,6-hexachlorocyclohexane, 99% or more gamma isomer.