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A Study of Organochlorine Insecticides in Freshwater Crayfish -- Analytical Problems and Biomonitoring Survey

Robert C. Vanderjack
Eastern Illinois University

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A STUDY OF ORGANOCHLORINE INSECTICIDES IN FRESHWATER CRAYFISH -- ANALYTICAL PROBLEMS AND BIOMONITORING SURVEY

(TITLE)

BY

ROBERT C. VANDERJACK

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF SCIENCE

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1973
YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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A STUDY OF ORGANOCHLORINE INSECTICIDES IN FRESHWATER CRAYFISH -- ANALYTICAL PROBLEMS AND BIOMONITORING SURVEY

Presented by

ROBERT C. VANDERJACK

a candidate for the degree of Master of Science and hereby certify that in their opinion it is acceptable.

ABSTRACT

Crayfish were collected from 3 sites on Polecat Creek, Coles County, Illinois in early spring, early summer, and late summer of 1972. Pesticides were extracted from the crayfish tissue in a one step procedure utilizing a Florisil elution column. Analysis was by electron capture gas chromatography. Pesticide residues in the nannogram range were analyzed and background contamination was an important consideration. Sources of background contamination were investigated and teflon stopcocks, Florisil, and residues on "cleaned" glassware were found to be major contamination sources. Distilled water used in flushing glassware should also be considered a potential contamination source.

Aldrin, dieldrin, and pp' DDE were identified and quantitated in all samples tested. Heptachlor epoxide was identified in several samples as were peaks that corresponded to either heptachlor or lindane. No pp' DDT was found. No significant differences were found in pesticide levels of aldrin, dieldrin, or pp' DDE with respect to time or location.

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TABLE OF CONTENTS

	Page
ABSTRACT	i
ACKNOWLEDGMENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
INTRODUCTION	1
LITERATURE REVIEW	2
MATERIALS AND METHODS	9
RESULTS	24
DISCUSSION AND CONCLUSIONS	41
LITERATURE CITED	51

LIST OF TABLES

Table		Page
1	Crayfish collected at site 1, Polecat Creek, Coles County, Illinois	34
2	Crayfish collected at site 2, Polecat Creek, Coles County, Illinois	35
3	Crayfish collected at site 3, Polecat Creek, Coles County, Illinois	36
4	Fat content of 10 gram portions of homogenized crayfish samples	37
5	Percent recoveries of aldrin, dieldrin, and pp' DDE from homogenized crayfish samples	37
6	Pesticide residue levels found in chemical blanks that were run periodically during the monitoring series of extractions	37
7	Aldrin levels in homogenate samples of crayfish	38
8	Dieldrin levels in homogenate samples of crayfish	38
9	pp' DDE levels in homogenate samples of crayfish	38
10A	Water quality parameters and relative water level data taken at three crayfish collection sites on Polecat Creek, Coles County, Illinois	39
10B	Water quality parameters and relative water level data taken at three crayfish collection sites on Polecat Creek, Coles County, Illinois	40

LIST OF FIGURES

Figure		Page
1	Map of Polecat Creek, Coles County, Illinois showing crayfish collection sites	10
2	Drawing of Chromaflex elution column with separatory funnel attached	12
3	Drawing of Kuderna-Danish evaporative concentrator	13
4	Chromatograms verifying the presence of pesticides in crayfish tissue by spiking of an unknown sample with known pesticides	26
5	Chromatograms of chemical blanks taken before and after efforts to eliminate background contamination	27
6	Chromatogram of residues obtained by soaking the teflon stopcock from the elution column in the extraction solvent mixture	28
7	Chromatogram of residues obtained by passing the extraction solvent mixture through a normally packed column minus the crayfish tissue and minus the teflon stopcock to see if the florisil is a contamination source	28
8	Chromatogram of residues obtained by soaking fiber glass in the extraction solvent mixture	29
9	Chromatogram of residues obtained by soaking glass wool in the extraction solvent mixture	29

Figure		Page
10	Chromatogram of residues obtained from the extraction solvent mixture after it had been used to flush the cleaned extraction glassware just prior to use in an extraction	30
11	Chromatogram of residues obtained from the extraction solvent mixture	30
12	Chromatograms of residues obtained from distilled water used in flushing glassware after acid cleaning. Distilled water stored in a plastic carboy, distilled water straight from the still, and distilled water straight from the still but with potassium permanganate added to the boiling pot are compared	31
13	Chromatograms of residues obtained from benzene stored in a plastic squirt bottle and from benzene stored in a "Mini-vial"	32
14	Chromatograms of residues obtained from the extraction solvent mixture stored in a "Mini-vial"	32
15	Chromatogram of residues obtained from the extraction solvent mixture after having been used in an extraction and then condensed for re-use	33
16	Chromatogram of a standard mixture of five pesticides	33

INTRODUCTION

A pesticide biomonitoring study was conducted on Polecat Creek (Coles County, Illinois) using the freshwater crayfish (three genera) as an indicator organism. A similar study had previously been conducted using the clam Amblema plicata (Hogan, 1972). Crayfish were selected as an indicator because of their availability, size, limited range, and feeding habits.

When contamination problems were encountered during the tissue extraction portion of the analysis, it was decided to devote the first part of the study to the documentation of these problems as an aid to future studies utilizing this method of analysis.

LITERATURE REVIEW

Aldrin, dieldrin, and DDT are synthetic organic insecticides more specifically referred to as organochlorine insecticides. Aldrin can break down into dieldrin and DDT can be metabolized by insects into the noninsecticidal DDE. The most famous of these is DDT.

DDT was first described by the German chemist Othmar Zeidler in 1874, but its insecticidal value was not discovered until 1939 by Paul Müller while working for the Geigy dye company in Basel, Switzerland (Whitten, 1966). When supplies of natural organic pesticides used for louse and rat control were largely cut off because of World War II it became necessary to find substitutes for military purposes. DDT thus came into use and demonstrated great success in halting a Typhus epidemic in Italy in 1943 and 1944 (U. S. Dept. of Health, Education, and Welfare, 1969). Thereafter, little 2-ounce cans of DDT became the constant companions of the American soldiers and their allies. As World War II drew to a close the use of DDT became routine in checking epidemics in liberated concentration camps and in protection of groups of wandering refugees. Other early uses of DDT were antimosquito campaigns in the United States and Italy to combat malaria (Whitten, 1966).

DDT became commercially available to the public about 1945. Research continued on other clorinated hydrocarbon compounds in the quest for even better insecticides, eventually resulting in the appearance of aldrin and dieldrin in 1948 (Greenslade, 1960). As of 1969 there were some 900 active pesticidal chemicals formulated into over

60,000 preparations (U. S. Dept. of Health, Education, and Welfare, 1969). These include insecticides, fungicides, herbicides, and plant growth regulators. Information concerning the amounts of pesticides produced in the United States indicate that one billion pounds are produced yearly (USDA, 1971) and in 1969 insecticides accounted for about one-half of the total amount. Relatively large percentages of these insecticides were exported. The production of DDT, though, has generally declined since hitting a production peak in 1959.

On October 21, 1972 the "Federal Environmental Control Act of 1972" was signed into law by the President of the United States. This is a comprehensive program to regulate pesticides for the protection of human beings and the environment (Congressional Quarterly Almanac, 1972). The act gives the Environmental Protection Agency (established in 1970) regulatory power over the use of pesticides. The EPA has banned all but essential uses of DDT effective December 31, 1972. DDT can still be used though for public health and disease control (under stringent conditions) and also for certain crop uses for which there are no effective alternatives.

A survey of the State Department of Agriculture in Minnesota showed that by May 1971, at least 29 states had taken some action to restrict the use of some pesticides (Environmental Protection Agency. Office of Water Programs, 1972). As of July 1, 1969 the State of Illinois has a "Pesticides Control Law" that controls the labeling, sale, and use or application of pesticides in the state of Illinois to prevent the undesirable contamination of water and the environment (Illinois Revised Statues, 1971).

In the past DDT seems to have been used as an all around pesticide including forest insect control programs, mosquito and bat control,

and more than 200 federally recommended agricultural crop applications. Aldrin has been extensively used as a soil insecticide especially with respect to corn production. It is effective against a variety of pests including the corn rootworm larvae. Dieldrin is effective against a variety of pests, especially when a long lasting residual effect is desired. Its primary uses seem to have been against termites and corn soil insects (U. S. Dept. of Health, Education, and Welfare, 1969).

The benefits obtained from the synthetic pesticides are impressive. In the area of public health there are at least 27 diseases, including some of the worlds deadliest, that can be controlled partly or completely by DDT and its allies (Whitten, 1966). Malaria is a prime example. In 1953 there were 75 million cases of malaria in India with an accompanying life expectancy of 32 years. By 1962, 147 million pounds of DDT had been used and life expectancy had increased to 47 years. In 1967 there were fewer than 100,000 cases of malaria in India (Burnside, Furrer, and Roselle, 1971). The USDA Agricultural Handbook 291 published in 1965 estimates that the annual loss caused by pests to agricultural commodities in the United States during 1951 to 1960 amounted to over 15 million dollars per year or the equivalent of about 33% of the total agricultural output (Burnside et al., 1971). Pesticides are an effective tool in reducing these losses.

Right from the start the effects of pesticides (i.e., DDT) on the environment were questioned. Eide, Deonier, and Burrell (1945), Ginsburg (1945), Cottam and Higgins (1946), Surber (1946), and Hess (1947) all investigated the toxic effects of DDT on nontarget organisms such as fish, fish food organisms, and wildlife. Hess (1947) was the only one who concluded that the effects were not significantly injurious, but he

did qualify this with the conjecture that heavier doses or different methods of application might make a difference. Taking into account the variables of habitat, dose rate, and method of application, the other investigators all found the effects of DDT to be deleterious. Cottam and Higgins (1946) specifically concluded that invertebrates and cold-blooded vertebrates were more readily affected than birds and mammals.

During August of 1950 there were fish kills in at least 15 streams in the Tennessee River Valley of Alabama. Investigations indicated that the kills were the indirect result of insecticide applications for the control of cotton insects. This was probably one of the first instances where extensive pollution of streams had resulted from the use of insecticides in agriculture (Young and Nicholson, 1951). The publication of "Silent Spring" by Carson in 1962 did much to focus public attention on the potential problems of pesticide residues even though the hazards may have been greatly exaggerated (Edwards, 1970).

Determination of pesticide residues is usually by chemical methods or by bioassay. Bioassay can generally be considered to be the response of an organism to a chemical. Several organisms have been investigated in this respect; e.g., houseflies, mosquito larvae, Drosophlia, other insects, microcrustaceans, fish, microorganisms, and plants. It is a simple, swift, versatile, and highly sensitive technique but often it must be used in conjunction with a chemical method in order to obtain general acceptance of the data (Dewey, 1958).

Gas chromatography is prominent among the several chemical methods of pesticide residue analysis. The theory of gas chromatography was proposed by James and Martin (1952), and one of the first uses of gas

chromatographic analysis of residues was with the microcoulmetric detector developed by Coulson and Cavanagh (1960) (Lisk, 1966). Residues of less than 0.1 ppm of halogenated and organophosphorus insecticides have been detected with this system (Lisk, 1966). The theory of the electron capture detector was published by Lovelock and Lipsky (1960). This is the most widely used and most sensitive detector for analysis of halogenated, nitro, and certain other pesticide compounds (Lisk, 1966). Insecticides in the nannogram (0.001 μg) and picogram (0.001 nannogram) ranges are readily detected with electron capture gas chromatography (Langlois, Stemp, and Liska, 1963).

When working with animal tissues the pesticides usually have to be extracted from the tissue, partitioned into a suitable solvent, and then the solvent cleaned up (e.g., lipid impurities) prior to gas chromatographic analysis. A method was introduced by Mills (1959) utilizing a florisil elution column for the cleanup portion of the procedure. For animal tissue samples of low fat content Langlois, Stemp, and Liska (1964) reported the use of a similar florisil column in a one step cleanup procedure.

Moore (1966) indicates five criteria that should be considered when choosing an indicator organism for pesticide residue analysis: (1) easily available, (2) analysis can be made of single animals or of bulked samples, (3) age of the indicator animal can be determined by inspection, (4) the indicator animal should ordinarily show a relatively high pesticide residue level (too high and an increase might have toxicological effects, too low and pesticide identification may not be reliable), and (5) the geographic range of the indicator species should be known. For the monitoring of freshwater habitats the fresh-

water crayfish would seem to fit three of these criteria very well. With respect to age, the general consideration that the older the crayfish is, the larger it will be, might suffice. The criterion that the indicator should ordinarily show a relatively high pesticide level seems to be a moot point in that this might be determined by the previous exposure of the indicator. Meeks (1968) conducted an extensive study of DDT accumulation in a freshwater marsh. He felt that good indicator species should reflect the current dynamics of the chemicals themselves. He thought that omnivorous species could do this better than organisms with restricted food habits. It was desirable if detritus formed a part of their diet because of the affinity between detritus and DDT residues. A low amount of sample variation was also important. He concluded that crayfish and small carp are often abundant and appear to satisfy these requirements.

The freshwater crayfish has been analyzed for pesticide residues several times. In some of these studies the residues found in crayfish were directly compared with other organisms. Bridges, Kallman, and Andrews (1963), Cope (1966), and Cole, Barry, and Frear (1967) conducted studies that compared pesticide residues between fish and crayfish. They generally found that fish contained about twice the residues that crayfish did and that trout seemed to accumulate higher pesticide concentrations than bottom feeding fish like bullheads and suckers. Hannon et al. (1970) compared several organisms with respect to their ability to concentrate pesticides using the pesticide level in the water as a reference of one. Crayfish exhibited a concentration factor over water of 18X, plankton and algae 37X, fish 790X, and aquatic insects 7300X. This concentration factor exhibited by aquatic insects

could explain why the insect eating trout would exhibit higher pesticide concentrations than the bottom feeding suckers and bullheads. Johnson et al. (1971) found similar results, with microcrustaceans and aquatic insects being able to concentrate pesticides many, many times greater than the crayfish.

Fredeen and Duffy (1970) compared fish to snails and clams and found that fish seemed to exhibit at least two to three times the pesticide levels of snails and clams. Fish such as suckers and catfish contained about twice the residues of fish like perch and sunfish. Bedford, Roelofs, and Zabik (1968) used the freshwater mussel as a pesticide monitoring organism and concluded that it would be an excellent monitoring organism.

Thus crayfish do not seem to be as efficient concentrators of pesticides as many other members of the aquatic environment but because of their availability, size, limited range, eating habits, and the pesticide concentration characteristics that they do possess they are in many situations the best choice for an indicator organism.

MATERIALS AND METHODS

Collection of specimens:

Crayfish were collected at three sites on Polecat Creek (Figure 1). Site 1 had a slow current, silty bottom, and was closely bordered by agricultural fields. Sites 2 and 3 had a faster current, sand-gravel-rock type bottoms, and were bordered by wooded areas as much as by agricultural fields. Collections were made mostly by hand and to a lesser extent by seining or by traps with bait obtained directly from the creek (e.g., clams, fish, etc.). Collections were made at all three sites early in the spring (before the usual spring rains), early in the summer, and again in late summer (nine samples total, Tables 1, 2, and 3).

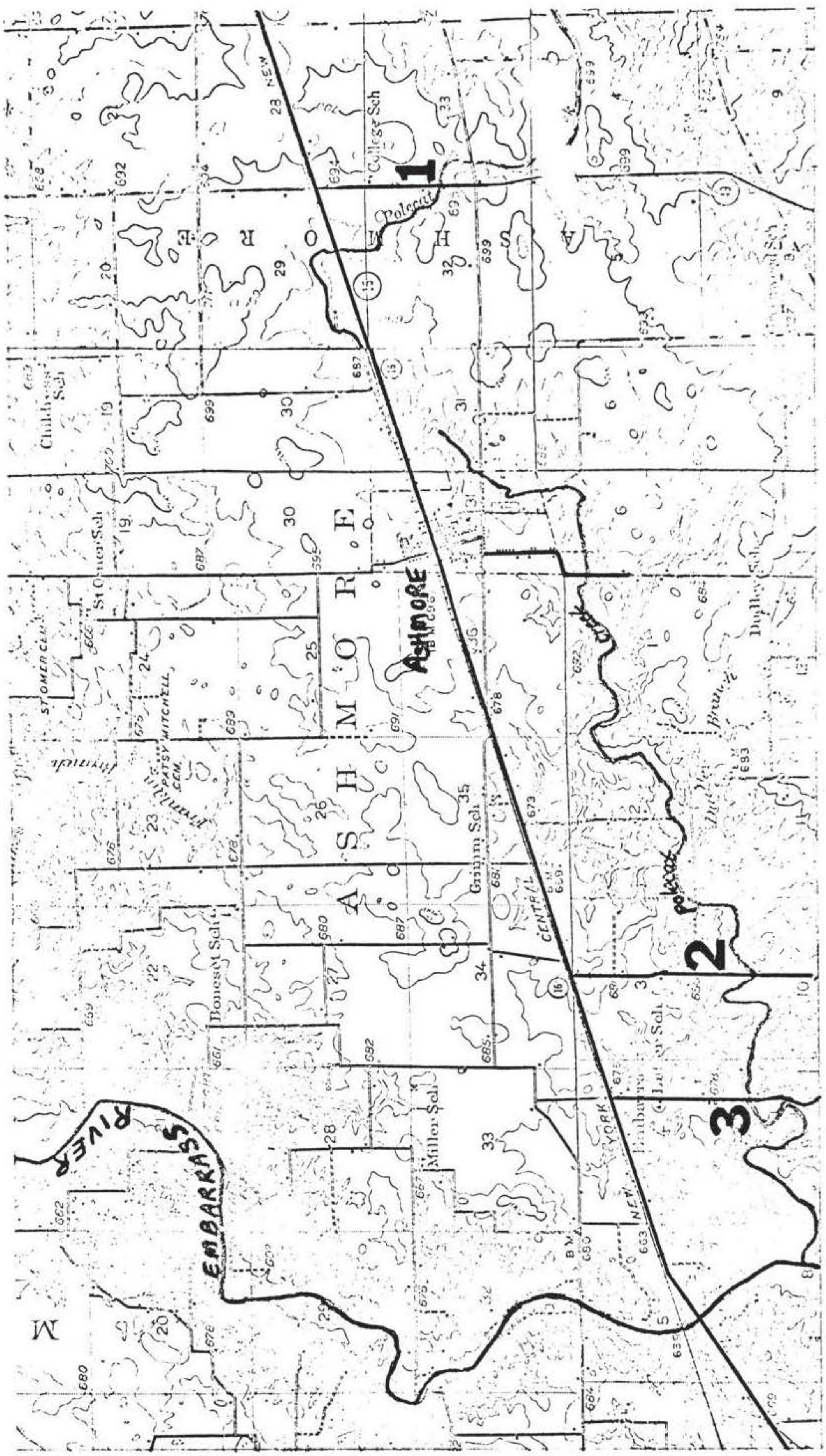
Specimens from each site were identified, homogenized in a blender, double wrapped in aluminum foil, sealed in "Baggies", and then frozen. Each sample is thus a homogenate of several whole crayfish. Specimens were identified using the taxonomic keys of Pennak (1953), and Eddy and Hodson (1961).

Extraction of insecticides from tissues:

Extraction of insecticides from the crayfish tissue was as per a modification of a one step procedure reported by Langlois, Stemp, and Liska (1964) that had been applied to a variety of animal product samples.

The size of the sample to be analyzed is limited by its fat content and by the size of the Florisil cleanup column used. A

FIGURE 1. Map of Polecat Creek, Coles County, Illinois showing crayfish collection sites.


ASHMORE
EMBARRASS RIVER
1
2
3
Polecat
Polecat Creek
College Sch
St Omer Sch
ST OMER CEM.
MITCHELL
PATSY CEM.
Gramm Sch
Bonesett Sch
Miller Sch
Dudley Sch
Childress Sch
NEW YORK CENTRAL
Brush Branch

pesticides analysis workshop at Argonne National Laboratory, Argonne, Illinois (Argonne Center for Educational Affairs) had specified that for the size Florisil column used in this study one gram of fat was the maximum allowable for any size sample.

Fat analysis of tissue:

Fat content of the crayfish tissue was ascertained by modification of a method described by Mills (1959). Four 10 gram samples of homogenized crayfish tissue were analyzed. Each sample was ground with a nominal amount of anhydrous sodium sulfate to combine with any water present and to disintegrate the sample. Twenty-five ml of petroleum ether (B.P. 30 - 60°C) was added to the sample which was shaken vigorously for one minute and then centrifuged for five minutes at 1500 R.P.M. The solvent layer was poured off and saved. The tissue was extracted two more times in a similar manner with 15 ml of petroleum ether each time resulting in 55 ml of extract. The solvent was boiled off in a water bath (60 + °C) and the residue was taken as the fat content of the original 10 grams of tissue (Table 4).

Glassware used:

Assembled glassware (Figures 2 and 3).

500 ml cylindrical separatory funnel with pressure equalizing line, ground glass stopcock, and ground glass joints on both ends.

Kontes Chromaflex column, size 224, ground glass joint at top, originally with teflon stopcock and fritted plate but modified with a glass stopcock and with the fritted plate removed. The fritted plate was removed to facilitate cleaning of the column and was replaced by a glass wool plug (angel hair) during use.

1000 ml Kuderna-Danish evaporative concentrator, all parts with ground glass joints.

Glass mortar and pestle.

FIGURE 2. Chromaflex elution column with separatory funnel attached. The eluant containing the pesticide residues was collected directly into the Kuderna-Danish evaporative concentrator. Not drawn to scale.

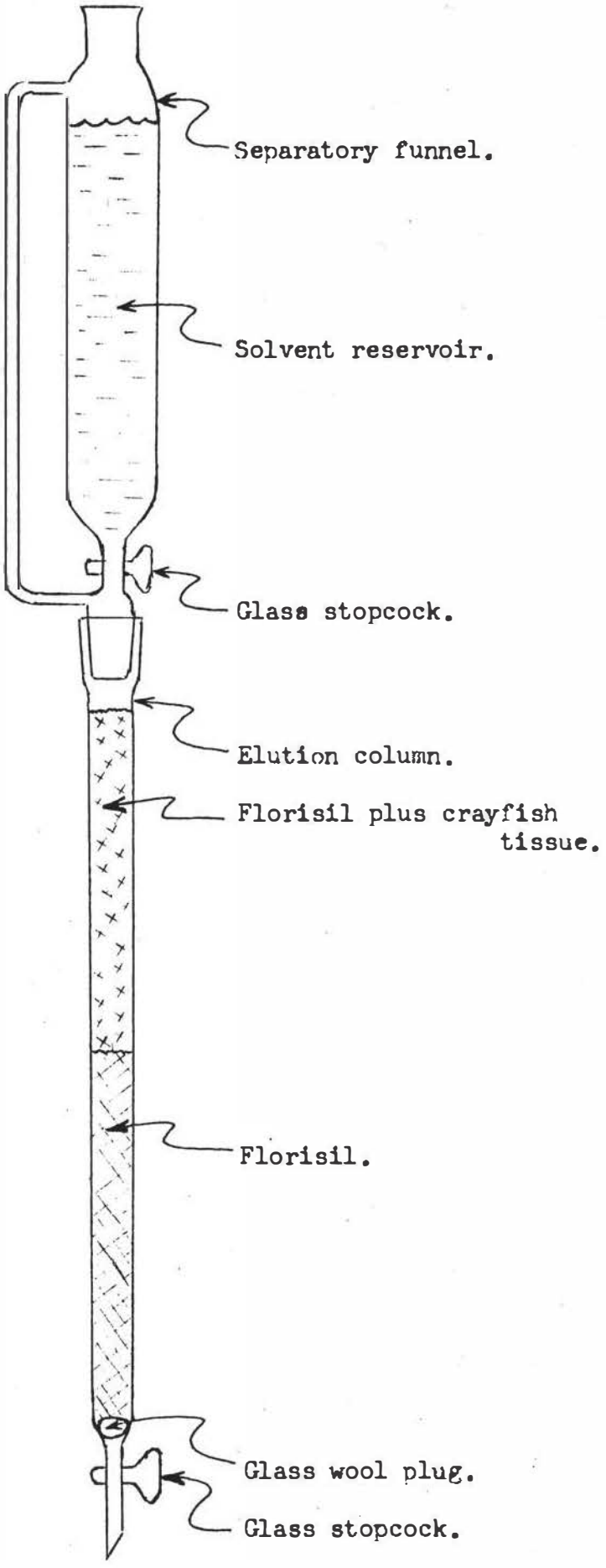
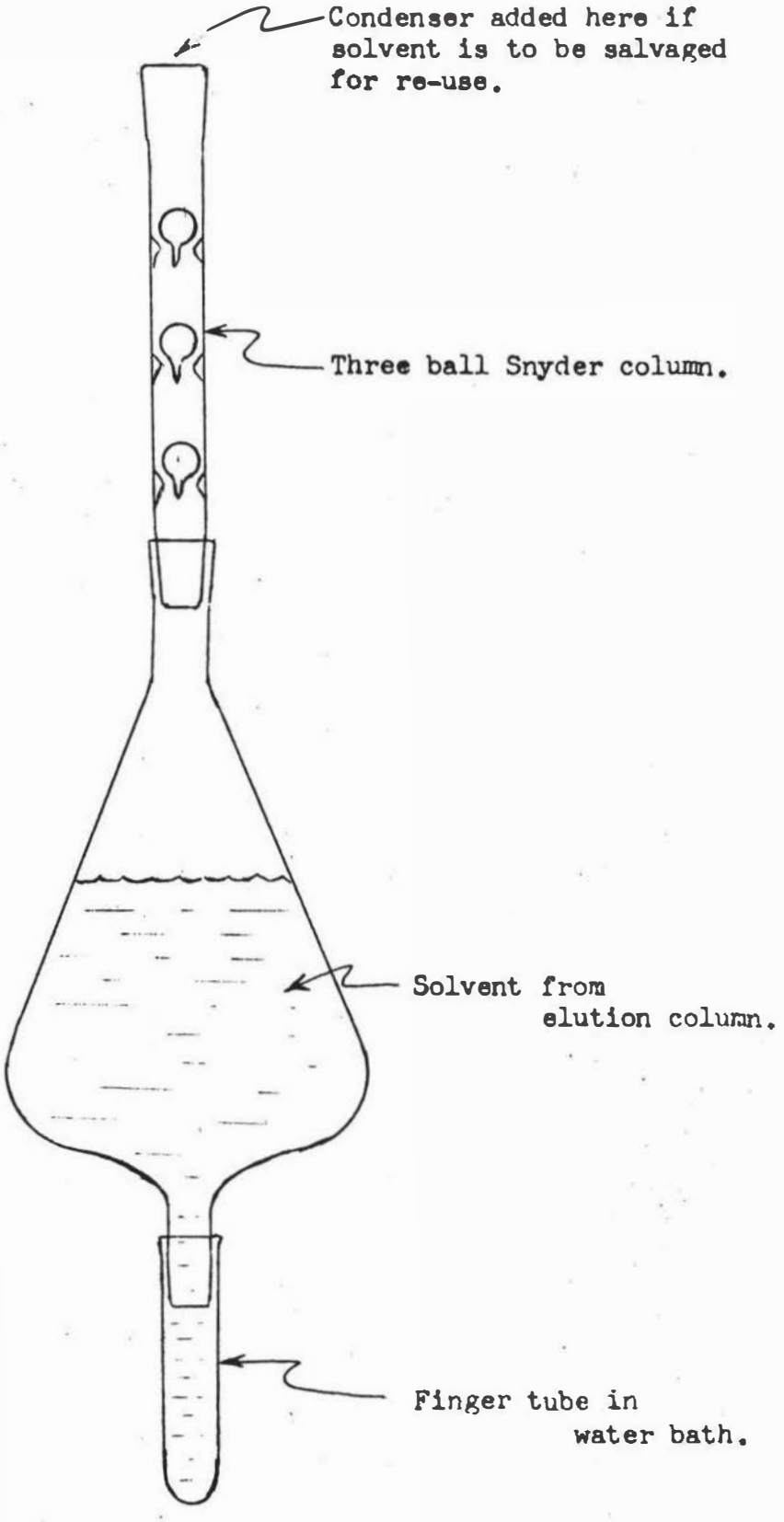

Separatory funnel.
Solvent reservoir.
Glass stopcock.
Elution column.
Florisil plus crayfish tissue.
Florisil.
Glass wool plug.
Glass stopcock.

FIGURE 3. Kuderna-Danish evaporative concentrator. To enhance evaporation the Snyder column was wrapped in asbestos and aluminum foil. All other glassware was wrapped in aluminum foil only. Not drawn to scale.


Condenser added here if solvent is to be salvaged for re-use.
Three ball Snyder column.
Solvent from elution column.
Finger tube in water bath.

Solvents:

Petroleum ether (B.P. 30 - 60°C) . . . . Nannograde quality, manufactured by Mallinckrodt.

Methylene chloride (dichloromethane) . . Nannograde quality, manufactured by Mallinckrodt.

Benzene . . . . . Nannograde quality, manufactured by Mallinckrodt.

Solvent mixtures:

80/20 = 80% petroleum ether (B.P. 30 - 60°C), 20% methylene chloride (dichloromethane) by volume.

50/50 = 50% petroleum ether, 50% methylene chloride by volume.

Column packing material:

Florisil, 60/100 mesh, manufactured by the Floridin Company and used as an absorbent for water and lipids. Florisil is a synthetic magnesium silicate compound.

General procedure:

Before each extraction all glassware was thoroughly washed in soap (Liquinox) and water. The glassware was then soaked in chromic acid (Chromomerge), a sulfuric acid-potassium dichromate mixture (preferably for several hours), flushed well with distilled water, and thoroughly dried. Immediately before use all glassware was flushed with a nominal quantity of 80/20 (pet. ether/meth. chl.).

Florisil was activated at 130 - 140°C for 12-14 hours, taken to a controlled level of deactivation by adding 5% distilled water to 95% activated Florisil (by weight) and then keeping the mixture in an air tight container at room temperature for approximately 48 hours. This allowed the Florisil-water mixture to reach equilibrium. Immediately before use, the Florisil water mixture was swirled in 30 - 50 ml of 50/50 (pet. ether/meth. chl.) to flush impurities out of the Florisil.

In preparation, the column was first filled approximately half way with 50/50 (pet. ether/meth. chl.) and a slurry of 25 grams Florisil and 50/50 was poured in. The side of the column was constantly tapped so the Florisil would settle compactly to the bottom. Thirty to fifty ml of 50/50 was then drawn through the column to further rid the Florisil of impurities. The column was not allowed to go dry.

Ten grams of crayfish tissue was ground with 25 grams of Florisil to a free flowing powder in a glass mortar and pestle. The 80/20 (pet. ether/meth. chl.) was added to the top of the column and the Florisil-crayfish tissue mixture was poured in, tapping the side of the column in the same manner as before.

Six hundred ml of 80/20 (pet. ether/meth. chi.) was eluted through the column at approximately 20 drops every ten seconds. The eluant was collected directly into the Kuderna-Danish evaporative concentrator and then evaporated in a water bath at 60 - 80°C (in an exhaust hood) until approximately 30 ml remained in the finger tube. Two or three small pieces of broken glass were used as boiling chips. Dry nitrogen was passed directly over the remaining eluant until about 2 ml remained. This was then transferred to a 5 ml "Mini-vial" (distributed by Altech Associates) and the finger tube flushed three times with small quantities of 80/20 (pet. ether/meth. chl.) to remove any residue. The sample was then taken to complete dryness in the "Mini-vial" with dry nitrogen and the residue redissolved in 1 ml of nannograde benzene. The benzene solution was injected into a gas chromatograph for qualitative-quantitative analysis.

Because of a high background level of impurities various aspects of the extraction procedure were examined separately in order to determine and eliminate the source(s) of the impurities.

Qualitative-quantitative analysis:

Equipment used - Varian Aerograph series 2800 gas chromatograph equipped with an electron capture detector. Detector operating temperature 200 - 210°C. Injector operating temperature 215 - 220°C. Carrier gas (Nitrogen) pressure 60 (-).

QF-1 column. Liquid phase 3% QF-1. Solid support 80/100 mesh Chromosorb W, acid washed and treated with dimethyldichlorosilane.

DC-11 column. Liquid phase 5% DC-11. Solid support 80/100 mesh Chromosorb W, acid washed and treated with dimethyldichlorosilane.

Pesticide standards - 1000 mg pesticide standards were obtained from the Environmental Protection Agency, Perrine Primate Laboratory, Perrine, Florida. The following standards were prepared using nanno-grade benzene as a solvent.

1. 1 ppm aldrin, 1 ppm lindane, 2 ppm pp' ODE, 2 ppm dieldrin, 2 ppm pp' DDT. Only used qualitatively.
2. 1 ppm aldrin, 1 ppm heptachlor, 1 ppm heptachlor epoxide, 2 ppm pp' DDT. Only used qualitatively.
3. 1 ppm aldrin, 2 ppm dieldrin. Used qualitatively and quantitatively.
4. 1 ppm aldrin, 2 ppm pp' DDE. Used qualitatively and quantitatively.
5. 1 ppm aldrin, 1 ppm dieldrin, 1 ppm pp' DDE. Used in determining percent recovery of these pesticides using this extraction scheme.

Qualitative determinations were made by comparing retention times of known standards to retention times of unknown peaks. Confirmation of the presence of the pesticides was made by preparing a 91.7% unknown - 8.3% standard injection (by volume) and comparing this to the pure unknown (Figure 4).

Quantitative determinations for aldrin and dieldrin were made by comparing peak areas of standards to peak areas of unknowns. Injection volumes were varied to keep the peaks of the standards and the unknown

samples of similar size and correspondingly similar concentration. This is necessary because the response of the detector to different concentrations of pesticides is not linear. The variation in injection volume was then compensated for by dividing the quantity of pesticide residues in the unknown sample obtained from the chromatogram by 12; i.e., the injection volume for the monitoring samples was 12 times greater than that of the standards. This quantity was then corrected for again by subtracting the corresponding background contamination. Quantitative determination for pp' DDE was made by comparing peak heights rather than the more accurate peak area method (except those samples spiked for percent recovery determinations). This was necessary because the background contamination that corresponded to pp' DDE did not exhibit itself as a peak all the time but sometimes only as a plateau or level of contamination. As before the background contamination was subtracted as a correction. Peak areas that were overlapped were determined by using the minimum between peaks as a dividing line and utilizing a disc integrator. Brace (1959) made a study of the quantitative evaluation of overlapping peaks and found this method to show adequate agreement with completely resolved peaks of the same components. Nonoverlapping peak areas were determined by extending the straight line portion of the peak to the baseline with subsequent utilization of the disc integrator. To determine background contamination a chemical blank was run after every third unknown. A total of four blanks was run and an average was taken.

Quantities of pesticides were then correlated to original crayfish specimens by the following conversion:

Definition of ppm used for mixing standards = 10^{-6}gm/ml of solution

Then 1 ppm from chromatogram = 10^{-6}gm/ml benzene

= 10^{-6}gm/ml solvent mixture

= 10^{-6}gm/10 gm of crayfish tissue.

Redefining ppm as 10^{-6}gm/gm of crayfish tissue

Then 10^{-6}gm/10 gm of crayfish tissue

= 0.1 x 10^{-6}gm/gm of crayfish tissue

= 0.1 ppm in crayfish tissue.

Thus 1 ppm from chromatogram = 0.1 ppm in crayfish tissue.

The QF-1 column was used for all qualitative-quantitative determinations. A DC-11 column was tried to see if a better resolution on aldrin could be obtained. It could not. The DC-11 column is not suitable for dieldrin-pp' DDE determinations because they emerge from the column simultaneously and yield the same peak on the chromatogram.

Comparison of pesticide residues found in each season:

The pesticide residues found at each site were combined and the average was taken to represent the season. Each season was then compared to each other season to see if significant differences existed. The comparison standard used was Student's "t" test:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2 + s_2^2}{N-1}}}$$

Where - $\bar{X}$ = Mean pesticide residue values for each season.

s^2= Variance (the square of the avg. deviation from the mean).

N = Number of analyses represented by the mean.

Comparison of pesticide residues found at each site:

The pesticide residues found in each season were combined and the average was taken to represent the particular site. Each site was then compared to each other site to see if significant differences existed. The comparison standard used was Student's "t" test as specified in the preceding section.

Percent recovery of pesticides:

Three samples were used (the three samples from early summer). Each sample was spiked with 1 ppm aldrin, 1 ppm dieldrin, and 1 ppm pp' DDE. When the quantity of one of these pesticides found in the unspiked sample of the same tissue was subtracted from its spiked counterpart the difference was taken as the percent recovery of this pesticide being achieved in the extraction; i.e., a difference of 1 ppm aldrin would indicate 100% recovery of aldrin and a difference of 0.9 ppm aldrin would indicate 90% recovery (Table 5). Monitoring samples were not corrected for percent recovery.

Water quality:

Several parameters of water quality along with relative water levels in the creek were checked periodically during the summer (Tables 10A and 10B).

Turbidity, ppm nitrate, ppm phosphate (ortho), and conductivity were measured with a direct reading, portable, colorimeter supplied by the Hach Chemical Company. pH was measured with a Sargent-Welch pH meter. Dissolved oxygen and five-day B.O.D. values were determined by Winkler analysis (Welch, 1948). Five-day B.O.D. samples were incubated in the dark at 20°C. All water samples were taken in relatively

deep and relatively slow-moving water away from riffle areas. Temperature was measured with a mercury thermometer and relative water levels were determined by painting marks on bridge foundations at sites 1 and 3.

Check of various components of extraction scheme for source(s) of contamination:

All samples, except the distilled water samples, were taken to dryness in the Kuderna-Danish evaporative concentrator followed by exposure to a direct flow of dry nitrogen. The distilled water samples were allowed to evaporate in an exhaust hood before being exposed to nitrogen. The QF-1 column was used for all analyses.

1. Teflon stopcock - A teflon stopcock used in several extractions was soaked in approximately 75 ml of 80/20 (pet. ether/meth. chl.) for about 20 hours. The solvent was taken to dryness, redissolved in 0.5 ml nannograde benzene, and the benzene solution was injected into the gas chromatograph (Figure 6).
2. Florisil - A Florisil column was prepared as usual except that a teflon stopcock was not used and no crayfish tissue was used. Three hundred ml of 80/20 (pet. ether/meth. chl.) was passed through the column at approximately 20 drops every ten seconds. A head of solvent was not maintained over the column because it could not be maintained without the stopcock at the bottom of the column. The eluant was taken to dryness, redissolved in 0.5 ml of nannograde benzene, and then injected into the gas chromatograph (Figure 7).

3. Fiber glass (used as a plug to replace fritted plate in the elution column) - An egg sized wad of fiber glass was soaked in approximately 75 ml of 80/20 (pet. ether/meth. chl.) for about four days. The solvent was taken to dryness, redissolved in 0.5 ml of nannograde benzene, and then injected into the gas chromatograph (Figure 8).
4. Glass wool (used instead of fiber glass to replace the fritted plate in the elution column) - An egg sized wad of glass wool was soaked in approximately 75 ml of 80/20 (pet. ether/meth. chl.) for about four days. The solvent was taken to dryness, redissolved in 1.0 ml of nannograde benzene, and injected into the gas chromatograph. For purposes of comparing this to the fiber glass the injection volume of this sample was twice as large as for the fiber glass thus compensating for the different volumes of benzene; i.e., 0.5 ml for the fiber glass and 1.0 ml for the glass wool (Figure 9).
5. Glassware flushings - Clean glassware that had been used for many extractions was flushed with 200 - 250 ml of 80/20 (pet. ether/meth. chl.). The flushings were taken to dryness, redissolved in 0.5 ml nannograde benzene, and then injected into the gas chromatograph (Figure 10).
6. 80/20 (pet. ether/meth. chl.) - Three hundred ml of 80/20 was taken to dryness, redissolved in 0.5 ml nannograde benzene, and the benzene solution was injected into the gas chromatograph (Figure 11).
7. Distilled water stored in a plastic (Nalgene) carboy - An acid jar that was used to prepare the chromic acid glass

cleaning solution was flushed with distilled water and then flushed with a moderate amount of 80/20 (pet. ether/meth. chl.). The jar was filled with approximately 2.2 liters of distilled water and 100 ml of 80/20 was added. The jar was shaken vigorously for one hour. Approximately 75 ml of 80/20 was recovered. This was mixed in a beaker with a nominal amount of anhydrous sodium sulfate, taken to dryness, redissolved in 0.5 ml nannograde benzene and then injected into the gas chromatograph (Figure 12A).

8. Distilled water collected directly from the still (not stored in a plastic carboy) - The sample was prepared and analyzed in the same manner as the preceding distilled water sample (Figure 12B).

9. Distilled water collected directly from the still and $KMnO_4$ (potassium per manganate) in the boiling pot. All non-glass components that could be removed from the still were removed. The sample was prepared and analyzed in the same manner as the preceding two distilled water samples (except no anhydrous sodium sulfate was used). The purpose of the $KMnO_4$ is to oxidize any organic molecules that may be present in the water before distillation (Figure 12C).

Check of other possible sources of contamination:

1. Nannograde benzene stored in a plastic (Nalgene) squirt bottle and used for cleaning the injection syringe - the benzene was injected "as is" into the gas chromatograph (Figure 13A).

2. Nannograde benzene stored in a "Mini-vial" - The benzene was injected "as is" into the gas chromatograph (Figure 13B).

3. 80/20 (pet. ether/meth. chl.) stored in a "Mini-vial" - The 80/20 was injected "as is" into the gas chromatograph (Figure 14).

Chemical blanks:

All the glassware was used and all the steps were performed as in a regular extraction except that no crayfish tissue was used. Four chemical blanks were run as a part of the monitoring sequence of extractions (Table 6). There was also a chemical blank run before any efforts were made to correct contamination sources (Figure 5A) and another was run after contamination correction attempts were made (Figure 5B).

Condensing and re-using solvents:

The 80/20 (pet. ether/meth. chl.) was collected and condensed as it left the Kuderna-Danish evaporative concentrator. Six hundred ml of the condensate was then taken to dryness again via the Kuderna-Danish concentrator followed by a stream of dry nitrogen. The residue was redissolved in 1.0 ml of nannograde benzene and this was then injected into the gas chromatograph. The purpose was to see if the solvent mixture would be free of carried over pesticides and thus suitable for re-use (Figure 15).

RESULTS

The presence of aldrin, dieldrin, and pp' DDE were confirmed by comparing the chromatogram of an unknown sample to the chromatogram of the same sample after it has been spiked with these same pesticides. If spiking produces an increase in the size of a particular peak this confirms that this represents the pesticide in question (Figure 4-A, B, and C).

Heptachlor epoxide was identified in some samples along with peaks that corresponded to either heptachlor or lindane. No pp' DDT was found. There was a large inconsistent peak with approximately 12 times the retention time of aldrin that was often observed. This was never identified and its source could not be ascertained.

A chemical blank was run to determine background levels of contamination (Figure 5A). Several aspects of the extraction scheme and associated factors were then analyzed separately (Figures 6 - 14) to see if the sources of contamination could be isolated. After all controllable sources of contamination were eliminated another chemical blank was run to compare to the original (Figure 5B). There was a worthwhile reduction in background contamination.

Figure 15 illustrates the feasibility of re-using the extraction solvent mixture. The condensed vapors of used solvent mixtures are free of carried over pesticide residues. Figure 16 is used to illustrate the type of chromatogram obtained from a mixture of pesticide standards.

Ten gram portions of homogenized crayfish samples were analyzed for fat content (Table 4). The average value for four samples tested was 0.203 ± 0.084 gm. This is well within the one gram limitation for fat content imposed by the use of the one step extraction scheme.

Percent recoveries were obtained for aldrin, dieldrin, and pp' DDE on three different samples (Table 5). Recoveries of over 100% were obtained for dieldrin and pp' DDE which places some doubt on the accuracy of the total analytical scheme. The percent recoveries are fairly consistent and are thus indicative that results obtained are fairly repeatable. This in turn would indicate that the relative quantities of pesticide residues found are reliable.

Four chemical blanks were run during the monitoring series of analyses (Table 6). These values were subtracted from the pesticide residue values found in the unknown samples to correct for background contamination.

Pesticide residue levels were calculated for three collection sites for (1) early spring, (2) early summer, and (3) late summer and are listed in Tables 7, 8, and 9.

Student's "t" test was used as a standard to determine if significant differences in pesticide residue levels were found with respect to time (early spring, early summer, and late summer) or location (sites 1, 2, and 3). No significant differences were indicated for pesticide residue levels with respect to either time or location ($P > 0.05$).

Tables 10A and 10B give water quality and water level data taken periodically during the monitoring interval. No drastic changes in the aquatic environment are evidenced. General changes or trends that seem explainable can be observed; i.e., the increase in nitrate levels that occurs during April, May, and June could be attributed to local agricul-

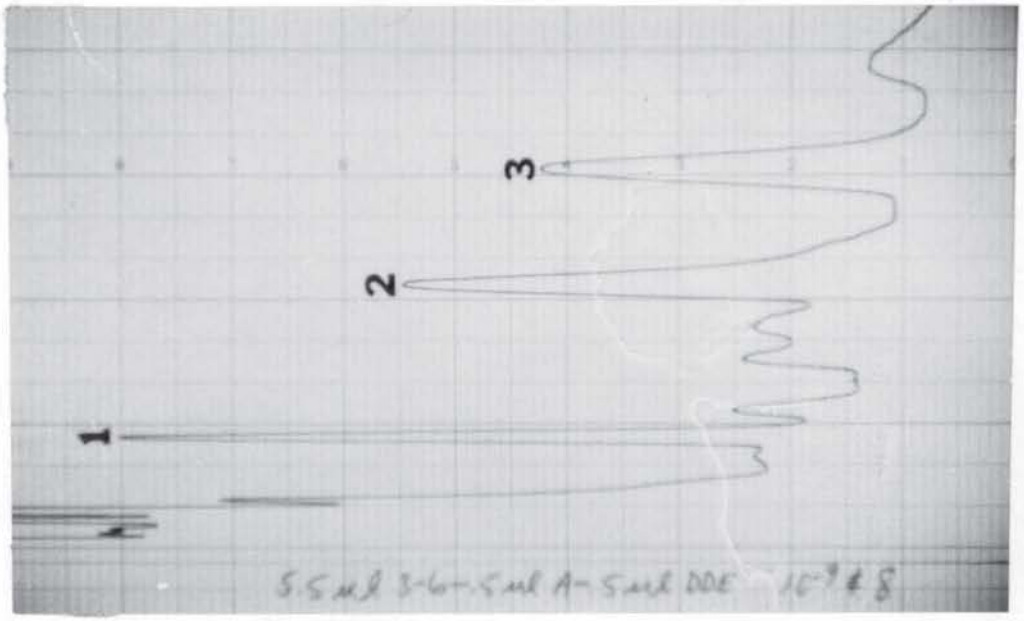
FIGURE 4. Verification of the presence of pesticides in a crayfish tissue sample from Polecat Creek, Coles County, Illinois (site 3, early summer) analyzed by electron capture gas chromatography. The sample is spiked with pesticides and the presence of these pesticides is confirmed by an increase in peak area and height of an existing peak.

A. Plain sample.

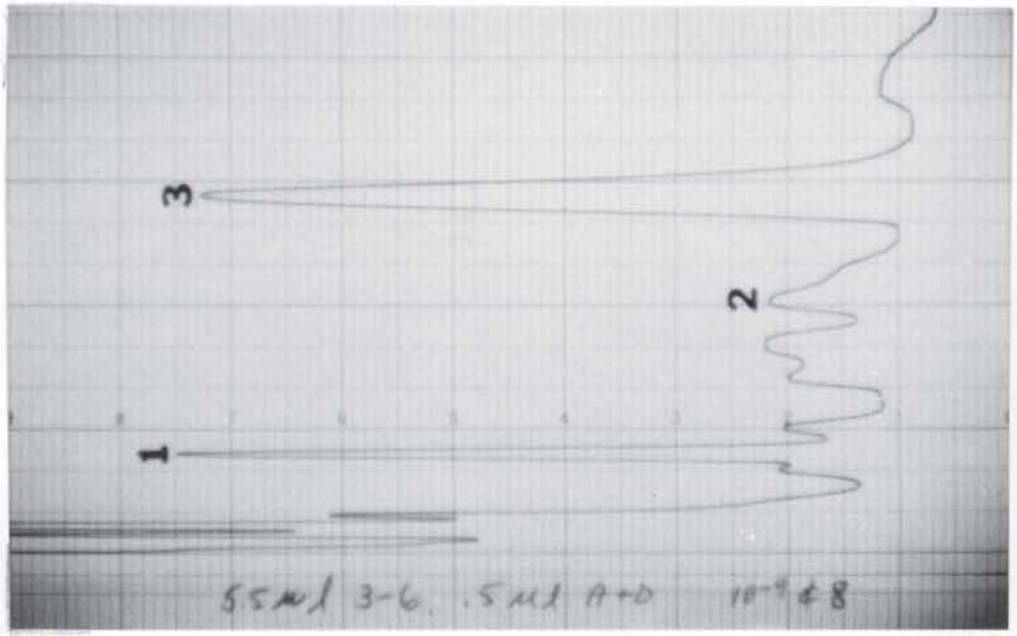
B. Sample spiked with aldrin (1) plus dieldrin (3).

c. Sample spiked with aldrin (1) plus pp' DDE (2).

C.


3
2
1
5.5 µl 3-6 .5 µl A- 5 µl DDE 10⁻⁹ # 8

B.


3
2
1
5.5 µl 3-6 .5 µl A+D 10⁻⁹ # 8

A.

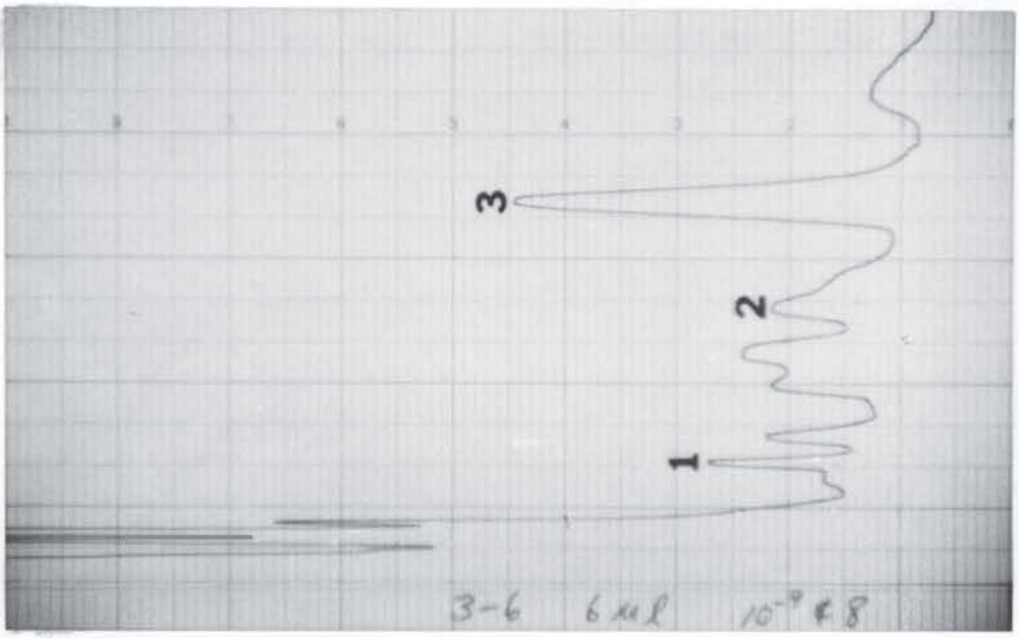
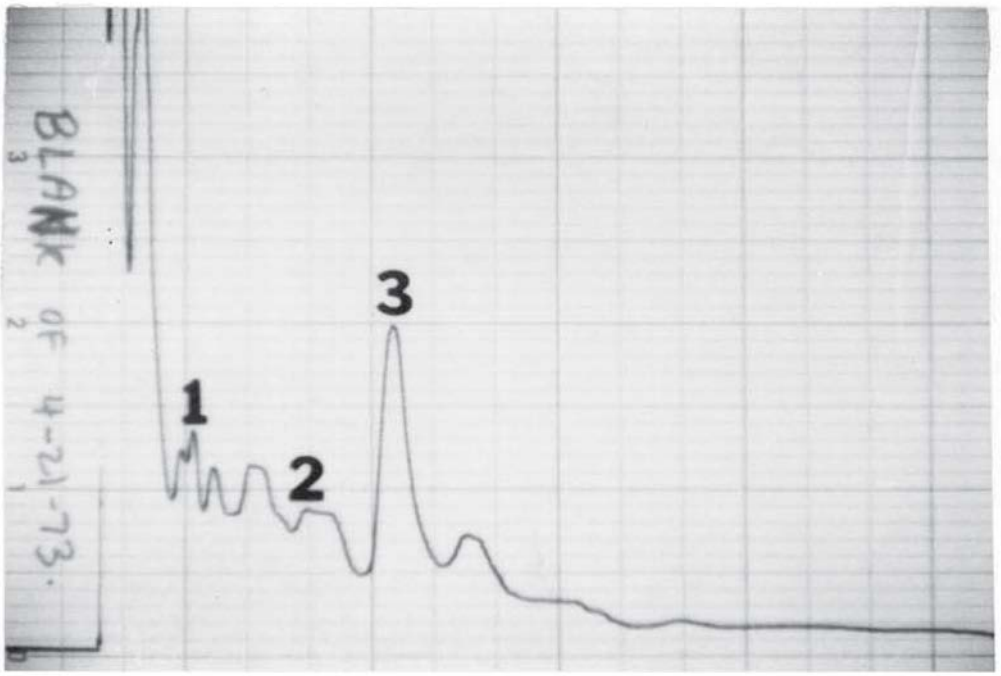

3
2
1
3-6 6 µl 10⁻⁹ # 8

FIGURE 5. Electron capture gas chromatograms of chemical blanks (i.e., normal pesticide extractions minus the crayfish tissue) to illustrate background contamination. The numbers 1, 2, and 3 demark the respective retention times of aldrin, pp' DDE, and dieldrin.

A. Initial blank.

B. Blank after all controllable sources of contamination were eliminated.

A.



B.

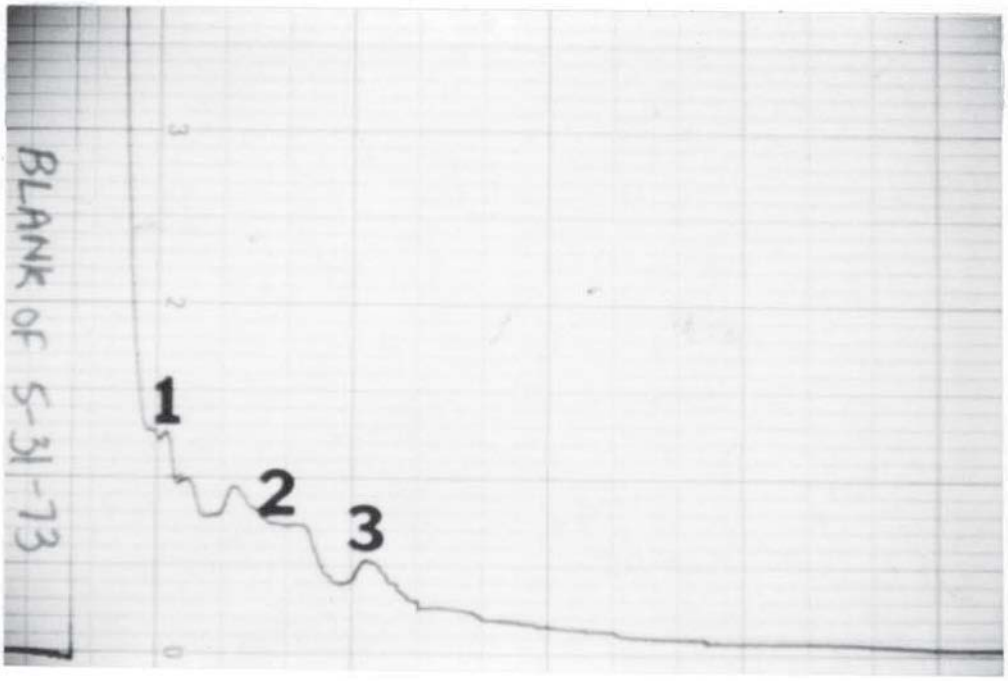
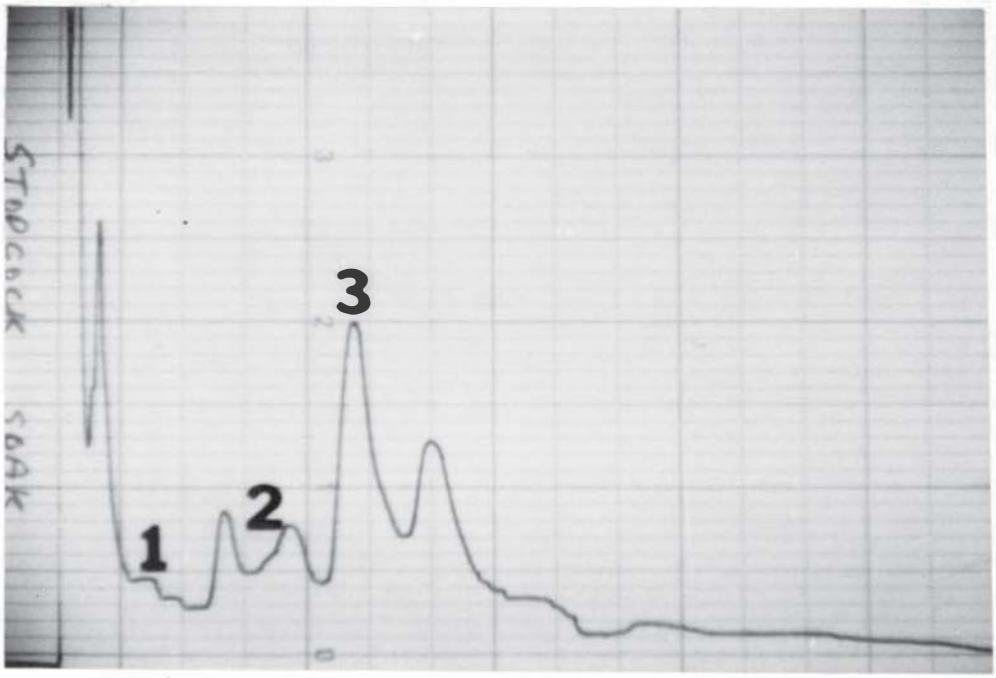


FIGURE 6. Electron capture gas chromatogram of residues obtained by soaking the teflon stopcock from the elution column used in the pesticide extraction in the solvent mixture used in the extraction. The numbers 1, 2, and 3 represent the respective retention times of aldrin, pp' DDE, and dieldrin.

FIGURE 7. Electron capture gas chromatogram of residues obtained by passing the extracting solvent mixture through a normally packed column minus the crayfish tissue and minus the teflon stopcock. The purpose was to see if the florisil is a source of contamination. The numbers 1, 2, and 3 represent the respective retention time of aldrin, pp' DDE, and dieldrin.


STOPCOCK SOAK
1
2
3

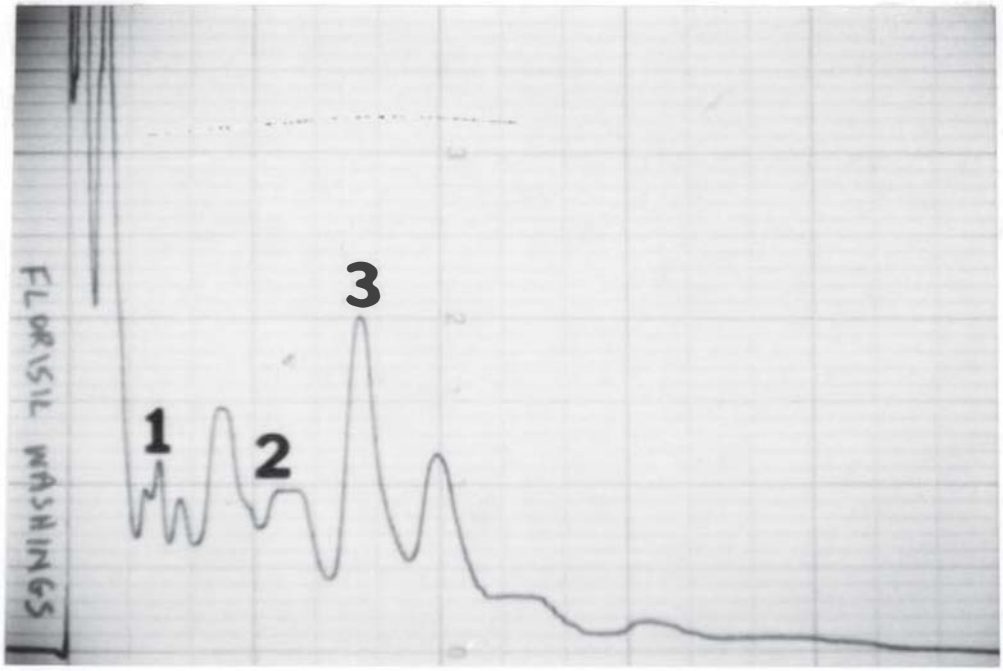
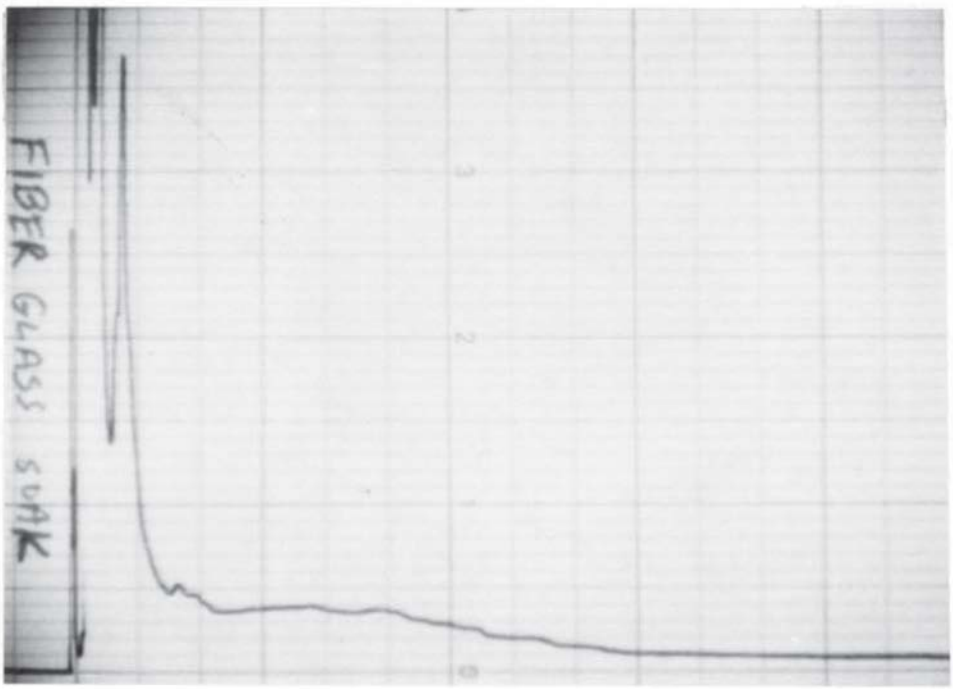

FLORISIL WASHINGS
1
2
3

FIGURE 8. Electron capture gas chromatogram of residues obtained by soaking fiber glass in the extraction solvent mixture. The fiber glass was used to plug the bottom of the pesticide extraction column.

FIGURE 9. Electron capture gas chromatogram of residues obtained by soaking glass wool (angel hair) in the extraction solvent mixture. The glass wool was used in place of fiber glass to plug the bottom of the pesticide extraction column.


FIBER GLASS SLAB

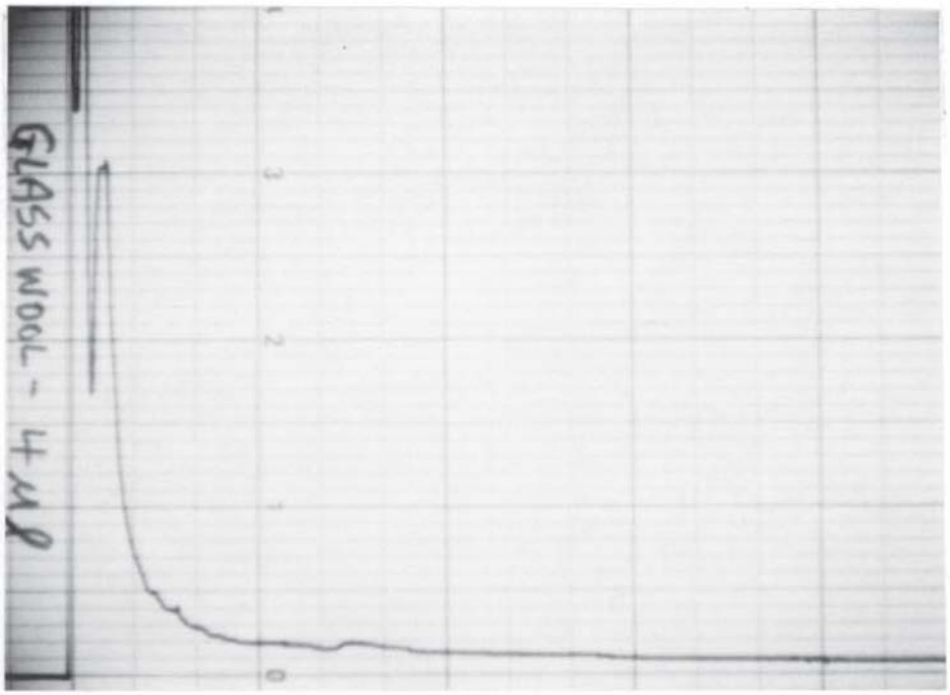
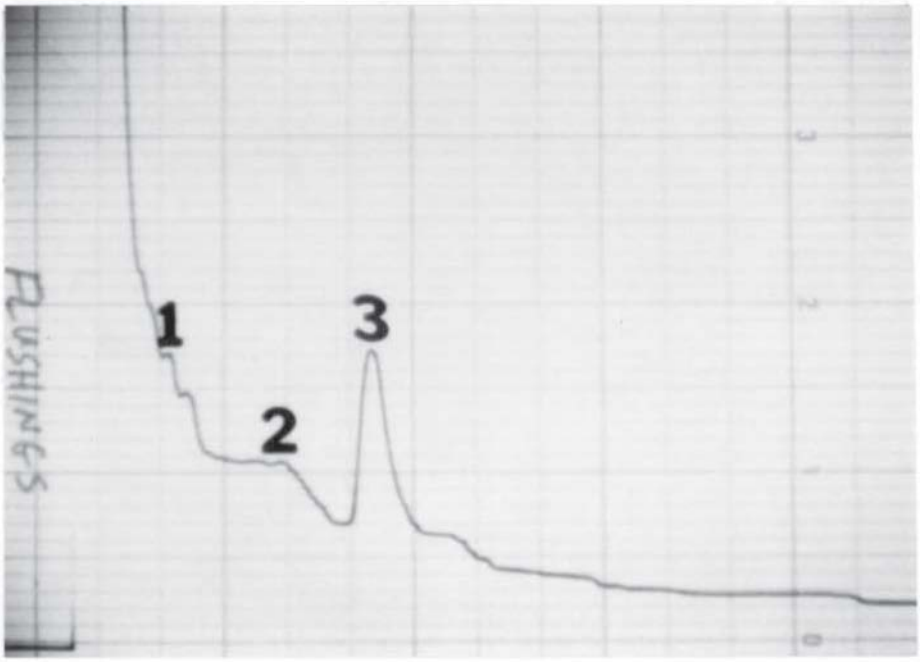

GLASS WOOL - 4 in

FIGURE 10. Electron capture gas chromatogram of residues obtained from the extraction solvent mixture after it had been used to flush the cleaned extraction glassware just prior to use in an extraction. The numbers 1, 2, and 3 represent the respective retention times of aldrin, pp' DDE, and dieldrin.

FIGURE 11. Electron capture gas chromatogram of residues obtained from the solvent mixture itself.


FLUSHINGS
1
2
3

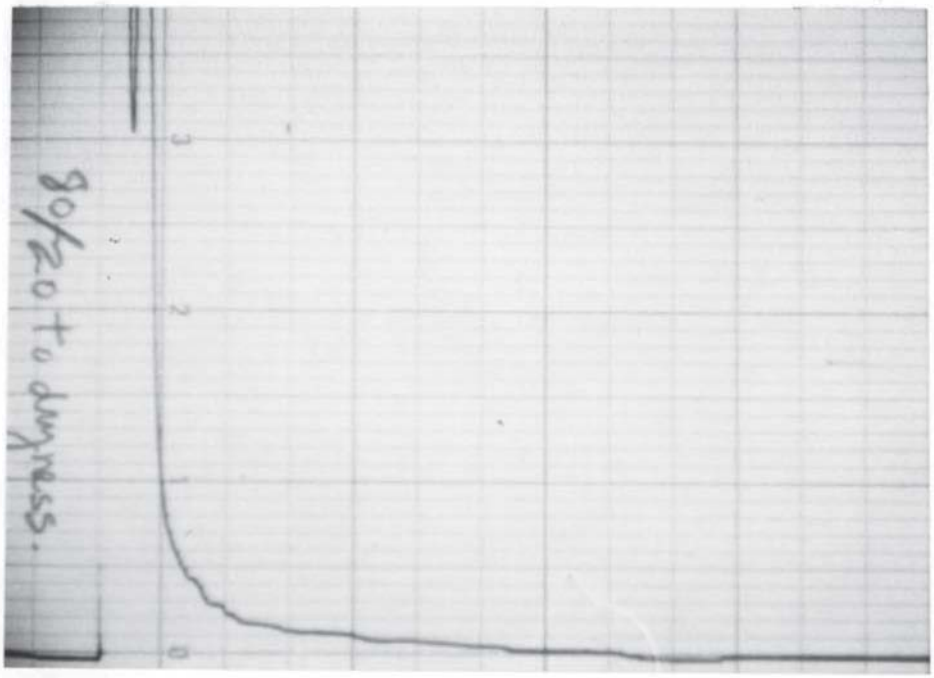

80/20 to dryness.

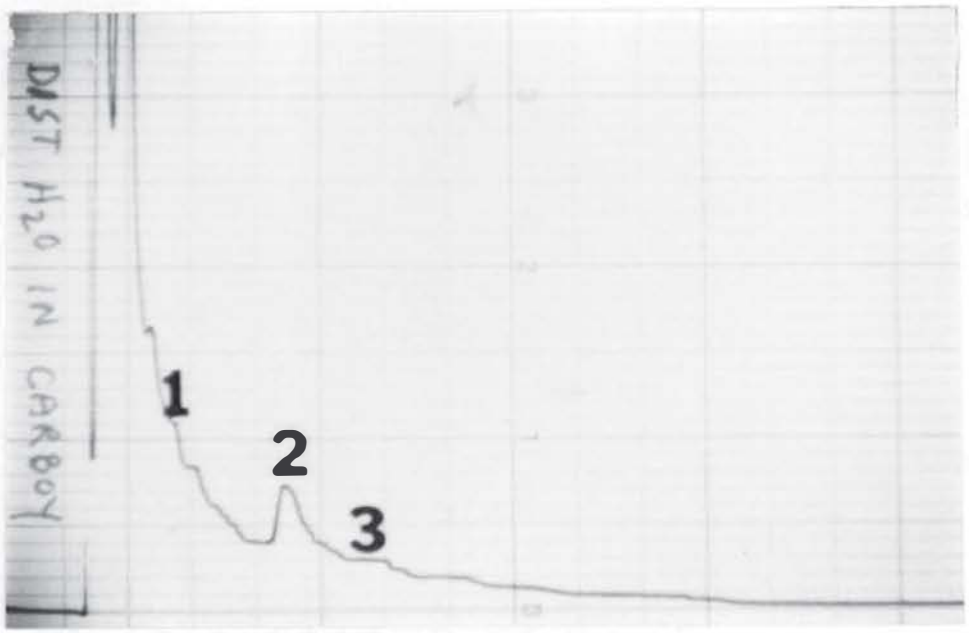
FIGURE 12. Electron capture gas chromatograms of residues obtained from the distilled water used to flush the extraction glassware after it has been acid cleaned. The numbers 1, 2, and 3 represent the respective retention times of aldrin, pp' DDE, and dieldrin.

A. Distilled water that has been stored in a plastic carboy prior to use.

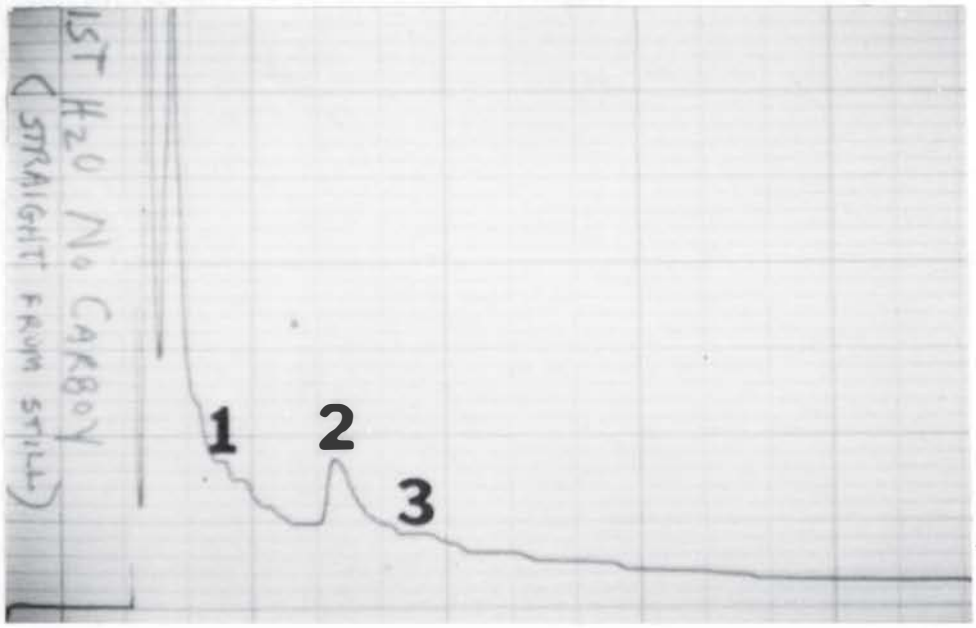
B. Distilled water collected straight from the still. Not stored in a plastic carboy.

C. Distilled water collected straight from the still with potassium permanganate added to the boiling pot to oxidize any organic molecules present in the water prior to distillation. Not stored in a plastic carboy.

A.


DIST H2O IN CARBOY
1
2
3

B.


1ST H2O No CARBOY
(STRAIGHT FROM STILL)
1
2
3

C.

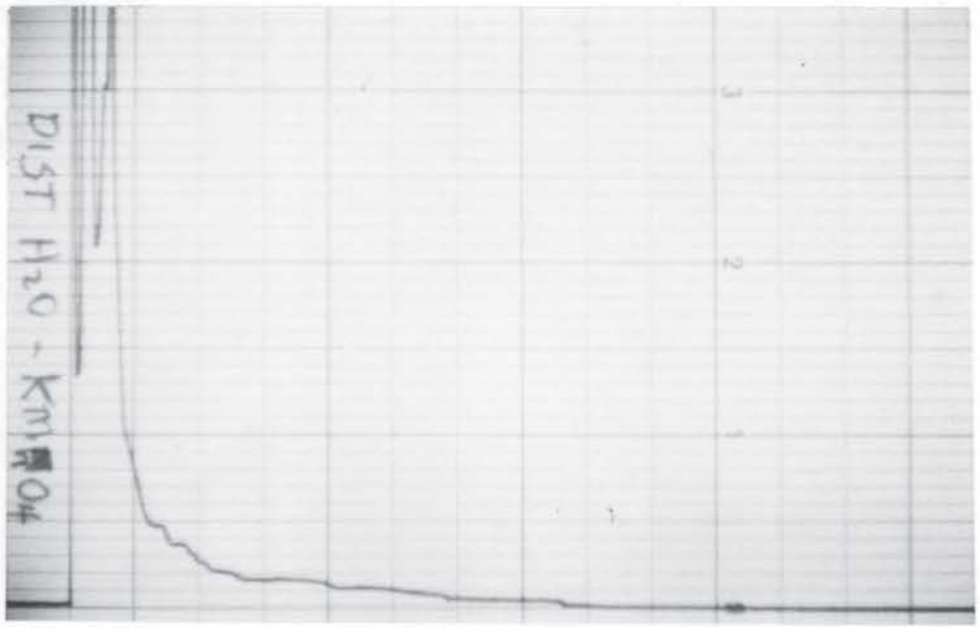

DIST H2O - KMnO4

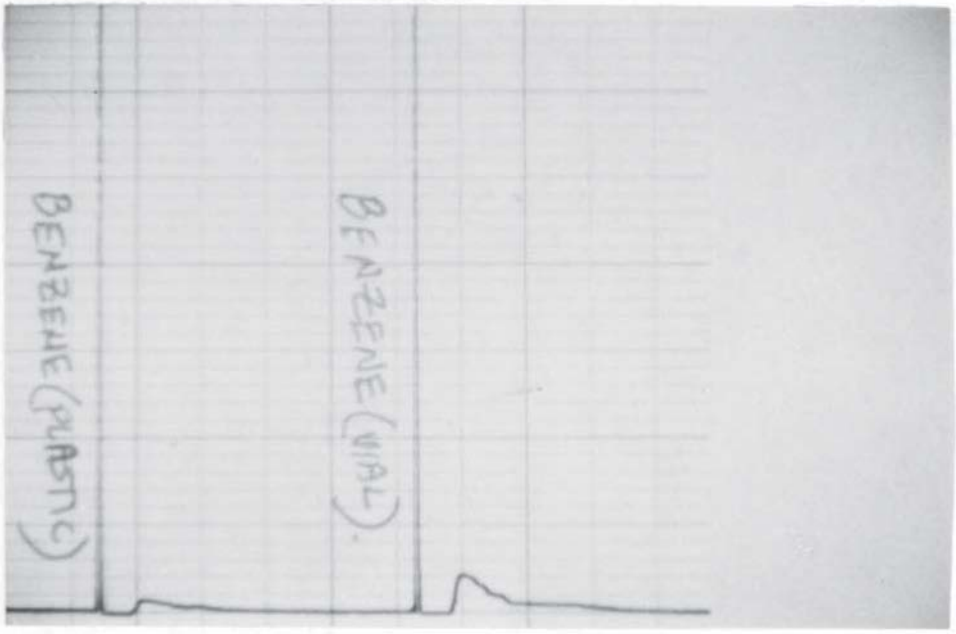
FIGURE 13. Electron capture gas chromatograms of residues obtained from nannograde benzene.

A. Benzene stored in a plastic squirt bottle and used for cleaning the gas chromatograph injection syringe.

B. Benzene stored in a "Mini-vial" to simulate the storage of a sample between the time of extraction and the time of analysis.

FIGURE 14. Electron capture gas chromatogram of the extraction solvent mixture stored in a "Mini-vial" to simulate the presence of the solvent mixture in the "Mini-vial" just prior to final dryness of the extraction eluant and the consequent presence of any solvent mixture residues in the "Mini-vial" after being redissolved in benzene.

A. B.



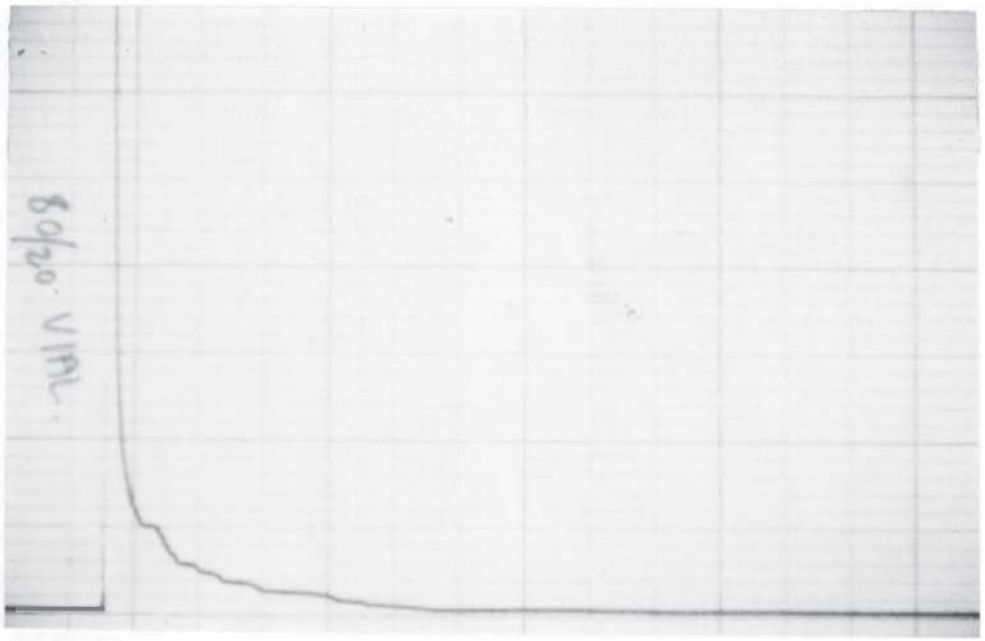
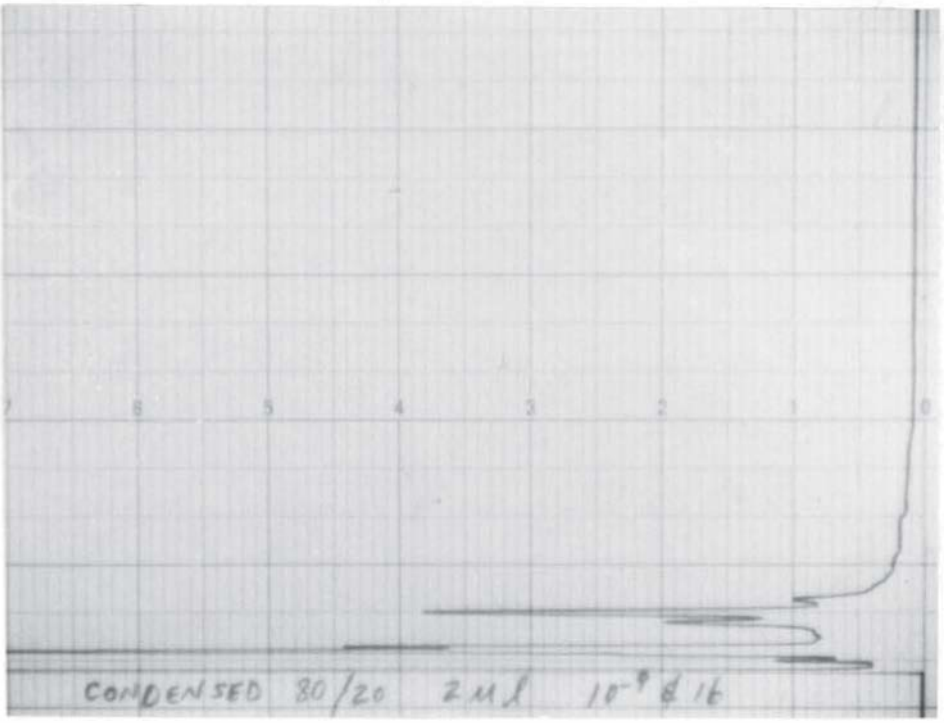


FIGURE 16. Electron capture gas chromatogram of a standard mixture of five pesticides. (1) 1 ppm lindane. (2) 1 ppm aldrin. (3) 2 ppm pp' DDE. (4) 2 ppm dieldrin. (5) 2 ppm pp' DDT.

FIGURE 15. Electron capture gas chromatogram of residues obtained from the condensed extraction solvent mixture after it had already been used in an extraction. The purpose is to see if the solvent mixture is free of carried over pesticides accrued during the drying operation using the Kuderna-Danish evaporative concentrator and thus suitable for re-use.


CONDENSED 80/20 2 µl 10⁻⁹ @ 16

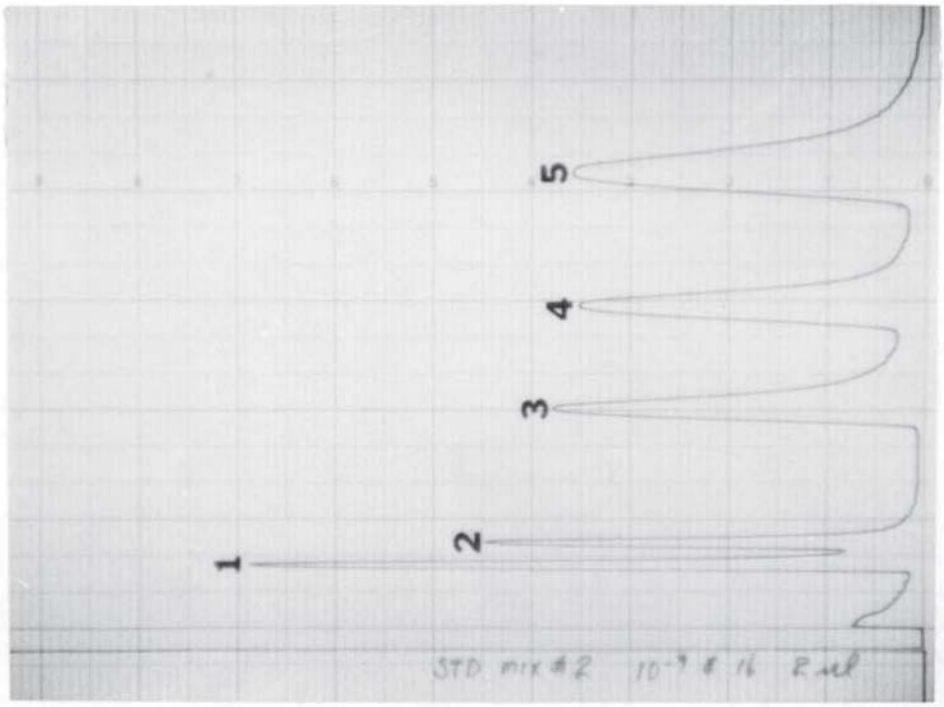

1
2
3
4
5
STD mix #2 10⁻⁹ @ 16 2 µl

TABLE 1. Crayfish collected at site 1, Polecat Creek, Coles County, Illinois.

Season	Number of specimens	Total wet weight of composite sample (gm)	Wet weight of specimens (gm)	Identification of specimens*
Early spring (March 20, 1972)	5	76.7	Lightest - 13.0 Heaviest - 18.3 Avg. wt. - 15.3	1 male - Procambarus blandingi (Girard) 4 females - unidentified
Early summer (June 4, 6, 1972)	5	95.0	Lightest - 9.4 Heaviest - 35.0 Avg. wt. - 19.0	2 males - Orconectes immunis (Hagen) 2 males - Orconectes 1 female - unidentified
Late summer (August 12, 1972)	11	52.2	Lightest - 3.8** Heaviest - 6.9 Avg. wt. - 4.7	2 males - Orconectes obscurus 2 males - Orconectes rusticus 4 females - Orconectes 2 males - Procambarus 1 female - Procambarus

*Genus identifications are positive, species identifications are uncertain. Only 1st form males can be positively identified. Thus identification of females is based on general overall appearance.

**These specimens were not all weighed individually. Specimens were selected by eye that appeared to represent the lightest and the heaviest specimens.

TABLE 2. Crayfish collected at site 2, Polecat Creek, Coles County, Illinois.

Season	Number of specimens	Total wet weight of composite sample (gm)	Wet weight of specimens (gm)	Identification of specimens*
Early spring (March 17, 1972)	5	54.0	Lightest - 8.8 Heaviest - 14.0 Avg. wt. - 10.8	5 males - Orconectes rusticus or propinquus
Early summer (June 9,10,11, 1972)	14	189.4	Lightest - 4.7 Heaviest - 43.4 Avg. wt. - 13.5	2 males - Orconectes virilis or obscurus 1 male - Orconectes propinquus 7 males - Orconectes 3 females - Orconectes 1 female - Cambarus
Late summer (August 12, 1972)	23	183.9	Lightest - 5.3** Heaviest - 12.7 Avg. wt. - 8.0	3 males - Orconectes obscurus 13 males - Orconectes propinquus 7 females - Orconectes

*Genus identifications are positive, species identifications are uncertain. Only 1st form males can be positively identified. Thus identification of females is based on general overall appearance.

**These specimens were not all weighed individually. Specimens were selected by eye that appeared to represent the lightest and the heaviest specimens.

TABLE 3. Crayfish collected at site 3, Polecat Creek, Coles County, Illinois.

Season	Number of specimens	Total wet weight of composite sample (gm)	Wet weight of specimens (gm)	Identification of specimens*
Early spring (March 20, 1972)	7	77.6	Lightest - 5.9 Heaviest - 15.0 Avg. wt. - 11.1	7 males - Orconectes rusticus or propinquus
Early summer (June 9,10,11, 1972)	10	127.1	Lightest - 7.2 Heaviest - 41.1 Avg. wt. - 12.7	1 male - Orconectes obscurus (Hagen) 2 males - Orconectes virilis (Hagen) 2 males - Orconectes 5 females - Orconectes
Late summer (August 11, 1972)	17	111.2	Lightest - 4.8** Heaviest - 11.1 Avg. wt. - 6.5	2 males - Orconectes propinquus 2 males - Orconectes rusticus 1 male - Orconectes virilis 1 male - Orconectes 11 females - Orconectes

*Genus identifications are positive, species identifications are uncertain. Only 1st form males can be positively identified. Thus identification of females is based on general overall appearance.

**These specimens were not all weighed individually. Specimens were selected by eye that appeared to represent the lightest and the heaviest specimens.

TABLE 4. Fat content of 10 gram portions of homogenized crayfish samples analyzed by modification of a method described by Mills (1959).

Sample Origin	Fat (gm)	Fat (w/o)
Site 2, Early summer	0.233	2.33
Site 2, Late summer	0.119	1.19
Site 2, Late summer	0.243	2.43
Site 1, Late summer	0.218	2.18

TABLE 5. Percent recoveries of aldrin, dieldrin, and pp' DDE from homogenized crayfish samples using a one step extraction and clean-up procedure of Langlois, Stemp, and Liska (1964).

Insecticide	Sample from site 1, Early summer	Sample from site 2, Early summer	Sample from site 3, Early summer
Aldrin	86%	75%	83%
Dieldrin	154%	124%	139%
pp' DDE	139%	116%	134%

TABLE 6. Pesticide residue levels found in chemical blanks that were run periodically during the monitoring series of extractions (N-4). Levels have been converted to their equivalents in crayfish tissue.

Insecticide	Mean (ppm)	Range	Standard deviation
Aldrin	0.00474	0.00294	0.00134
Dieldrin	0.0126	0.0142	0.00637
pp' DDE	0.00559*	0.00551	0.00294

*Only three blanks were used in this average. The chromatogram of the fourth appeared erroneous and was not counted.

TABLE 7. Aldrin levels (ppm) in homogenate samples of crayfish collected from Polecat Creek, Coles County, Illinois.

Collection period	Collection sites		
	1	2	3
Early spring	0.00193	0.00335	0.00173
Early summer	0.00150	0.00375	0.00077
Late summer	0.00453	0.00165	0.00208

TABLE 8. Dieldrin levels (ppm) in homogenate samples of crayfish collected from Polecat Creek, Coles County, Illinois.

Collection period	Collection sites		
	1	2	3
Early spring	0.0128	0.0132	0.0108
Early summer	0.0063	0.0235	0.0100
Late summer	0.0168	0.0129	0.0090

TABLE 9. pp' DDE levels (ppm) in homogenate samples of crayfish collected from Polecat Creek, Coles County, Illinois.

Collection period	Collection sites		
	1	2	3
Early spring	0.00434	0.00340	0.00386
Early summer	0.00234	0.00581	0.00301
Late summer	0.0117	0.00781	0.00701

TABLE 10A. Water quality parameters and relative water level data taken at three crayfish collection sites on Polecat Creek, Coles County, Illinois.

Parameters measured and relative water level	3-25-72			4-8-72			5-5-72		
	site 1	site 2	site 3	site 1	site 2	site 3	site 1	site 2	site 3
pH	8.1	8.2	8.1	6.7	6.9	6.8	8.3	8.2	8.2
Turbidity (JTU)	10	28	11	0	7	21	17	21	32
D.O. (ppm)	25.9	18.0	15.6	13.7	11.8	11.9	13.1	10.9	9.4
B.O.D. (5 day, ppm)	-	-	-	1.6*	2.7*	3.1*	-	-	-
NO_3 (ppm, includes NO_2)	39.6	30.8	35.2	68.2	41.8	39.6	77.0	46.2	55.0
PO_4 (ortho-, ppm)	0.03	0.00	0.05	0.06	0.06	0.05	0.05	0.08	0.05
Conductivity	210	220	230	230	220	225	260	250	240
Temperature (°C)	15.0	11.5	10.5	12.5	10.5	9.2	22.0	21.0	20.0
Relative water level**	-	-	+ 3	+ 12	-	+ 12	+ 10	-	+ 10

*These B.O.D. readings taken after 6 days.
**Based on a relative water level of "0" on 8-10-72.

TABLE 10B. Water quality parameters and relative water level data taken at three crayfish collection sites on Polecat Creek, Coles County, Illinois.

Parameters measured and relative water level	6-7-72			7-20-72			8-10-72		
	site 1	site 2	site 3	site 1	site 2	site 3	site 1	site 2	site 3
pH	8.2	8.3	7.8	8.2	8.7	8.6	8.3	8.6	8.5
Turbidity (JTU)	2	7	10	43	20	9	47	22	14
D.O. (ppm)	16.5	8.7	8.3	6.4	9.2	9.5	7.1	9.6	10.2
B.O.D. (5 day, ppm)	1.5	0.8	1.0	-	-	-	5.3	1.0	1.1
NO_3 (ppm, includes NO_2)	63.8	50.6	19.8	26.4	26.4	17.6	26.4	17.6	26.4
PO_4 (ortho-, ppm)	0.03	0.13	0.02	0.65	0.22	0.14	0.13	0.18	0.15
Conductivity	280	290	290	280	270	260	250	255	300
Temperature (^{o}C)	24.0	23.0	24.0	32.0	30.0	28.8	22.2	22.8	21.8
Relative water level*	+ 14	-	+ 6	+ 4	-	+ 2	+ 2	-	0

*Based on a relative water level of "0" on 8-10-72.

DISCUSSION AND CONCLUSIONS

The background contamination that occurred during the extractions was in the nannogram range (10^{-9}) (Table 6). If the pesticide residue levels found were in the microgram range (10^{-6}) this background level would be insignificant. The pesticide residue levels found in the crayfish tissue are also in the nannogram range thus making background contamination a crucial factor to evaluate and control. Various aspects of the extraction scheme and associated steps were analyzed in an effort to determine the source(s) of the contamination.

The elution columns used here for extractions were originally equipped with teflon stopcocks which were never acid cleaned but were washed with soap and water and flushed with solvent mixture after being placed on the column. One of the stopcocks was soaked in extraction solvent mixture and the mixture was analyzed for pesticide residues. Figure 6 demonstrates that this was a source of contamination especially with respect to dieldrin residues. It is possible that more thorough soaking with solvents before use could have eliminated this as a contamination source but to eliminate the variable the teflon stopcocks were replaced by glass stopcocks that could be acid cleaned. Bevenue et al. (1971a) soaked a teflon stopcock overnight in hexane and after concentration and gas chromatographic analysis concluded that the teflon was not wholly inert and did contain hexane-soluble contaminants (apparently in the 0.1 nannogram range) that were sensitive to an electron capture detector.

In order to evaluate Florisil as a contamination source extraction solvent mixture was passed through a normally packed column minus crayfish tissue and minus the teflon stopcock. The chromatogram shown in Figure 7 indicates that the Florisil is indeed a potential source of contamination. It should be kept in mind that some of the extraction glassware is also used in an evaluation such as this and the glassware itself might be the source or co-source of the contamination. Radomski and Rey (1970) mentioned constant problems with contamination primarily associated with the Florisil adsorption column when they were attempting to analyze pesticides in human and animal tissue by gas chromatography. Zweig (1967) states that a chemical blank from an extraction scheme will often contribute a series of peaks on a gas chromatogram and further states that studies have indicated that a cleanup column containing Florisil and sodium sulfate is a likely source for this contamination. To minimize the possibility of contaminants being introduced by the Florisil it should be flushed before use with a nominal amount of extraction solvent mixture (approx. 50 ml per 25 gm of Florisil) and the solvent mixture discarded. As a further preventive the bottom half of the column (pure Florisil) should again be flushed by running approximately 50 ml of solvent mixture through it before adding the Florisil-crayfish tissue mixture to the top of the column. Langlois et al. (1964) recommended a 50/50 mixture (pet. ether/meth. chl.) of solvents by volume for this Florisil flush.

An egg sized wad of the fiber glass used to prevent Florisil from running out the bottom of the elution column was soaked in extraction solvent mixture and the solvent mixture was analyzed for residues (Figure 8). The fiber glass was a minor source of contamination.

Glass wool (angel hair) was analyzed in a similar manner (Figure 9) and contributed almost no background contamination at all. Glass wool is recommended as a plugging material in place of fiber glass.

Just prior to use in an extraction the glassware was flushed with extraction solvent mixture. These flushings were collected and analyzed for pesticide residues (Figure 10). Relatively large amounts of pesticide residues were found in the flushings. The acid soaking may leave pesticide residues on the glassware and may not be an adequate cleansing procedure for the kind of study undertaken here. Lamar, Goerlitz, and Law (1965) recommend heat treating glassware at 300°C overnight (after conventional cleaning) to rid the glassware of contaminants. The temperature to be used and the duration may be arbitrary but if equipment is available it is certainly an avenue to be explored. Glassware with odd configurations may be subject to breakage from stresses from heat.

Three hundred ml of the extraction solvent mixture (80/20, pet. ether/meth. chl.) were analyzed to see how much of the background contamination might be attributed to it (Figure 11). Detector response to residues derived from the solvent mixture were minimal in the areas where pesticide peaks might be observed even at what is apparently a nannogram level.

Distilled water used to flush the glassware after acid soaking was analyzed. Distilled water stored in a plastic carboy, and distilled water collected directly from the still with and without potassium permanganate in the boiling pot were compared (Figure 12). A variable was inadvertantly introduced into this comparison and should be mentioned. In the two evaluations not utilizing potassium permanganate in the still boiling pot, anhydrous sodium sulfatew as used to

absorb any water present prior to concentration and analysis. The anhydrous sodium sulfate was not used when the evaluation of the use of potassium permanganate was made. The possibility exists that the anhydrous sodium sulfate might have been a contaminant source itself. Aside from this the use of potassium permanganate in the boiling pot to oxidize any organic molecules present appears to be quite effective especially with reference to a background peak that corresponds to pp' DDE. Lamar et al. (1965) recommend the use of an alkaline permanganate in conjunction with an all glass still to obtain organic-free distilled water. Bevenue et al. (1971a) also advocate the use of potassium permanganate in the still boiling pot in addition to an all glass system.

Three other associated factors that are not a part of the extraction scheme proper were checked as possible sources of contamination; (1) benzene stored in a plastic squirt bottle and used for cleaning the gas chromatographic injection syringe, (2) benzene stored in a "Mini-vial" to simulate the storage of a sample between the time of extraction and the time of analysis, and (3) extraction solvent mixture stored in a "Mini-vial" to simulate the presence of the solvent mixture in the "Mini-vial" just prior to final dryness of the extraction eluant and the consequent presence of any solvent mixture residues in the "Mini-vial" after being redissolved in benzene. As seen in Figures 13 and 14 none of these three factors are a source of contamination.

Lamar et al. (1965) suggest as another possible source of contamination the plastic screw caps or plastic cap liners used on sample bottles and reagent bottles. Thus any plastic materials associated with the analytical scheme or used in the packaging of materials should

be viewed with suspicion with respect to being possible sources of contamination. Bevenue, Kelley, and Hylin (1971b) suggest that glassware with ground glass sections are another contamination source in that the ground glass areas would be difficult to clean even with heating. Neither of these possible contamination sources were investigated in this study.

Condensed vapors of solvent mixture used in an extraction were analyzed for pesticides to see if the solvent mixture was suitable for re-use (Figure 15). There is no evidence of pesticides carried over and the solvent mixture is apparently suitable. The question of whether the relative proportions of the two solvents in the mixture has changed is unanswered. Figure 16 is used to illustrate the type of chromatogram obtained from a standard mixture of five pesticides.

Crayfish used in this study were of three genera and apparently several species (Tables 1, 2, and 3). They were not plentiful enough to restrict samples to one species or even to one genus. It is assumed that the method of uptake and the ability to concentrate pesticides is similar for freshwater crayfish and justifies the grouping of these three genera into homogenate samples for use as indicators.

The one step extraction procedure used here (Langlois et al., 1964) imposes a limitation of one gram of fat on whatever size sample is being extracted. Fat analysis of ten gram portions of crayfish tissue used in this study (Table 4) show that homogenate samples of whole crayfish are well under this limitation, i.e., 0.203 ± 0.084 gm (N = 4).

Percent recoveries of aldrin, dieldrin, and pp' DDE added to three different samples were obtained (Table 5). Recoveries of over

100% for dieldrin and pp' DDE may cast some doubt on the accuracy of the quantities of pesticide residues found but the results seem fairly consistent which is indicative of fair repeatability. This in turn indicates that relative comparisons of pesticide residues found would be reliable. Langlois et al. (1964) and Hogan (1972) utilized the same extraction method as in this study. Langlois et al. (1964) reported percent recoveries for dieldrin of approximately 92% (approx. 47% lower than the results of this study). Hogan (1972) reported on the same three pesticides as this study but found about an 8% lower recovery for aldrin, a 14% lower recovery for dieldrin, and about a 40% lower recovery for pp' DDE. The percent recovery found for aldrin in this study seems proper but no explanation can be offered at this time for the high percent recoveries of dieldrin and pp' DDE.

Crayfish were collected at three different sites in early spring, early summer, and late summer. The crayfish tissue was kept frozen for approximately one year prior to analysis during which time the tissue had been inadvertently thawed three or four times resulting in some fluid loss from the tissue. Some investigators have kept tissue frozen for extended periods of time prior to pesticide extraction (Dimond et al., 1968; Dimond, Getchell, and Blease, 1971) while others (Spencer, 1967) advocate shortening the period between collection of sample and chemical analysis due to conversion of pesticides like DDT to metabolites.

Aldrin, dieldrin, and pp' DDE were found in all samples in the study. Heptachlor epoxide was identified in several samples as were peaks that corresponded to either heptachlor or lindane. No pp' DDT was found. pp' DDE is the metabolite of pp' DDT and the explanation

of its presence alone is conjecture. Bridges et al. (1963) studied the persistence of DDT and its metabolites in a farm pond and found that within four months DDT had essentially disappeared from crayfish and after eleven months the metabolite DDE was the only component found in significant amounts. There may be a parallel in this study. An alternate explanation is that a high metabolite level and a low parent insecticide level may come about by accumulation via the food chain (Butler, 1969).

Pesticide levels found here averaged in the 2 nannogram area for aldrin (Table 7), and 13 nannogram area for dieldrin (Table 8), and the 5 nannogram area for pp' DDE (Table 9). Hogan (1972) conducted a similar study on Polecat Creek in 1971 using the clam Amblema plicata as an indicator. Two of the collection sites in this study were the same as two of the three collection sites in his study. His collections started at about the time of summer when collections in this study terminated. He found pesticide levels averaged in the 36 nannogram area for aldrin, the 47 nannogram area for dieldrin, and in the 43 nannogram area for pp' DDE. The pesticide residue levels found by Hogan were several fold higher than the pesticide levels found in this study. Water quality data taken during this study (Tables 10A and 10B) generally agree with the environmental data taken during his study. There were temperature changes that corresponded to the season and higher turbidities in some areas but in neither study was there any drastic alteration of the aquatic environment. It would seem then that, barring much speculation, the difference in pesticide levels found is more attributable to the different indicator organisms used than anything else. Several investigators have assayed crayfish tissue

for pesticide residues under a variety of situations and conditions. Some of the higher pesticide levels reported were by Dimond et al. (1968) who found 2.725 ppm of combined DDT and metabolites in crayfish collected from streams sprayed in the year of analysis, and by Meeks (1968) who found 3.8 ppm (avg.) of DDT (and metabolites) in crayfish obtained from a marsh treated with DDT. Various pesticide levels from these down to less than 0.001 ppm of aldrin and dieldrin (Hannon et al., 1970) have been reported. When crayfish have been compared to other organisms in the same study the pesticide levels in crayfish were comparable to other invertebrates (i.e., scuds, dragonfly naiads, damselfly naiads, backswimmers, bloodworms, and pond snails) (Meeks, 1968). Red leeches though accrued notably higher levels than the other invertebrates (Meeks, 1968). Fish seem to accumulate higher pesticide levels than crayfish (Cole et al., 1967; Cope, 1966; Bridges et al., 1963; Hannon et al., 1970). No studies were found where freshwater crayfish and freshwater clams were directly compared but the comparison of this study to the results of Hogan's (1972) study in 1971 is highly indicative that clams concentrate pesticide residues to an appreciably higher degree than crayfish.

Pesticides can be taken up by aquatic organisms either through the food web or directly from the water. It is open to question as to which is the major pathway. Chadwick and Brocksen (1969), investigated dieldrin uptake by sculpins via both pathways and concluded that accumulation directly from the water was the major uptake pathway except maybe when the pesticide is present in the water for only a short period of time or if the concentration is extremely low. Macek and Korn (1970) on the other hand investigated the uptake of DDT by

brook trout and concluded that the food chain was the major uptake pathway primarily because there is generally a higher concentration of DDT in the food web than in the water. Crayfish are omnivorous feeders (Crocker and Barr, 1968) and would be likely to accumulate pesticides from several sources via the food web.

It is taken as general knowledge that erosion and runoff are major means by which organic pesticides may enter streams, ponds, or lakes (Epstein and Grant, 1968). It was hoped that this study would support this belief if low pesticide levels were found in the early spring before the heavy spring rains with higher levels then being found in early summer or late summer. No such support was found. Student's "t" test was used to compare samples from early spring, early summer, and late summer but no significant differences were found in levels of pesticide residues between any of these three seasons. It was also hoped that differences in residue levels could be found between site 1 (silty bottom) and sites 2 and 3 (sand-gravel-rock bottoms). Student's "t" test was used again but as before no significant differences were found in pesticide residue levels between the three different sites.

Polychlorobiphenyls (PCB's) and related compounds are a group of industrial chemicals with a multitude of applications that are of growing interest with respect to pesticide residue analysis. They are not used as pesticides but because of their similarities in structure and properties to the DDT pesticide group they can (if present) be carried through the usual extraction and screening procedures, and since they possess electron absorbing properties, will interfere with electron capture gas chromatographic analysis of organochlorine compounds

(Reynolds, 1969). It seems that PCB's are becoming as ubiquitous as DDT and Maugh (1973) reports on a theory proposed by K. W. Moilanen and D. G. Crosby whereby PCB's may be a breakdown product of DDT via irradiation with ultraviolet light of the same wavelengths present in sunlight in the lower atmosphere. No efforts were taken in this study to identify any polychlorobiphenyl compounds and there were no indications of interference by any such compounds.

In summary the major sources of background contamination were found to be teflon stopcocks, Florisil, and residues on "cleaned" glassware. Distilled water used in flushing glassware should also be considered a potential contamination source. Aldrin, dieldrin, and pp' DDE were identified and quantitated in all samples tested. Heptachlor epoxide was identified in several samples as were peaks that corresponded to either heptachlor or lindane. No pp' DDT was found. No significant differences were found in pesticide levels of aldrin, dieldrin, or pp' DDE with respect to time or location.

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EXAMINATION CERTIFICATE

Master's Degree Certificate
for
Comprehensive Examination

I certify that Robert C. Vanderjack has successfully passed a comprehensive examination.

The examining committee consisted of:

Signatures of the Committee

11 September 1973
DATE