REPORT FOR SECOND YEAR OF THE PROJECT ON DEVELOPMENT AND IMPLEMENTATION OF ANALYTICAL METHODOLOGY TO DETERMINE NONPOINT-SOURCE POLLUTION-RELATED PESTICIDES IN FISH AND MUSSEL TISSUE AND IN SEDIMENTS FROM THE TENNESSEE RIVER

WORK ACCOMPLISHED BY WRIGHT STATE UNIVERSITY
UNDER TENNESSEE WILDLIFE RESOOURCES AGENCY
CONTRACT NO. GU-0-01830-0-00

Prepared By

Thomas O. Tiernan, Daniel J. Wagel, Garrett F. VanNess,
Joseph G. Solch, John H. Garrett, Carl Reynolds and Mark S. Hanes
Department of Chemistry and Toxic Contaminant Research Program
175 Brehm Laboratory
Wright State University
Dayton, Ohio 45435

Submitted To

Aubrey D. McKinney, Chief
Environmental Services Division
Tennessee Wildlife Resources Agency
Ellington Agricultural Center
P.O. Box 40747
Nashville, Tennessee 37204

January 6, 1991

TABLE OF CONTENTS

I. INTRODUCTION

During the second year of work under Contract No. GU-0-01830-0-00 with the Tennessee Wildlife Resources Agency, Wright State University has continued to develop and apply analytical methodology to measure a specified set of pesticides and related compounds in mussel and fish tissue samples from various waterways which were provided by the Agency. This report describes the results of the studies conducted by Wright State during the second year of the contract.

II. SUMMARY OF RESULTS OF THE FIRST YEAR OF THE PROJECT

As a prelude to describing the achievements during the second year of the project, it is useful to summarize the results of the first year of this program. The goal of this effort was to develop an overall analytical scheme capable of measuring the compounds specified by the Tennessee Wildlife Resources Agency in mussels, fish and sediments. The compounds specified as target analytes included, (1) the thirty-seven (37) chemicals which were monitored using the previously developed methods which were applied in the U.S. EPA's National Bioaccumulation Study; (2) twenty-four (24) additional pesticides, for which concerted methodology had not been demonstrated; (3) PCBs, for which Wright State analytical methods were already available; and (4) 2,3,7,8-TCDD and 2,3,7,8-TCDF, for which Wright State methods were also already available. All of these target analytes are listed in Table 1 of Attachment 1 to this report. During the first year of the project, the previously demonstrated methodologies just mentioned for items (1), (3) and (4) were successfully applied to mussel tissues, and since these procedures had already been applied in other studies for the analyses of fish and sediments, no further methods development was required for these analytes. Therefore, the major developmental efforts during the first year of the project were focused on the 24 additional pesticides for which concerted analytical methodology was not available. Specific accomplishments relating to this task during the first year of the project included the following:

A. It was demonstrated that 10 of the 24 pesticides (Diethyl Phthalate, Di-n-Butyl Phthalate, Atrazine, Metolachlor, Diazinon, Methyl Parathion, Ethoprop, Disulfoton, Simazine and Hexazinone) could be extracted from mussel tissue by using Soxhlet extraction with methylene chloride/hexane, and that these could be separated from the lipid fraction using Gel Permeation Chromatography (GPC), and quantitated using coupled Gas Chromatography-Mass Spectrometry (GC-MS). It

was also shown that some, but not all of these ten compounds could be efficiently recovered when the sample extract was subjected to silica gel column chromatography cleanup, following the GPC separation. Those compounds which were irreversibly sorbed on silica gel were successfully determined by GC-MS in a fraction of the extract which had not been subjected to silica gel cleanup. These analytical procedures, which are quite similar to those applied to measure 37 target analytes in the National Bioaccumulation Study protocol, were described in detail in the annual report for the first year of the project.

B. It was also shown that 9 of the remaining 14 pesticides (24 minus the ten discussed in item A above) could be derivatized with a trifluoroacetic anhydride/ trifluoroethanol (TFAA/TFE) procedure and that the derivatives could be successfully analyzed by GC-MS. These pesticides were Benzoic Acid, Carbofuran, 2,4-D, Dinoseb, Acifluorfen, Carbaryl, Endothal, Picloram and Glyphosate. Again, the procedures implemented were described in detail in the project's first year annual report. It remained to be demonstrated at the end of the first year of the project that these compounds could be efficiently extracted from the mussel tissue matrix and separated from the lipid fraction using GPC.

C. It was shown that the five remaining pesticides (Alachlor, Cyanazine, Acephate, Diquat Dibromide and Fluometuron) could not be derivatized using the TFAA/TFE procedure and were not amenable to measurement using the procedures applied in the National Bioaccumulation Study protocol. No alternative procedures had been developed at the conclusion of the first year of the project.

III. CONTINUATION OF ANALYTICAL METHODOLOGY DEVELOPMENT FOR PESTICIDES REMAINING TO BE SUCCESSFULLY MEASURED - RESULTS OF THE SECOND YEAR OF THE PROJECT

As a follow-on to the methodology development tests accomplished during the first year of the project, a series of experiments were conducted during the second year which, it was hoped, would culminate in the final analytical scheme for measuring all target analytes. These experiments are described in the following subsections. In most of these experiments, GC-MS was the instrumental analytical technique utilized and the GC-MS instrumentation, operating parameters and calibration procedures were the same as those which were described in Section II.D. of our annual report for the first year of this project.

A. Evaluation of the Efficiency of Various Extraction Processes, Followed by GPC, for Recovery from Spiked Mussel Tissue of the Nine Pesticides Which Are Effectively Derivatized by TFAA/TFE and the Derivatives Measured by GC-MS (Benzoic Acid; Carbofuran; 2,4-D; Dinoseb; Acifluorfen; Carbaryl; Endothal; Picloram; and Glyphosate).

As a continuation of the experiments summarized in Section II.B. above, various extraction procedures were evaluated (followed by GPC fractionation) to determine their efficiency for recovery of the nine pesticides amenable to TFAA/TFE derivatization and GC-MS quantitation.

1. Soxhlet Extraction of Spiked Mussel Tissue With Methylene Chloride/Hexane (1/1). This procedure was initially tested since it is the same procedure used to extract the 37 National Bioaccumulation Study analytes, and if it proved effective for the nine additional pesticides, then a separate aliquot of the former extract could be removed, derivatized and analyzed. This would reduce the number of separate extractions required and the quantity of sample needed for the overall analyses. The procedures used in these tests are described below.

a. Prepare triplicate aliquots by transferring 20.00 g of control mussel tissue TENOC-4 to each of three 400 mL beakers.

b. Add purified sodium sulfate to each beaker and thoroughly mix the sodium sulfate with the tissue; continue adding sodium sulfate and mixing until the mixture is dry and free flowing.

c. Transfer the three dry samples to separate cleaned Soxhlet extraction thimbles.

d. Add 25 µL of surrogate standard 109097-3 (see Appendix A, Table 1 of this report) to each mussel tissue sample and to a laboratory blank, LB09200-1, which is an aliquot of the Soxhlet extraction solvent.

e. Add the native pesticides to the three mussel tissue samples in the indicated quantities:

TENOC-4F-S	25 µL 146094-1 and 12.5 µL 146094-3 (see Appendix A, Table 7). (6250 ng each pesticide)
TENOC-4G-S	50 µL 146094-1 and 25 µL 146094-3 (12500 ng each pesticide)
TENOC-4H-S	250 µL 146094-1 and 125 µL 146094-3 (62500 ng each pesticide)

f. Extract each sample for 16 hours in a Soxhlet apparatus, using hexane/methylene chloride (1/1) as the refluxing solvent.

g. Concentrate each extract and the blank solution to a volume of approximately 5 mL in a KD apparatus and then transfer each concentrated solution to a separate tared 15 mL vial.

h. Reserve 25% of each concentrated extract for other uses, and concentrate the remaining portion of each extract to a volume of approximately 2 mL.

i. Filter the latter extract portions through separate syringe filters, rinse the filters, and reconcentrate each filtrate and combined rinsate to a volume of approximately 2 mL.

j. Chromatograph the concentrated and filtered extracts on the GPC column, using procedures described previously.

k. Concentrate each of the collected GPC eluate fractions to a volume of approximately 2 mL and then transfer the concentrated solution to separate tared 3 mL vials.

l. Reserve 25% of each concentrated GPC eluate (based on the original extract volume), and transfer the remaining eluate portions to separate 1 mL vials.

m. Add 25µL of the Reaction Surrogate Solution, 109142-17 (see Appendix A, Table 4) to each vial and concentrate the solutions in the vials further, to minimum volumes.

n. Add 200 µL TFAA and 100 µL TFE to each vial.

o. Heat the vials to a 100°C water bath for 1 hour, and then concentrate the liquids in the vials to minimum volumes.

p. Add 180 µL of toluene and 20 µL of Internal Standard Solution 109142-12 (see Appendix A, Table 3) to each vial.

q. Analyze an aliquot of the solution in each vial using GC-MS.

The results of the analyses of the derivatized extracts of the nine pesticides are summarized in Table 2 of Attachment 1 to this report. The percent recoveries which are indicated by these data are excessively large for four of the pesticides evaluated, and quite low for four other pesticides. Moreover, the identifications of the mass chromatographic peaks detected, as compared to the pesticide standards in the mass spectral library, and as indicated by the "Fit" parameters shown in parentheses beside the percent recoveries in Table 2, are unacceptably low (<0.50) for most of the peaks detected. It must be concluded that the peaks detected are due primarily to interfering components in the derivatized sample extracts, and that recoveries of the pesticides using these methods are

unacceptable. Since it was shown previously that this set of pesticides could be efficiently derivatized by using TFAA/TFE and the derivatives could be detected by GC-MS, it must be further concluded that Soxhlet extraction with 1/1 methylene chloride/hexane is not effective, and/or the pesticides are lost in the course of GPC fractionation. Consequently, this procedure was not investigated further. Copies of the Intralaboratory Sample Tracking Forms and GC-MS Quantitation reports resulting from preparation and analyses of the samples generated in these tests are provided in Appendix B to this report.

2. Soxhlet Extraction of Spiked Mussel Tissue With Ethyl Acetate. Soxhlet extraction with a more polar solvent, ethyl acetate, was next evaluated in an attempt to improve recoveries of the nine pesticides amenable to TFAA/TFE derivatization and GC-MS analyses. In addition, the pH of the samples was adjusted prior to extraction so that most of the pesticides would not be in the ionized state. The procedures implemented are described below.

a. Prepare triplicate aliquots by transferring 10.0 g of control mussel tissue Sample TENOC-1 to each of three 400 mL beakers.

b. While mixing the tissue, slowly add concentrated HCl to the tissue in each beaker until the pH ≤2.

c. Add purified sodium sulfate to each beaker and thoroughly mix the sodium sulfate with the tissue; continue adding sodium sulfate and mixing until the mixture is dry and free flowing.

d. Transfer the three dry samples to separate clean Soxhlet extraction thimbles and add 25 µL of Surrogate Standard Solution 109142-14 (see Appendix A, Table 3) to each mussel tissue sample, and to a laboratory blank, 1B-B, which is an aliquot of the Soxhlet extraction solvent.

e. Add the native pesticides to the three mussel tissue samples in the indicated quantities:

TENOC-1C-S	25 µL 109142-19 and 50 µL 109142-22 (see Appendix A, Table 3) (6250 ng each pesticide
TENOC-1D-S	125 µL 109142-19 and 31.3 µL 109142-21 (see Appendix A, Table 3) (31250 ng each pesticide)

f. Extract each sample for 16 hours in a Soxhlet apparatus, using ethyl acetate as the refluxing solvent.

g. Concentrate each extract and the blank solution to a volume of approximately 5 mL in a KD apparatus and then transfer each concentrated solution to a separate tared 15 mL vial.

At this point in the procedure, it was found that the volume of the extracts could not be reduced below about 5 mL using either Kuderna-Danish (KD) or conventional nitrogen blow-down concentration. Moreover, the concentrate at this stage exhibited a very strong acetic acid order, suggesting that the ethyl acetate had decomposed to ethanol and acetic acid in the course of heating in the Soxhlet apparatus. Further processing of the samples was terminated since the acidic solution would have been expected to damage the GPC column.

3. Extraction of Nine Pesticides from Spiked Mussel Tissue by Blending with Ethyl Acetate. This test was intended to evaluate the efficiency of the more polar ethyl acetate solvent under conditions which would not degrade the solvent. To compensate for not using Soxhlet extraction, the tissue sample was more thoroughly ground to facilitate extraction by simply blending it with the solvent. The procedures used in these tests are described below.

a. Prepare triplicate aliquots by transferring 10.0 g of control mussel tissue Sample TENOC-1 to each of three 125 mL bottles fitted with Teflon-lined caps.

b. Mix the tissue in each bottle while slowly adding concentrated HCl until the pH ≤2.

c. Add 25 µL of surrogate standard 109142-14 (see Appendix A, Table 3) to each mussel tissue sample.

d. Add the native pesticides to the three mussel tissue samples in the indicated quantities:

TENOC-1E-S	25 µL 109142-19 and 50 µL 109142-22 (see Appendix A, Table 3) (6250 ng each pesticide)
TENOC-1F-S	25 µL 109142-19 and 50 µL 109142-22 (6250 ng each pesticide)
TENOC-1G-S	125 µL 109142-19 and 31.3 µL 109142-21 (see Appendix A, Table 3) (31250 ng each pesticide)

e. Add 50 mL of ethyl acetate to each sample bottle and blend each mixture with a Tissuemizer for a period of about 10 minutes. Then, cap and seal each bottle.

f. Agitate each sealed sample bottle for a period of 10 minutes on a wrist action shaker, then place the sample bottles in an ultrasonic bath for an additional 10 minutes.

g. Remove the sample bottles from the ultrasonic bath and centrifuge each bottle at 1500 rpm for a period of 10 minutes.

h. Pour off the ethyl acetate supernatant extract, passing it through a funnel containing a glass wool plug and collecting the filtrate in a 125 mL bottle. Rinse the original extraction bottle and the funnel with two additional 5 mL portions of ethyl acetate, each time centrifuging and collecting the supernatant extracts, and combining these with the original extract in the 125 mL bottle. Discard the residues from centrifugation.

i. Add approximately 10 g of sodium sulfate to the filtered extract in the 125 mL bottle, and allow the extract to remain in contact with the sodium sulfate overnight.

j. Quantitatively transfer each of the extracts to separate test tubes, using methylene chloride rinses, and then concentrate the extracts in the tubes to near dryness by immersing the tubes in an ambient temperature water bath and applying a gentle stream of nitrogen. Then, immediately redilute the samples with 5 mL of methylene chloride.

k. Quantitatively transfer each of the extracts to a separate tared 15 mL vial and reserve 25% of each of the extracts, placing these in separate vials.

l. Filter each of the residual extracts through a syringe filter, rinsing with methylene chloride, and reconcentrate the combined filtrate and rinsate to a volume of approximately 2 mL, using a gentle stream of nitrogen.

m. Chromatograph the concentrated and filtered extracts on the GPC column using procedures described previously.

n. Concentrate each of the collected GPC eluate fractions to a volume of approximately 2 mL, and then transfer the concentrated solutions to tared 15 mL vials.

o. Reserve 25% of each concentrated GPC eluate which was collected (based on the original extract volumes) and transfer the remaining eluate portions (50%) to 1 mL vials.

p. Add 25 µL of the Reaction Surrogate Solution 109142-17 (see Appendix A, Table 3) to each vial, and concentrate the solutions in the vials to minimum volumes.

q. Add 200 µL TFAA and 100 µL TFE to each vial and heat the vials at 100°C for 1 hour.

r. Concentrate the solutions in the vials to minimum volumes, and then add 45 µL of toluene to each vial, as well as 5.0 µL of Internal Standard Solution, 109142-12 (see Appendix A, Table 3).

s. Analyze the derivatized samples using GC-MS.

The recoveries of the nine pesticides which were added to one of the control mussel tissue samples (TENOC-1G-S) using the procedures just described are shown in Table 3 of Attachment 1. As can be seen from these results, the recoveries achieved ranged from 64 to 250% for eight of the nine pesticides with which the sample had been fortified. Obviously, interfering components are still present in the derivatized sample extract which result in apparent recoveries exceeding 100% for several of the pesticides added to the sample. It may be possible to further reduce the levels of these interferences by using additional clean-up and fractionation procedures. It is clear, however, that the procedures applied are completely ineffective for determining Glyphosate, and it is concluded that this compound is not extracted from the mussel tissue matrix by this extraction method. At this point, further attempts to determine Glyphosate, as part of the unified analytical procedure developed for the other target compounds in this study, were abandoned. Copies of the Intralaboratory Sample Tracking Form and GC-MS Quantitation Report resulting from preparation and analyses of the sample discussed above are provided in Appendix C to this report. In view of the results obtained for spiked sample TENOC-1G-S, which were just discussed, preparation and analyses of the other two spiked samples prepared for this test phase (TENOC-1E-S and TENOC-1F-S) were not warranted.

4. Extraction of Nine Pesticides from Spiked Mussel Tissue by Blending Tissue with Chloroform and Water. This test was an attempt to adapt previously published procedures for extracting Glyphosate from fruits and vegetables (1-4) to extract all nine pesticides of interest in this phase of the study from mussel tissue. A chloroform/water solvent was tested, the rationale being that the aqueous component should be good for extracting the very polar Glyphosate while the remaining eight pesticides are expected to be soluble in the chloroform component at an acidic pH. The procedures used in these tests are described below.

a. Transfer 10.0 g of control mussel tissue to a tared 125 mL bottle.

b. Add surrogate (25 µL of standard 109142-14, 25 µL of standard 109142-19 and 50 µL of standard 109142-22) to the thawed tissue sample in the bottle.

c. Add 25 mL of water and 0.25 mL of phosphoric acid to the bottle containing the tissue sample.

d. Agitate the sealed bottle on a wrist action shaker for a period of 5 minutes and verify that the pH ≤ 2.

e. Add 25 mL of chloroform to the sample bottle.

f. Blend the sample and liquids, using a Tissuemizer, for a period of 10 minutes.

g. Agitate the sample bottle for a period of 20 minutes.

h. Place the sample bottle in an ultrasonic bath for a period of 10 minutes.

i. Centrifuge the sample bottle for 5 minutes at 1500 rpm.

j. Transfer the separated organic and aqueous fractions of the extract to separate 125 mL bottles.

Unfortunately, when these procedures were implemented, an emulsion formed during the blending step, and this emulsion could not be broken by centrifuging or cooling the emulsion, or by altering the pH. Consequently, the aqueous and chloroform phases could not be separated, and recovery of these individually was therefore impossible. In view of this, the sample was not processed further and this test was terminated.

5. Evaluation of Silica Column for Further Separation of Pesticides from Matrix Interferences. As discussed in Section III. 3. above, it was established in earlier tests that eight of the nine target pesticides of interest in this phase of the study could be extracted from mussel tissue spiked with these compounds by blending the tissue with ethyl acetate. However, as also discussed, with the method as described in Section III. 3., substantial quantities of interfering compounds extracted from the sample matrix also gave responses at the same retention times and ion masses which were monitored as indicators of several of these pesticides, resulting in apparent recoveries exceeding 100%. It was decided to determine whether the silica gel column which is utilized in the National Bioaccumulation Study analytical protocol would reduce these matrix interferences while still permitting recovery of the pesticides. In an initial test, using the same elution solvents and sequence as applied in the method just mentioned, the observed percent recoveries of five of the eight pesticides were either zero or very low. Consequently, a second test was conducted, but using a stronger elution solvent mixture. The procedures used in these tests are described below.

a. Combine 10 µL of standard solution 109143-1 (a mixture of the derivatized target pesticides) and 25 µL of surrogate solution 109142-14, (see Appendix A, Tables 3 and 4), and dilute this mixture with 500 µL of hexane.

b. Introduce this solution onto a 2 mm. ID glass column packed with 300 mg of silica gel-60, previously activated at 400°C for 4 hours and deactivated with 1% (w:w) water.

c. Elute the target analytes (the derivatized pesticides) from the column with 20 mL of 50% methylene chloride-in-hexane, collecting the entire eluate.

d. Concentrate the eluate to a volume of 90 µL, and then add 10 µL of internal standard solution 109142-12 (see Appendix A, Table 5).

e. Analyze the collected eluate using GC-MS, as described previously.

The results of this experiment are summarized in Table 4 of Attachment 1. As can be seen from these data, the recoveries of six of the eight target pesticides from the silica gel column range from 43% to 133%, well within an acceptable range. Clearly, under these elution conditions, the silica gel column will pass most of these target pesticides, and this column could also be expected to remove significant quantities of the matrix interferences extracted from mussel tissue by ethyl acetate. However, it is apparent that Carbofuran and Endothal cannot be recovered from the silica gel column and alternative procedures must be applied for these compounds. Further applicability of the silica gel column will be examined in future tests. Copies of the Intralaboratory Sample Tracking Forms and GC-MS Quantitation Report resulting from preparation and analyses of the test sample discussed here are provided in Appendix D.

B. Evaluation of Overall Analytical Scheme for Determination of Seventeen Pesticides in Mussel Tissue Starting with Ethyl Acetate Extraction.

As described in an earlier section, it was demonstrated that eight of the target pesticides (Benzoic Acid, Carbofuran, 2,4-D, Dinoseb, Acifluorfen, Carbaryl, Endothal and Picloram) not amenable to Soxhlet extraction from spiked mussel tissue using methylene chloride/hexane as a solvent could be effectively extracted by blending the sample with ethyl acetate. It was further shown that once extracted, these eight pesticides could be efficiently derivatized and the derivatives could be detected and quantitated using GC-MS. As also mentioned earlier in this report, five of the pesticides which were shown previously to be effectively extracted from spiked mussel tissue using Soxhlet extraction with methylene chloride/hexane were irreversibly sorbed by the silica gel cleanup procedure following extraction, thereby requiring that these be measured in separate GC-MS analyses (Ethoprop, Hexazinone, Metolachlor, Simazine, Atrazine). It was decided therefore, in an effort to reduce the number of separate GC-MS analyses required, to see if the latter five pesticides could be extracted with ethyl acetate, along with the eight target pesticides already shown to be amenable to this procedure. In addition, it was decided to determine whether procedures for determining Alachlor, Cyanazine, Acephate and Fluometuron could be incorporated in an overall analytical scheme, starting with ethyl acetate extraction. To develop and demonstrate the optimum analytical scheme, mussel tissue and Reagent Water (QA/QC) samples were spiked with all seventeen of these pesticides and several tests were conducted concurrently to evaluate the effectiveness of the several different steps in the analytical procedure. These tests were conducted concurrently in order to minimize the time required for this evaluation. The overall sample preparation and spiking scheme used for these tests is summarized in Table 5, Attachment 1, and a sample flow chart showing the various analytical procedures implemented is

provided in Figure 3 of Attachment 2 to this report. A detailed description of these procedures follows.

a. Prepare six mussel tissue samples by transferring 10 g aliquots of mussel tissue sample number TENOC-4 to 125 mL bottles. Also, prepare four water samples by adding 4 mL of reagent water to 125 mL bottles.

b. Add the surrogate standards, the two native compound solutions and fluometuron to the mussel tissues, (4O, 4P, 4Q, 4R and 4S), and to the water samples (QI, QJ, QK and QL), and to the empty vials (QM, QN, QO, QP, QQ, QR, 4TE-S and 4TD-S), as shown in Table 5, Attachment 1.

c. While mixing the samples in the bottles, slowly add 0.75 mL of concentrated HCl to each tissue and water sample, and verify that the pH of each samples is ≤2.

d. Add 50 mL of ethyl acetate to each tissue and water sample, and blend each tissue sample using a Tissuemizer for a period of 6 minutes, at a speed which does not allow the Tissuemizer probe to heat the solution.

e. Seal the bottles and agitate them for 15 minutes on a wrist action shaker.

f. Place each sample bottle in an ultrasonic bath for 10 minutes, then centrifuge each bottle for 10 minutes at 1500 rpm.

g. Remove the organic liquids from each sample bottle (the extract) and transfer these to new 125 mL bottles (tare the bottles for Samples 4R and QJ).

h. Add 20 mL of ethyl acetate to the remaining samples in the original sample bottles, seal the bottles and agitate these for 10 minutes, then place the bottles in an ultrasonic bath for 10 minutes.

i. Centrifuge each sample bottle for 10 minutes, then remove and transfer the organic phase in each bottle to the 125 mL bottle containing the initial ethyl acetate extract.

j. For samples 4R and QJ, remove aliquot (~7 mL) from each ethyl acetate extract, transferring these to vials, and designate these by the Suffix A following the sample number (for example, 4RA).

k. Stop preparation of samples 4R, QJ, QK and QL at this point. Reserve these for possible HPLC analyses.

l. Place the 125 mL bottles containing the other sample extracts in a water bath at room temperature, and concentrate the extracts using a gentle stream of nitrogen to a value of about 5 mL.

m. Quantitatively transfer the concentrated extracts to 15 mL vials using methylene chloride to rinse the bottles (tare the bottles for samples 4R and QJ).

n. For samples 4R and QJ, remove aliquots (~1 mL) of each extract and designate these by the Suffix B following the sample number (e.g. 4RB).

o. Concentrate each of the other sample extracts to near dryness in a water bath at room temperature and immediately redilute each extract with 5 mL methylene chloride.

p. Filter the concentrated extracts into 15 mL vials using methylene chloride to rinse the original 15 mL vial and the filter.

q. Concentrate the filtered extracts to a volume of approximately 2 mL in a water bath at room temperature.

r. Add samples QM and QN diluted with 2 mL of methylene chloride to the set of other samples being processed.

s. Fractionate all of the samples except those in Sets A and B, using Gel Permeation Chromatography (Sample Nos.4O, 4P, 4Q, 4R, 4S, 4T, QI, QJ, QM and QN).

t. Concentrate the GPC eluate for each sample which was collected in a 250 mL bottle to a volume of approximately 2 mL in a water bath at room temperature while passing a gentle stream of nitrogen over the sample.

u. Quantitatively transfer each concentrated sample to a tared 7 mL vial.

v. Transfer aliquots of the GPC eluates (~1 mL) to 1 mL vials (add aliquot 4T to a vial already containing standards), concentrate the contents of the vials to ~20 µL, and adjust to a final volume of 22.5 µL by adding toluene. Designate these fractions by the suffix D following the sample numbers.

w. Add Internal Standard (109142-12, 75 ng/µL) as shown in Table 5, Attachment 1.

x. Analyze these fractions using GC-MS (Sample Nos. 4OD, 4QD, 4RD, 4SD, 4TD, QID, QJD, QMD and QND).

y. Transfer additional aliquots of the GPC eluates (~1.5 mL) to 2 mL vials (add aliquot 4T to a vial already containing standards). Designate these aliquots by the suffix E following the sample number.

z. Concentrate aliquots 4RB and QJB to a volume of ~0.5 mL in a water bath at room temperature, and transfer the concentrated extracts to 1 mL vials.

aa. Insert samples QO and QP, diluted to 0.5 mL of methylene chloride in the set of samples being processed at this point (4O, 4P, 4Q, 4R, 4S, 4T, QI, QJ, QM, QN, QO, QP, 4RB and QJB).

ab. Concentrate these samples to a minimum volume (near dryness), then add 200 µL of TFAA and 100 µL of TFE to each sample.

ac. Heat these samples for 1 hour at 100°C, then concentrate each sample to a minimum volume (near dryness).

ad. Add the required volume of toluene to the concentrated samples, then add Internal Standards (109142-12, 75 ng/µL) to each sample, as indicated in Table 5, Attachment 1.

ae. Analyze these fractions using GC-MS (Sample Nos. 4OE, 4PE, 4QE, 4RE, 4SE, 4TE, QIE, QJE, QME, QNE, QOE, QPE, 4RB and QJB).

af. Reserve the remaining portions of the GPC eluates for HPLC analyses. Designate these aliquots by the Suffix C following the sample numbers (Sample Nos. 4OC, 4PC, 4QC, 4RC, 4SC, 4TC, QIC, QJC, QMC and QNC).

The results of analyses of the several sets of spiked test samples prepared as described in the foregoing discussion (see Figure 3 in Attachment 3 for the overall analytical scheme) are presented in Tables 6-14 in Attachment 1 to this report. Supporting data for these analyses (Intralaboratory Sample Tracking Forms and GC-MS Quantitation Reports) are provided in Appendices E-N.

This set of experiments with spiked samples was intended to provide information on the loss of target analytes and/or the introduction of interfering compounds as a result of applying the several steps in the analytical sequence which entails extraction by blending with ethyl acetate, cleanup or prefractionation of the extract using GPC, derivatization with TFAA/TFE, and analysis of the derivatized pesticides by GC-MS. Table 6 in Attachment 1 shows the percent recoveries of the eight target pesticides (Benzoic Acid, Carbofuran, 2,4-D, Dinoseb, Acifluorfen, Carbaryl, Endothal and Picloram) which were achieved when solutions of these compounds in an organic solvent were subjected to TFAA/TFE derivatization and the derivatized products were analyzed by GC-MS. As can be seen, the recoveries are generally quite good with the exception of Benzoic Acid, which exhibited very low recovery, and Acifluorfen, for which the recovery was obviously impacted by an unknown interference. The next experiment utilized the same solution of pesticides, but this time, GPC fractionation was accomplished first, followed by TFAA/TFE derivatization and GC-MS detection and quantitation of the derivatives. The results of this experiment, shown by the data in Table 7 in Attachment 1, indicate that very low recoveries were achieved for five of the eight pesticides (Benzoic Acid, 2,4-D, Acifluorfen, Endothal and

Picloram). It is probable that this results from irreversible adsorption of these pesticides on the GPC column which occurs in the absence of matrix constituents such as those which would be present in a mussel or fish tissue sample extract. The same effect was observed when the same analytical sequence was applied to a water sample (HPLC Grade Water) spiked with the eight target pesticides, as indicated by the data presented in Table 8 in Attachment 1. However, in this case, only three of the eight pesticides (Benzoic Acid, Endothal and Picloram) exhibited very low recoveries. When both control mussel tissue and water samples spiked with the eight target pesticides were extracted and analyzed using the procedures just described, but without GPC fractionation prior to TFAA/TFE derivatization, then the apparent recoveries of the pesticides, as shown by the data in Table 9 of Attachment 1, were severely affected by interferences arising from matrix components in the sample extracts which were analyzed by GC-MS. These interferences made it very difficult to interpret the mass chromatograms resulting from the GC-MS analyses. Finally, in this set of tests, control mussel tissue samples spiked with the eight target pesticides were subjected to the full analytical sequence (ethyl acetate extraction, GPC, TFAA/TFE derivatization, and GC-MS analysis), and the results are summarized in Table 10 of Attachment 1. As can be seen from these results, recoveries of the eight target pesticides were greatly acceptable, except for Benzoic Acid and Carbofuran, which exhibit the effects of interferences. In the case of Benzoic Acid, the tests described earlier in this report with a silica gel column suggest that the interference to Benzoic Acid might well be reduced by a final cleanup of the processed sample extract with silica gel prior to GC-MS analysis. Experiments to confirm this with the mussel tissue matrix are still in progress. As also indicated by the results in Table 10, the laboratory blank analyzed with this set of samples showed no detectable quantities of the eight target pesticides, as should be the case, of course.

Results obtained for the set of eight pesticides which do not require TFAA/TFE derivatization (Ethoprop, Simazine, Atrazine, Alachlor, Cyanazine, Metolachlor, Hexazinone and Acephate) prior to GC-MS analyses, are summarized in Tables 11-13 in Attachment 1. Table 11 shows the percent recoveries of these eight pesticides which were achieved when an organic solution of these was chromatographed on the GPC column and the appropriate GPC eluate fraction was analyzed using GC-MS. As can be seen, from the results shown in Table 11, quite acceptable recoveries of all of these eight pesticides were achieved. Consequently, if these pesticides can be efficiently extracted by ethyl acetate, then this overall procedure would be workable. However, when an aqueous solution of these eight pesticides was extracted with ethyl acetate, chromatographed on the GPC column and analyzed by GC-MS, acceptable recoveries were achieved only for Metolachlor and Hexazinone, as shown by the data presented in Table 12, Attachment 1. Somewhat better results were obtained when aliquots of mussel tissue spiked with these eight pesticides were extracted with ethyl acetate, fractionated using GPC, and

quantitated by GC-MS. As shown by the results in Table 13 in Attachment 1, these procedures yield acceptable recoveries for Alachlor, Metolachlor, Hexazinone and Ethoprop. The triazines (Simazine, Atrazine, Cyanazine) and Acephate were not effectively recovered by these methods. It was concluded that the triazines must be separately determined by another procedure. Methods have been described in the literature for determining Cyanazine in water by extracting this pesticide with methylene chloride and quantitating Cyanazine in the extract using HPLC. It seems evident that Atrazine and Simazine can also be determined by this procedure, since separation and detection of these by an HPLC column has been reported. Fluometuron can also be determined by HPLC, as demonstrated by the results of the present tests, which are described below. There are also existing HPLC methods for the determination of Carbofuran. Tests are still in progress in our laboratory to determine whether all five of these pesticides can be determined in a single HPLC analysis sequence.

Mussel tissue samples which were spiked or fortified with Fluometuron, as indicated in Table 5 in Attachment 1, were extracted by blending with ethyl acetate and the extract was separated by filtration and then concentrated as described in the foregoing, and in Figure 2 in Attachment 2 of this report. The extract was fractionated initially using the GPC procedures described earlier and the collected eluate fraction from the GPC was analyzed using High Performance Liquid Chromatography (HPLC). The HPLC instrumentation and operating parameters used in these analyses are described below.

HPLC Instrument System:

DuPont Instruments Series 8800 Gradient Controller
DuPont Model 8800 Pump Module
DuPont Manually Operated Column Compartment
Varian Varichrom Model VUV-10 OV/VIS Detector
Nelson Analytical Chromatographic Data System

HPLC Operating Parameters for Analysis of Samples for Fluometuron:

HPLC Column:	Zorbax ODS, 6.2 mm x 25.0 cm
Mobile Phase:	60% Methanol, 40% Water (isocratic)
Flow Rate:	2.00 mL/min (112 bar pressure)
Column Temperature:	35°C
Detector Wave Length:	240 nm (Band width = 16)
Detector Sensitivity:	0.005 AVFS
Volume of Sample Introduced into Sample Loop:	25 µL

Additional supporting documentation relating to sample preparation, calibration of the HPLC and analyses of the Fluometuron-spiked mussel tissue samples is presented in Appendix N to this report. The recoveries of Fluometuron which were achieved by using these procedures are shown in Table 14 of Attachment 1 to this report. As can be seen from the results

presented in Table 14, reasonably acceptable recoveries of Fluometuron were achieved by the methods, and this appears to be a useful procedure for determining this pesticide in mussel, and presumably, fish tissues. As noted above, demonstration of similar HPLC methods for determining Atrazine, Simazine, Cyanazine and Carbofuran is in progress.

IV. FINAL ANALYTICAL SCHEME

Although the final experiments are still in progress to demonstrate successful analyses of the last six remaining target pesticides (Atrazine, Simazine, Cyanazine, Carbofuran, Benzoic Acid and Acephate) for which analytical methodology development has yet to be completed, progress achieved at this point permits us to define the probable overall analysis scheme. This scheme is shown schematically in Figure 3 of Attachment 2 to this report, and should yield analytical data for all of the target pesticides and related compounds identified by the Tennessee Wildlife Resources Agency for this study, with the exception of Glyphosate and Diquat. As discussed elsewhere in this report, while methods to detect these compounds in organic solutions have been demonstrated in our laboratory, we have been unable, despite intensive effort, to successfully extract these two pesticides from mussel tissue. Consequently, at this point, it seems prudent to remove these two compounds from the target compound list for this study.

V. RESULTS OF ANALYSES OF MUSSEL AND FISH SAMPLES FROM VARIOUS SITES WHICH WERE SUBMITTED BY THE TENNESSEE WILDLIFE RESOURCES AGENCY (TWRA) FOR DETERMINATION OF THE TARGET PESTICIDES AND RELATED COMPOUNDS

Several sets of mussel and fish tissue samples were submitted to Wright State University by the TWRA during the second year of the Contract for analyses to determine the presence of the target pesticides and related compounds. The samples received were composited into seven samples for analysis, as shown in the sample receipt documentation provided in Appendix O to this report. These samples were analyzed using the procedures described in detail in this report and in the Annual Report for the first year of the contract, according to the overall analytical scheme shown in Figure 3 of Attachment 2 to the present report.

The results of the analyses of the five mussel and two fish samples submitted by TWRA, as well as results obtained in the analyses of one Laboratory Blank and one mussel sample spiked with target analytes, for the thirty-seven compounds from the National Bioaccumulation Study and for several other target pesticides specified by TWRA are summarized in Table 15 of Attachment 1 to this report. More detailed supporting data obtained in the GC-MS analyses for these compounds are presented in Appendices Q through X to this report. As can be seen from

the results shown in Table 15, the detection limits achieved in the present analyses for the target analytes were generally lower than those achieved in the analyses completed during the first year of this project. Also, the recoveries of the target compounds achieved in the analysis of the native-spiked mussel sample in the present case are generally much better than those achieved in the earlier analyses. Finally, the quality of the identifications of target analytes made by comparisons with the MS computer library of spectra (as indicated by the Fit parameters shown in the tables in Appendix Q) are generally superior to those achieved in the earlier data. All of these improvements in the data are attributable to major improvements which were made in the GC-MS data processing software used in these analyses during the second year of the TWRA project. These enhanced analytical capabilities have resulted in the detection and quantitation of a much larger group of pesticide and related compound residues in the mussel and fish samples analyzed during this project year than were detected in the analyses reported at the conclusion of the first year of this effort. As can be seen from the data reported in Table 15, Attachment 1, one or more of the mussel samples analyzed in the second year of the project were found to contain measurable quantities of α-BHC, Lindane, Pentachloronitrobenzene, Alachlor, Heptachlor, Chloropyrifos, Oxychlordane, Butachlor, pp'-DDE, Dieldrin, Perthane, Chlorobenzilate, Endrin, Diethylphthalate, Diazinon, Methyl parathion and Di-n-butyl phthalate. One or more of the fish samples analyzed here were found to contain these same pesticides, with the exception of Pentachloronitrobenzene, Perthane and Chlorobenzilate. In addition the fish samples analyzed exhibited measurable quantities of Trifluralin, Octachlorostyrene, trans-Chlordane, cis-Chlordane and trans-Nonachlor.

The results of the analyses of the mussel and fish samples submitted by TWRA for PCBs are summarized in Table 16 in Attachment 1. Additional supporting data are provided in Appendices Y and Z. As is the case for all GC-MS analyses reported herein, the raw and processed data files for the PCB analyses are quite voluminous and most of these are not included in this report. All raw and processed data for these and other analyses reported herein are on file at our laboratory and will be retained indefinitely. As can be see from the data presented in Table 16, both of the fish samples and one mussel sample analyzed here were found to contain readily measurable levels of penta- and hexachlorinated biphenyls.

The results of the analyses of the mussel and fish samples submitted by TWRA for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) are summarized in Table 17 in Attachment 1. Additional supporting data are provided in Appendix AA. As can be seen from the data presented in Table 17, both 2,3,7,8-TCDD and 2,3,7,8-TCDF were found in the two fish samples analyzed, although the concentrations are quite low (~1.5-5.5 ppt). Only 2,3,7,8-TCDF was found in the mussel samples analyzed here, again at low levels.

VI. SUMMARY

The results obtained by our laboratory at Wright State on the TWRA project during the first two years indicate that it will be possible to analyze aquatic samples (fish, mussels, sediments) for all of the target pesticides and related compounds which were specified by TWRA with the exception of Glyphosate and Diquat Dibromide. While methods for quantitation of even these two pesticides have been demonstrated, it has not been possible to efficiently extract these two compounds from mussel tissue, and so these have been eliminated from the list of target compounds for future studies. Final analytical methods have been developed and demonstrated for all of the other target compounds (see Table 1 in Attachment 1) except for Benzoic Acid, Carbofuran, Atrazine, Simazine, Cyanazine and Acephate. While methods have been demonstrated for some of the latter, efficiently incorporating the analyses of these into the analytical scheme shown in Figure 3 of Attachment 2 required changes in the methodology initially envisioned. The methods to be used for these in the final scheme (as shown in Figure 3, Attachment 2) are in hand, and are undergoing final testing. There is good reason to believe that these will be acceptable procedures, and the overall analytical scheme shown in Figure 3, Attachment 2 will then be fully demonstrated and will be implemented for future analyses of TWRA samples during the third and fourth years of this project.

VII. REFERENCES

1. Seiber, J. N., M. M. McChesney, R. Kon and R. A. Leavitt, "Analysis of Glyphosate Residues in Kiwi Fruit and Asparagus Using High-Performance Liquid Chromatography of Derivatized Glyphosate as a Cleanup Step," *J. Agric. Food Chem.* 32, 678-681, 1984.

2. Moye, H. A., C. J. Miles and S. J. Scherer, "A Simplified High-Performance Liquid Chromatographic Residue Procedure for the Determination of Glyphosate Herbicide and (Aminomethyl)phosphonic Acid in Fruits and Vegetables Employing Post Column Fluorogenic Labeling," *J. Agric. Food Chem.*, 31, 69-72, 1983.

3. Deyrup, C. L. S-M Chang, R. A. Weintraub and H. A. Moye, "Simultaneous Esterification and Acylation of Pesticides for Analysis by Gas Chromatography. 1. Derivatization of Glyphosate and (Aminomethyl)phosphonic Acid with Fluorinated Alcohols - Perfluorinated Anhydrides," *J. Agric. Food Chem.*, 33, 944-947, 1985.

4. Roy, D. N. and S. K. Konar, "Development of an Analytical Method for the Determination of Glyphosate and (Aminomethyl)phosphonic Acid Residues in Soils by Nitrogen-Selective Gas Chromatography," *J. Agric. Food Chem.*, 37, 441-443, 1989.

ATTACHMENT 1

TO

WRIGHT STATE UNIVERSITY REPORT
TO THE TENNESSEE WILDLIFE RESOURCES AGENCY
ON WORK ACCOMPLISHED DURING THE SECOND YEAR OF THE PROJECT
CONDUCTED UNDER TENNESSEE WILDLIFE RESOURCES AGENCY
CONTRACT NO. GU-0-01830-0-00

TABLES OF DATA AND RESULTS OF ANALYSES OF FISH AND MUSSEL TISSUES

TABLE 1

TARGET ANALYTES TO BE MEASURED
IN MUSSEL AND FISH TISSUES AND IN SEDIMENTS
IN THE TENNESSEE WILDLIFE RESOURCES AGENCY PROJECT

A. Thirty-seven (37) Compounds Monitored in U.S. EPA's National Bioaccumulation Study:

1,3,5-Trichlorobenzene
1,2,4-Trichlorobenzene
1,2,3-Trichlorobenzene
1,2,3,5-/1,2,4,5-Tetrachlorobenzene
1,2,3,4-Tetrachlorobenzene
Pentachlorobenzene
Hexachlorobenzene
Hexachlorobutadiene
Biphenyl
Trifluralin
Alpha-BHC
Pentachloroanisole
Lindane
Pentachloronitrobenzene
Diphenyldisulfide
Alachlor
Heptachlor
Chloropyrifos
Isopropalin
Octachlorostyrene
Heptachlorepoxide
Oxychlordane
Butachlor
Trans-Chlordane
Cis-Chlordane
Trans-Nonachlor
Cis-Nonachlor
p,p'-DDE
Dieldrin
Perthane
Nitrofen
Chlorobenzilate
Endrin
Triphenylphosphate
Methoxychlor
Dicofol
Mirex

B. Twenty-four (24) Additional Pesticides Specified by Tennessee Wildlife Resources Agency:

Diethylphthalate
Di-n-Butylphthalate
Atrazine
Metolachlor
Diazinon
Methyl Parathion
Ethoprop
Disulfoton
Simazine
Hexazinone
Benzoic Acid
Carbofuran
2,4-D
Dinoseb
Acifluorfen
Carbaryl
Endothal
Picloram
Glyphosate
Alachlor
Cyanazine
Acephate
Diquat Dibromide
Fluometuron

TABLE 1 (con't)

TARGET ANALYTES TO BE MEASURED
IN MUSSEL AND FISH TISSUES AND IN SEDIMENTS
IN THE TENNESSEE WILDLIFE RESOURCES AGENCY PROJECT

C. Polychlorinated Biphenyls (PCBs):

Monochlorobiphenyls
Dichlorobiphenyls
Trichlorobiphenyls
Tetrachlorobiphenyls
Pentachlorobiphenyls
Hexachlorobiphenyls
Heptachlorobiphenyls
Octachlorobiphenyls
Nonachlorobiphenyls
Decachlorobiphenyl

D. Polychlorinated Dibenzo-p-Dioxins and Polychlorinated Dibenzofurans:

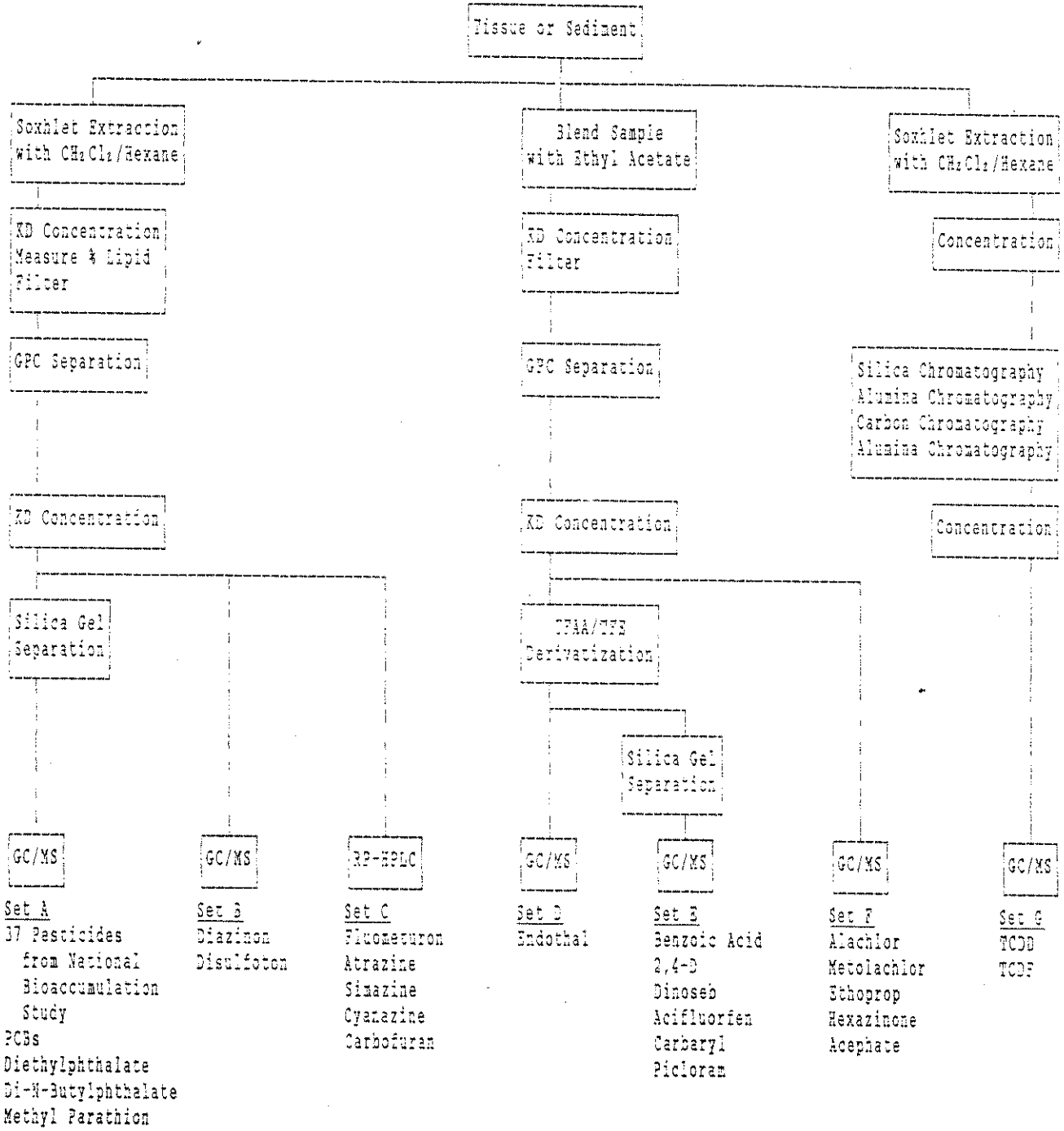
2,3,7,8-TCDD
2,3,7,8-TCDF

FIGURE 1

WRIGHT STATE UNIVERSITY

ANALYTICAL SCHEME FOR THE DETERMINATION OF BIOACCUMULATING PESTICIDES
IN AQUATIC BIOLOGICAL TISSUES AND RELATED SAMPLES

DEVELOPED FOR THE TENNESSEE WILDLIFE RESOURCES AGENCY



THE OCCURRENCE OF ORGANIC CONTAMINANTS IN BLEMISHED AND UNBLEMISHED CATFISH FROM KENTUCKY LAKE

AUGUST 1990

Table . Summary of tissue analysis performed on two species of catfish collected from the New Johnsonville area of Kentucky Lake Reservoir, 1990. Tissue blemishes were present in all blue catfish. No tissue blemishes were present in channel catfish. ND signifies NONE DETECTED. All data are given as ppb, except dioxin and difuran are given as ppt.

River Mile	100.0	100.0
Species	Blue Catfish	Channel Catfish
Sample Date	21 Aug 90	20 Aug 90
Compound		
Iodobenzene	44.3	102.0
Iodonapthalane	141.0	217.0
4,4'-Diiodobiphenyl	182.0	713.0
1,3,5-Trichlorobenzene	ND	ND
1,2,4-Tricxhlorobenzene	ND	ND
Hexachlorobutadiene	ND	ND
1,2,3,-Trichlorobenzene	ND	ND
1,2,3,5-Tetrachlorobenzene	ND	ND
1,2,4,5-Tetrachlorobenzene	ND	ND
Biphenyl	ND	ND
1,2,3,4-Tetrachlorobenzene	ND	ND
Pentachlorobenzene	ND	ND
Trifluralin	5.1	41.8
Alpha-BHC	28.3	ND
Hexachlorobenzene	ND	ND
Pentachloroanisole	ND	ND
Lindane	ND	ND
Pentachloronitrobenzene	ND	ND
Diphenyldisulfide	ND	ND

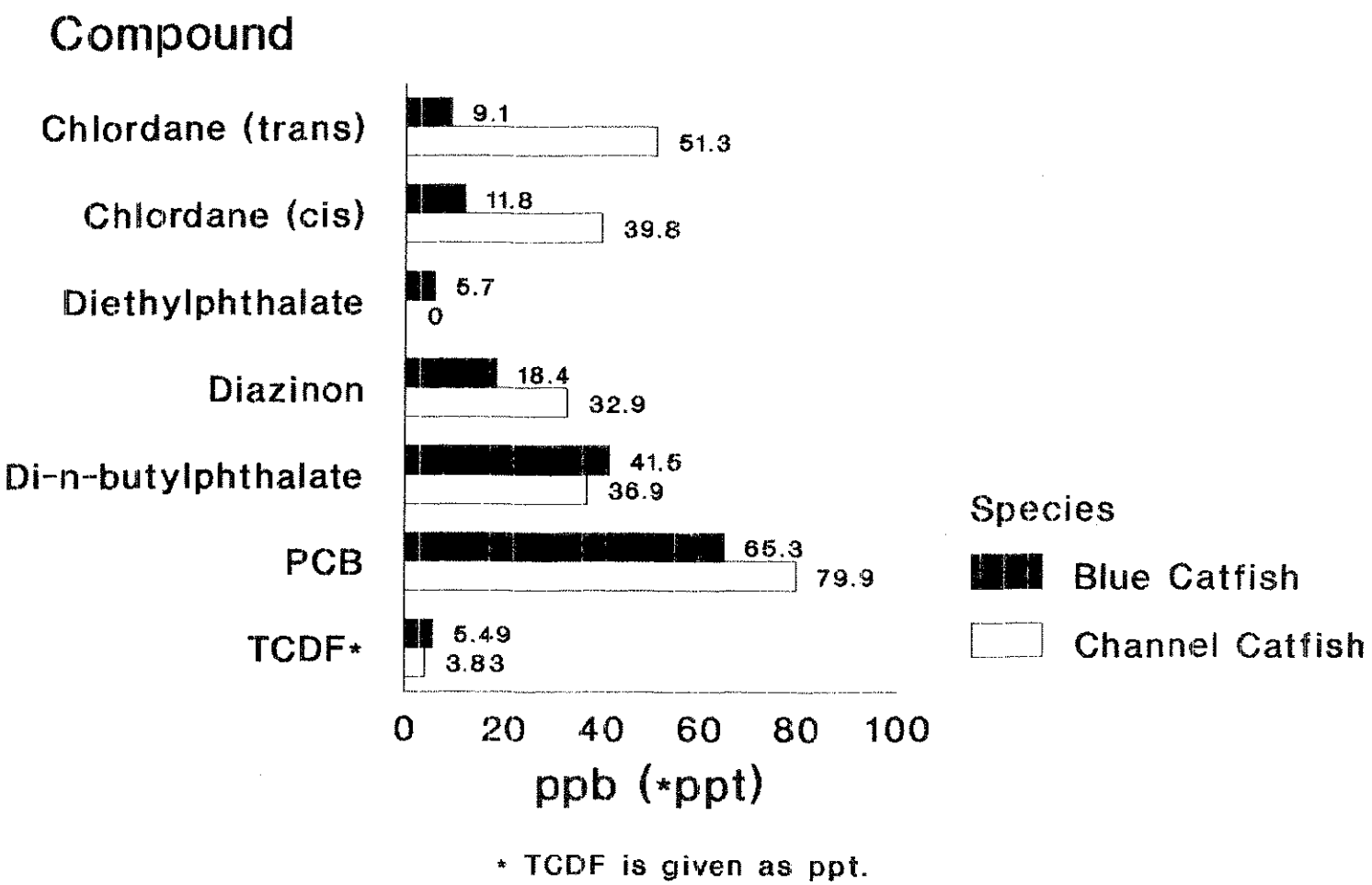
Table . ...continued.

River Mile	100.0	100.0
Species	Blue Catfish	Channel Catfish
Sample Date	21 Aug 90	20 AUG 90
Compound		
Alachlor	ND	73.0
Heptachlor	1.4	139.0
Chloropyrifos	3.0	ND
Isopropalin	ND	ND
Octachlorostyrene	ND	ND
Heptachlorepoxide	ND	ND
Oxychlordane	154.0	229.0
Butachlor	35.9	45.8
Chlordane (trans)	9.1	51.3
Chlordane (cis)	11.8	39.8
Nonachlor (trans)	11.3	ND
DDE-p-p'	217.0	805.0
Dieldrin	60.5	629.0
Perthane	ND	ND
Nitrofen	ND	ND
Chlorobenzilate	ND	ND
Endrin	9.6	132.0
Nonachlor (cis)	ND	ND
Triphenylphosphate	ND	ND
Methoxychlor	ND	ND
Dicofol	ND	ND
Mirex	ND	ND
Diethylphthalate	5.7	ND

Table . ...continued.

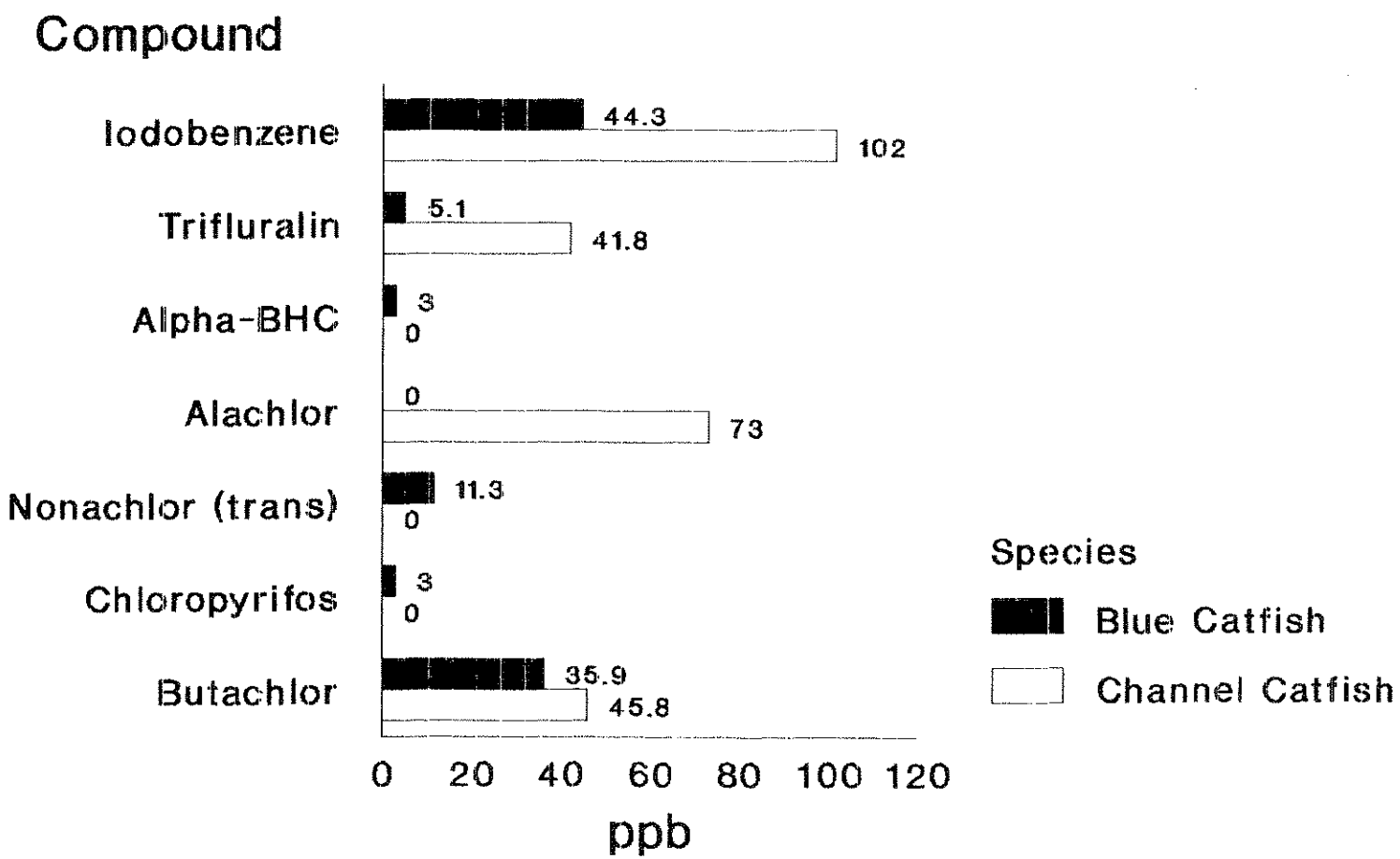
River Mile	100.0	100.0
Species	Blue Catfish	Channel Catfish
Sample Date	21 Aug 90	20 Aug 90
Compound		
Diazinon	18.4	32.9
Disulfoton	ND	ND
Methylparathion	468.0	140.0
Di-n-butylphthalate	41.5	36.9
PCB	65.3	79.9
TCDD	ND	ND
TCDF	5.49	3.83
Compounds Detected	22	19

Comparisons of tissue analysis of the blue catfish and the channel catfish collected at TRM 100.0, 20-21 Aug 1990.



* TCDF is given as ppt.

Comparisons of tissue analysis of the blue catfish and the channel catfish collected at TRM 100.0, 20-21 Aug 1990.



Comparisons of tissue analysis of the blue catfish and the channel catfish collected at TRM 100.0, 20-21 Aug 1990.

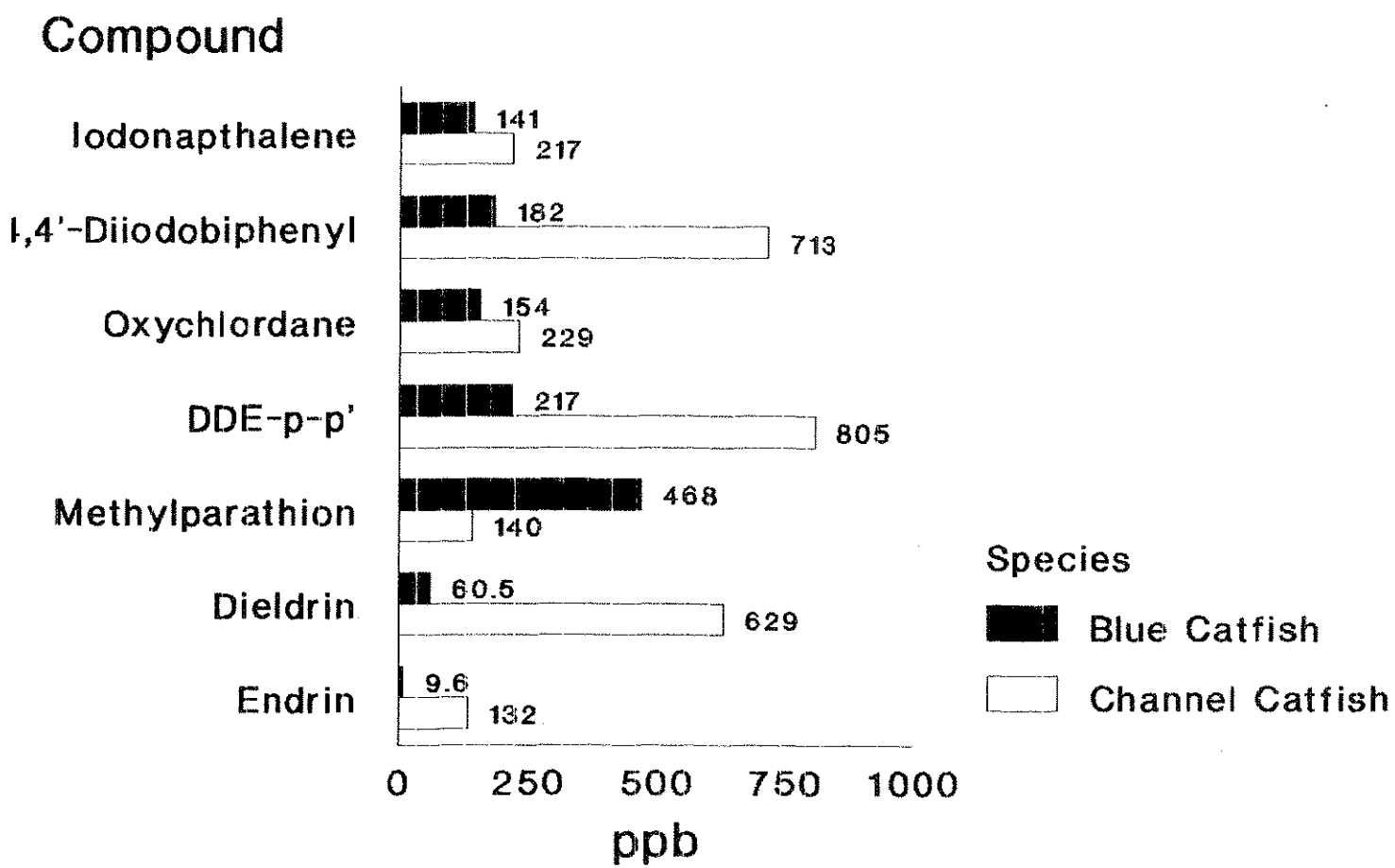


TABLE 15

RESULTS OF ANALYSES OF TWRA MUSSEL AND FISH SAMPLES FOR TARGET PESTICIDES AND RELATED COMPOUNI

Wright State University - Dayton, Ohio 45435

Ebony S. Trm 88.1

	WSU I.D. NUMBERS	Concentration in ng/g							
COMPOUND	L805141-1 METHOD BLANK	TRA1-1B TENN RIVER @ RICHLAND CREEK IN CHANNEL, 50', WASHBOARD	TRA1-2B BLUE CATFISH KENTUCKY LAKE	TRA1-3B TENN RIVER @ RICHLAND CREEK IN CHANNEL, 50' EBONY SHELL	TRA1-4B TRM 197.5, UPSTREAM DIAMOND ISLAND, RIGHT BANK 20', EBONY SHELL	TRA1-5B TENN RIVER @ RICHLAND CREEK OVERBANK, 14'	TRA1-5C-S TENN RIVER @ RICHLAND CREEK OVERBANK, 14' (SPIKE)	TRA1-6B BIG SANDY EMBAYMENT MAPLE LEAF	TRA1-7B CHANNEL CAT KENTUCKY CONTROL SAM
IODOBENZENE	42.3 [27]	21.7 [14]	44.3 [28]	12.9 [8]	14.3 [9]	12.6 [8]	11.5 [7]	7.1 [5]	102 [65]
IODONAPTHALENE	72.6 [46]	112 [72]	141 [90]	38.6 [25]	63.5 [41]	83.1 [53]	57.4 [37]	38.6 [25]	217 [139]
4,4'-DIIODOBIPHENYL	122 [78]	151 [96]	182 [116]	66.2 [42]	125 [80]	173 [111]	152 [97]	153 [98]	713 [455]
1,3,5-TRICHLOROBENZENE	ND(1.3) (c)	ND(1.3) (c)	ND(2.4) (c)	ND(1.3) (c)	ND(1.3) (c)	ND(1.3) (c)	1.0 [20]	ND(1.3) (c)	ND(19.6) (c)
1,2,4-TRICHLOROBENZENE	ND(1.3) (d)	ND(1.3) (c)	ND(2.4) (d)	ND(1.3) (c)	ND(1.3) (c)	ND(1.3) (d)	1.1 [22]	ND(1.3) (c)	ND(19.6) (d)
HEXACHLOROBUTADIENE	ND(1.3) (c)	ND(1.3) (c)	ND(2.4) (c)	ND(1.3) (c)	ND(1.3) (c)	ND(1.3) (c)	1.0 [20]	ND(1.3) (c)	ND(19.6) (c)
1,2,3-TRICHLOROBENZENE	ND(1.3) (d)	ND(1.3) (c)	ND(2.4) (d)	ND(1.3) (c)	ND(1.3) (c)	ND(1.3) (c)	1.2 [24]	ND(1.3) (c)	ND(19.6) (d)
1,2,3,5- and 1,2,4,5-TETRACHLOROBENZENE	ND(1.3) (c)	ND(1.3) (c)	ND(2.4) (c)	ND(1.3) (c)	ND(1.3) (c)	ND(1.3) (c)	2.0 [41]	ND(1.3) (c)	ND(19.6) (c)
BIPHENYL	ND(1.3) (d)	ND(1.3) (d)	ND(2.4) (d)	ND(1.3) (d)	ND(1.3) (d)	ND(1.3) (d)	2.7 [53]	ND(1.3) (d)	ND(19.6) (d)
1,2,3,4-TETRACHLOROBENZENE	ND(1.3) (c)	ND(1.3) (c)	ND(2.4) (c)	ND(1.3) (c)	ND(1.3) (c)	ND(1.3) (c)	2.5 [50]	ND(1.3) (c)	ND(19.6) (c)

Comment Key:

* ND(x), indicates the target compound was not found, and the parentheses contain the minimum detectable quantity.

[X] Bracketed values represent per cent recoveries.

(a) Analyst confirmed target analyte supressed by chemical noise.

(b) Quantitation ion appears outside five scan window relative to standard

(c) Peak detected in retention time window does not meet 0.40 library fit requirement for target compound.

(d) Peak detected in retention time window met 0.40 library fit requirement for target compound but quantification ion did not meet required signal to noise value.

TABLE 15 - continued

RESULTS OF ANALYSES OF TWRA MUSSEL AND FISH SAMPLES FOR TARGET PESTICIDES AND RELATED COMPOUNDS

Wright State University - Dayton, Ohio 45435

	WSU I.D. NUMBERS			Concentration in ng/g					
COMPOUND	L805141-1 METHOD BLANK	TRA1-1B TENN RIVER RICHLAND CREEK IN CHANNEL, 50', WASHBOARD	TRA1-2B BLUE CATFISH KENTUCKY LAKE	TRA1-3B TENN RIVER RICHLAND CREEK IN CHANNEL, 50', EBONY SHELL	TRA1-4B TRM 197.5, UPSTREAM DIAMOND ISLAND, RIGHT BANK 20', EBONY SHELL	TRA1-5B TENN RIVER @ RICHLAND CREEK OVERBANK, 14'	TRA1-5C TENN RIVER @ RICHLAND CREEK OVERBANK, 14' (SPIKE)	TRA1-6B BIG SANDY EMBAYMENT MAPLE LEAF	TRA1-7B CHANNEL CAT KENTUCKY L CONTROL SAM
PENTACHLOROBENZENE	ND(1.3) (d)	ND(1.3) (c)	ND(2.4) (c)	ND(1.3) (c)	ND(1.3) (c)	ND(1.3) (d)	3.8 [77]	ND(1.3) (c)	ND(19.6) (d)
TRIFLURALIN	ND(2.5) (d)	ND(2.5) (c)	5.1	ND(2.5) (c)	ND(2.5) (c)	ND(2.5) (c)	4.3 [87]	ND(2.5) (c)	41.3
ALPHA-BHC	ND(1.3) (d)	8.2 (b)	28.3 (b)	3.0 (b)	5.2 (b)	11.4 (b)	2.7 [53]	3.2 (b)	ND(19.6) (c)
HEXACHLOROBENZENE	ND(1.3) (c)	ND(1.3) (c)	ND(2.4) (d)	ND(1.3) (c)	ND(1.3) (d)	ND(1.3) (d)	5.2 [105]	ND(1.3) (c)	ND(19.6) (d)
PENTACHLOROANISOLE	ND(2.5) (c)	ND(2.5) (d)	ND(4.8) (d)	ND(2.5) (c)	ND(2.5) (c)	ND(2.5) (c)	5.1 [102]	ND(2.5) (c)	ND(39.2) (d)
LINDANE	ND(1.3) (c)	ND(1.3) (d)	6.8	4.1 (b)	9.3 (b)	17.0 (a)	3.5 [70]	ND(1.3) (d)	51.9
PENTACHLORONITROBENZENE	ND(2.5) (c)	ND(2.5) (d)	ND(4.8) (c)	ND(2.5) (d)	ND(2.5) (d)	3.2 (b)	5.7 [114]	ND(2.5) (c)	ND(39.2) (c)
DIPHENYLDISULFIDE	ND(2.5) (c)	ND(2.5) (c)	ND(4.8) (c)	ND(2.5) (c)	ND(2.5) (c)	ND(2.5) (c)	4.4 [88]	ND(2.5) (c)	ND(39.2) (d)
ALACHLOR	ND(1.3) (c)	ND(1.3) (d)	ND(2.4) (d)	ND(1.3) (d)	3.4	13.1 (a)	0.8 [16]	ND(1.3) (d)	73.0
HEPTACHLOR	ND(1.3) (c)	1.4 (b)	ND(2.4) (d)	ND(1.3) (d)	ND(1.3) (d)	1.4	2.4 [48]	ND(1.3) (d)	139 (b)

Comment Key:

* ND(x), indicates the target compound was not found, and the parentheses contain the minimum detectable quantity.

[X] Bracketed values represent per cent recoveries.

(a) Analyst confirmed target analyte supressed by chemical noise.

(b) Quantitation ion appears outside five scan window relative to standard

(c) Peak detected in retention time window does not meet 0.40 library fit requirement for target compound.

(d) Peak detected in retention time window met 0.40 library fit requirement for target compound but quantification ion did not meet required signal to noise value.

TABLE 15 - continued

RESULTS OF ANALYSES OF TWRA MUSSEL AND FISH SAMPLES FOR TARGET PESTICIDES AND RELATED COMPOUNDS

Wright State University - Dayton, Ohio 45435

	WSU I.D. NUMBERS			Concentration in ng/g					
COMPOUND	L805141-1 METHOD BLANK	TRA1-1B TENN RIVER RICHLAND CREEK IN CHANNEL, 50', WASHBOARD	TRA1-2B BLUE CATFISH KENTUCKY LAKE	TRA1-3B TENN RIVER RICHLAND CREEK IN CHANNEL, 50', EBONY SHELL	TRA1-4B TRM 197.5, UPSTREAM DIAMOND ISLAND, RIGHT BANK 20', EBONY SHELL	TRA1-5B TENN RIVER @ RICHLAND CREEK OVERBANK, 14'	TRA1-5C TENN RIVER @ RICHLAND CREEK OVERBANK, 14' (SPIKE)	TRA1-6B BIG SANDY EMBAYMENT MAPLE LEAF	TRA1-7B CHANNEL CATF KENTUCKY LA CONTROL SAMP
CHLOROPYRIFOS	ND(5.4) (c)	3.0 (b)	3.5 (b)	4.7 (b)	ND(1.3) (d)	ND(1.3) (d)	24.6 [491]	6.3	ND(19.6) (d)
ISOPROPALIN	ND(6.3) (c)	ND(6.3) (c)	ND(12.0) (d)	ND(6.3) (c)	ND(6.3) (c)	ND(6.3) (d)	6.3 [126]	ND(6.3) (c)	ND(98.1) (d)
OCTACHLOROSTYRENE	ND(1.3) (c)	ND(1.3) (d)	5.0	ND(1.3) (d)	ND(1.3) (c)	ND(1.3) (d)	4.9 [98]	ND(1.3) (c)	ND(19.6) (d)
HEPTACHLOREPOXIDE	ND(2.5) (c)	ND(2.5) (c)	ND(4.8) (c)	ND(2.5) (c)	ND(2.5) (c)	ND(2.5) (c)	4.7 [93]	ND(2.5) (c)	ND(39.2) (c)
OXYCHLORDANE	ND(1.3) (c)	ND(1.3) (d)	154	ND(1.3) (a)	26.2 (b)	9.1 (a)	17.3 [345]	ND(1.3) (d)	229
BUTACHLOR	ND(2.5) (c)	ND(2.5) (d)	35.9 (b)	ND(2.5) (c)	ND(2.5) (d)	3.5 (b)	3.4 [69]	ND(2.5) (d)	45.9
CHLORDANE(TRANS)	ND(2.5) (c)	ND(2.5) (c)	9.1	ND(2.5) (c)	ND(2.5) (d)	ND(2.5) (c)	5.4 [109]	ND(2.5) (c)	51.3
CHLORDANE(CIS)	ND(1.3) (c)	ND(1.3) (c)	11.8	ND(1.3) (c)	ND(1.3) (d)	ND(1.3) (d)	5.2 [104]	ND(1.3) (c)	39.5
NONACHLOR(TRANS)	ND(1.3) (c)	ND(1.3) (c)	11.3	ND(1.3) (d)	ND(1.3) (c)	ND(1.3) (c)	5.5 [109]	ND(1.3) (c)	ND(33.5) (d)
DDE-p-p'	ND(1.3) (c)	8.6	217	4.9	12.8	16.3	0.1 [2]	ND(1.3) (d)	805

Comment Key:

* ND(x), indicates the target compound was not found, and the parentheses contain the minimum detectable quantity.

[X] Bracketed values represent per cent recoveries.

(a) Analyst confirmed target analyte supressed by chemical noise.

(b) Quantitation ion appears outside five scan window relative to standard

(c) Peak detected in retention time window does not meet 0.40 library fit requirement for target compound.

(d) Peak detected in retention time window met 0.40 library fit requirement for target compound but quantification ion did not meet required signal to noise value.

TABLE 15 - continued

RESULTS OF ANALYSES OF TWRA MUSSEL AND FISH SAMPLES FOR TARGET PESTICIDES AND RELATED COMPOUNDS

Wright State University - Dayton, Ohio 45435

	WSU I.D. NUMBERS								
	Concentration in ng/g								
COMPOUND	LB05141-1 METHOD BLANK	TRA1-1B TENN RIVER RICHLAND CREEK IN CHANNEL, 50', WASHBOARD	TRA1-2B BLUE CATFISH KENTUCKY LAKE	TRA1-3B TENN RIVER RICHLAND CREEK IN CHANNEL, 50', EBONY SHELL	TRA1-4B TRM 197.5, UPSTREAM DIAMOND ISLAND, RIGHT BANK 20', EBONY SHELL	TRA1-5B TENN RIVER @ RICHLAND CREEK OVERBANK, 14'	TRA1-5C TENN RIVER @ RICHLAND CREEK OVERBANK, 14' (SPIKE)	TRA1-6B BIG SANDY EMBAYMENT MAPLE LEAF	TRA1-7B CHANNEL CAT KENTUCKY L CONTROL SAM
DIELDRIN	ND(1.3) (d)	3.6	60.5	29.1	10.4	ND(54.2) (c)	53.7 (a)	ND(55.4) (c)	609
PERTHANE	ND(1.3) (d)	ND(1.3) (d)	ND(2.4) (d)	ND(1.3) (d)	3.4 (b)	ND(1.3) (d)	6.0 [119]	ND(1.3) (d)	ND(19.6) (d)
NITROFEN	ND(6.3) (d)	ND(6.3) (d)	ND(12.0) (d)	ND(6.3) (d)	ND(6.3) (d)	ND(6.3) (d)	3.1 [62]	ND(6.3) (d)	ND(98.1) (d)
CHLOROBENZILATE	ND(1.3) (d)	ND(1.3) (d)	ND(2.4) (d)	ND(1.3) (d)	ND(1.3) (d)	ND(1.3) (d)	7.2 [145]	5.7 (b)	ND(19.6) (d)
ENDRIN	ND(2.5) (c)	ND(2.5) (c)	9.6 (b)	ND(2.5) (c)	ND(2.5) (d)	14.1 (a)	5.1 (a)	ND(2.5) (c)	132
NONACHLOR(CIS)	ND(2.5) (c)	ND(2.5) (c)	ND(4.8) (d)	ND(2.5) (c)	ND(2.5) (c)	ND(2.5) (c)	2.5 [49]	ND(2.5) (c)	ND(39.2) (d)
TRIPHENYLPHOSPHATE	ND(2.5) (c)	ND(2.5) (d)	ND(4.8) (d)	ND(2.5) (d)	ND(2.5) (d)	ND(2.5) (d)	1.4 [27]	ND(2.5) (d)	ND(39.2) (d)
METHOXYCHLOR	ND(1.3) (d)	ND(1.3) (d)	ND(2.4) (d)	ND(1.3) (c)	ND(1.3) (d)	ND(1.3) (d)	6.0 [119]	ND(1.3) (d)	ND(19.6) (d)
DICOFOL	ND(2.5) (c)	ND(2.5) (c)	ND(4.8) (c)	ND(2.5) (c)	ND(2.5) (c)	ND(2.5) (d)	2.1 (a)	ND(2.5) (c)	ND(48.1) (c)
MIREX	ND(1.3) (c)	ND(1.3) (c)	ND(2.4) (d)	ND(1.3) (d)	ND(1.3) (d)	ND(1.3) (d)	5.4 [108]	ND(1.3) (c)	ND(19.6) (d)

Comment Key:

* ND(x), indicates the target compound was not found, and the parentheses contain the minimum detectable quantity.

[X] Bracketed values represent per cent recoveries.

(a) Analyst confirmed target analyte supressed by chemical noise.

(b) Quantitation ion appears outside five scan window relative to standard

(c) Peak detected in retention time window does not meet 0.40 library fit requirement for target compound.

(d) Peak detected in retention time window met 0.40 library fit requirement for target compound but quantification ion did not meet required signal to noise value.

TABLE 15 - continued

RESULTS OF ANALYSES OF TWRA MUSSEL AND FISH SAMPLES FOR TARGET PESTICIDES AND RELATED COMPOUND

Wright State University - Dayton, Ohio 45435

	WSU I.D. NUMBERS			Concentration in ng/g					
COMPOUND	LB05221-1 METHOD BLANK	TRA1-1B TENN RIVER RICHLAND CREEK IN CHANNEL, 50', WASHBOARD	TRA1-2B BLUE CATFISH KENTUCKY LAKE	TRA1-3B TENN RIVER RICHLAND CREEK IN CHANNEL, 50', EBONY SHELL	TRA1-4B TRM 197.5, UPSTREAM DIAMOND ISLAND, RIGHT BANK 20', EBONY SHELL	TRA1-5B TENN RIVER @ RICHLAND CREEK OVERBANK, 14'	TRA1-5FB TENN RIVER @ RICHLAND CREEK OVERBANK, 14' (SPIKE)	TRA1-6B BIG SANDY EMBAYMENT MAPLE LEAF	TRA1-7 CHANNEL C KENTUCKY CONTROL SA
DIETHYLPHTHALATE	ND(1.3) (c)	1.4	5.7	ND(1.3) (d)	2.0	4.5	37.5 [75]	ND(1.3) (d)	ND(3.9 (d)
DIAZINON	ND(1.3) (c)	2.6	18.4	4.1	2.1 (b)	2.3	32.9 [66]	ND(1.3) (d)	32.9
DISULFOTON	ND(1.3) (c)	ND(1.3) (c)	ND(2.4) (c)	ND(1.3) (c)	ND(1.3) (c)	ND(1.3) (c)	6.7 [13]	ND(1.3) (c)	ND(3.9 (c)
METHYLPARATHION	ND(1.3) (c)	15.8	458	ND(1.3) (d)	61.5	ND(710) (c)	60.5 [121]	ND(17.0) (c)	140
DI-N-BUTYLPHTHALATE	6.5	9.0	41.5	3.8	10.0	11.6	56 [112]*	6.4	76.9

Comment Key:

* ND(x), indicates the target compound was not found, and the parentheses contain the minimum detectable quantity.

[X] Bracketed values represent per cent recoveries.

(a) Analyst confirmed target analyte supressed by chemical noise.

(b) Quantitation ion appears outside five scan window relative to standard

(c) Peak detected in retention time window does not meet 0.40 library fit requirement for target compound.

(d) Peak detected in retention time window met 0.40 library fit requirement for target compound but quantification ion did not meet required signal to noise value.

TABLE 16

Results of PCB Analysis for Tennessee Wildlife Resources Agency

Concentration in Actual Samples (ng g)

WIC Sample ID	Customer ID	Mono PCB	Di PCB	Tri PCB	Tetra PCB	Penta PCB	Hexa PCB	Hepta PCB	Octa PCB	Deca PCB
TRA1-1B	Tenn River @ Richland Creek In Channel, 50', Washboard	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00
TRA1-2B	Blue Catfish KY Lake	< 9.30	< 9.30	< 9.30	< 9.30	11.15	54.19	< 9.30	< 9.30	< 9.30
TRA1-3B	Tenn. River @ Richland Creek In Channel, 50', Ebony Shell	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01
TRA1-4B	TRM 197.5, Upstream Diamond Island, Right Bank, 20', Ebony Shell	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01
TRA1-5B	Tenn. River @ Richland Creek Overbank, 14'	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01
TRA1-5C	Tenn. River @ Richland Creek Overbank, 14'	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01
TRA1-5D	Tenn. River @ Richland Creek Overbank, 14'	< 5.01	< 5.01	< 5.01	< 5.01	25.11 [33.4]	8.93 [11.9]	< 5.01	< 5.01	< 5.01
TRA1-5E	Tenn. River @ Richland Creek Overbank, 14'	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01
TRA1-6B	Big Sandy Embayment Maple Leaf	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01
TRA1-7B	Channel Catfish KY. Lake Control Sample	< 15.00	< 15.00	< 15.00	< 15.00	37.83	42.10	< 15.00	< 15.00	< 15.00
L3005141-1		< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00
TRA1-8P	Tenn. River @ Richland Creek Overbank, 14'	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01	< 5.01
L3005211-1		< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00	< 5.00

Comment Key:

* ND(x), indicates the target compound was not found, and the parentheses contain the minimum detectable quantity.

[X] Bracketed values represent percent recoveries.

TABLE 17

RESULTS OF ANALYSES OF TWRA MUSSEL AND FISH SAMPLES
FOR 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN AND 2,3,7,8-TETRACHLORODIBENZOFURAN
Wright State University, Dayton, Ohio 45435

Column - DB-DIOXIN 60M , 0.25u

Analysis for Substituted 2378 Dioxins and Furans

Concentrations Found (picograms per gram of sample or parts-per-trillion)a.

TENNESSEE WRA 2327 Sample Number	2378 TCDF	2378 TCDD
TENN RIVER @ RICH.	1.79	ND 0.497
BLUE CATFISH KY LAKE	5.49	1.51
TENN. RIVER @ RICH.	2.55	ND 0.808
TRM 197.5, UPSTREAM	1.99	ND 0.287
TENN. RIVER @ RICH.	2.87	ND 0.581
BIG SANDY EMBAYMENT	ND 0.507	ND 0.363
CHANNEL CATFISH KY LAKE	3.83	1.90
LAB BLANK	ND 0.261	ND 0.363

a. The designation ND indicates "None Detected" in excess of the minimum detectable concentration which is listed directly below the ND designation.

THE OCCURRENCE OF ORGANIC CONTAMINANTS IN MUSSELS FROM KENTUCKY LAKE

1990

Table . Summary of tissue analysis performed on freshwater mussels collected from the Tennessee River, Kentucky Lake Reservoir, 1990. ND signifies NONE DETECTED. All data are given as ppt except dioxin and difuran are given as ppt.

Reservoir	Kentucky Lake	Kentucky Kake	Kentucky Lake	Kentucky Lake	Kentucky Lake
River Mile	TRM 197.5	TRM 88.1	TRM 88.1	TRM 88.1	B. Sandy Embay.
Species	F. ebena	F. ebena	F. ebena	M. nervosa	Q. quadrula
Area (Depth in ft.)	Tailwater (25)	Overbank (14)	Channel (50)	Channel (50)	Overbank (14)
Sample Date	11 Sep 90	11 Dec 90	11 Dec 90	11 Dec 90	18 Sep 90
Compound					
Iodobenzene	14.3	12.6	12.9	21.7	7.1
Iodonapthalene	63.5	83.1	38.6	112.0	38.6
4,4'-Diiodobiphenyl	125.0	173.0	66.2	151.0	153.0
1,3,5-Trichlorobenzene	ND	ND	ND	ND	ND
1,2,4,-trichlorobenzene	ND	ND	ND	ND	ND
Hexachlorobutadiene	ND	ND	ND	ND	ND
1,2,3-Trichlorobenzene	ND	ND	ND	ND	ND
1,2,3,5-Tetrachlorobenzene	ND	ND	ND	ND	ND
1,2,4,5-Tetrachlorobenzene	ND	ND	ND	ND	ND
Biphenyl	ND	ND	ND	ND	ND
1,2,3,4-Tetrachlorobenzene	ND	ND	ND	ND	ND
Pentachlorobenzene	ND	ND	ND	ND	ND
Trifluralin	ND	ND	ND	ND	ND
Alpha-BHC	5.2	11.4	3.0	8.2	3.2
Hexachlorobenzene	ND	ND	ND	ND	ND
Pentachloroanisole	ND	ND	ND	ND	ND
Lindane	9.3	17.0	4.1	ND	ND
Pentachloronitrobenzene	ND	3.2	ND	ND	ND
Diphenyldisulfide	ND	ND	ND	ND	ND
Alachlor	3.4	13.1	ND	ND	ND

Table . ...continued.

Reservoir	Kentucky Lake	Kentucky Lake	Kentucky Lake	Kentucky Lake	Kentucky Lake
River Mile	TRM 197.5	TRM 88.1	TRM 88.1	TRM 88.1	B. Sandy Embay.
Species	F. ebena	F. ebena	F. ebena.	M. nervosa	Q. quadrula
Area (Depthin ft.)	Tailwater (25)	Overbank (14)	Channel (50)	Channel (50)	Overbank (14)
Sample Date	11 Sep 90	11 Dec 90	11 Dec 90	11 Dec 90	18 Sep 90
Compounds					
Heptachlor	ND	1.4	ND	1.4	ND
Chloropyrifos	ND	ND	4.7	3.0	6.3
Isopropalin	ND	ND	ND	ND	ND
Octachlorostyrene	ND	ND	ND	ND	ND
Heptachlororepoxide	ND	ND	ND	ND	ND
Oxychlordane	26.2	9.1	ND	ND	ND
Butachlor	ND	3.5	ND	ND	ND
Chlordane (trans)	ND	ND	ND	ND	ND
Chlordane (cis)	ND	ND	ND	ND	ND
Nonachlor (trans)	ND	ND	ND	ND	ND
DDE-p-p'	12.8	16.3	4.9	8.6	ND
Dieldrin	10.4	ND	29.1	3.6	ND
Perthane	3.4	ND	ND	ND	ND
Nitrofen	ND	ND	ND	ND	ND
Chlorobenzilate	ND	ND	ND	ND	5.7
Endrin	ND	14.1	ND	ND	ND
Nonachlor (cis)	ND	ND	ND	ND	ND
Triphenylphosphate	ND	ND	ND	ND	ND
Methaoxychlor	ND	ND	ND	ND	ND
Dicofol	ND	ND	ND	ND	ND
Mirex	ND	ND	ND	ND	ND

Table . ...continued.

Reservoir	Kentucky Lake	Kentucky Lake	Kentucky Lake	Kentucky Lake	Kentucky Lake
River Mile	TRM 197.5	TRM 88.1	TRM 88.1.	TRM 88.1	B. sandy Embay.
Species	F. ebena	F. ebena	F. ebena	M. nervosa	Q. quadrula
Area (Depth in ft.)	Tailwater (25)	Overbank (14)	Channel (50)	Channel (50)	Overbank (14)
Sample Date	11 Sep 90	11 Dec 90	11 Dec 90	11 Dec 90	18 Sep 90
Compounds					
Diethylphthalate	2.0	4.5	ND	1.4	ND
Diazinon	2.1	2.3	4.1	2.6	ND
Disulfoton	ND	ND	ND	ND	ND
Methylparathion	61.5	ND	ND	15.8	ND
Di-n-butylphthalate	10.0	11.6	3.8	9.0	6.4
PCB	ND	ND	ND	ND	ND
Dioxin	ND	ND	ND	ND	ND
Difuran	1.99	2.87	2.55	1.79	ND
Compounds Detected	15	16	11	13	7

Table . Comparison of tissue analysis of the freshwater mussel Fusconia ebena, from two locations in Kentucky Lake Reservoir for two consecutive years. ND signifies NONE DETECTED. All data are given as ppb, except dioxin and difuran are given as ppt.

Reservoir Area	Pickwick Reservoir Tailwaters		Harmon Creek	
River Mile	200.0		89.0	
Sample Year	1989	1990	1989	1990
Compounds				
Iodobenzene	36.3	14.3	25.8	12.6
Iodonapthalene	72.0	63.5	89.4	83.1
4,4'-Diiodobiphenyl	152.0	125.0	185.0	173.0
1,3,5-Trichlorobenzene	ND	ND	ND	ND
1,2,4-Trichlorobenzene	ND	ND	ND	ND
Hexachlorobutadiene	ND	ND	ND	ND
1,2,3-trichlorobenzene	ND	ND	ND	ND
1,2,3,5-Tetrachlorobenzene	ND	ND	ND	ND
1,2,4,5-tetrachlorobenzene	ND	ND	ND	ND
Byphenyl	ND	ND	ND	ND
1,2,3,4-Tetrachlorobenzene	ND	ND	ND	ND
Pentachlorobenzene	ND	ND	ND	ND
Trifluralin	ND	ND	ND	ND
Alpha-BHC	ND	5.2	ND	11.4
Hexachlorobenzene	ND	ND	ND	ND
Pentachloroanisole	ND	ND	ND	ND
Lindane	ND	9.3	ND	17.0
Pentachloronitrobenzene	ND	ND	19.6	3.2
Diphenyldisulfide	ND	ND	ND	ND
Alachlor	ND	3.4	ND	13.2

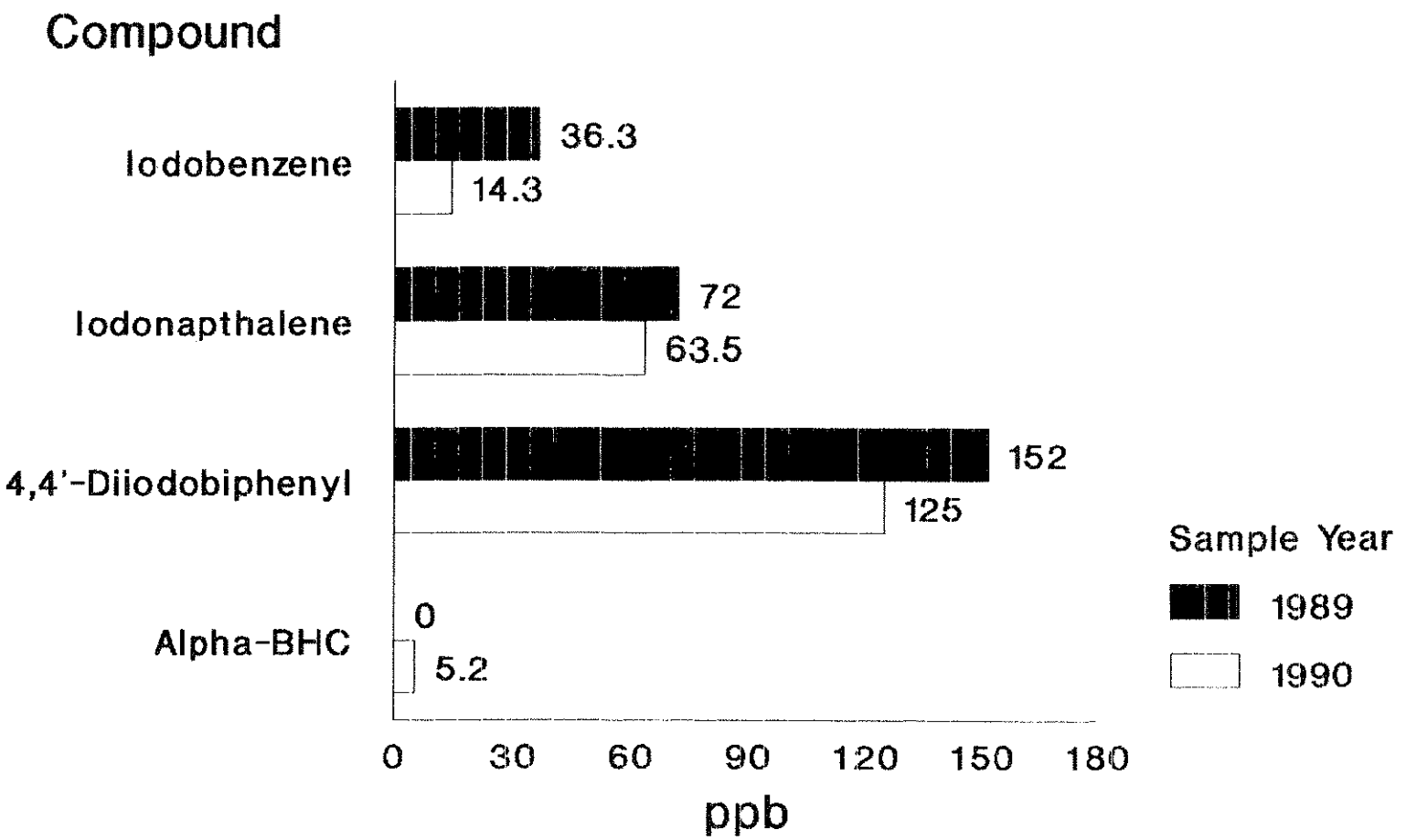
Table . ...continued.

Reservoir Area	Pickwick Res. Tailwater		Harmon Creek	
River Mile	200.0		89.0	
Sample Year	1989	1990	1989	1990
Heptachlor	ND	ND	ND	1.4
Chloropyrifos	ND	ND	ND	ND
Isopropalin	ND	ND	ND	ND
Octachlorostyrene	ND	ND	ND	ND
Heptachlorepoxide	ND	ND	ND	ND
Oxychlordane	ND	26.2	ND	9.1
Butachlor	ND	ND	ND	3.5
Chlordane (trans)	ND	ND	ND	ND
Chlordane (cis)	ND	ND	ND	ND
Nonachlor (trans)	ND	ND	ND	ND
DDE-p-p'	16.5	12.8	8.3	16.3
Dieldrin	21.9	10.4	199.0	ND
Perthane	ND	3.4	ND	ND
Nitrofen	ND	ND	ND	ND
Chlorobenzilate	ND	ND	ND	ND
Endrin	ND	ND	ND	14.1
Nonachlor (cis)	ND	ND	ND	ND
Triphenylphosphate	ND	ND	ND	ND
Methoxychlor	ND	ND	ND	ND
Dicofol	ND	ND	ND	ND
Mirex	ND	ND	ND	ND
Diethylphthalate	15.5	2.0	20.4	4.5
Diazinon	ND	2.1	ND	2.3
Disulfoton	35.5	ND	32.8	ND

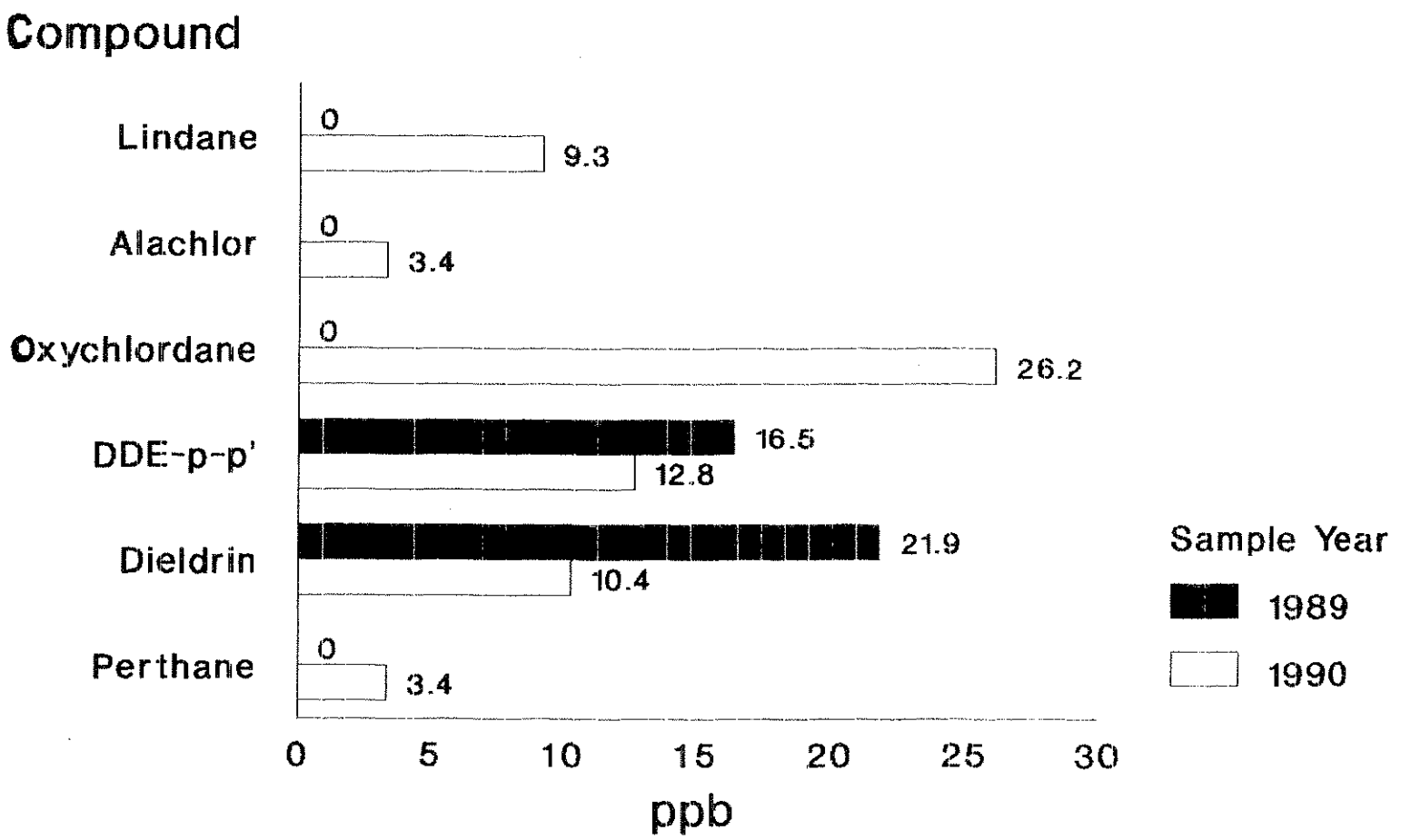
Table . ...continued.

Reservoir Area	Pickwick Res. Tailwater		Harmon Creek	
River Mile	200.0		89.0	
Sample Year	1989	1990	1989	1990
Methylparathion	237.0	61.5	350.0	ND
Di-n-butylphthalate	3.1	10.0	18.8	11.6
PCB	ND	ND	ND	ND
TCDD	ND	ND	ND	ND
TCDF	2.11	1.99	1.85	2.87
Compounds Detected	10	15	11	16

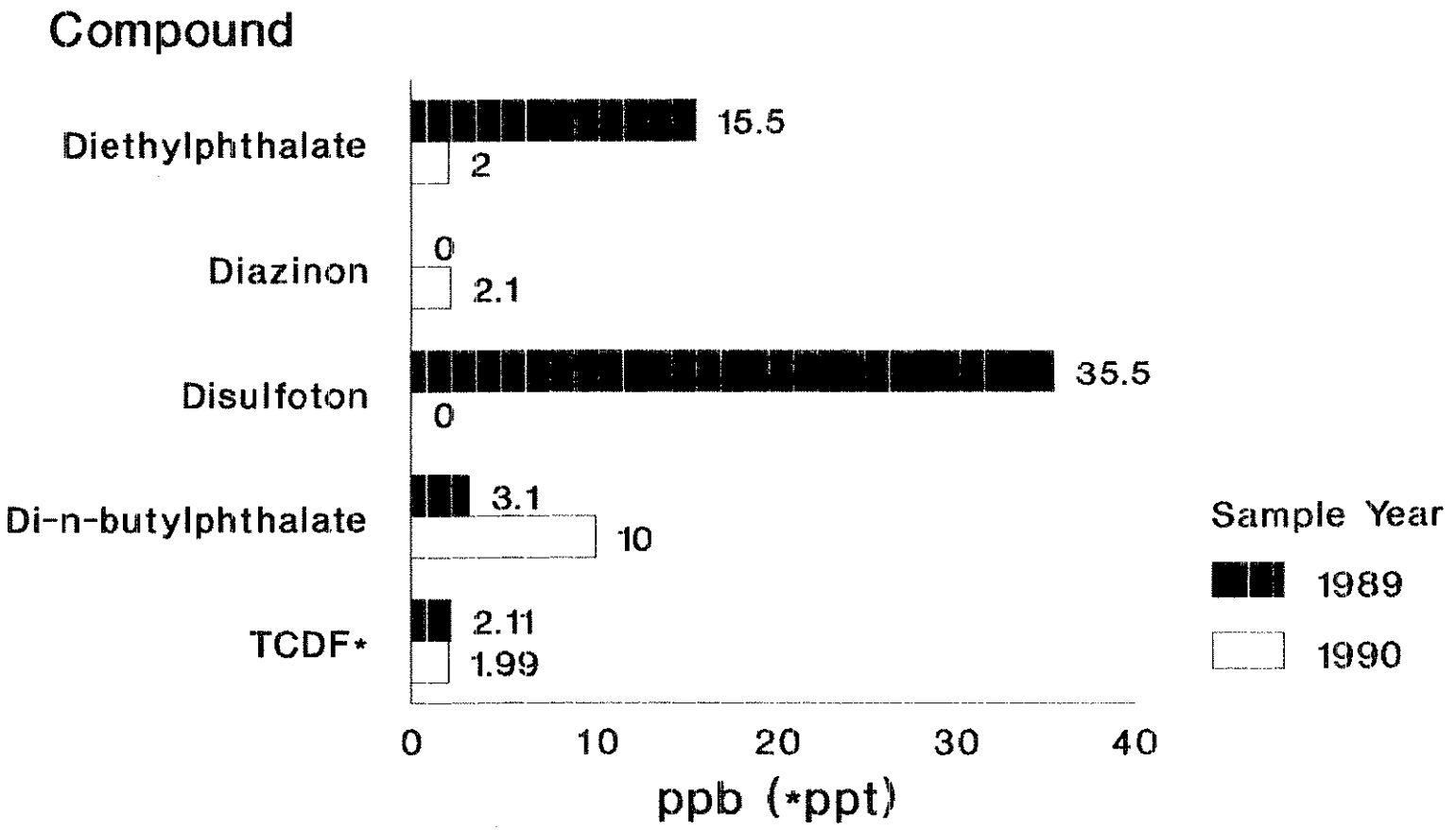
Comparison of tissue analysis of the freshwater mussel Fusconaia ebena from the Pickwick tailwater area, 1989-90.



Comparison of tissue analysis of the freshwater mussel Fusconaia ebena from the Pickwick tailwater area, 1989-90.

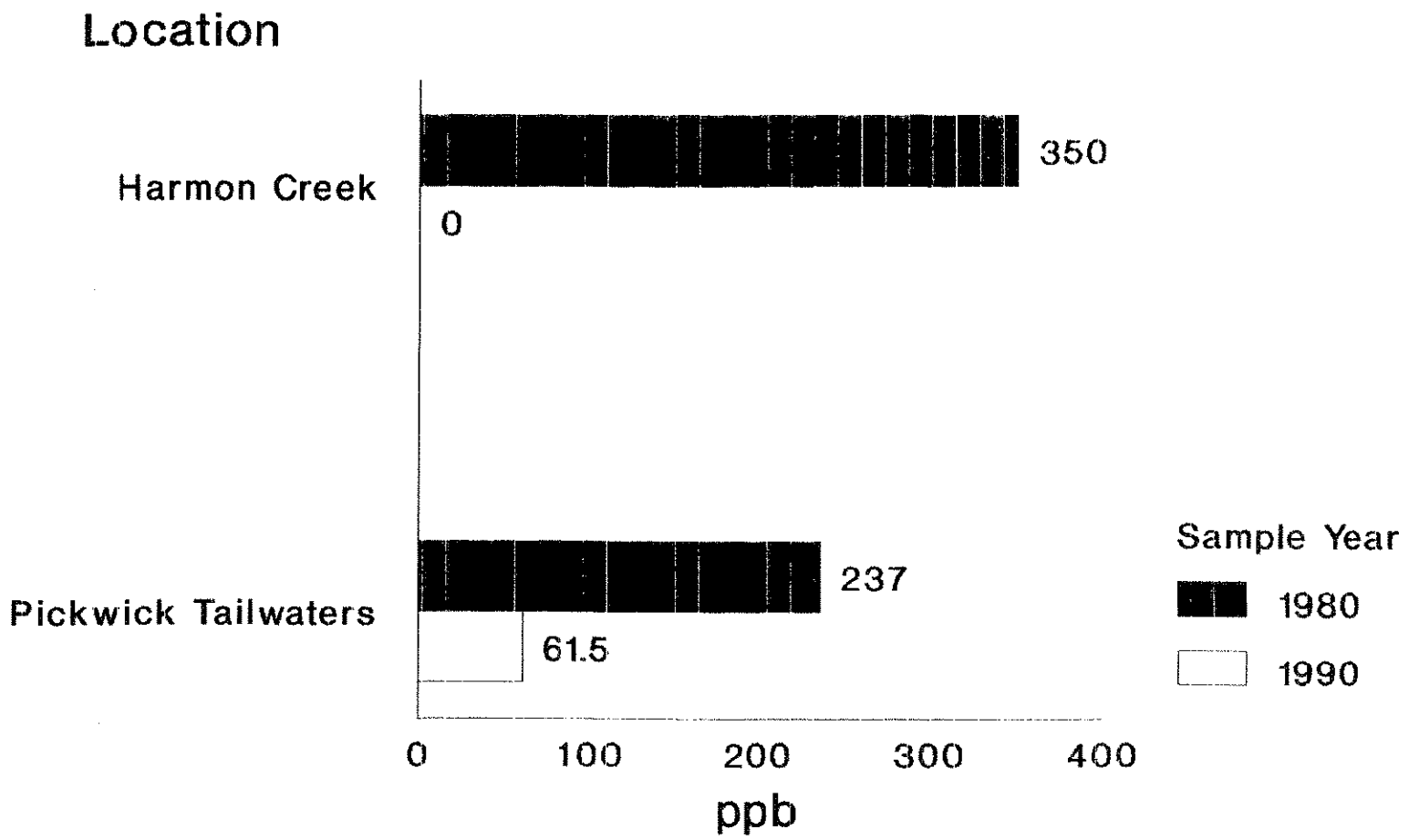


Comparison of tissue analysis of the freshwater mussel Fusconaia ebena from the Pickwick tailwater area, 1989-90.

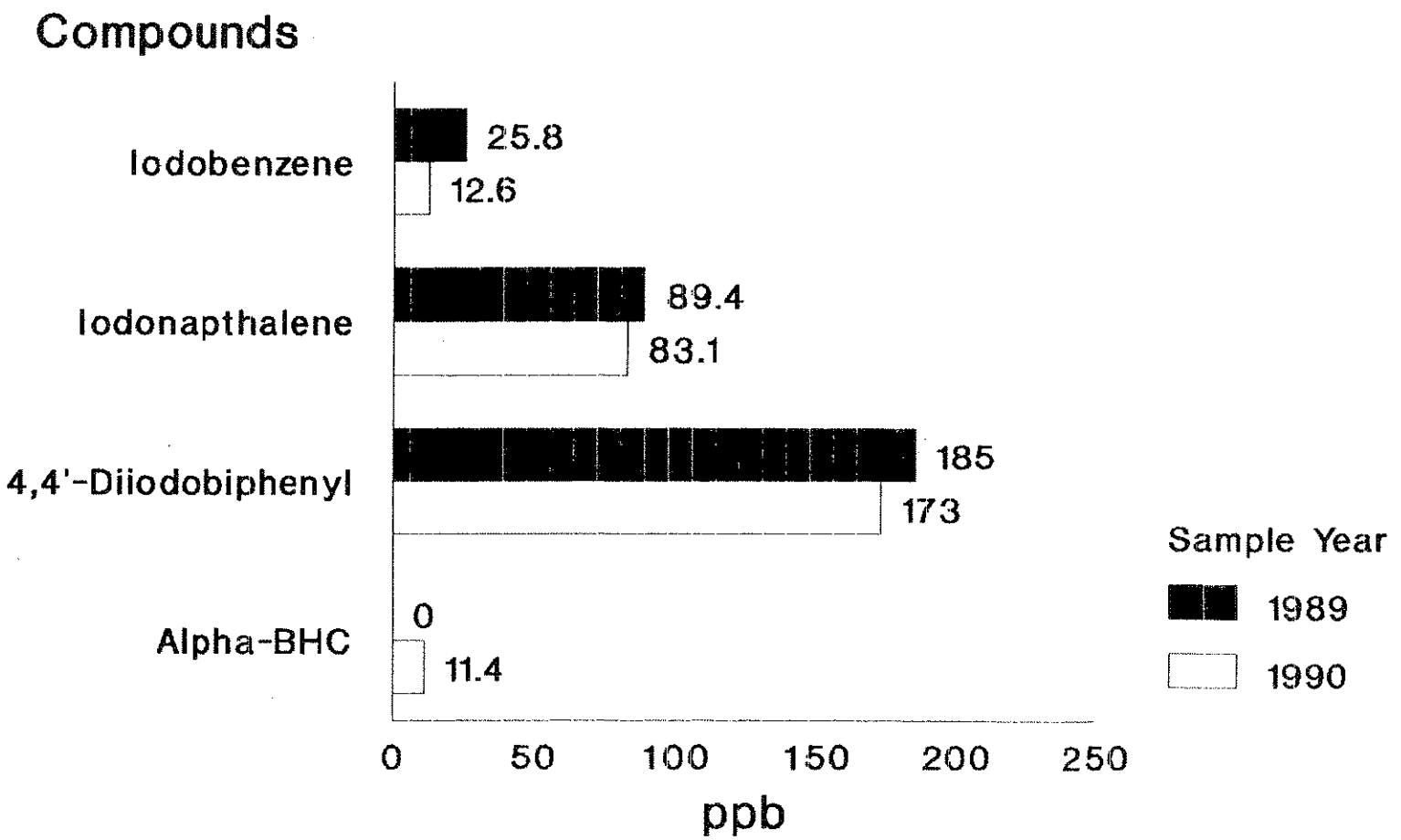


* TCDF given as ppt.

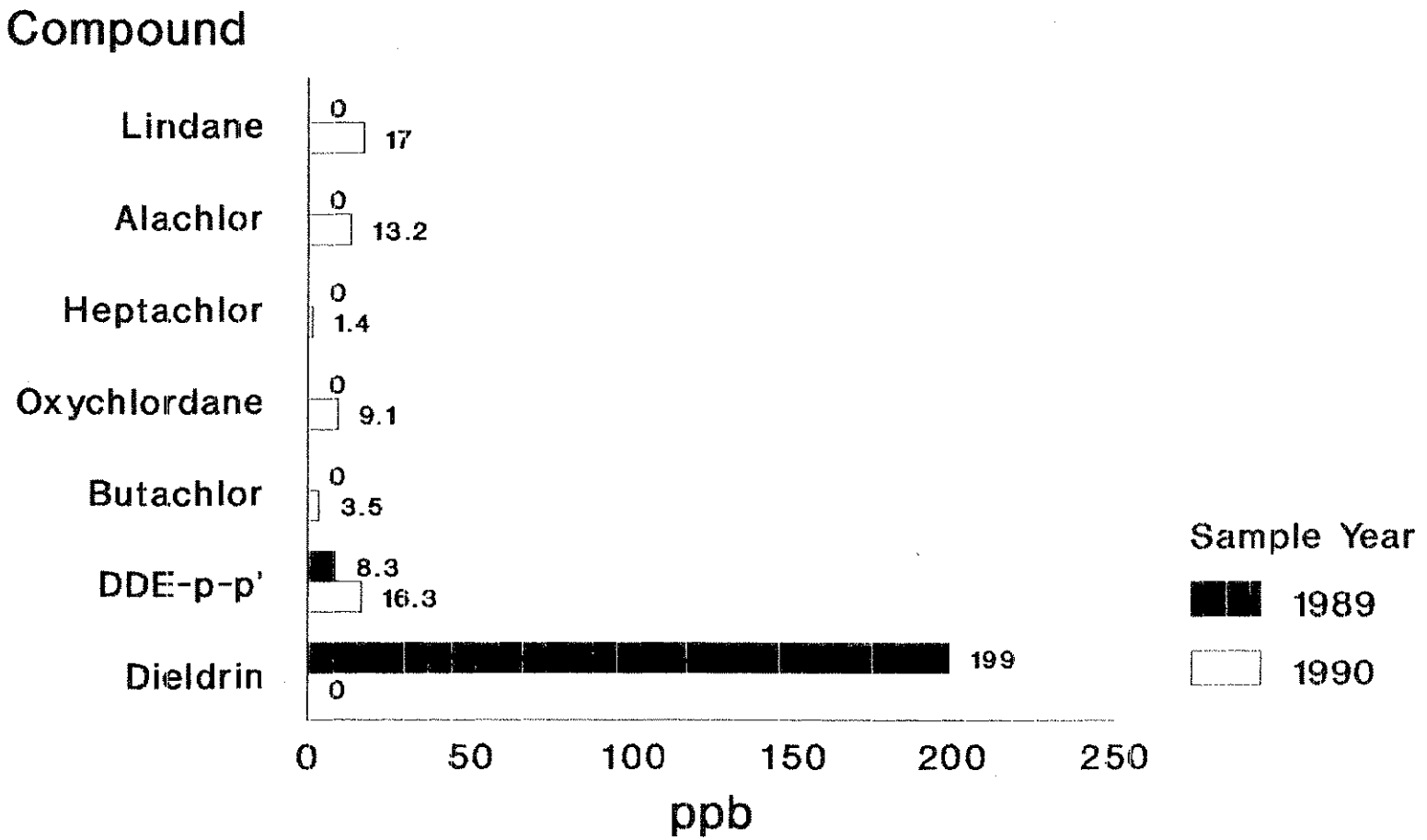
Comparison of the concentration of methylparathion in Fusconaia ebena from two locations in Kentucky Lake, 1989--90.



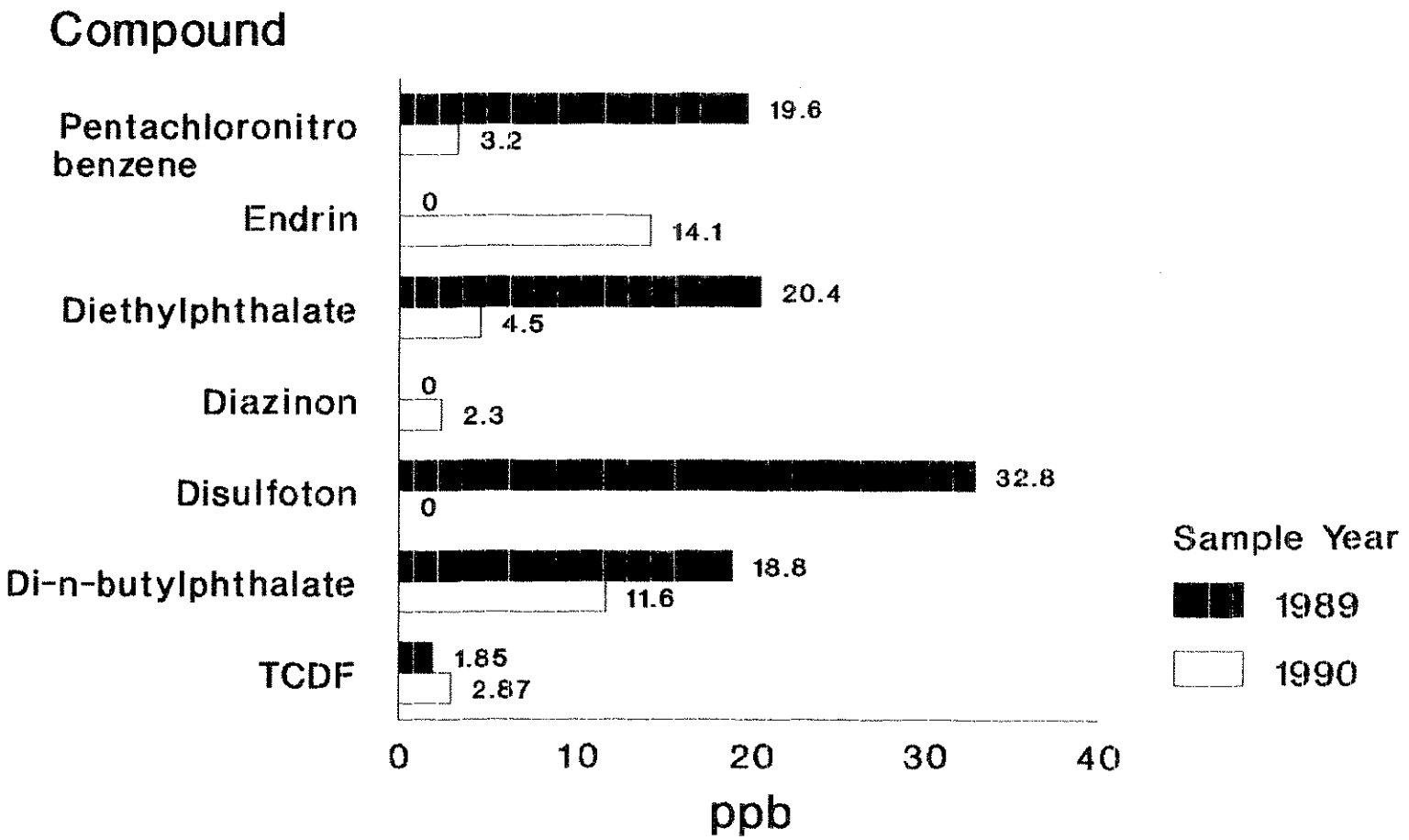
Comparison of tissue analysis of the freshwater mussel Fusconaia ebena from the Harmon Creek area, 1989-90.



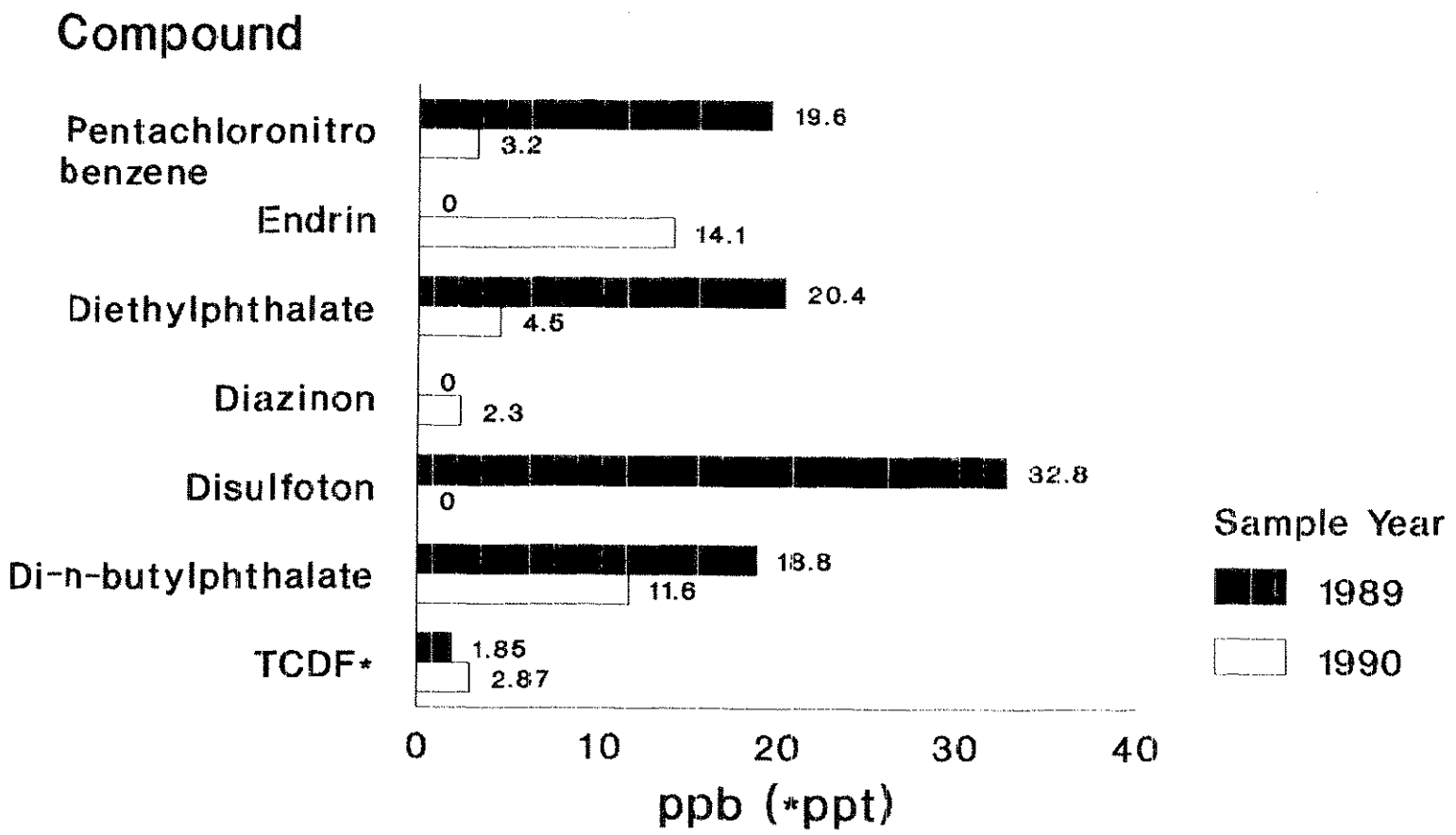
Comparison of tissue analysis of the freshwater mussel Fusconaia ebena from the Harmon Creek area, 1989-90.



Comparison of tissue analysis of the freshwater mussel Fusconaia ebena from the Harmon Creek area, 1989-90.



Comparison of tissue analysis of the freshwater mussel Fusconaia ebena from the Harmon Creek area, 1989-90.



* TCDF is given as ppt.