

Feasibility of *Corbicula flumina* as a Biomarker for Environmental Pollutants in the Willamette River

By Douglas Lauderbach

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Faculty Mentor:

Max J. Dennis

Date:

6/26/96

Advising Mentor:

Douglas F. Bouff

Date:

6/26/96

BRR Director :

John B. Hay

Date:

26 Jun 96

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DESCRIPTIVE ABSTRACT

This report examines the feasibility of using the bivalve *Corbicula flumina* as a biomarker for environmental contaminants in the Willamette River. Compounds such as DDT, dioxins, furans, and PCBs persist in the environment, pose health related concerns, and due to their lipophilic nature accumulate in biological organisms. The success of *Corbicula flumina* as a biomarker will be based on;

1. Detection of DDT, dioxins, furans, and PCBs.
2. Ease of Collecting *Corbicula flumina* from the Willamette River.
3. Abundance of *Corbicula flumina* in the Willamette River.

INTRODUCTION

Environmental studies show that chlorinated organic compounds such as DDT, dioxins, furans, and polychlorinated biphenyls (PCBs) are persistent environmental pollutants ^(1,2). DDT was a widely used insecticide due to its effectiveness and inexpensive cost. It was banned in 1972 after studies indicated DDT and its metabolite DDE were persistent environmental pollutants that accumulated in biological organisms.

Dioxins and Furans are byproducts of municipal waste incineration, the bleaching process in pulp and paper mills, and waste water treatment facilities ⁽¹⁾. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is considered the most toxic dioxin compound. Overall toxicity of all other dioxins and furans are compared to TCDD ⁽³⁾. The Environmental Protection Agency (EPA) and the International Agency for Research on Cancer have classified TCDD as a probable human carcinogen, based on animal studies ^(4,5). In addition, exposure to TCDD has been linked to reproductive dysfunction and immune system suppression in laboratory animals ^(3,6,7). Dioxins and Furans can accumulate in biological organisms such as fish, bald eagles, and humans ^(8,9,10,11).

Polychlorinated biphenyls are chemically stable compounds that were used in industry as electrical insulation, hydraulic fluids, and flame retardants ⁽¹²⁾. The

chemical stability of PCBs have made them persistent environmental pollutants that can accumulate in biological organisms such as fish ^(1,8). Laboratory studies examining health effects associated with PCBs have shown a link between PCB exposure and immune system and neurotransmitter suppression in animals ^(13,14).

The abilities of chlorinated organic compounds such as DDT, dioxins, furans, and PCBs to accumulate in biological organisms can be categorized as bioconcentration, bioaccumulation, or biomagnification. Bioconcentration is the uptake of a chemical by a biological organism through direct contact with its environment. Bioaccumulation is the uptake of a chemical by a biological organisms through bioconcentration and ingestion of chemically tainted food. Biomagnification is a direct relationship between increased concentrations of a chemical and the higher a biological organism is on the food chain.

Biological organisms which concentrate environmental pollutants are often used as biomarkers. By analyzing the tissue of a biomarker organism it is possible to determine the presence of a compound in the environment. For aquatic environments the criteria for biomarkers are;

1. The organism should bioaccumulate the pollutant without mortality.
2. The organism should be sedentary, which allows for specific geographical information.
3. The organism should be present in large quantities.

4. The organism should have a life span exceeding one year.
5. The organism should be of sufficient size so as to provide sufficient tissue for analysis.
6. Collection of the organism should be relatively easy ⁽¹⁵⁾.

Examples of aquatic biomarkers are fish, crayfish, and bivalves. In a study analyzing fish from the Willamette River DDE, dioxins, furans, and PCBs were detected ⁽⁸⁾. A study examining the Lower Columbia River Basin detected DDE, dioxins, furans, and PCBs in fish and crayfish ⁽⁴⁾. Bivalves have been used to detect the presence of chlorinated organic compounds ^(15,16). Oregon's Department of Environmental Quality and Washington's Department of Ecology have identified the bivalve *Corbicula flumina* as a possible biomarker for chlorinated organic compounds, for the Columbia and Willamette Rivers ⁽⁴⁾.

Feasibility of *Corbicula flumina* as a Biomarker for the Willamette River

Corbicula flumina also known as *C. manilensis* or *C. laena* ranges from Northern California to Washington and as far East as Florida. *Corbicula flumina* was introduced into the United State from Asia ⁽¹⁷⁾. The filter feeding characteristics of bivalves should expose them to chemicals that are present in the water and those associated with suspended particles or organic material. Bivalves are sedentary organisms which allow for site specific information about the presence of environmental pollutants. *Corbicula flumina* has a life span exceeding one year and

ranges in size from approximately 3 to 5 cm in width, which should supply ample tissue for analysis ⁽¹⁷⁾. A study of the Kaskaskia River in Illinois indicated that *Corbicula flumina* is a successful biomarker for Organochlorine pesticides ⁽¹⁵⁾.

Research Objective

This report examines the feasibility of using the bivalve *Corbicula flumina* as a biomarker for DDT, dioxins, furans, and PCBs in the Willamette River. Samples will be acquired from the Willamette River north of Willamette Falls in the Portland Metropolitan Area. Past studies indicate this area has high concentrations of chlorinated organic compounds ^(4,8). The feasibility of *Corbicula flumina* as a biomarker for the Willamette River is based on three criteria.

1. The presence of DDT, dioxins, furans, and PCBs in the bivalve tissue.
2. The relative ease of collecting *Corbicula flumina* samples from the Willamette River.
3. The abundance of *Corbicula flumina* in the Willamette River.

MATERIALS AND METHODS

Sample Collection

Bivalves were collected from four sites along the Willamette River between the Willamette Falls in Oregon City and the mouth of the Willamette River. Site A (river km 41.2) consisted of gravel, site B (river km 27.8) had a narrow gravel bar adjacent to a tidal flat (river km 7.2), site C (river km 7.8) consisted of gravel, site D (river km 7.0) was a combination of gravel and firm soil resembling clay. All samples were collected from the west bank of the Willamette River between September 10 to 15, 1995.

Collection sites were deemed suitable after a minimum of 30 bivalves had been visually accounted for within an approximate 50 meters of river bank. Bivalves were collected by hand and initially stored in a mesh net. Upon collection of 30 plus bivalves they were removed from the mesh net and placed inside an igloo cooler. The bivalves were then transferred to screw top glass jars and stored in a freezer at -10°C until tissue analysis. Average time elapse of 3.0 hours between initiating bivalve collection and storage in freezer.

Sample Identification

Bivalve samples collected from sites A through D were identified as *Corbicula flumina* using the taxonomy book Fresh Water Invertebrates of the United States by R. W. Pennal ⁽¹⁶⁾.

Laboratory Materials Preparation

All Metalware and glassware was washed, rinsed with distilled water, and then baked in a pesticide oven at 470° C for 10 hours. After cooling, glassware was sealed with aluminum foil and stored in designated cabinets. Glassware which I was unable to seal with aluminum foil was baked immediately prior to its use.

Plastic and Teflon items were washed, then rinsed with distilled water. Teflon lid liners were rinsed with dichloromethane. Teflon stopcocks were rinsed with methanol when using columns to prepare reagents, cyclohexane when using the defatting column, and hexane when using the alumina column.

Sample Analysis Parameters

Samples from location A through D were divided into two sets, experimental run 1 and run 2. Each experimental run included a sample from locations A through

D, a blank, and a control. The blank consisted of 100 g of sodium sulfate. The control consisted of nonfortified tissue from marine mussels purchased from Cub Foods in Corvallis, Oregon. Marine mussels should have lower concentrations of chlorinated organic compounds than the *Corbicula flumina* samples collected from the Willamette River. The larger water volume of the marine environment should result in environmental pollutants at lower concentrations than in the Willamette River.

Experimental Procedure Overview

The preparation for the analysis of the bivalve tissue consists of six steps;

1. Tissue Preparation
2. Tissue Extraction
3. Defatting Column
4. Decolorizing and Alumina Column
5. Carbon Column
6. GC-MS Preparation

The bivalves tissue is prepared by grinding 25 g of tissue and 100 g of sodium sulfate to a homogenized mixture. The resulting sodium sulfate plus tissue mixture is a dry powder . The sodium sulfate plus tissue mixture is then fortified with two internal standards C¹³- 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and 2,2',3,4,4',5,6,6' Octachlorobiphenyl (PCB 204). The internal standards are used to determine percent recovery and to quantify the compounds in the tissue.

The sodium sulfate plus tissue mixture is placed in a 100 ml Soxhlet extraction thimble. A Soxhlet apparatus with 700 ml of a 1:1 ratio of cyclohexane and dichloromethane is used to extract the tissue. Voltage supplied by a Variac regulator to a heating mantle is set so the solvent cycles every 15 minutes. The Soxhlet is run for 24 hours resulting in approximately 100 cycles. When finished the sample is a bright yellow.

Next the sample is applied to a defatting column consisting of sodium sulfate, potassium silicate, and silica gel. The sodium sulfate removes any water from the sample. The polar sites on the potassium silicate and silica gel bind with the polar sites on the lipids in the sample. The lipids remain associated with the reagents while DDT, dioxins, furans, and PCBs elute out of the column. When finished the sample is a pale yellow.

The sample is then applied to a decolorizing and alumina column. The two columns are set up in tandem so elutant from the decolorizing column enters the alumina column. The decolorizing column contains cesium silicate and 40% sulfuric acid in silica gel. Cesium silicate has a basic pH. The 40% sulfuric acid in silica gel has an acidic pH. The acidic and basic properties of these reagents cause the breakdown of various organic compounds into small polar constituents. These bind with the polar sites on the silica gel. When the sample elutes from the decolorizing column it is clear.

The alumina column contains alumina and sodium sulfate. Alumina interacts with polar compounds. The alumina column is used to separate DDT and PCBs from the dioxins and furans. DDT and PCBs are less polar so they elute out of the column earlier. They are collected and prepared for GC-MS analysis. Dioxins and furans are more polar so they elute out of the column later. They are collected and applied to a carbon column.

The carbon column contains silica gel and a carbon silica gel mixture. Carbon interacts with planar compounds. Dioxins and furans are planar compounds so they associate with the carbon. Nonplanar compounds elute out of the column. The column is inverted and toluene is used to elute out the dioxins and furans.

Samples are now prepared for GC-MS analysis. The sample containing DDT and PCBs and the sample containing dioxins and furans are reduced to 100 μ l. A automatic pipette is used to transfer the sample to a 100 μ l glass ampoule. The sample is then reduced to less than 1 μ l. It is then reconstituted with 10 μ l of nonane. The glass ampoule is placed in a 1 ml screw top vial, and sealed with a cap containing a Teflon septum. The sample is now ready for GC-MS analysis.

Tissue Preparation

The sample container was removed from the freezer immediately before tissue extraction. Removing the bivalve tissue from the shell is easiest when the tissue is frozen.

Wearing latex gloves which were previously rinsed with distilled water, working in a vacuum hood, I removed 20 bivalves from the sample container and placed them in a 400 ml beaker. Grasping the bivalve with one hand the shell is pried open with a scoopula and the tissue is removed. The bivalve tissue is placed in a Buchner funnel to allow the tissue to thaw and to collect excess water into a 250 ml Erlenmeyer flask. The Buchner funnel is covered with aluminum foil and left for approximately 1 hour.

25 grams of wet tissue are collected in a mortar and mixed with 100 grams of sodium sulfate. The sodium sulfate bivalve tissue mixture is ground in the mortar with a pestle. After achieving a homogenous mixture, it is covered with aluminum foil and left for 10 minutes after which it is ground again; this is repeated four times.

Sodium sulfate was baked at 600° C for 48 hours and then stored at 130° C. Sodium sulfate anhydrous granular, (12-60 Mesh), was obtained from J.T.Baker Inc., Philipsburg, NJ.

Tissue Fortification

Tweezers are used to place a 100 ml Soxhlet extraction thimble into a beaker. The sodium sulfate tissue mixture is transferred from the mortar into the Soxhlet extraction thimble with a scoopula. The mortar, pestle, and scoopula are rinsed with three 1 ml washes of cyclohexane which are transferred into the extraction thimble with a disposable glass pipette. The sodium sulfate plus tissue mixture is then covered with a thin layer of glass wool.

The sodium sulfate tissue mixture is fortified with 250 pg of a C¹³-2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) and 25 ng of a 2,2',3,4,4',5,6,6' Octachlorobiphenyl (PCB 204) internal standard. The C¹³-2,3,7,8-Tetrachlorodibenzo-p-Dioxin internal standard is a carbon 13 isotope obtained from Cambridge Isotope Laboratories Inc., Cambridge, MA. 50 µl of a 50 ng/ml solution of C¹³ TCDD in nonane is used. The 2,2',3,4,4',5,6,6' octachlorobiphenyl internal standard was obtained from ULTRA Scientific, North Kingstown, RI. 25 µl of a 10 µg/ml solution of PCB 204 in nonane is used. The Soxhlet extraction thimble is then placed into the Soxhlet apparatus.

Glass wool is placed in a beaker then rinsed with distilled water and baked in a pesticide oven at 475° C for ten hours. After cooling the beaker is covered with aluminum foil.

Nonane was obtained from Aldrich Chemical Company, Inc., Milwaukee, WI.

C^{13} TCDD and PCB 204 internal standard solutions are stored in specified refrigerators in glass stoppered volumetric flasks sealed with parafilm. Solutions are allowed to warm to room temperature prior to sample fortification

A 25 μ l and 50 μ l Wiretrol pipette is used to pipette the internal standards. Each internal standard has a specified Wiretrol pipette plunger. After each use the plunger is rinsed with toluene and the glass pipette is discarded.

Wiretrol pipettes are obtained from Drummond Scientific Company, Broomall, PA.

Tissue Extraction

The sodium sulfate tissue mixture is extracted in a Soxhlet apparatus with 700 ml of a 1:1 ratio of cyclohexane and dichloromethane. The extraction solvent and six boiling chips are placed in a 1000 ml round bottom flask. Boiling chips are used to ensure smooth evaporation of the extraction solvent. Voltage supplied by the Variac to the heating mantle is set to allow for a 15 minute extraction cycle for the Soxhlet apparatus. The Soxhlet is run for 24 hours resulting in approximately 100 total extractions.

Boiling chips are placed in a beaker, rinsed with distilled water, and baked in a pesticide oven at 475° C for ten hours. After cooling the beaker is covered with aluminum foil.

Cyclohexane and dichloromethane, glass distilled solvents were obtained from EM Science, Gibbstown, NJ.

The volume of the solvent extract solution is reduced on a rotary evaporator to approximately 250 ml. Under vacuum the sample is heated to 35° C. The boiling chips in the solution cause violent boiling if the sample is put under strong vacuum..

Defatting Column

A 20 ml glass pipette is used to transfer the 250 ml concentrated solution onto a defatting column. The 3.4 cm X 50 cm column is packed from bottom to top with a glass wool plug, 50 g of sodium sulfate, 30 g of silica gel 60, 30 g of potassium silicate, and 50 g of sodium sulfate.

The 1000 ml round bottom flask is rinsed with a 1:1 ratio of cyclohexane and dichloromethane using two 50 ml rinses. Solvent rinses are transferred to the defatting column by the 20 ml glass pipette used to transfer the 250 ml concentrated sample solution. A 500 ml round bottom flask is used to collect the elutant from the column.

Batches of 300 g of Silica Gel 60 are washed in a 5 cm X 100 cm column plugged with glass wool. The silica gel is washed with 600 ml of methanol followed by 600 ml of dichloromethane. The silica gel is dried while remaining in the column. An electric ribbon heater supplied with 60 volts by a Variac regulator is used to heat the silica gel while it dries under a nitrogen jet. The drying process takes approximately 4 hours. When dried the silica gel is poured into a beaker and covered with aluminum foil. The silica gel is stored at 130° C. It must activate at this temperature for 24 hours before it can be used. Silica Gel 60, particle size 0.063-0.200 mm (70-230 mesh ATSM), is obtained from EM Science, Gibbstown, NJ.

Potassium silicate is prepared from activated silica gel 60 and potassium hydroxide pellets. 168 g of potassium hydroxide pellets are dissolved in 700 ml of methanol in a 2 L round bottom flask. The dissolution of potassium hydroxide in methanol is exothermic. When the solution has cooled to room temperature 300 g of activated silica gel 60 are added. The mixture is heated at 55° C while rotating on a rotary evaporator for 90 minutes. No vacuum is applied. The potassium silicate mixture is poured into a 5 cm X 100 cm column plugged with glass wool. After draining, the potassium silicate is washed with 600 ml of methanol then 600 ml of dichloromethane. The potassium silicate is dried while remaining in the column by a nitrogen jet. The potassium silicate is stored at 130° C. It must activate at this temperature for 24 hours before it can be used. Potassium hydroxide pellets are obtained from Aldrich Chemical Company, Inc., Milwaukee, WI.

Methanol, glass distilled solvent was obtained from EM Science, Gibbstown, NJ.

The volume of the defatting column elutant is reduced on a rotary evaporator to approximately 1 ml. Under vacuum the elutant is heated to 35° C. A disposable glass pipette is used to transfer the concentrated elutant from the 500 ml round bottom flask to a 10 ml round bottom screw top test tube. The round bottom flask is then rinsed with four 1 ml rinses of hexane which are transferred to the test tube.

The 10 ml round bottom screw top test tube is placed in a water bath heated by a hot plate to 45° C. A nitrogen jet is used to reduce the volume of the elutant to approximately 0.5 ml. The elutant is reconstituted to 1 ml with hexane.

Hexane, glass distilled solvent was obtained from EM Science, Gibbstown, NJ.

Decolorizing and Alumina Column

A disposable glass pipette is used to transfer the defatting column extract onto a decolorizing column in tandem with an alumina column. A disposable glass pipette with the tapered end cut off is used for the 0.6 cm X 9 cm decolorizing column . The column is packed from bottom to top with a glass wool plug, 0.5 g of 40% sulfuric

acid in silica gel, and 0.5 g of cesium silicate. The decolorizing column is washed with 10 ml of hexane immediately before use. The decolorizing column is set up to elute into the alumina column.

40% sulfuric acid in silica gel is prepared from concentrated sulfuric acid and activated silica gel 60. 100 g of concentrated sulfuric acid and 150 g of activated silica gel 60 are placed in a screw top bottle. The bottle is capped with a cap lined with a Teflon liner. The mixture is shaken in the bottle until no lumps remain. Sulfuric acid is obtained from J.T. Baker Chemical Co., Philipsburg, NJ.

Cesium silicate is prepared from cesium hydroxide monohydrate and activated silica gel 60. 33.6 g of cesium hydroxide monohydrate is dissolved in 100 ml of methanol in a 500 ml round bottom flask. 50 g of activated silica gel 60 are added to the 500 ml round bottom flask. The mixture is heated at 55° C while rotating on a rotary evaporator for 90 minutes. No vacuum is applied. The cesium silicate mixture is poured into a 3.4 cm X 50 cm column plugged with glass wool. After draining, the cesium silicate is washed with 100 ml of methanol then 100 ml of dichloromethane.

The cesium silicate is dried while remaining in the column by a nitrogen jet. Cesium silicate is stored in a screw top bottle at room temperature. The bottle is capped with a cap lined with a Teflon liner. Cesium hydroxide monohydrate, is obtained from Aldrich Chemical Company, Inc., Milwaukee, WI.

The alumina column is a 0.6 cm X 50 cm tulip column packed from bottom to top with a glass wool plug, 3.65 g of alumina, and 0.5 g of sodium hydroxide. The alumina column is packed wet. 3.65 g of alumina is placed in a beaker with 15 ml of hexane, then it is stirred with a glass rod, and then finally poured into the column. The beaker is rinsed with 5 ml of hexane to ensure total transfer of the alumina into the column. Next 0.5 g of sodium sulfate is added to the column. The alumina column is washed with 20 ml of hexane after the initial solvent has been drained.

Alumina is washed in 100 g batches in a beaker with 200 ml of methanol. The solution is stirred with a glass rod. Methanol is decanted out of the beaker. The alumina is then washed with 200 ml of dichloromethane. The solution is stirred with a glass rod. Dichloromethane is decanted out of the beaker. The beaker is covered with aluminum foil and the alumina allowed to dry at room temperature. When dry, the alumina is activated for 24 hours at 190° C. Alumina is stored at 130° C after activation. Acidic alumina, AG-4, was obtained from Bio-Rad Laboratories, Richmond, CA.

The extract from the defatting column is applied to the decolorizing column. Four 1 ml washes of hexane are used to rinse the sample through the column. The 10 ml round bottom screw top test tube is rinsed with the four hexane washes prior to application to the column. Each rinse is allowed to completely enter the column before the next rinse is applied. As the elutant from the decolorizing column runs onto the

alumina column the flow through the alumina column is regulated so no air enters the column. When the last rinse passes through the decolorizing column into the alumina column the decolorizing column is discarded.

The alumina column is washed with 5 ml of hexane followed by 15 ml of 2% dichloromethane in hexane. These elutants which contain DDT and PCB compounds are collected in a 50 ml round bottom flask. Next, the alumina column is rinsed with 15 ml of 5% dichloromethane in hexane and 20 ml of 8% dichloromethane in hexane. These elutants which contain dioxin and furan compounds are collected in a 100 ml round bottom flask.

The sample containing the dioxin and furan compounds are reduced on a rotary evaporator to approximately 0.5 ml. Under vacuum the sample is heated to 35° C. A disposable glass pipette is used to transfer the concentrated sample to a 10 ml round bottom screw top test tube. The 100 ml round bottom flask is then rinsed with four 1 ml rinses of hexane which are transferred to the test tube.

The 10 ml round bottom screw top test tube is placed in a water bath heated by a hot plate to 45° C. A nitrogen jet is used to reduce the volume of the elutant to less than 0.5 ml. The elutant is reconstituted to 0.5 ml with hexane.

Carbon Column

The sample containing the dioxin and furan compounds is applied to a dispersed carbon column. A disposable glass pipette with the tapered end cut off is used for the 0.6 cm X 9 cm dispersed carbon column. The column is packed from bottom to top with a glass wool plug, 0.5 cm of silica gel 60 (approximately 0.15 g), 1.75 cm 2.5% activated carbon in silica gel 60 (approximately 0.5g), and 0.1 cm of glass wool.

Activated carbon is purchased as a wet powder. It is dried in an aluminum foil covered beaker at 130° C for 24 hours prior to use. Super-A activated carbon (AS-21) was obtained from Anderson Development Co., Adrian, MI.

0.25 g of activated carbon is mixed with 9.75 g of activated silica gel 60 to form dispersed carbon. The dispersed carbon is stored at 130°C.

After packing the dispersed carbon column is washed from top to bottom with 5 ml of dichloromethane, 5 ml of a 1: 1 ratio of benzene and dichloromethane, and 5 ml of toluene. The column is then inverted and washed from bottom to top with 10 ml of hexane. The column may be stored in trimethyl pentane until used. If stored in trimethyl pentane, the column is washed from bottom to top with 5 ml of hexane before use.

The sample containing the dioxin and furan compounds is applied to the top of the dispersed carbon column. Three 1 ml washes of hexane are used to rinse the sample through the column, the 10 ml round bottom screw top test tube is rinsed with the three hexane washes prior to application to the column. The column is washed with 10 ml of dichloromethane, 10 ml of a 1:1 ratio of benzene and dichloromethane. These elutants are discarded. The column is inverted and the sample eluted from bottom to top into a 50 ml pear shaped flask with 15 ml of toluene.

GC-MS Preparation

The sample containing DDT and PCBs is reduced on a rotary evaporator to approximately 100 μ l. Under vacuum the sample is heated to 50° C. A automatic pipette is used to transfer the concentrated sample to a 100 μ l glass ampoule. A 100 μ l syringe is used to rinse the 50 ml pear shaped flask with three 50 μ l rinses of hexane, which are transferred to the ampoule. While heated to 50° C the sample volume is reduced to less than 1 μ l under a nitrogen jet. A automatic pipette is used to reconstitute the sample with 10 μ l of nonane. The glass ampoule is placed in a metal spring of proportional diameter. This is placed in a 1 ml screw top vial, and sealed with a cap containing a Teflon septum. The sample is now ready for GC-MS analysis.

The sample containing dioxins and furans is reduced on a rotary evaporator to approximately 100 μ l. Under vacuum the sample is heated to 50° C. A automatic

pipette is used to transfer the concentrated sample to a 100 μ l glass ampoule. A 100 μ l syringe is used to rinse the 50 ml pear shaped flask with three 50 μ l rinses of hexane, which are transferred to the ampoule. While heated to 50° C the sample volume is reduced to less than 1 μ l under a nitrogen jet. A automatic pipette is used to reconstitute the sample with 10 μ l of nonane. The glass ampoule is placed in a metal spring of proportional diameter. This is placed in a 1 ml screw top vial, and sealed with a cap containing a Teflon septum. The sample is now ready for GC-MS analysis.

The automatic pipette tips are washed with dichloromethane and hexane.

The 100 μ l syringe is washed prior to use with dichloromethane and hexane. A different syringe is dedicated to GC-MS preparation of PCB compounds and dioxin and furan compounds.

GC-MS Analysis

The GC-MS analysis was performed with a Varian 3400 Gas Chromatograph and a Finnigan 4000 Mass Spectrometer Instrument. The Varian 3400 instrument used a 30 m SE 54 Column with a 0.25 internal diameter; settings were;

	<u>Initial</u>	<u>Final</u>	<u>Rate</u>
Column Temperature	140° C	280° C	4° C per minute

Injector 300° C, Detector 300° C, carrier gas high purity helium at 20 psi. The Finnigan 4000 instrument has a 4,500 source; settings were Source Temperature of 120° C, methane negative chemical ionization (pressure of methane 0.555 torr). The standard used for quantification was in decane.

RESULTS

Sample Collection

According to Fresh-Water Invertebrates of the United States by R.W. Pennal⁽¹⁶⁾ and from my field observations the bivalve *Corbicula flumina* appears to prefer habitats consisting of gravel, sand, or a gravel sand mixture. Bivalves were present in depths varying from approximately 5 cm to 1 m, allowing collection to be done by hand. Sample collection is best during late August and early September, at other times the water level of the Willamette River is too high.

I found *Corbicula flumina* at various locations along the Willamette River north of Willamette Falls in the Portland Metropolitan Area. I located four places where bivalves were present in quantities sufficient to support an environmental analysis. At these four locations *Corbicula flumina* varied in number from approximately 90 to 200.

At collection site A approximately 90 bivalves were present, collection site B contained approximately 120, collection site C contained approximately 120, and collection site D contained approximately 200 bivalves.

Outside the Portland Metropolitan Area it was difficult to locate sufficient numbers of *Corbicula flumina* to support an environmental analysis. I examined two areas south of the Willamette Falls for the presence of *Corbicula flumina*. South of the Willamette Falls in Oregon City to Newberg the Willamette River resembles a lake environment, slow moving water with muddy shores containing organic material. I was unable to locate any bivalves in this area. According to R.W. Pennal and from my field observations, *Corbicula flumina* does not favor habitats consisting of mud and organic material.

I examined the Willamette River from Corvallis to Salem for the presence of *Corbicula flumina*. I located *Corbicula flumina* at various locations. However, I was unable to locate sufficient numbers to support an environmental analysis.

Percent Recovery

Samples were fortified with an internal standard of 1 ppb of PCB 204. The internal standard was compared with a 50 ppb external standard of PCB 204 to determine the percent recovery. Percent recovery of PCB 204 ranged from 268% to

607 %. This indicates that PCB 204 was present at concentrations of 2.68 ppb to 6.07 ppb in the samples, which exceeds the 1 ppb internal standard.

Percent Recovery	Site A	Site B	Site C	Site D
Run 1	356%	268%	643%	518%
Run 2	372%	445%	334%	607%

* Each run included samples from sites A,B,C, and D, a blank, and a control. Run 1 & 2 refer to the first and second set of samples that were taken through the experimental procedure.

Quantification of the samples was performed assuming a percent recovery of 100 %.

Possible Reasons Why Percent Recovery Exceeded 100%

1. The solution containing the PCB 204 internal standard used to fortify the tissue may have been inaccurate.
2. Improper fortification of the tissue with the PCB 204 internal standard.
3. Conditions present in the samples may have effected the GC-MS.
4. The external standard of 50 ppb of PCB 204 used to determine percent recovery may have been inaccurate.
5. PCB 204 may be an environmental pollutant that is present in the bivalves tissue.

A tissue blank was not analyzed to determine the presence of natural occurring PCB 204 in *Corbicula flumina* from the Willamette River in the Portland Metropolitan Area.

Blank Analysis

No DDE or DDT was detected in the blank from run 1. However, a pentachlorinated biphenyl and two heptachlorinated biphenyls were detected in the blank from run 1. Their retention time did not match any pentachlorinated or heptachlorinated biphenyls detected from samples A,B,C, or D from run 1. Analysis of the blank from run 1 indicates that possible contamination occurred during the experimental procedure.

No DDE, DDT, or PCBs were detected in the blank from run 2. Analysis of the blank from run 2 indicates no contamination occurred during the experimental procedure.

Control Analysis

No DDE, DDT, or PCBs were detected in either control from run 1 or run 2. Analysis of the controls indicates that compounds detected in the samples collected from the Willamette River are due to their accumulation from the environment.

PCB Analysis

Quantification of pentachlorinated biphenyls was estimated by comparison to a 50 ppb external standard of PCB 118. Quantification of hexachlorinated biphenyls was estimated by comparison to a 50 ppb external standard of PCB 138. Quantification of heptachlorinated biphenyls was estimated by comparison to a 50 ppb external standard of PCB 187. Quantification of octachlorinated biphenyls was estimated by comparison to a 50 ppb external standard of PCB.

Total Concentration of Penta and Hexachlorinated Biphenyl

Compound	Collection Site	Run 1 (ng/g tissue)	Run 2 (ng/g tissue)
PCB-5	A	301	234
	B	174	120
	C	445	297
	D	304	428
PCB-6	A	520	634
	B	522	778
	C	955	707
	D	800	1,191

* Each run included samples from sites A,B,C, and D, a blank, and a control. Run 1 & 2 refer to the first and second set of samples that were taken through the experimental procedure.

Total Concentration of Hepta and Octachlorinated Biphenyl

Compound	Collection Site	Run 1 (ng/g tissue)	Run 2 (ng/g tissue)
PCB-7	A	484	307
	B	241	450
	C	633	277
	D	469	682
PCB-8	A	379	72
	B	304	42
	C	42	65
	D	44	57

* Each run included samples from sites A,B,C, and D, a blank, and a control. Run 1 & 2 refer to the first and second set of samples that were taken through the experimental procedure.

DDE and DDT Analysis

Quantification of op DDE and pp DDE was estimated by comparison to a 50 ppb external standard of pp DDE. Quantification of op DDT and pp DDT was estimated by comparison to a 50 ppb external standard of op DDT.

Concentrations of op DDE and pp DDE

Compound	Collection Site	Run 1 (ng/g tissue)	Run 2 (ng/g tissue)
op DDE	A	ND	ND
	B	ND	159
	C	ND	58.5
	D	368	270
pp DDE	A	1.15	1.56
	B	ND	414
	C	4.83	2.75
	D	2.88	3.1

* ND = Not Detected

** Each run included samples from sites A,B,C, and D, a blank, and a control. Run 1 & 2 refer to the first and second set of samples that were taken through the experimental procedure.

Concentrations of op DDT and pp DDT

Compound	Collection Site	Run 1 (ng/g tissue)	Run 2 (ng/g tissue)
op DDT	A	ND	ND
	B	ND	ND
	C	372	ND
	D	429	220
pp DDT	A	ND	ND
	B	ND	.773
	C	.712	ND
	D	1.65	.613

* ND = Not Detected

** Each run included samples from sites A,B,C, and D, a blank, and a control. Run 1 & 2 refer to the first and second set of samples that were taken through the experimental procedure.

Dioxin and Furan Analysis

Dioxin and furan samples were prepared but were not analyzed. The high resolution mass spectrometer required for dioxin and furan analysis was not available, so no analysis was performed. However, a preliminary analysis of a nonfortified sample from collection site B using the Varian 3400 and Finnigan 4000 instrument indicated that the following dioxin and furan isomers were present.

Preliminary Dioxin Analysis

Compound	Isomer	Number of Isomers Detected
Dioxin	Penta	1
	Hexa	1
	Octa	1

Preliminary Furan Analysis

Compound	Isomer	Number of Isomers Detected
Furan	Penta	3
	Hexa	3
	Hepta	1

DISCUSSION

The ability to locate and collect bivalves from the Willamette River is effected by the rivers' water level. Late August and early September are ideal times to collect samples, throughout the rest of the year bivalve collection would be difficult.

When located in the Portland Metropolitan Area, bivalves were present in numbers ranging from approximately 90 to 200. This should allow for sufficient quantities of *Corbicula flumina* to be collected for future studies of this area.

Outside the Portland Metropolitan Area I had difficulty locating *Corbicula flumina* in the Willamette River. Between Oregon City and Newberg the Willamette River offers unfavorable habitat for *Corbicula flumina*. From Corvallis to Salem *Corbicula flumina* is present in the Willamette River but at insufficient quantities to support an environmental analysis.

The presence of PCBs, DDT, and DDE in the bivalve tissue indicates that *Corbicula flumina* does accumulate these compounds from the environment. Preliminary analysis of a blank sample from location site B indicates that penta, hexa, and octachlorinated dioxin isomers and penta, hexa, and heptachlorinated furan isomers are present in the bivalve tissue, the concentration and the isomers present is unknown.

The percent recovery for the internal standard PCB 204 exceeded 100% for all samples. Sample quantification was performed assuming a percent recovery of 100%. Possible sources for the percent recovery exceeding 100% could be a result of improper technique during the experiment procedure, variables present in the GC-MS, or incorrect quantification. It is also possible that PCB 204 may be a naturally occurring environmental contaminant present in *Corbicula flumina* from the Willamette River. A tissue blank was not analyzed so the natural occurrence of PCB 204 in *Corbicula flumina* is unknown.

Seasonal variation of the water level of the Willamette River affects sample collection. *Corbicula flumina* are present in the Willamette River in the Portland Metropolitan area in sufficient quantity to supply samples for future environmental studies of this area. Outside the Portland Metropolitan Area *Corbicula flumina* are difficult to locate. The detection of DDT, PCBs and the preliminary detection of various dioxin and furan isomers indicate that *Corbicula flumina* does accumulate these compounds from the environment. In conclusion, results indicate that *Corbicula flumina* is a feasible biomarker for DDT, PCBs, and potentially dioxins and furans in the Willamette River in the Portland Metropolitan Area.

Future Work

The analysis of the dioxin and furan samples should be completed. The presence of dioxins and furans in the samples would determine the feasibility of *Corbicula flumina* as a biomarker for those compounds.

A further examination of the Willamette River south of the Willamette falls for the presence of *Corbicula flumina* should be done. Until *Corbicula flumina* is located in abundant quantities in the Willamette River south of the Willamette Falls their success as a biomarker is limited to the Portland Metropolitan Area.

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FIGURE 1

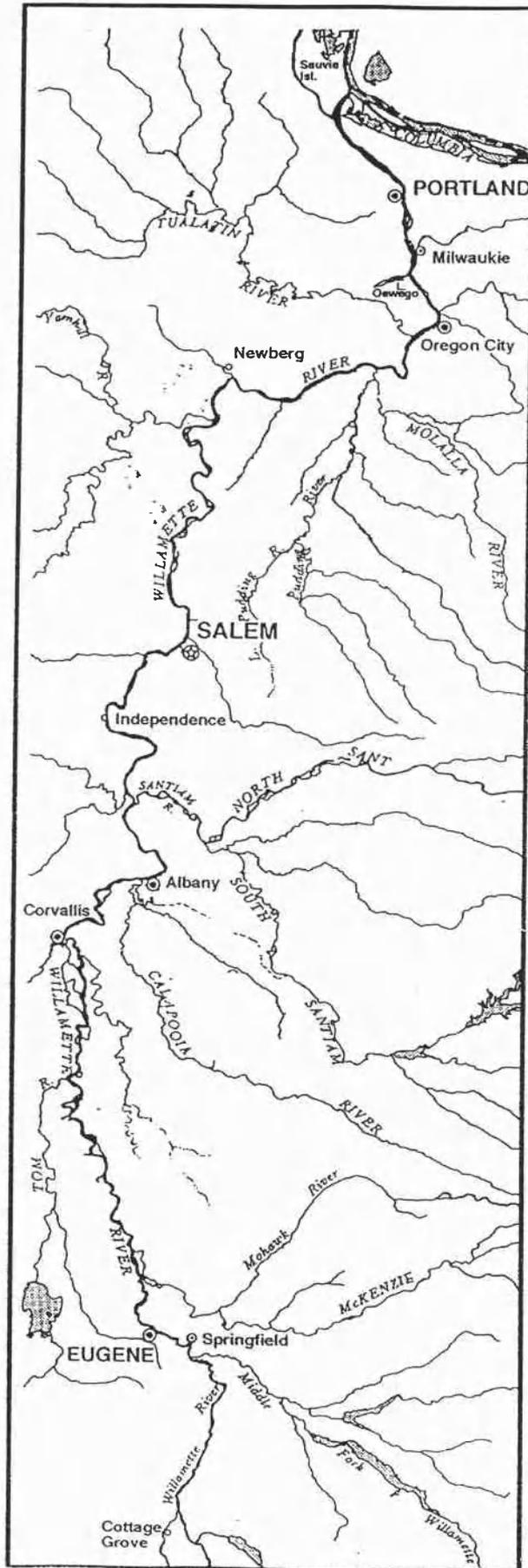


FIGURE 2

Concentration of op DDE

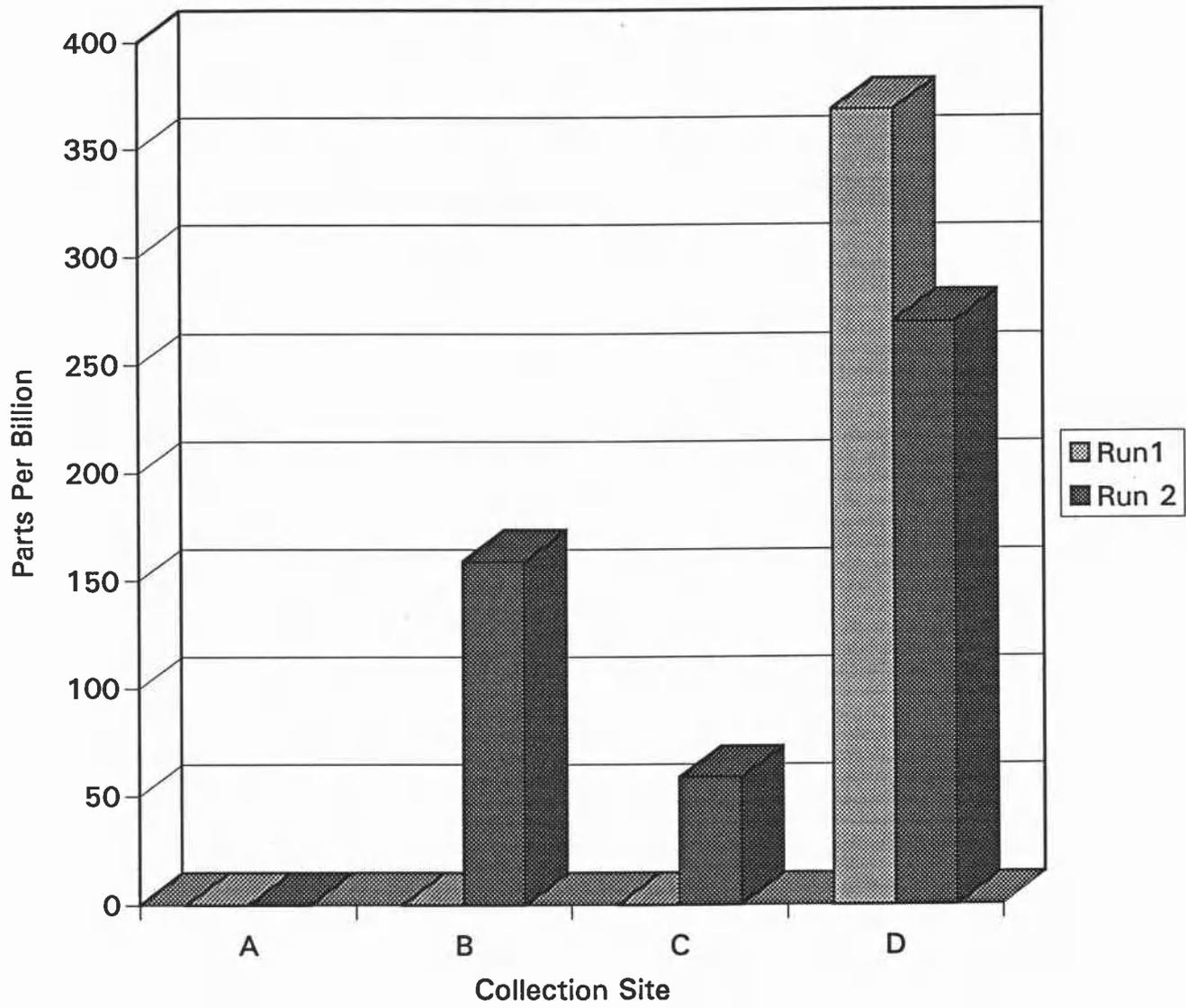


FIGURE 3

Concentration of pp DDE

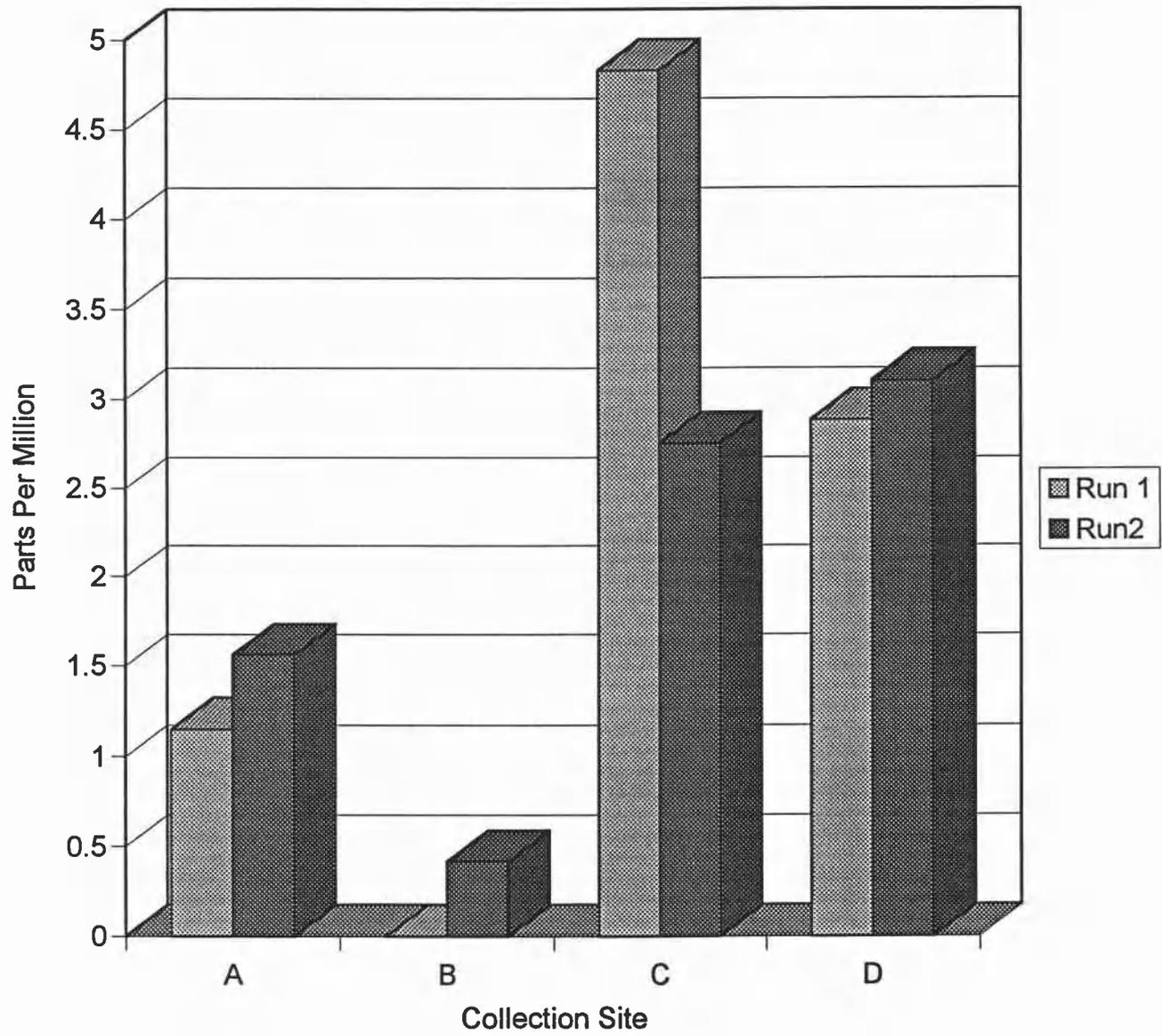


FIGURE 4

Concentration of op DDT

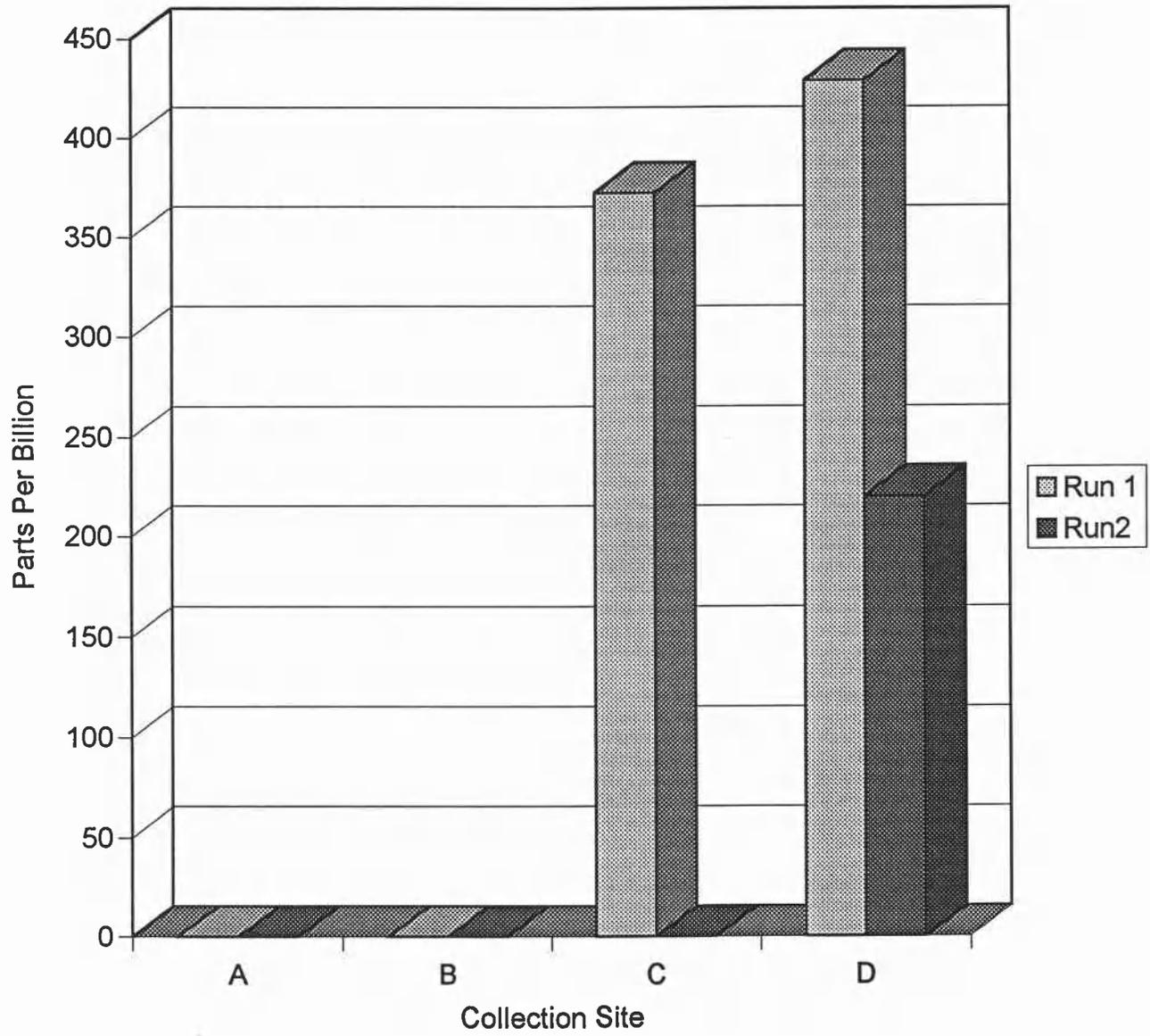


FIGURE 5

Concentration of pp DDT

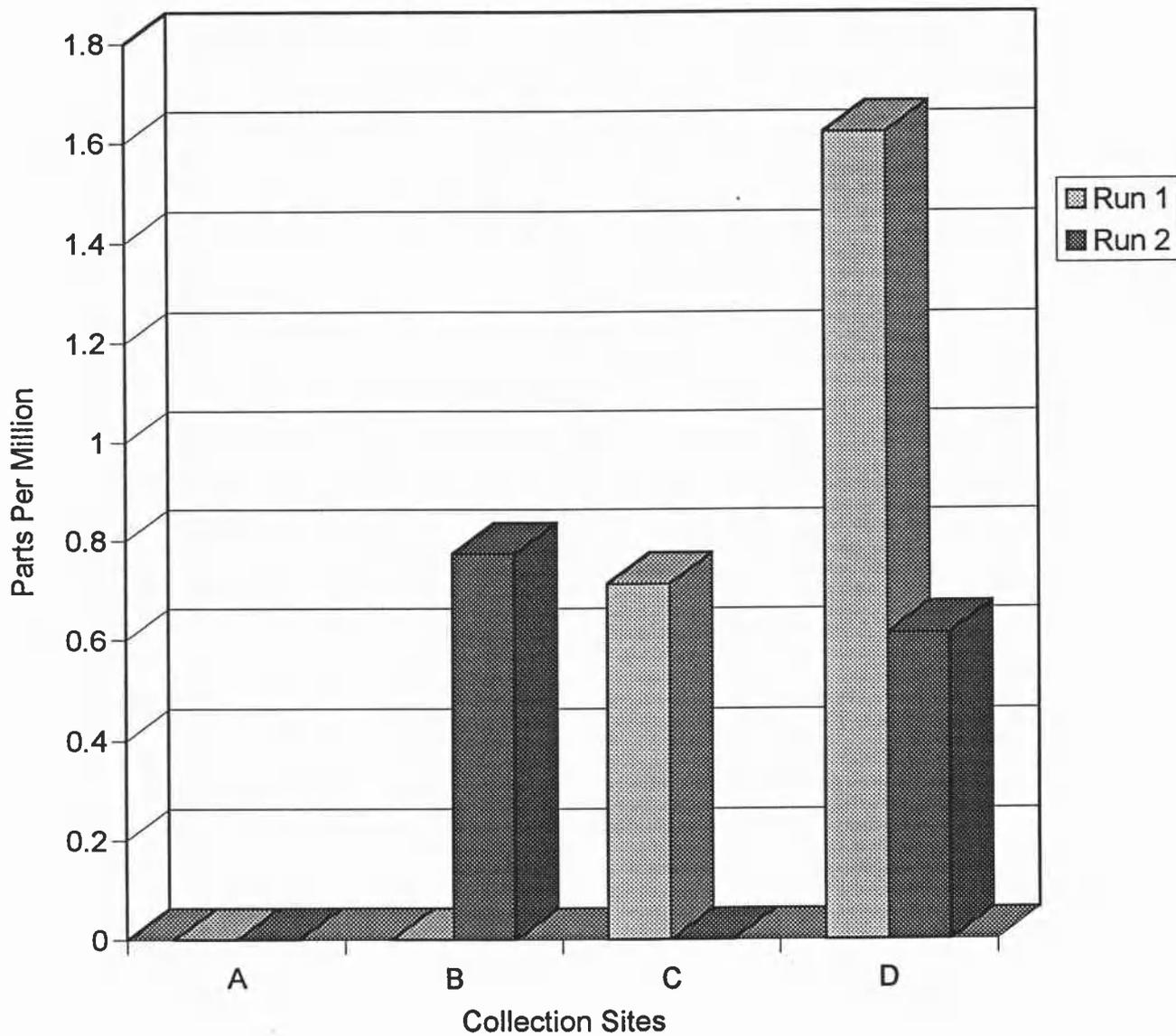


FIGURE 6

Total Pentachlorinated Biphenyls

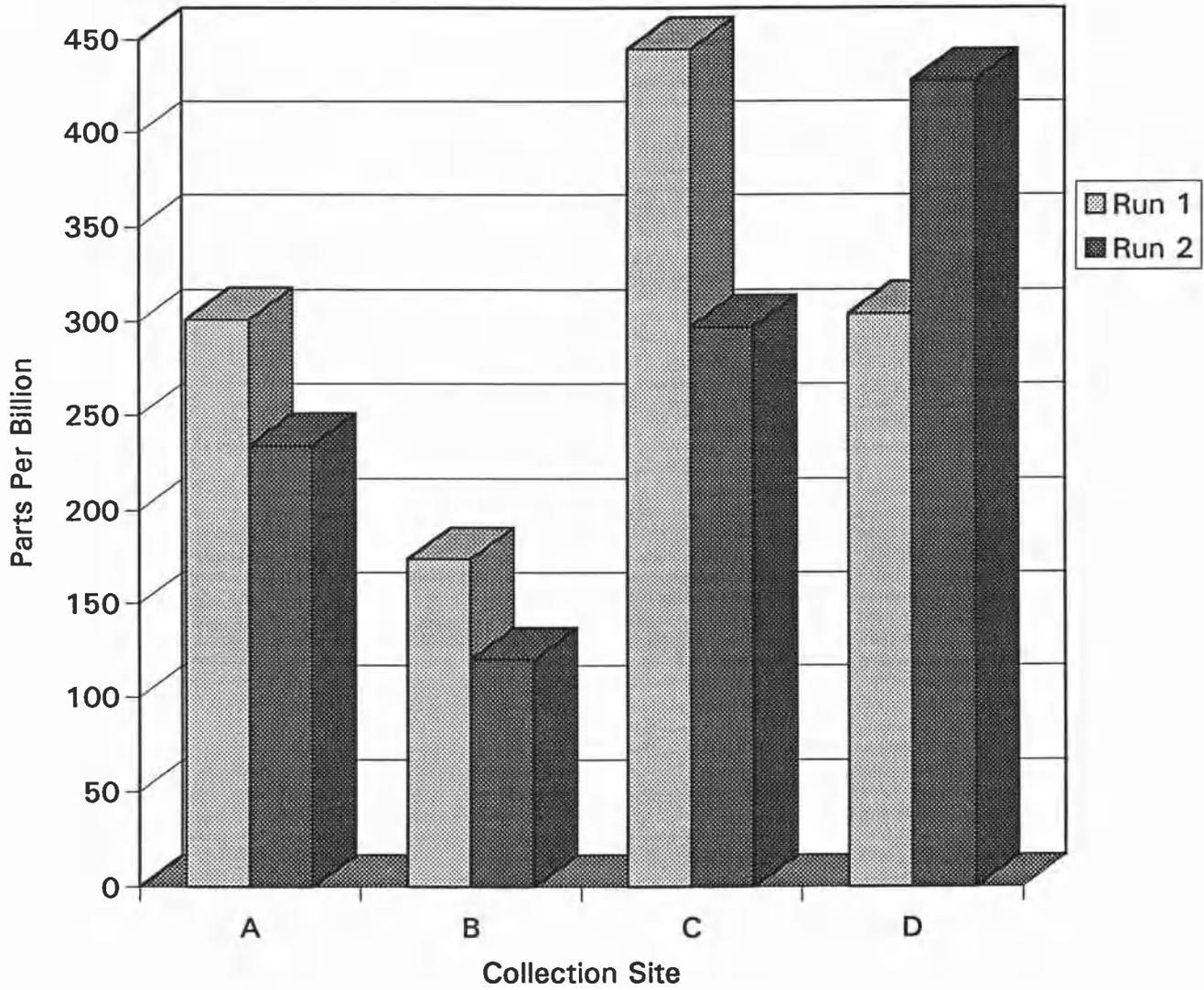


FIGURE 7

Total Hexachlorinated Biphenyls

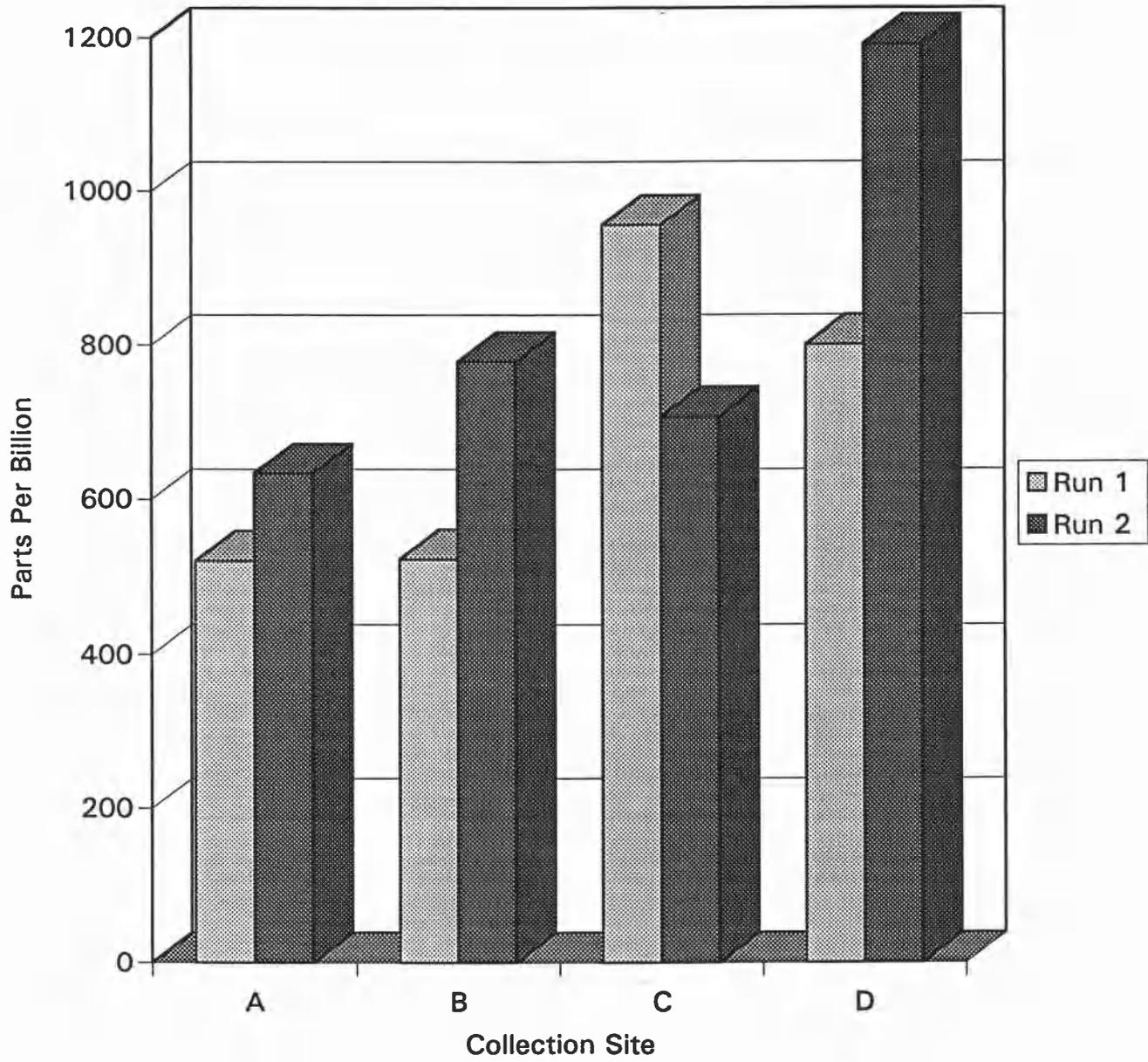


FIGURE 8

Total Heptachlorinated Biphenyls

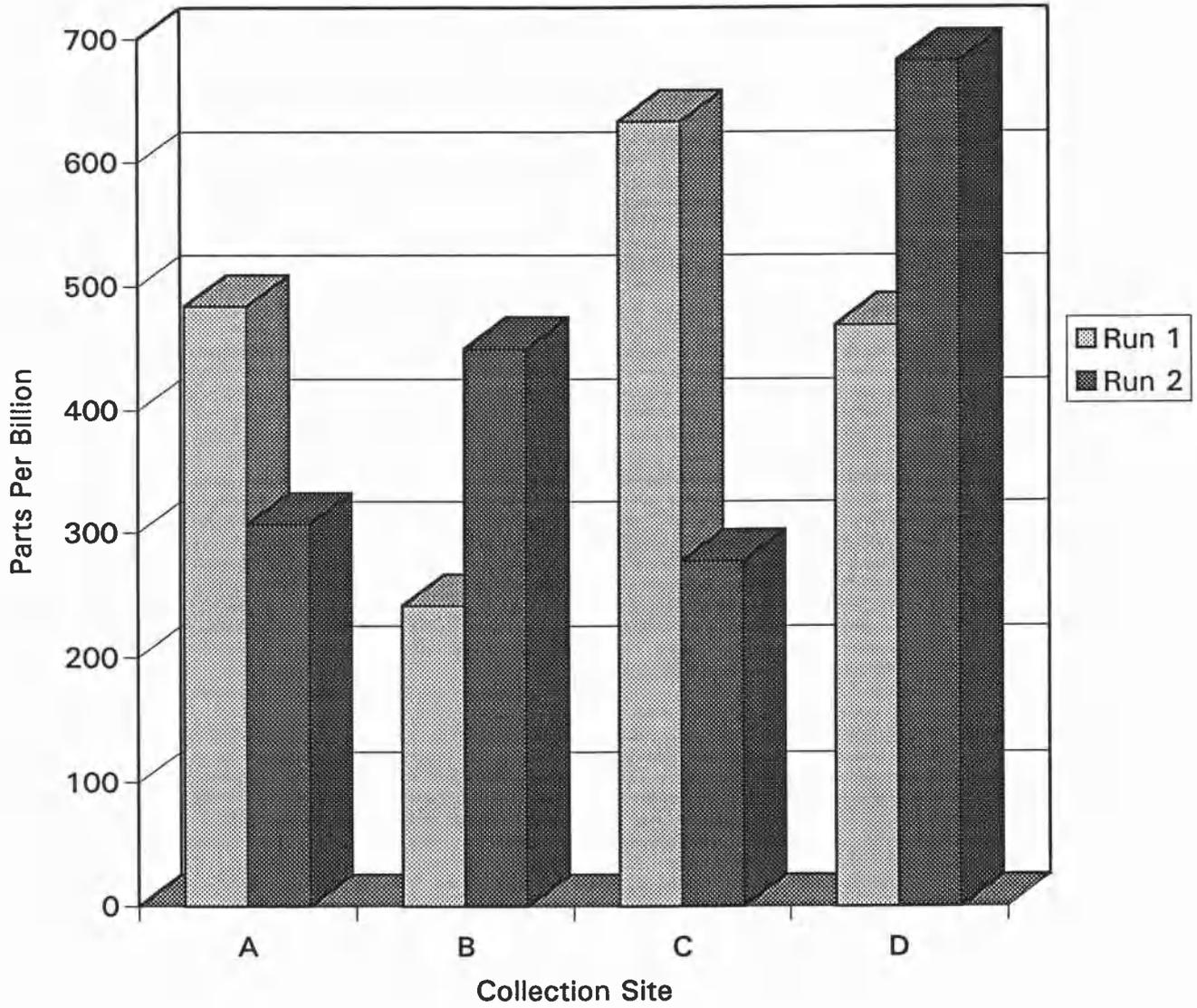


FIGURE 9

Total Octachlorinated Biphenyls

