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thesis entitled

**ANALYSIS OF FISH EGGS
FROM FISH OF THE GREAT LAKES REGION
FOR 2,3,7,8-TETRACHLORODIBENZOFURAN AND
2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN**

presented by

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By

Holly Fortnum Adamsons

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ABSTRACT

ANALYSIS OF FISH EGGS FROM FISH OF THE GREAT LAKES REGION FOR 2,3,7,8-TETRACHLORODIBENZOFURAN AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

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A method for analyzing fish eggs containing 2,3,7,8-tetrachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzo-p-dioxin at the part per trillion level was adapted to existing laboratory equipment. The method utilizes a combination of silica gel and potassium hydroxide treated silica gel column extraction of the fish egg sample. Additional clean-up for interfering chemical compounds took place on a carbon/celite column followed by a tandem set of columns. These columns were prepared with sulfuric acid treated silica gel and potassium hydroxide treated silica gel, followed by an acid alumina column. Column efficiencies were examined using ^{14}C-2,3,7,8-tetrachlorodibenzo-p-dioxin and liquid scintillation counting. Recoveries ranged from 8-80%.

2,3,7,8-tetrachlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzofuran were recovered at the part-per-trillion level from spiked fish and fish egg samples, and detected using capillary gas chromatography methane negative chemical ionization mass spectrometry. Actual environmental fish egg samples had non-detectable levels of these two compounds using this method.

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I. INTRODUCTION

The Great Lakes region, which encompasses the states of Michigan, Wisconsin, Minnesota, Indiana, Illinois, Ohio, and New York, along with the Canadian province of Ontario, has long been recognized for its commercial and sports fishing. The contamination of fish in the Great Lakes Region with polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) has caused a great deal of concern to the fishing industry in terms of fish reproduction and human health safety. These compounds are neither pesticides nor industrial chemicals, but are found in several industrial and agricultural chemicals as trace contaminants. Polychlorinated dibenzo-p-dioxins and dibenzofurans are well-known toxic compounds thought to be ubiquitous in the environment. These compounds enter the environment through two main paths, either a waterborne path or an airborne path. Evidence suggests that the atmospheric path is most likely, except in cases where the toxic compounds are directly discarded into the water.[1]

Not much is known concerning the long term effects of these compounds. Most of the present data concerns PCDDs, and very little is known about PCDFs.

Assessment of the impact of PCDDs and PCDFs in aquatic ecosystems is difficult because only limited chronic toxicity data are available for aquatic organisms.[2-10] Few values for PCDDs and PCDFs are available for fish tissues, and no values are available for salmonid eggs. More evidence is needed to determine the presence of these toxic compounds to assess the risks involved to both aquatic organisms and humans.

In 1983, a method was developed for use in our laboratory by Swiatoslav Kaczmar.[11] The basis for this method was an analytical method developed by the Dow Chemical Company Michigan Division, known as method ML-AM-78-63. This method was used to maximize the chromatographic resolution of the TCDD isomers to allow specific detection of 2,3,7,8-TCDD. The detection limit was 20 pg. of 2,3,7,8-TCDD in a 20 g. sample of fish (1 ppt). As in most methods for the determination of trace chemical residues in biological media, the TCDD is isolated from the sample through a series of extraction and cleanup steps. However, analysis for a specific isomer at the part per trillion level requires that the clean-up steps and final chromatographic resolution be extremely efficient. The entire sample extract is injected for GC/MS quantification as a single aliquot of about 2 ul. The final extract must

be free of any materials such as PCBs, DDE or phthalates that are initially present at a million-fold excess over TCDD and which might interfere during mass spectrometric detection. A high background signal results in a loss of sensitivity while non-TCDD components of the extract with a retention time and mass fragments corresponding to TCDD would result in false positive determinations. Ideally, the final extract should contain only 2,3,7,8-TCDD along with a few of the other isomers of TCDD which can be completely resolved from 2,3,7,8-TCDD during final GC/MS quantification.

In analyzing for these compounds in fish, fish eggs, and in samples from remote areas, it is necessary to have very specific and sensitive clean-up and detection techniques. Most of the methods for determining dibenzofurans and dibenzodioxins are complicated and time-consuming.

II. STATEMENT OF OBJECTIVES

The goal of this research project was to adapt methodology for the determination of 2,3,7,8-tetrachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzo-p-dioxin in fish fillets to existing equipment in our laboratory, in order that these compounds may also be determined in fish eggs. This was a first step in a larger project where isomer-specific analysis of sample for a wide range of chlorinated dioxins and furans would be necessary to chart the maternal deposition of these compounds from fish to eggs. Another goal of this research project was to determine if there were trace levels of these toxic compounds out in the environment. The method previously used in the laboratory was specific for 2,3,7,8-tetrachlorodibenzodioxin in fish [11]. However, that method also took as much as a week to perform, due to its many steps including high performance liquid chromatography. In order to perform isomer-specific analysis for the wide range of dibenzo-p-dioxins and dibenzofurans, a method needs to be used which omits acid extraction procedures, and the use of a Florisil column. Specifically, octachlorodibenzodioxin and octachlorodibenzofuran are not easily recovered from

Florisil. Since the method developed here would be eventually used for a broad range of chlorinated dioxin and furan congeners, it was important to the project to use such a method. This method would also have to be less time consuming than the method previously used in our lab.

Specifically, optimization of the clean-up steps and of the separation were desired in order to separate these two compounds from each other, the biological matrix, and from interferences. Also, the efficiency of each step was followed in the procedure using a ^{14}C label and liquid scintillation counting. Finally, fish eggs would be analyzed for 2,3,7,8-tetrachlorodibenzo-p-dioxin, and 2,3,7,8-tetrachlorodibenzofuran.

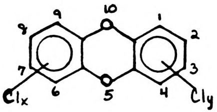
III. LITERATURE REVIEW

A. CHEMICAL AND PHYSICAL PROPERTIES OF CHLORINATED DIOXINS AND DIBENZOFURANS

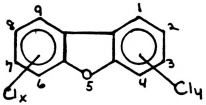
Dibenzo-p-dioxins and dibenzofurans are two families of compounds with similar structures. Most environmental interest has concerned the chlorinated dioxins and dibenzofurans. These are nearly planar compounds with substituents numbered as in Figure 1. Theoretically, there are 75 different chlorinated dioxins and 135 different chlorinated dibenzofurans.

Because of their structures, PCDDs and PCDFs are extremely stable, non-reactive compounds. These compounds are resistant to the action of concentrated acids and bases.[12] Polychlorinated dioxins and furans can maintain their chemical stability to temperatures as high as 700° C.[13] TCDD does not undergo hydrolysis in water and is resistant to microbial attack. Only aerobic biodegradation of special mammals and bacteria has been reported to degrade TCDD.[14]

Figure 1. Substituent numbering for chlorinated dibenzo-p-dioxins and dibenzofurans.



Dibenzo-p-dioxin



Dibenzofuran

Figure 1.

The 2,3,7,8-TCDD isomer exhibits an extremely low vapor pressure (7.4 x 10^{-10} torr at 25° C) [15], is soluble in water to only 7.91 ng/l [16] and has an estimated octanol/water partition coefficient of 1 x 10^{6} g/g. Studies done by Sarna et.al.[17] showed octanol/water partition coefficients for a variety of dioxins and furans ranging from 1 x 10^{4} for unsubstituted dibenzofuran, 3.14 x 10^{4} for unsubstituted dibenzodioxin to 1 x 10^{12} to 1 x 10^{13} for OCDD and 1 x 10^{13} to 1 x 10^{14} for OCDF. Vapor pressures of dibenzofurans were studied confirming their similarity to dioxins. When the chlorinated substitution is increased, the vapor pressure decreases.[18] Photolysis of TCDD in organic solvents is rapid with half-lives of 3-4 hours and causes formation of lower chlorinated congeners.[15] TCDD photolyzes in water with a half life of about 4-5 days in the summer at 40° latitude.[19] The environmental dynamics of chlorinated dioxins are similar to some of the longer-lived chlorinated hydrocarbons (such as DDT).

B. TOXICOLOGY OF CHLORINATED DIOXINS AND DIBENZOFURANS

Polychlorinated dibenzo-p-dioxins (PCDDS) and polychlorinated dibenzofurans (PCDFs) are known for their toxicity due to the known effects of several congeners.[20] These compounds have been found to be teratogenic, embryotoxic, and carcinogenic. Polychlorinated dibenzofurans and dibenzodioxins elicit a number of common biological and toxic responses which are triggered by their initial binding to a receptor protein. The receptor binds with TCDD, then travels to the nucleus and to a structural gene (Ah locus in mice) initiating a pleiotropic response resulting in the induction of a number of coordinately expressed and possibly repressed critical proteins or enzymes. The affinity a compound has for this receptor protein determines the dose required to achieve a particular degree of toxicity. The 2,3,7,8-TCDD congener has been shown to have the highest degree of affinity for the receptor and the lowest LD_{50}. In spite of extensive investigations, the cause of liver injury and lethality, the mode of action and the mechanism of action of 2,3,7,8-tetrachlorodibenzo-p-dioxin are not completely known. Recently, it has been proposed that interaction between thyroid hormones and brown adipose

tissues make the differences in species toxicities.[21,22] These toxic responses include induction of several cytochrome P-448 dependent monooxygenases(aryl hydrocarbon hydroxylase, AHH, and ornithine decarboxylase, ODC), body weight loss and thymic atrophy and /or immunotoxicity, endocrine disorders, gastric lesions, hepatoxicity, chloracne, and other dermal lesions. Specifically, the 2,3,7,8-tetrachlorodibenzodioxin congener induces chloracne (a dermatological disorder),polyneuropathy (multiple lesions of peripheral nerves), mystagmus (involuntary rapid movement of the eyeball), and liver dysfunction.[22] A large proportion of administered 2,3,7,8-TCDD persists in the unmetabolized form in the liver partially concentrated in the microsomal fraction in all species studied. This finding implies that the unmetabolized compound, rather than a metabolite, is responsible for its toxic effects in mammals. 2,3,7,8-TCDD is slowly excreted via the biliary tract in the form of glucuronide and other more polar metabolites.[23]

Most of the notoriety of dioxins and dibenzofurans is due to the toxicity of the 2,3,7,8-TCDD and 2,3,7,8-TCDF congeners which kill guinea pigs at low doses. The acute toxicities of 2,3,7,8-TCDD vary over 5,000

-fold from highly sensitive guinea pigs (LD_{50} 0.6-2.0 ug/kg) to the hamster(LD_{50} 1157-5051 ug/kg.) [24] Consequently, most of the toxicological research performed with PCDDs and PCDFs has focussed on these particular congeners. Other congeners of known toxicity are 1,2,3,7,8-PCDD; 1,2,3,4,7,8-HCDD; 1,2,3,6,7,8-HCDD; 1,2,3,7,8,9-HCDD; 1,2,3,7,8-PCDF; 2,3,4,7,8-PCDF; 2,3,4,6,7,8-HCDF; 1,2,3,4,7,8-HCDF.[25] More studies are being conducted to assess the toxic effects of other PCDD and PCDF congeners such as 1,2,3,7,8-pentachlorodibenzodioxin [26], and 1,2,3,4,6,7,8-heptachlorodibenzodioxin [27], which elicit responses similar to those of the 2,3,7,8-TCDD. More emphasis has also been directed to the study of effects of the chlorinated dibenzofurans which also induce AHH in rats.[28] A systematic study of each of the four different positions for chlorine substitution in the dibenzofuran ring system showed that the toxic and biologic potencies of these compounds varied with respect to differential chlorine substitution at all four positions C-3(7) > C-2(8) > C-4(6) > C-1(9). [29] Structural activity relationships (SARS) for polychlorinated dibenzofurans are different than for dibenzodioxins due to the assymmetric structure of the furans.

Because of the toxicity of these compounds, their presence in widely used herbicides, and their extreme stability, it is important to assess the environmental fate, risks of human exposure, and food chain contamination by PCDDs and PCDFs. Some evidence suggests that there is a background level of PCDDs and PCDFs in the general human population, but the route of exposure and absorption of these compounds are not well known. Most human tissue samples analyzed so far have been reported to contain 2,3,7,8-TCDD at concentrations greater than 3 ppt.[30] A single dose of 1.14 ng. of ^{3}H-2,3,7,8-TCDD/kg body weight, ingested by a human volunteer , was absorbed almost completely from the intestine. The resulting adipose tissue levels, measured 13 and 69 days after dosage were 3.09 and 2.85 ppt, respectively. The half life of elimination was 2120 days.[31] Another study found that the pattern of concentrations found for 2,3,7,8-substituted tetra- through octachlorodibenzodioxins and tetra- and pentachlorodibenzofurans is consistent with airborne particulates being the ultimate source of these compounds.[32] Therefore, the dangers to humans which are posed by the presence of these compounds in air, water, soils, and sediments, and food (fish, for example) are still uncertain.

C. SOURCES OF CONTAMINATION

There are several sources of PCDDs and PCDFs, as well as several pathways of entrance into the environment. Dioxins and dibenzofurans enter the environment through the atmosphere or through the water. Explosions such as the one in a chemical plant at Seveso,Italy cause atmospheric contamination. Incineration of chlorinated wastes is another source of aerial input. Industrial discharge into rivers and streams or leaching at hazardous waste sites may contaminate water supplies. It is difficult to discern which source is the major factor in contamination and which is most threatening to humankind.

The ubiquitous occurrence of polychlorinated biphenyls(PCBs) is already well known.[1,33] Since dioxins and dibenzofurans are chemically similar to PCBs, they most likely are ubiquitous in the environment, too. Evidence for this effect can be found in the results of studies of sediment from Siskiwit Lake in Isle Royale located in northern Lake Superior.[34,35] Because the lake is landlocked and the water level is 17 m. higher than that of Lake Superior, there is no movement of water from Lake

Superior into Siskiwit Lake. It appears that the atmosphere is the only source of anthropogenic material. Once the inputs cross the air-water interface as vapors or particulates, the nonvolatile compounds will move through the water column to the sediment.

There is much data already given to show that PCDDs and PCDFs are emitted from several combustion sources. There are at least two probabilities proposed for the formation of these compounds in combustion and in chemical manufacturing. First, PCDDs and PCDFs are formed from pyrolysis of chlorinated aromatic precursors (such as chlorobenzenes, chlorophenols, or PCBs) present in fuel.[36-38] PCDDs and PCDFs are also formed from the reaction of organic compounds with inorganic chloride, which may have been present in fuel.[39] In 1978, DOW Chemical Company researchers proposed that PCDDs are ubiquitous and formed as trace level byproducts of natural combustion.[40] Dioxins and dibenzofurans can also be formed in flyash and incinerators or be already present there and volatilized upon heating. Photochemical reactions may also take place.[41] There is debate as to whether dioxins are a result of natural processes or due soley to combustion sources.

Dioxin contamination has been found in phenoxy herbicides. 2,3,7,8-TCDD is the major congener found in the 2,4,5-T formulations.[42] 1,3,6,8-TCDD is found in 2,4-D esters and amine salts.[43] Hexachlorophene, a bactericide prepared from the sodium chlorophenate salts used as starting material in 2,4,5-T has 0.2-0.5 ng/g of 2,3,7,8-TCDD.[44] PCDFs have been found in PCBs, specifically in Phenochlor DP-6 and Clophen A 60, but not in Arochlor 1260.[45] PCDFs and PCDDs are also found in commmercial diphenyl ether herbicides. Isomers found were 1,3,6,8-TCDD and 1,3,7,9-TCDD.[46] Again, the starting material is 2,4,6-trichlorophenol. There was no 2,3,7,8-TCDD found.

Chlorophenols are most widely used as wood preservatives, but also as fungicides, mold inhibitors, antiseptics, disinfectants, and insecticides. They are also used in slime control in the manufacture of pulp for tanning leather, and in synthetic cutting fluids, paint glues, and outdoor textiles. Most common are 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorophenol or their sodium or potassium salts. The entire range of dioxins and furans are found in these compounds ranging from <0.1 ug/g to 500 ug/g.[25]

Thermal reactions cause formation of dioxins and dibenzofurans at various temperatures. 2,3,7,8-TCDD is formed from 2,4,5-T but not from 2,4,5-T acids or esters at 400-500° C in 30 minutes.[47] At higher temperatures 500-850° C, 2,4,5-T esters will produce 2,3,7,8-TCDD.[48] Burning of material impregnated with salts of chlorophenols purified 2,4,6-trichlorophenate and pentachlorophenate form mostly lower TCDDs and PCDDs.[49] PCBs convert to PCDFs under pyrolytic conditions (10%).[50] Pyrolysis of chlorobenzenes forms PCDDs and PCDFs. Thermal decomposition of polychlorinated diphenyl ethers at 500-600° C yields 0.1% to 4.5% PCDDs and PCDFs.[51] Recently, an investigation for new syntheses for PCDDs and PCDFs during combustion from simple compounds showed that the interaction of benzene in the gas phase with $FeCl_3$ on a supporting surface is demonstrated to produce trace quantities of PCDD and PCDF over a wide range of conditions.[52]

Evidence for formation of PCDDs and PCDFs at trace levels in fly ash and incineration products has been found in the Netherlands. 0.2 ug/g PCDD and 0.1 ug/gPCDF were found in a municipal incinerator.[53] They also found 0.6 ug/g PCDD and 0.3 ug/g PCDF in an industrial heating facility. Variations have been found in incinerator samples of levels of 2,3,7,8-TCDD. Mostly lower chlorinated PCDFs (Cl_4 and Cl_5) and higher chlorinated PCDDs (Cl_7 and Cl_8) are found.[54] Tiernan et.al.[55] detected all 22 isomers of fly ash from a municipal incinerator in the United States.

Accidents and explosions are a major contribution of dioxins and dibenzofurans into the atmosphere. In a fire in a State Office Building in Binghamton, N.Y. where PCBs make up 65% of the dielectric fluid in transformers and chlorinated benzenes 35% , the PCDFs in ashes were found at high concentrations (2000 ug/g).[54,56] The most toxic isomers were the major components of each group of congeners present. There were also a series of capacitor accidents in Scandinavia. Wipes taken from capacitor explosions in Stockholm, Sweden found high levels of biphenylenes (PCBPs) which are closely related to the most toxic PCDDs and PCDFs, particularly 2,3,6,7-TCBP.[57] In 1976, a reactor at Industrie Chemiche Meda

Societa,Anonima (ICMSA), Seveso, Italy, making 2,4,5-trichlorophenol for use hexachlorophene production, went out of control, releasing several pounds of dioxins in a densely populated area.[58]

D. AQUATIC BACKGROUND

The presence of PCDDs and PCDFs in foods is of human concern because these compounds are highly lipophilic, resistant to biological degradation, and tend to accumulate in the food chain. Bioaccumulation is the uptake of a compound by an organism from its environment. The bioaccumulation takes place in areas where sediment acts as the reservoir from which aquatic organisms gradually pick up residues. Bioaccumulation ratios ranging from 2000-26,000 have been found in laboratory experiments on the uptake of 2,3,7,8-TCDD from water by various aquatic organisms, including fish.[59-62] Consumption of fish could be an important route for ingestion of these toxic compounds. It is important to have an abundance of data to compare with that of other aspects of the surrounding environment.

Biological half-lives of chlorinated dibenzo-p-dioxins and dibenzofurans in rainbow trout (Salmo Gairdneri) were determined for 5 PCDD and 2 PDCF congeners following a single oral exposure. Estimated half lives ranged from 2 days for 2,7-dichlorodibenzodioxin to 43 days for 1,2,3,4-tetrachlorodibenzodioxin and 12 days for octachlorodibenzofuran and 24 days for 3,6-dichlorodibenzofuran. No consistent relationship between half-lives and number of chlorines appears to exist.[63] The biological half-life of 2,3,7,8-TCDD was estimated to be 58 days in trout and greater than 100 days in guppies.[64,65] No detectable levels of di-, tri-, and tetrachlorodibenzofurans and only low levels of octachlorodibenzofuran present in salmon fed diets containing 3 to 9 ng/kg of each congener for 140 days.[66] Only trace levels of octachlorodibenzodioxin were present in guppies fed 50 ng/kg for 70 days.[67] The bioconcentration factor of 2,3,7,8-TCDD in adipose tissue of humans was calculated to be 153.[68]

After aqueous exposure of fish to fly ash extract only a few highly chlorinated dibenzo-p-dioxins and dibenzofurans are accumulated. The elimination rates were high except for the 2,3,7,8-TCDD and 2,3,7,8-TCDF

congeners. Those compounds found were 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,7,9-PeCDD, 1,2,3,7,8,9-HCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and 2,3,4,7,8-PeCDF. No hexa-, hepta-, or octa- chlorinated dibenzofurans were found in the fish, unlike mammals.[69] In fish sampled in the natural environment and fish exposed to fly ash from municipal incinerator only TCDD and TCDF were found.[70,71] Preference to selectively bioaccumulate PCDD/PCDF congeners substituted in 2,3,7,8 positions has been observed. Depuration half-life for 2,3,7,8-TCDD is 300-325 days. The rate appears to decrease as the chlorine substitution increases.[72] In comparing the levels of the heptachlorinated dioxin isomers (1,2,3,4,6,7,8 and 1,2,3,4,6,8,9) from fly ash to the fish exposed to the fly ash , there is more evidence for the preference of the substitution positions that are selectively taken up by the fish. In the fly ash the levels of the two isomers are almost equal. However, in the fish the two isomers are found in a 50:1 ratio in favor of the 1,2,3,4,6,7,8 isomer.[73] Fish exposed to sediment with levels of 2,3,7,8-TCDD of 39 pg/g for 55 days accumulated 7.5 pg/g.[74]

Assessment of the impacts of PCDDs and PCDFs in aquatic ecosystems is difficult because only limited chronic toxicity data are available for aquatic organisms. Presently, there is no information available on the dose-response relationship for PCDDs and PCDFs in fish eggs from the Great Lakes region. No information on bioconcentration factors exists for the entire PCDF class of compounds. The primary concern for contaminants in Great Lakes fish is not so much the short term acute toxic effects but rather the long term effects such as effects on reproduction. Because of this concern, research must be conducted to determine what fish contaminants are present, at what levels, and their distribution throughout the Great Lakes. It is also vital to identify the source of these contaminants.

Based on the few studies, which are available, 2,3,7,8-TCDD appears to be extremely toxic to fish. No studies have been conducted to determine chronic effects of 2,3,7,8-TCDD on fish reproduction, under ecologically-relevant exposure conditions. The data base to conduct an assessment of the hazard to Great Lakes fish populations, is inadequate. Silver salmon and guppies were given 23 mg/g for 24 hours, and rainbow trout were given 6.3 ug/wk/10 animals. Toxic

effects observed were death within 10-80 days of exposure for the salmon and guppies, and trout died after 33 days of exposure. [75] In another study, pike and rainbow trout , freshly fertilized eggs, yolk sac fry, and juveniles were exposed to 0.1 to 100 ppt in tap water. This resulted in induced retardation of embryonic development and growth, dose-related incidences of hemorrhages, edema and hepatic injury, followed by death. Those that survived exhibited skeletal malformations, inclusion bodies in stomach, pancreas, and liver.[76] Hawkes and Norris exposed rainbow trout to 2,3,7,8-TCDD via contaminated food and found that mortality resulted when the whole body concentration reached 1.57 ng/g due to exposure to 2.3 ug/g in the food.[77] One day exposure to levels as low as 7.1 ng/l caused mortality for several weeks in fathead minnows (Pimephales Promelas).[78] Guppies (Poecilia reticulatus) treated with 0.1,1.0, and 10.0 ppb 2,3,7,8-TCDD for 120 hours exhibited 100% mortality within 37 days after the exposure. The average survival time was 21 days.[79] Coho salmon treated for 96 hours at 0.54 ng/g showed no effects up to 60 days postexposure. Those exposed to 5.4 ng/g for 96 hrs. showed reduced growth and survival over 114 days postexposure. Guppies treated for 96 hours at 0.08

ng/g exhibited no observable effects, but those exposed to 0.8 ng/g for 96 hours developed fin disease 42 days after exposure.[80] Mosquito fish exposed in a model ecosystem died at all exposure levels (0.1, 1.0, and 10.0 mg/kg applied to sediment; water concentration was 1.0, 10.0, and 100.0 ng/l at 3 days). Snails, algae, and water fleas were unaffected. [81]

These studies of the effects of PCDDs and PCDFs on aquatic organisms have been conducted by exposing juvenile or adult organisms to residues directly from water. This does not allow one to determine the dose-response relationship for eggs and fry which have been exposed by deposition into eggs subsequent to maternal exposure. Presently, there is no information available on the dose-response relationship for PCDDs and PCDFs in fish eggs from the Great Lakes region. It is known that toxic substances become concentrated in the lipids of fish eggs and become more concentrated in the remaining egg yolk as the fry absorbs the yolk, so that the fry receives a large dose of xenobiotics at the "swim- up" stage as the last of the yolk is absorbed. [82] Recently, a correlation has been demonstrated between concentration of residues, presently in chinook salmon eggs, collected from Lake Michigan, and their viability and survivorship.[83] Helder studied exposure of eggs to 2,3,7,8-TCDD, which showed that the exposure to parts per trillion concentrations in water caused a decrease in growth and survival, including delayed mortality. However, the levels of TCDD in the eggs were not quantitated, making it impossible to assess maternal deposition.[84]

Not many values for PCDDs and PCDFs are available for fish tissues and no values are available for salmonid eggs. Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans have been measured in fish from the Great Lakes at concentrations ranging from 1 pg/g to greater than 1 ug/g. Several studies have been conducted concerning fish contamination in the Great Lakes area. [2-6] Levels of 2,3,7,8-TCDD and 2,3,7,8-TCDF of up to 150 ng/kg have been reported in fish from the Great Lakes.[7-9] PCDD were not detected in all of the fish analyzed. Mostly 2,3,7,8-TCDD was found. The predominant PCDF found was the 2,3,7,8-TCDF congener. In some areas the concentrations of 2,3,7,8-TCDD and 2,3,7,8-TCDF in fish from the Great Lakes were as great as 417 and 1015 ppt, respectively. [9] Findings in a survey of 13 Michigan Rivers for 2,3,7,8-TCDD in carp and sucker ranged from undetectable to 530 pg/g. Most of the fish

had less than 8.6 pg/g except in the Tittabawassee River.[3] Another study monitoring TCDD in fish from the Great Lakes and Michigan Rivers showed concentrations of 2,3,7,8-TCDD in 6 of 20 carp to be 15-46 ppt and 8 of 19 catfish in a range of 18-102 ppt.[6]

Eleven coho salmon were examined and no detectable 2,3,7,8-TCDD was present. The concentrations of PCDD and PCDF were examined in 6 species of Great Lakes fish by Petty,1983.[2] This study included all isomers of PCDD and PCDF but the number of fish analyzed from any one location was small. Isomers of dichlorodibenzo-p-dioxin and tri- and penta-chlorodibenzofuran were present, but not hexa- or hepta-chlorodibenzofuran in fish from waters or watersheds of Great Lakes.[10] Concentrations of 2,3,7,8-TCDD in lake trout (weighing 1.03 kg) from Lake Ontario were on an average of 30.8 ppt(SD=20.0) and 22ppt (SD=10) in liver and fillets, respectively.[4] There were no detectable residues of TCDD and the lowest concentrations of PCDFs were observed in samples from Lake Siskiwit, on an island in Isle Royale National Park, Lake Superior [11].

IV. METHODOLOGY REVIEW

A.EXTRACTION AND CLEAN UP OF BIOLOGICAL SAMPLES

Clean up of fish extracts for PCDDs and PCDFs is a messy, difficult, time-consuming process. The fish matrix is a lipid one, making it complicated to perform trace organochlorine analyses. In addition, analysis of samples for PCDDs and PCDFs is often complicated by the presence of other organochlorine compounds such as polychlorinated biphenyls, methoxy biphenyls, hydroxy biphenyls, diphenyl ethers, methoxy diphenyl ethers, hydroxy diphenyl ethers, benzyl phenyl ethers, naphthalene' biphenylene, phenylbenzoquinone, xanthene, and bis(phenoxy)methane. At the present time, the procedures for fish analysis require a highly skilled technician to perform them. This requires as much as $1000 and a week per sample. There are several procedures designed for fish sample clean-up and detection of PCDDs and PCDFs. It would be most effective to simplify these methods into a one-step automated method that would reduce sample loss, time, and would give improved detection limits with minimal interferences. The method would separate all 75 polychlorinated dibenzo-p-dioxins and 135

polychlorinated dibenzofurans and allow for trace determination of specific isomers at one part per trillion levels. The analytical methodology for PCDDs and PCDFs requires extraction of the PCDDs and PCDFs from the major matrix constituents, other chlorinated residues and chemical contaminants, and trace level detection of PCDD and PCDF in the cleaned up sample extract. In addition, the analytical methodology should allow data to be generated with adequate accuracy and precision, show low susceptibility to interferences and false-positive determinations, and minimize analysis time to allow for a large number of samples. The existing methodology for fish analysis and biological matrices is summarized here.

1. U.S. ENVIRONMENTAL PROTECTION AGENCY (EPA) [85]

There are two types of procedures utilized in processing fish samples: acid extraction and neutral extraction. Besides fish, these procedures can be used for adipose tissue, milk, water, soil, and sediment samples. With the acid extraction procedure a sample is spiked with ^{37}Cl-2,3,7,8-TCDD standard, and refluxed with KOH. The sample is extracted with hexane. This hexane sample is then extracted with

concentrated sulfuric acid dried on sodium carbonate. Chromatography is performed on neutral alumina using carbon tetrachloride and methylene chloride to elute. The neutral extraction procedure requires mixing the sample with sodium sulfate and dry ice. The powder resulting is spiked with ^{37}Cl-2,3,7,8-TCDD standard, and extracted with acetonitrile, then acetonitrile saturated with hexane. The acetonitrile layer is saved and concentrated, then replaced with hexane. Chromatography is performed using a Florisil column followed by neutral alumina column, eluted with 100% hexane; 10% methylene chloride in hexane; and 25% methylene chloride in hexane sequentially. Recoveries obtained using capillary gas chromatography/ high resolution mass spectrometry were 80% for 2.5 to 10 ng/g of ^{37}Cl-TCDD internal standard.

2. FOOD AND DRUG ADMINISTRATION (FDA) [86,87]

Samples are dissolved in an alkaline solution (ethanol and KOH) by agitating at room temperature for 2-3 hours. This solution is then extracted with hexane followed by extraction with concentrated acid. After drying on sodium carbonate, chromatography is performed on neutral alumina, eluting with 20% carbon tetrachloride in hexane, then methylene

chloride. Finally, HPLC is used to further clean up the sample on a Zorbax-ODS column at 40 °C using methanol as solvent. Detection levels for this method were 0.1 to 28 ppb total dioxins and 0.01 ppb for individual dioxins (hexa-, hepta-, and octa-chlorodioxins). Percent recoveries were 89-103% for dioxins and furans with the exception of OCDF(39%) and OCDD(70%) indicating possible loss of octachloro isomers using the acid extraction procedure.

3. DOW CHEMICAL COMPANY [88,89]

For fish and milk, DOW Chemical has utilized an acid extraction procedure. First, the sample is spiked with ^{13}C-2,3,7,8-TCDD internal standard, and shaken with concentrated HCl for one hour. This solution is extracted with hexane shaking overnight plus an additional 3 hours. The hexane extract is then run through a combined column and silica, concentrated sulfuric acid on silica, 1 M KOH on silica (using 22% sulfuric acid and 44% sulfuric acid on silica columns and NaOH on silica columns alternatively). Then the hexane extract goes through a second dual column of silver nitrate on silica and basic alumina. These dioxin fractions are then cleaned up using normal-phase silica (Zorbax-SIL) HPLC, then reverse-phase HPLC

(Zorbax ODS, methanol solvent). Detection limits were in the 10-100 parts per trillion range and percent recovery of TCDDs is 75±25%.

4. NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES [90]

In order to detect more than the tetra isomers, this method was developed to optimize relative to maximum recovery of PCDDs and PCDFs. The sample is extracted with anhydrous sodium sulfate and chloroform in a Soxhlet extractor. The lipid residue is leached from the fat sample into carbon tetrachloride and partitioned against concentrated sulfuric acid and centrifuged at 2000 rpm for 30 minutes. The carbon tetrachloride solution is passed through anhydrous sodium carbonate and concentrated by rotary evaporation. The residue is leached into n-hexane in methylene chloride 97:3. This solution is loaded onto an alumina column and eluted with n-hexane:methylene chloride 97:3, then n-hexane:methylene chloride 80:20. The percent recovery is 100 ± 2% for a wide range of dioxins. The Florisil step is omitted since OCDD is not completely eluted from a Florisil column.[91]

5. CENTER FOR LABORATORIES AND RESEARCH [92,93]
NEW YORK STATE DEPARTMENT OF HEALTH

A neutral clean up procedure for fish was developed and improved giving at least 80% recovery and parts-per-trillion level detection. Fish fillets were homogenized with methylene chloride and filtered through a ICN neutral alumina and Celite 545. A large-scale reverse-flow column using MgO/Celite 545 and sodium sulfate and alumina and elute with solvents 3% methylene chloride/hexane, then 80% methylene chloride/hexane. For high fat samples the first column in the reverse flow chromatography is Florisil and sodium sulfate with activated alumina added to the solvent layer. This column is eluted with hexane. After inverting the column the sample is eluted with 4% MeOH/carbon tetrachloride, the carbon tetrachloride, then 10% methylene chloride/ carbon tetrachloride, then methylene chloride. Finally, the sample is cleaned up using HPLC. A slightly different work up procedure is used if the fish is unskinned.[93]

6. SUMMARY

All of these methods have been used for dioxin analysis, primarily in analyzing for 2,3,7,8-TCDD from other chlorinated aromatic hydrocarbons in environmental samples. In order to perform isomer specific determination of the wide range of dioxins and dibenzofurans, a method needs to be developed which omits acid extraction procedures, and the use of Florisil columns[91]. Such a method has been developed by Stalling, Smith, and Johnson [94] for determining part-per-trillion levels of PCDDs and PCDFs in environmental samples for biological tissues and sediment samples. Interferences are kept at very low levels and false-positive determinations are very rare. Because of contaminant enrichment procedures several steps can be linked together, allowing this method to be automated.[94] This method utilizes an extraction column containing potassium silicate, and silica gel. Further clean up takes place on cesium silicate, silica gel, and activated carbon adsorbent on glass fibers. PCDDs and PCDFs, along with other chemical compounds such as polychlorinated naphthalenes (PCNs), polychlorinated biphenylenes, and polynuclear aromatic hydrocarbons (PAHs), remain on the carbon

adsorbent. They can be removed by reverse elution with toluene. The sample is cleaned up through a series of columns,with the final sample transferred for analysis on HRGC/LRMS.

B. DETECTION METHODS FOR TRACE LEVEL ANALYSIS

Several detection methods have been utilized in trace analysis of PCDDs and PCDFs. These include radioimmunoassay [95], ultraviolet spectroscopy, [96] and thin layer chromatography (TLC)[97] as screening methods. For parts-per-trillion level detection electron capture gas chromatography [98] has been used, as well as, low-resolution mass spectrometry (LRMS)[99], high resolution mass spectrometry (HRMS) [100], metastable ion monitoring [101], low- and high-pressure negative chemical ionization (NCI)[102], atmospheric pressure ionization (API) [103], and tandem mass spectrometry (MS/MS).[104] Several interferences must be avoided in the detection technique. These are 1,1-dichloro-2,2-bis (p-chlorophenyl) ethane (p,p'-DDE, a degradation product of DDT); a minor component of toxaphene; polychlorinated biphenyls; benzyl, phenyl ethers; tetrachlorinated methoxy biphenyls.[105] These

compounds have either similar gas chromatographic retention indices or interfering ions in mass spectrometry or both. To screen for planar polychlorinated aromatic compounds, such as PCDDs and PCDFs, nonplanar polychlorinated aromatic compounds must be eliminated because they usually exist at concentrations three times greater than the concentration of the planar aromatic compounds.

High-resolution gas chromatography (HRGC) with wall-coated open tubular columns (WCOT) is capable of separating all 75 PCDD compounds. Buser and Rappe [106] have studied the separation of all 22 TCDD isomers on glass WCOT columns with mass spectrometric detection. They had to use three different stationary phases, Silar 10C, OV-17, and OV-101 in order to resolve and identify all of the isomers. An SP-2330 column is also found to be very useful in separating a maximum number of isomers. Fused silica columns found to be useful in isomer separation as well are SP-2330, SP-2340, SILOV, SE-54, and DB-5.[55]

A GC/MS technique with high-resolution gas chromatography and low-resolution mass spectrometry (LRMS) appears to be ideal for obtaining complete and

specific analyses. Using the selected-ion-monitoring (SIM) mode of GC/MS operation gives higher sensitivity, since the mass spectrometer only detects ion currents at a few selected masses rather than scanning an entire spectrum. Ultra-trace analysis requires HRGC/HRMS for low parts-per-trillion or pg/g levels in a complex matrix. Early attempts to determine TCDD in biological samples using electron-capture gas chromatography had a detection limit of 50 ppb.[107] In 1973, a method for TCDD was described which had a detection limit close to 1 ppt. using direct probe introduction of a cleaned-up sample extract into a high resolution mass spectrometer.[108] One part-per-billion ^{37}Cl-2,3,7,8-TCDD was added to the sample for recovery data.

Several ionization methods have been used for dioxin analysis. These include electron impact (EI), positive chemical ionization (PCI), negative chemical ionization (NCI), and atmospheric pressure ionization (API). Electron impact mass spectra of PCDD and PCDF will give intense molecular ion peaks (M^+) and the two chlorine isotopes will provide the characteristic cluster of isotope peaks. PCDD mainly fragments to M^+-COCl and M^+COCl-Cl_2. Through using EI mass spectra PCDDs/PCDFs can usually be easily distinguished from

other chlorinated aromatic hydrocarbons possibly present in a sample. In some cases, such as PCBs and DDE, fragmentation produces ion masses that interfere with the detection of PCDDs and PCDFs. High resolution mass spectrometry must be used in these cases. Methane positive chemical ionization (MPCI) of PCDDs show the $[M+1]^+$ ion as the base peak, $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$ are also present. Other peaks will be due to loss of $Cl^\cdot$,$H^\cdot$, and combinations of $Cl^\cdot$ and $H^\cdot$ from the $[M+1]^+$ or $[M+C_2H_5]^+$ ions.[109]

Of the ionization methods, only oxygen negative chemical ionization (ONCI) using a Townsend discharge source and methane negative chemical ionization (MNCI) show significant differences between isomers.[110] Negative chemical ionization has proven to be a powerful tool for analysis of complex environmental matrices for trace levels of compounds that are oxidizing, alkylating agents, or both.[111] The technique is known for its high sensitivity. ONCI is said to be more sensitive than EI for PCDDs containing 5 or more chlorine atoms, and gives the most structural information. MNCI gives the greatest sensitivity of any technique for the higher chlorinated dioxins, but less information on isomers than with ONCI. With MNCI all spectra are unique but no information concerning the position of chlorine atoms on the ring is found. From an ONCI study of mass spectra of HCDDs, each fragmentation pattern is unique giving more information in addition to chromatography for isomer identification. Temperature is an important parameter to control if one wants reproducible data. The relative abundance of M^- ions increases with increasing temperature while total ionization yield decreases. If the temperature is too low, chromatographic resolution decreases due to band broadening.

The two major fragmentation pathways reported for PCDDs using ONCI are shown in Figure 2.[110] Pathway I produced a (M-19) ion due to the loss of a chlorine ion and the addition of an oxygen. Pathway II was first reported for 2,3,7,8-tetrachlorodibenzo-p-dioxin by Hunt and Harvey [112], and lead to the formation of either one or two product ions depending upon the chlorine distribution on the two aromatic rings and yielded information defining the number of chlorine atoms on each ring. Analytical conditions were found that allowed an isomer specific separation and detection of all the 10 HCDDs using HRGC, HPLC, and ONCIMS with a Townsend discharge as a selective mode of ionization.[113]

Figure 2. Fragmentation pathways for PCDDs and PCDFs.[110]

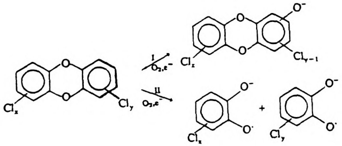


Figure 2.

The methane negative chemical ionization (MNCI) mass spectra of PCDDs exhibit loss of H, Cl, and Cl_2 from the negative molecular ion. PCDFs exhibit an intense negative molecular ion $[M]^-$ with limited fragmentation occurring via addition of H and loss of Cl to yield $[M-34]^-$ ions.[114]

In atmospheric pressure ionization mass spectrometry (APIMS), the ion source is at atmospheric pressure. There is an orifice (approximately 20-50 um) which ions go through to the mass analyzer. The system is differentially pumped to keep the quadrupole rods and electron multiplier in a high-vacuum (10^{-7} torr).[115] By using APIMS and monitoring the (M-19) product ions, and the ether cleavage product ions, the PCDDs should be able to be separated into 14 groups.[116] Use of APIMS with high-resolution capillary gas chromatography will give better isomer specificity for individual PCDDs than GC/EIMS. Low mass ions (m/z 76-143) can be used to show distinctions. The main advantages of using APIMS are low detection limits and ease of GC interfacing. API ionization efficiency is almost 100%, although not all ions pass into the mass analyzer.

In the negative ion mode, the conditions in the API source are similar to those of electron capture detection. Ionization occurs via high energy beta radiation emitted from a ^{63}Ni foil or via a corona discharge. The advantage of using a corona discharge is a larger dynamic range plus both equilibrated and nonequilibrated conditions may be used.[115] Problems with the discharge are continual erosion of the discharge tip and clogging of the orifice due to sputtered material. Ions in the carrier gas enter the mass analyzer through a free jet expansion through a source opening. The ion/molecule reactions that take place are due to the make up gas and reagents.

V. MATERIALS AND METHODS

A. Materials

1. Standards

$^{13}C_{12}$-2,3,7,8-tetrachlorodibenzo-p-dioxin (50 ug/ml), $^{13}C_{12}$-2,3,7,8-tetrachlorodibenzofuran (48 ug/ml), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (1 mg.) were obtained from Cambridge Isotope Laboratories, Woburn, MA. 2,3,7,8-tetrachlorodibenzofuran (1 mg.) was obtained from Ultrascientific, Hope,RI.

2. Glassware

Chromatography Columns were obtained from Spectrum Scientific Inc., Houston, TX. These columns had teflon end plates for use with organic solvent systems. Columns varied in size. For the extraction process (Column 1) part 125029 with dimensions 2.5 cm x 60.0 cm was used. For columns 3 and 5 part 125001 was used. This is a column made with precision bore glass and dimensions of 0.9 cm x 15 cm. For column 2 a Kimax column with dimensions of 22 mm X 500 mm, was used (American Scientific Products, McGaw Park, IL). Column 4 was a nine inch disposable pipet (VWR).

3. Glassware Cleaning Procedure

All glassware went through a rigorous cleaning procedure which included the following steps:

a. wash with soap

b. rinse with deionized water

c. acetone rinse

d. toluene rinse

e. cyclohexane/methylene chloride (1:1) rinse

f. burn in oven at 550° C for 12 hours

4. Solvents

All solvents were of glass distilled grade. Cyclohexane, methylene chloride, toluene, benzene, hexane, and methanol were all obtained from J.T.Baker, Phillipsburg, NJ.

5. Adsorbents

Silica Gel 60 (70-230 mesh) (EM Reagent, MC/B Cincinnatti, OH); Acid Alumina (AG-4,100-200 mesh) (Bio Rad Labs,Richmond,CA) ; Super A Activated carbon (AX-21 Lot 79-6)(Anderson Development Company, Adrian, MI); Celite 545 (Supelco,Bellefonte, PA); Hydrocarbon trap for nitrogen evaporation HT-200-2

(R&D Separations , Rancho Cordova, CA); Anhydrous sodium sulfate (Mallinckrodt, Paris,KY); KOH pellets (J.T.Baker, Phillipsburg, NJ).

6. Washing of adsorbents

Silica gel and alumina were washed with methanol and methylene chloride. Sodium sulfate was washed with hexane and methylene chloride prior to activation. Silica gel was activated at 135° C and alumina was activated at 190° C for 48 hours.

B. Methods

1. Preparation of Adsorbents

a. Potassium Hydroxide Silica Gel

Potassium silicate was prepared from the reaction of 64 g. of potassium hydroxide, 150 g. silica gel, and 400 ml of methanol. The reaction was carried out in a 1 liter round-bottom flask which was rotated and was heated with a rotary evaporation apparatus (no vacuum applied). 64 g. KOH was dissolved in 150 ml methanol, followed by an additional 100 ml of methanol. Then the 150 g. silica gel was added.

Following the reaction, the mixture was poured into a large glass column (5 cm. x 100 cm.) containing a 1 cm glass wool plug. The adsorbent was washed into the column with methanol, and then 200 ml of methylene chloride was applied for every 100 g. silica gel . The liquid was allowed to run through the column and the silicate dried. Potassium silicate was activated in an oven at 130° C, and stored there.

b. Sulfuric Acid Impregnated Silica Gel (40% w/w)

Sulfuric Acid silica gel was prepared by adding two parts of concentrated sulfuric acid to three parts by weight of 130° C activated silica gel in a screw capped bottle, then shaking until the mixture was completely free of lumps, 15 min. The silica gel was activated at 130° C; unactivated silica gel was not satisfactory for preparation of sulfuric acid-silica gel (SA-SG). The adsorbent was stored in a screw cap bottle in a desiccator.

2. Sample Collection

Samples of Chinook salmon were collected randomly, at the Manistee Wier, Manistee, MI. Spawning females were caught, their bodies slit open, and eggs, and fillets separated from the carcass. Eggs and fillets were placed in polypropylene bags, and stored in a freezer at -20^{o} C.

3. Method Development for Fish Eggs

The analysis of fish eggs for 2,3,7,8-tetrachlorodibenzofuran, and 2,3,7,8-tetrachlorodibenzo-p-dioxin was modeled after a method initially developed by Stalling, Smith, and Johnson [94] for the analysis of PCDDs and PCDFs in fish fillets.

a. Sample preparation

Samples were thawed, and the fillets were homogenized in a quart size Omni Mixer. Egg samples were homogenized in a pint size Omni Mixer. Initially, fish samples of 40 g were prepared by grinding the 40 g of fish with 200 g of anhydrous sodium sulfate, placed in the first column of the procedure, and extracted with solvent. This method

Table 1. Fish location log

Identification	Location	Species
LM-186	Manistee Wier	Chinook
LM-187	Manistee Wier	Chinook
LM-188	Manistee Wier	Chinook
LM-191	Manistee Wier	Chinook
LM-192	Manistee Wier	Chinook
LM-195	Manistee Wier	Chinook
LSLT-2	Lake Siskiwit	Lake Trout
LSLT-3	Lake Siskiwit	Lake Trout
LSLT-7	Lake Siskiwit	Lake Trout
CARP-71	Tittabawassee River	Carp
CARP-155	Tittabawassee River	Carp
CARP-246	Tittabawassee River	Carp

was very difficult to use since this quantity of material was hard to grind, and required a large amount of the adsorbents, and solvents, making this process expensive. The procedure was modified using a 10 g sample, which was blended with 40 g anhydrous sodium sulfate. These fish samples were spiked with sufficient amounts of 2,3,7,8-TCDF to allow detection on the electron capture detector gas chromatograph (ECD-GC) for rapid screening of the utility of the column. However, when tried with the fish egg samples, this procedure presented problems. First, when the sample was blended with 40 g anhydrous sodium sulfate , a paste like substance was formed in the mortar, making it virtually impossible to transfer the prepared sample from the mortar to the extraction column and to assure any notable recovery. A larger amount of the anhydrous sodium sulfate was necessary to work with the fish eggs samples. Secondly, the amount of fish egg sample was doubled to 20 g since it was hypothesized that the amounts of 2,3,7,8-TCDF in fish eggs were at very low concentrations (low parts per trillion). 100 g of anhydrous sodium sulfate was used with the fish egg samples in the preparation process. This resulted in an excess of sodium sulfate. Finally, 75 g of anhydrous sodium sulfate was found to be sufficient for a 20 g fish egg sample.

Fish egg samples were spiked to monitor the success of the procedure as it was developed. The results and levels of these spikes were compiled in Table 4. Spiking took place before the sample was added to the extraction column. It was finally determined that each sample should be spiked with 5 ng each of $^{13}C_{12}$-2,3,7,8-TCDF and $^{13}C_{12}$-2,3,7,8-TCDD for confirmation.

b. Extraction Procedure

Simultaneous extraction, including removal of acidic and highly polar coextractables took place on Column 1. Column 1 was prepared from bottom to top with: a 1 cm. plug of silanized glass wool, 2 cm. of anhydrous sodium sulfate, 30 g silica gel (70-230 mesh), 30 g potassium hydroxide treated silica gel, 2 cm anhydrous sodium sulfate, the sample mixture, and 2 cm anhydrous sodium sulfate. The column dimensions were 2.5 cm X 60 cm. Initially, this column contained 30 g each of the KOH silica gel and silica gel, as well as, the sample mixture. Since the amount of sample was divided into four equal portions, 15 g of KOH silica gel and silica gel were used for the adsorbents in this column. Since this extraction worked well, the procedure was not changed even for the larger egg samples. The following

summarizes the elution, collection, and evaporation sequence for the fish egg extraction method. The sample was eluted with 450 ml of methylene chloride/cyclohexane (50:50),collected in a 500 ml round bottom flask and evaporated in a rotary evaporator to a 3 ml volume. (The original method for fish suggested 650 ml, and a column 4.5 cm in diameter and 1 meter long.[94])

The second column, Column 2, was similar to the first, and was used to remove any lipids which may not have been removed in the first column. Column 2 was prepared from bottom to top with a 1 cm. plug of silanized glass wool, 15 ml of silica gel, 15 ml of potassium hydroxide treated silica gel, and another 1 cm. plug of glass wool. The dimensions of this column were 22 mm X 500 mm. This column was eluted with 250 ml of methylene chloride/cyclohexane (50:50). The effluent was collected in a 500 ml round bottom flask, and evaporated in a rotary evaporator to a 3 ml volume. Initially, a Kimax column with dimensions of 11 mm X 500 mm was used because of availability in the lab (Pesticide Research Center, Michigan State University). This column, however, did not do a sufficient job in cleaning up the lipids. This conclusion was drawn from visual observation of the

effluent color. A yellowish-green color indicates lipid content. Also, the column took an entire 12 hours to completely elute.

These two columns were necessary to extract the chlorinated compounds from the biological matrix, as well as, to facilitate the flow, due to the lipid removal,through the third column, the carbon column. Acidic compounds which could have interfered with the GC/MS analysis were phenols, carboxylic acids, sulfonamides, hydroxy polychlorinated biphenyls, and hydroxy phenyl ethers.[94]

c. Carbon clean up

The carbon clean up procedure eliminated potential interferences such as: p,p'-DDE (1,1'-(dichloroethenylidene)bis(4-chlorobenzene), PCB's (polychlorinated biphenyls), methoxy PCB's, PCDPE's (polychlorinated diphenyl ethers), and methoxy PCDPE's,(Figure 3). Biogenic materials which were not eliminated in the first two steps were now also removed. Polyhalogenated, planar, multi-ring aromatic molecules would be adsorbed onto the carbon column.

Figure 3. Interferences in chlorinated furan and dioxin analysis eliminated in the carbon clean-up procedure. A. p,p'-DDE (1,1'-(dichloroethenylidene)-bis(4-chlorobenzene)). B.PCB's (polychlorinated biphenyls. C. Methoxy PCB's (methoxy polychlorinated biphenyls). D.PCDPE's (polychlorinated diphenyl ethers). E. Methoxy PCDPE's (methoxy polychlorinated diphenyl ethers).

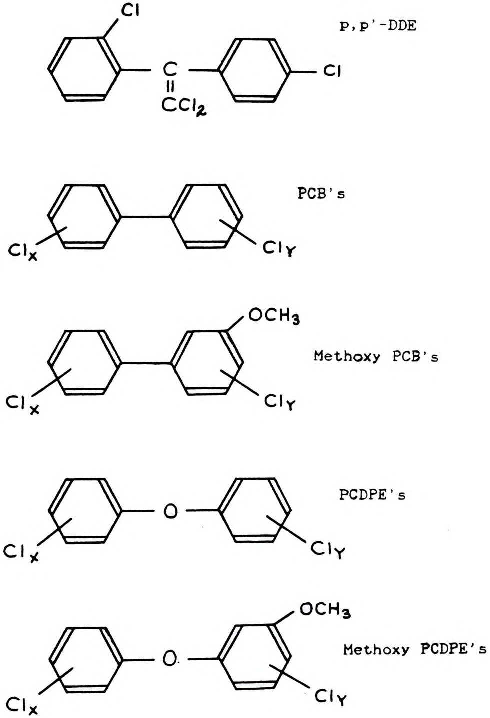

Cl
p,p'-DDE
C
CCl2
Cl
PCB's
Clx
Cly
OCH3
Methoxy PCB's
Clx
Cly
PCDPE's
O
Clx
Cly
OCH3
Methoxy PCDPE's
O
Clx
Cly

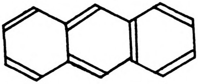
Figure 3.

Examples would include compounds such as PAH's (polyaromatic hydrocarbons), PCN's(polychlorinated naphthalenes), PCDD's and PCDF's (Figure 4).

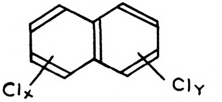
Initially, a column that was 0.9 cm in diameter and 15 cm long made of precision bore tubing was packed with activated carbon (Amoco AX-21). 4 cm of carbon was packed between two 1 cm. plugs of glass wool. Due to the inconsistency in flow and recovery using this column, continued work with this column was abandoned. Often no solvent flow would occur. This may have been the result of excessive compacting of the carbon particulates.

Instead of using carbon as the sole packing material, a second approach utilizing carbon dispersed on glass fibers was tried as suggested by Smith [116]. Glass microfiber filter (Whatman GF/D) material was cut into small pieces (3mm X 5mm). The glass fiber material was placed in methylene chloride (70 ml). Using a Polytron homogenizer the fibers were separated in the methylene chloride during a period of 25 seconds. The carbon was added (70 mg for every 700 mg of shredded glass fibers) and mixed thoroughly. Carbon was removed from suspension as it adhered to the settling glass fibers. The slurry of carbon/glass in

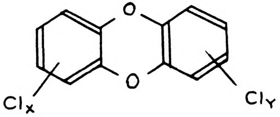
Figure 4. Polyhalogenated, planar, multi-ring, aromatic molecules which adsorb onto carbon columns. PCN's and PAH's will be interferences until clean-up takes place with sulfuric acid silica gel and alumina. A. a PAH (polyaromatic hydrocarbon, Anthracene). B. PCN's (polychlorinated naphthalenes). C. PCDD's (polychlorinated dibenzodioxins). D. PCDF's (polychlorinated dibenzofurans).



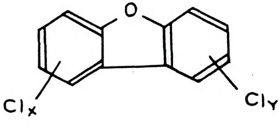
PAH (Anthracene)



PCN's



PCDD's



PCDF's

Figure 4.

methylene chloride was poured into the column, which had a 1 cm. plug of silanized glass wool in the end, and the other end was similarly plugged with the glass wool. Use of this column also presented problems with the solvent flow and poor recoveries of PCDD and PCDF were the result.

Finally, a carbon column was packed with 1 cm. silanized glass wool plugs on each end, and 4 cm of a carbon/celite (50:50) mixture in the middle. The column was eluted with the following solvents: 75 ml of the methylene chloride/cyclohexane (50:50), 50 ml of methylene chloride/methanol/benzene 75:20:5. The column was then reversed, and eluted with 45 ml of toluene to remove the adsorbed PCDDs and PCDFs off of the column. The amount of toluene used was determined by taking fractions off of the column, and analyzing them for 2,3,7,8-TCDF content by using GC/MS in the NCI mode with methane as the reagent gas (Figure 5), and selected ion monitoring was employed as a means for data collection (Table 3). The effluent was collected in a 250 ml round bottom flask and evaporated to 1 ml in a rotary evaporator.

Figure 5. Determination of 2,3,7,8-tetrachlorodibenzofuran I. 5 ml fractions were collected and analyzed by GC/MS using methane NCI/SIM to determine the elution of 2,3,7,8-tetrachlorodibenzofuran through the carbon/celite column.

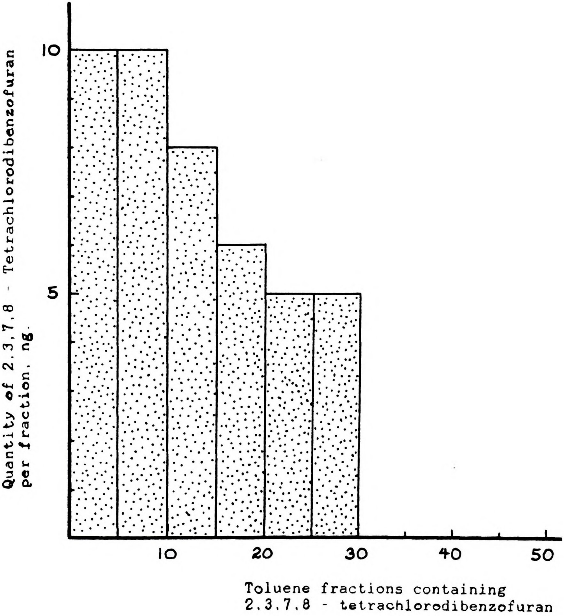

Quantity of 2.3,7,8 - Tetrachlorodibenzofuran per fraction. ng.
10
5
10
20
30
40
50
Toluene fractions containing 2.3.7.8 - tetrachlorodibenzofuran

Figure 5.

d. Tandem Separation

A tandem set of columns was used to remove trace acidic compounds, PAHs, PCN's, and trace PCB's. The first column was packed from bottom to top with a 1 cm. plug of glass wool, 3 cm sulfuric acid treated silica gel, 3 cm. KOH treated silica gel (unactivated), and 0.5 cm anhydrous sodium sulfate. The tip of this column rested on the top of the packing for column 5. Column five was packed with a 1 cm. plug of glass wool, 3.5 ml activated acid alumina, and 0.5 cm anhydrous sodium sulfate. This series of columns had remained relatively the same between fish and fish egg analysis.

The 1 ml effluent from column 3 was placed on the top of column four in small additions (quantitatively transfering) and allowed to pass completely through to column five before the next rinse with hexane was placed on column four. After all of the sample was allowed to flow through column four it could be discarded. Column 5 was eluted with a series of solvents: 15 ml of 2% methylene chloride in hexane, 15 ml of 5% methylene chloride in hexane, and 20 ml of 8% methylene chloride in hexane. Only the last 30 ml were collected as determined by collecting fractions (Figure 6) in a 250 ml round bottom flask and evaporated to 1

Figure 6. Determination of 2,3,7,8-tetrachlorodibenzofuran II. 5 ml fractions were collected from the tandem clean-up column (4 & 5) and analyzed by GC/MS using methane NCI/SIM for 2,3,7,8-tetrachlorodibenzofuran to establish elution collection.

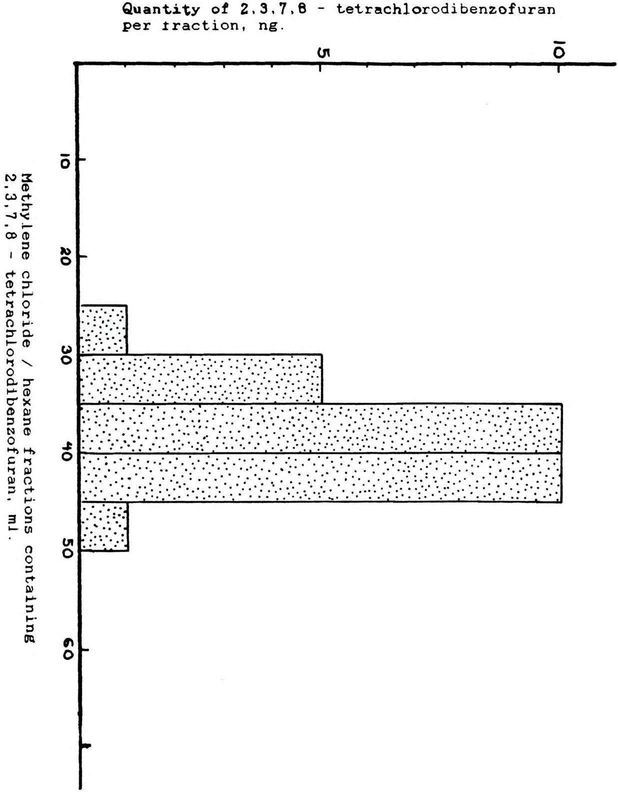

Quantity of 2,3,7,8 - tetrachlorodibenzofuran per fraction, ng.
5
10
10
20
30
40
50
60
Methylene chloride / hexane fractions containing 2,3,7,8 - tetrachlorodibenzofuran, ml.

Figure 6.

ml in a rotary evaporator. The contents were transferred to a conical vial and evaporated under nitrogen to near dryness and reconstituted in 10 ul of isooctane.

e. Summary

Table 2 provides an overview of the procedures discussed in the preceding section.

4. Liquid Scintillation Counting

Liquid scintillation counting was used to determine the amount of recovery of PCDDs and PCDFs at each step of the collection process. Using a ^{14}C labelled 2,3,7,8-TCDD, with a specific activity of 500 uCi/mg, spiked samples were run through the columns and collected at the points of the elution range that the furans and dioxins would be collected. These collected fractions were evaporated down to the specified volumes of the procedure, and a 200 ul aliquot was put in a liquid scintillation vial with 10 ml of PPO liquid

Table 2A. Extraction and Clean-up procedure.

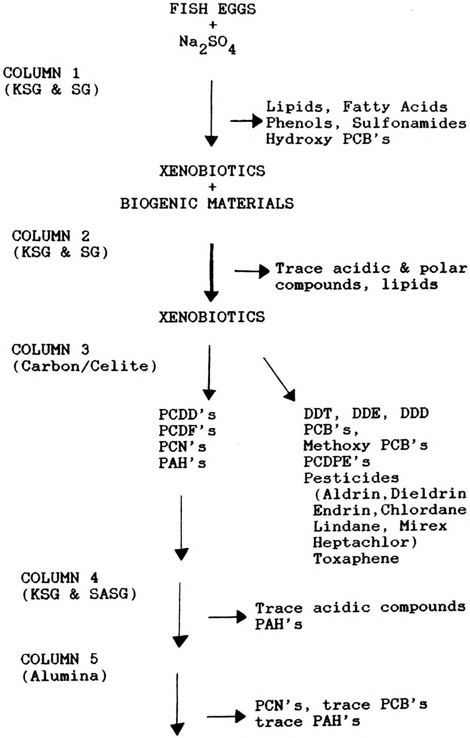


Table 2B. Extraction and Clean-up procedure.

SAMPLE PREPARATION:

Prepare homogenous egg sample from 20 g. tissue in Omni Mixer.

Mix 20 g. sample + 75 g. Na_2SO_4.

Add 5 ng $^{13}C_{12}$-2,3,7,8-TCDD and 5 ng $^{13}C_{12}$-2,3,7,8-TCDF

EXTRACTION:
COLUMN 1:
Elute Column 1 with 450 ml of
methylene chloride/cyclohexane 50:50.
Column 1: glass wool, 2 cm. Na_2SO_4, 30 g silica gel,
30 g KOH treated silica gel, 2 cm. Na_2SO_4,
sample mixture, 2 cm. Na_2SO_4.

Evaporate to 3 ml.

COLUMN 2:
Elute Column 2 with 250 ml
methylene chloride/cyclohexane 50:50.
Column 2: glass wool, 15 ml silica gel,
15 ml KOH treated silica gel,
glass wool.

Evaporate to 3 ml.

Table 2B. (continued)

CARBON CLEAN-UP:

Elute column 3 with:

1) 75 ml methylene chloride/cyclohexane 50:50.
2) 50 ml methylene chloride,methanol, benzene 75:20:5.

Column 3: glass wool, 4 cm carbon celite mixture, glass wool.

Reverse elute column 3 with 45 ml toluene.

Evaporate to 1 ml.

TANDEM SEPARATION:

Apply sample to column 4 in 0.5 ml washes with hexane.

Allow all of the sample to run from column 4 onto column 5

Column 4: glass wool, 3.5 ml activated acid alumina, 0.5 cm Na_2SO_4.

Column 5: glass wool, 3.5 ml activated acid alumina, 0.5 cm Na_2SO_4.

Evaporate.

Transfer to conical vial.

Evaporate to near dryness and reconstitute in

10 ul isooctane.

Perform GC/MS analysis.

scintillation cocktail. A 6895 Beta Trace liquid scintillation system was used to detect (count) the ^{14}C. The counter was set at 200K 0.5 % for 5 min. (meaning it would count at 95 % probability for 5 min.). A summary of these results has been compiled in Table 4.

5. Gas Chromatography

Gas chromatography was performed on spiked samples to monitor the success of the fish eggs method. Using a Varian 1400, equipped with an electron capture detector with a ^{3}H foil, and a capillary column, the 2,3,7,8-TCDF could be detected for presence at high parts per trillion levels (100 or more ppt). First, a DB-1 (methyl silicone) capillary column, 30 meters in length with a 0.25u phase thickness and 0.25 mm inner diameter was used. However, the 2,3,7,8-TCDF adsorbed so strongly to this column, that separation between 2,3,7,8-TCDD and 2,3,7,8-TCDF , was impossible. As a result, a DB-5 column (5% phenyl methyl silicone), and DB-1701 column (7% cyanopropyl phenyl silicone) were tried. The DB-1701 did not work any better than the DB-1 as far as the adsorption was concerned. The DB-5 could be used for the screening purposes of the GC, at a 30 meter length. However, in the final analysis, a

60 meter DB-5 column was necessary in order to separate, and quantitate low levels of 2,3,7,8-tetrachlorodibenzofuran from 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Other parameters that were set for the GC analysis were: Split ratio of 3:1, Detector temperature of 230°C, and Injector temperature of 260°C. Temperature programming for the GC analysis was designed to detect 2,3,7,8-TCDF in the shortest amount of time possible in relatively clean, spiked egg samples. The final temperature program used was as follows:

210°C for 1 min.(hold)

210-270°C, at 10°C/min.

6. Gas Chromatography/Mass Spectrometry

A Nermag R-10 10 S Gas Chromatograph/Mass Spectrometer was used in the NCI mode with methane as the reagent gas, samples were analyzed for the presence of 2,3,7,8-tetrachlorodibenzofuran, and 2,3,7,8-tetrachlorodibenzo-p-dioxin at low part per trillion levels. The source pressure was maintained at 1×10^{-1} torr. A DB-5 capillary column, 60 meter in length with a 0.25u phase thickness and 0.25 mm inner

diameter was used to separate 2,3,7,8,-tetrachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzo-p-dioxin. An on-column injector was used to efficiently introduce samples onto the column. Temperature program for the 60 meter column was set as follows:

100-260° C, at 30° C/min
260° C hold for 30 min.

The source temperature was set for 110° C, filament current at 0.100 mA., and electron volts at 72.6 eV (Figures 7 and 8). The ions that were monitored using selected ion monitoring have been shown in Table 3.

Response was measured as the total peak area of each ion monitored. A minimum signal equivalent to 2.5 times the baseline noise level is a requirement for quantitation. In addition the chlorine isotope ratio between the 304 to 306 peak for 2,3,7,8-TCDF and the 320 to 322 peak for 2,3,7,8-TCDD must be 70:100. The retention time for the $^{13}C_{12}$-2,3,7,8-TCDD and $^{13}C_{12}$-2,3,7,8-TCDF must match the retention time for the native 2,3,7,8-TCDD and 2,3,7,8-TCDF.

Figure 7. Optimization of reagent gas pressure in the source of the mass spectrometer for methane negative chemical ionization/SIM for determination of 2,3,7,8-tetrachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzo-p-dioxin.

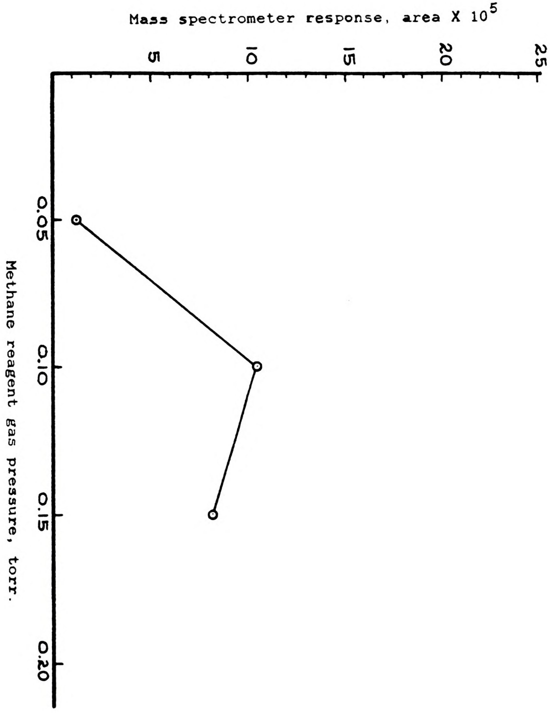

Mass spectrometer response, area X 10^5
5
10
15
20
25
0.05
0.10
0.15
0.20
Methane reagent gas pressure, torr.

Figure 7.

Figure 8. Optimization of filament current for methane negative chemical ionization /SIM determination of 2,3,7,8-tetrachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzo-p-dioxin.

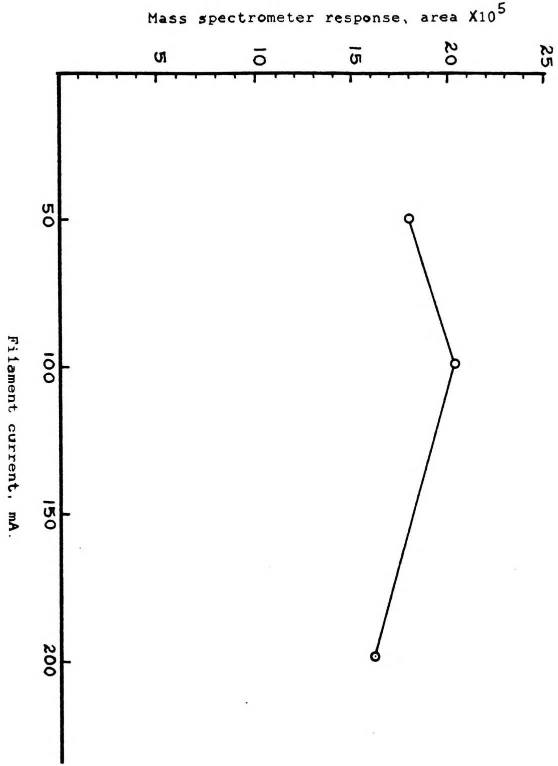

Mass spectrometer response, area $\times 10^5$
5
10
15
20
25
Filament current, mA.
50
100
150
200

Figure 8.

Table 3. Selected Ion Monitoring Masses

Compound	Masses Monitored
transchlordane	410
2,3,7,8-TCDF	304 306 308
$^{13}C_{12}$-2,3,7,8-TCDF	316 318
2,3,7,8-TCDD	320 322
$^{13}C_{12}$-2,3,7,8-TCDD	332 334

A. CALCULATIONS

The concentration of 2,3,7,8-tetrachlorodibenzofuran present in fish and fish egg samples was calculated by the following formula:

$$\text{ppt(ng/kg) 2,3,7,8-TCDF} = \frac{A \times E}{C \times H} \times \frac{B \times G}{D \times F}$$

A = Peak area of native 2,3,7,8-TCDF in the sample.

B = Peak area of added $^{13}C_{12}$-2,3,7,8-TCDF in the sample.

C = Peak area of ^{12}C-2,3,7,8-TCDF in the standard.

D = Peak area of $^{13}C_{12}$-2,3,7,8-TCDF in the standard.

E = Mass of ^{12}C-2,3,7,8-TCDF in the standard (ng).

F = Mass of $^{13}C_{12}$-2,3,7,8-TCDF in the standard (ng).

G = Mass of $^{13}C_{12}$-2,3,7,8-TCDF added to the sample (5 ng).

H = Mass of fish sample.

Using the first term, the concentration of native 2,3,7,8-TCDF in the injection aliquot was calculated, while the second term corrects for recovery of the internal standard. In samples which do not contain any native 2,3,7,8-TCDF, the limit of detection for that sample was calculated by multiplying the second term by the minimum mass of 2,3,7,8-TCDF which can be detected by GC/MS. Similar calculations were performed for 2,3,7,8-TCDD levels.

VI. RESULTS AND DISCUSSION

A. Recovery Study

Results of the liquid scintillation study done with ^{14}C are in Table 4. These data indicate that the extraction process has a very successful recovery rate, while the recoveries for the carbon column, and the transfer to GC/MS are low and inconsistent. Since PCDDs and PCDFs adsorb strongly to the carbon this results in the variability in recovery. The on-column injector was used in order to improve recovery in the transfer to the GC column, however, the evaporation process would also cause possible losses in recovery at this point. Other reasons for the losses of recovery in the GC/MS analysis were due to the variability in sensitivity using methane negative chemical ionization mass spectrometry. Optimum pressure and filament current were determined. However, if any of these parameters were slightly off, the possibility of reduced sensitivity existed.

Table 4. Working recoveries determined using ^{14}C-2,3,7,8-tetrachlordibenzo-p-dioxin (A-D). Method recovery determined by $^{13}C_{12}$-labeled standards (E).

Treatment	% Recovery
A. Column Extraction	100 ± 7
B. Column 2	98 ± 6
C. Carbon Clean-up	65 ± 33
D. Acid Alumina	97 ± 10
E. GC/MS transfer	57 ± 52

B. Spiking studies

The first five samples that were run prior to this study were fish samples using the standard procedure. Two samples were spiked with 50.5 ppb 2,3,7,8-TCDD and three others were not spiked at all. These samples were screened by GC/MS. The samples containing the dioxin spike were detectable (Table 5.).

Egg samples were spiked with 2,3,7,8-tetrachlorodibenzofuran. A standard curve was run and has been shown in Figure 9. Typical GC/MS conditions have been recorded in Appendix A. The results of the study have been compiled in Table 5. These studies were done in order to assess the success of the column clean-up as well as to assess the parameters of the GC/MS. An adequate level of separation and response had to be assured for analysis of environmental egg samples. No matrix interference was apparent. Recoveries varied, again reflecting the inconsistency of the carbon/celite column and the losses due to the transfer for GC/MS analysis.

Figure 9. Standard curve for 2,3,7,8-tetrachlorodibenzo furan (injected standards) for fish egg spiking experiments. The slope of this line is 3.6×10^6 and the correlation coefficient is 0.99. Standards were quantitated by GC/MS using methane NCI/SIM.

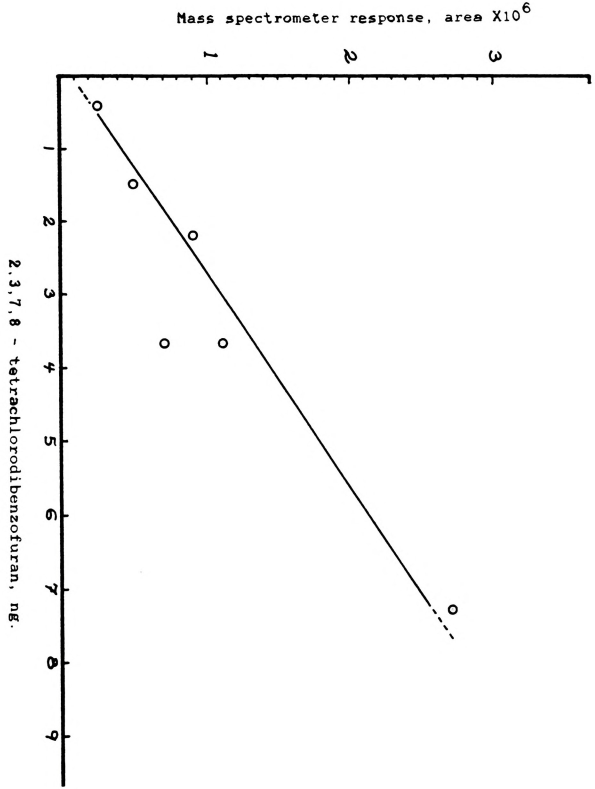

Mass spectrometer response, area X10^6
1
2
3
2,3,7,8 - tetrachlorodibenzofuran, ng.
1
2
3
4
5
6
7
8
9

Figure 9

Table 5. Fish egg spiking studies. Fish egg samples were spiked with varying levels of 2,3,7,8-tetrachlorodibenzofuran, and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Blanks were run intermittently, and were found to be non-detectable.

	SPIKE(PPB)		RECOVERY(PPB)	
Sample	TCDF	TCDD	TCDF	TCDD
1	26	NONE	9.4	NONE
2	30	NONE	17	NONE
3	26	NONE	5.1	NONE
4	4.9	NONE	4.9	NONE
5	13	92	0.5	72
6	11	78	0.4	41
7	76	NONE	24	NONE
8	62	NONE	27	NONE
9	0.04	NONE	0.01	NONE
10	0.4	NONE	0.1	NONE
11	0.4	NONE	0.1	NONE
12	0.4	NONE	0.2	NONE

Fish samples were also analyzed for 2,3,7,8-tetrachlorodibenzofuran, and 2,3,7,8-tetrachlorodibenzo-p-dioxin (Table 6). Standard curves were run for both compounds as shown in Figures 10 and 11. Typical GC/MS conditions have been recorded in Appendix B. Samples were spiked with a mixture of transchlordane, $^{13}C_{12}$-2,3,7,8-TCDD, and $^{13}C_{12}$-2,3,7,8-TCDF (Figures 12-14). These samples were of three groups. The first group represented the fish from which fish egg samples were taken. The second group of fish samples represented samples that were also analyzed in 1979 for 2,3,7,8-TCDD using the method developed by Kaczmar [12]. The third group of samples represented a remote environmental collection (Lake Siskiwit). Of these samples, only one of those previously analyzed for 2,3,7,8-TCDD had this compound present (Figure 15). No 2,3,7,8-TCDF was found. Recoveries ranged from 8 to 47 %, based on the amount of ^{13}C compounds detected.

Table 6. Fish spiking studies. Recoveries calculated based on response for $^{13}C_{12}$-labeled compounds.

Sample	2,3,7,8 TCDF(PPT)	2,3,7,8 TCDD(PPT)	% RECOVERY TCDF	% RECOVERY TCDD
FLM-186	N.D.	N.D.	37	42
FLM-188	N.D.	N.D.	33	37
FLM-192	N.D.	N.D.	47	19
LSLT-2	N.D.	N.D.	23	20
LSLT-3	N.D.	N.D.	13	12
LSLT-7	N.D.	N.D.	22	24
CARP 71	N.D.	120	-	31
CARP 155	N.D.	N.D.	-	8
CARP 246	N.D.	N.D.	-	22

Figure 10. Standard curve for 2,3,7,8-tetrachlorodibenzofuran (injected standards) for fish and fish egg samples spiked at picogram levels (see Tables 5,6). The slope of this line is 3800 and the correlation coefficient is 0.83. These standards were quantitated using GC/MS methane negative chemical ionization with selected ion monitoring.

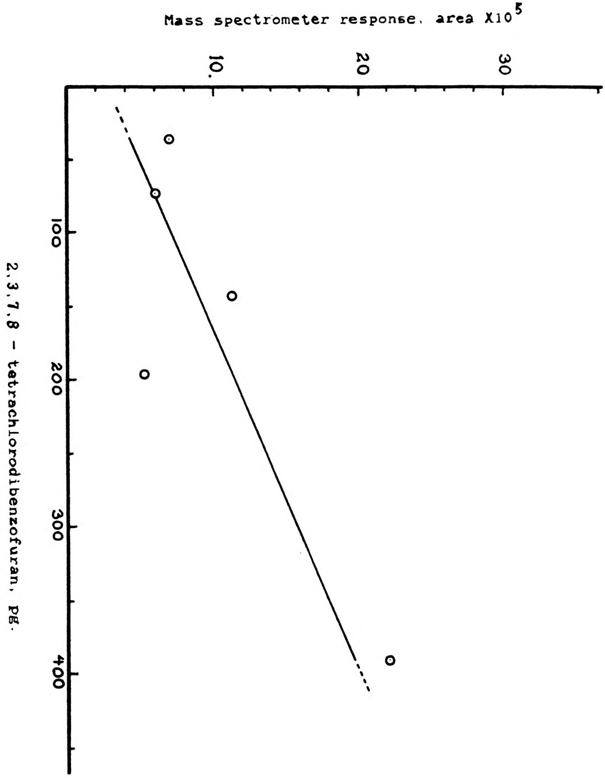

Mass spectrometer response, area X10^5
10.
20
30
100
200
300
400
2.3.7.8 - tetrachlorodibenzofuran, pg.

Figure 10.

Figure 11. Standard curve for 2,3,7,8-tetrachlorodibenzo-p-dioxin (injected standards) for fish and fish egg samples spiked at picogram levels (see Tables 5,6). The slope of this line is 66000 and the correlation coefficient is 0.53. These standards were quantitated using GC/MS methane negative chemical ionization with selected ion monitoring.

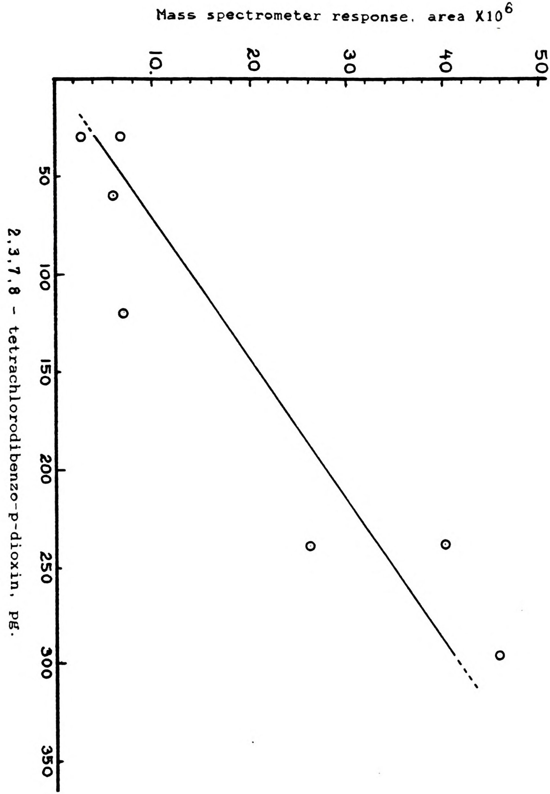

Mass spectrometer response, area X10^6
10.
20
30
40
50
50
100
150
200
250
300
350
2,3,7,8 - tetrachlorodibenzo-p-dioxin, pg.

Figure 11.

Figure 12. Mixture of transchlordane (A), $^{13}C_{12}$-2,3,7,8-tetrachlorodibenzofuran (B) and $^{13}C_{12}$-2,3,7,8-tetrachlorodibenzo-p-dioxin (C).

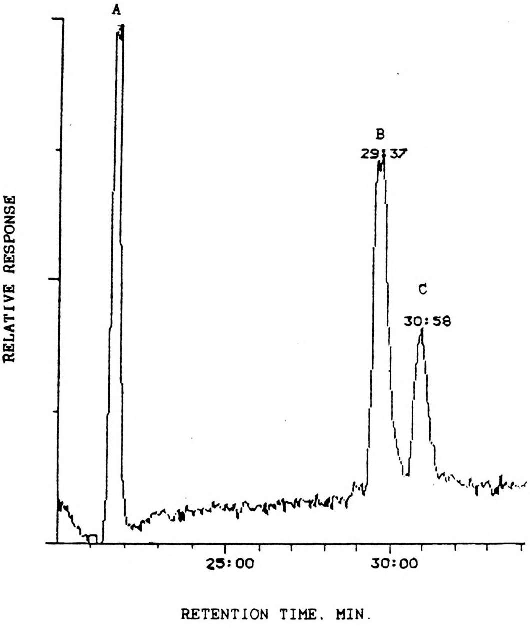

A
B
29:37
C
30:58
RELATIVE RESPONSE
25:00
30:00
RETENTION TIME, MIN.

Figure 12.

Figure 13. Mixture of transchlordane (410), $^{13}C_{12}$-2,3,7,8-tetrachlorodibenzofuran (316), $^{13}C_{12}$-2,3,7,8-tetrachlorodibenzo-p-dioxin (332) using selected ion monitoring.

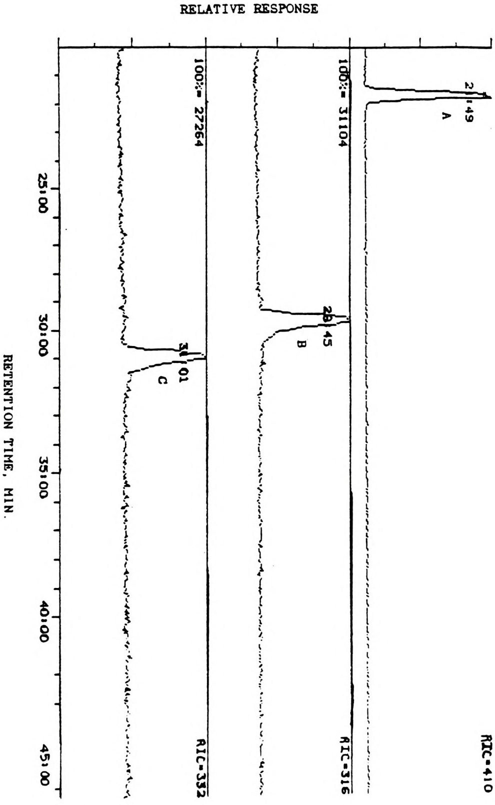
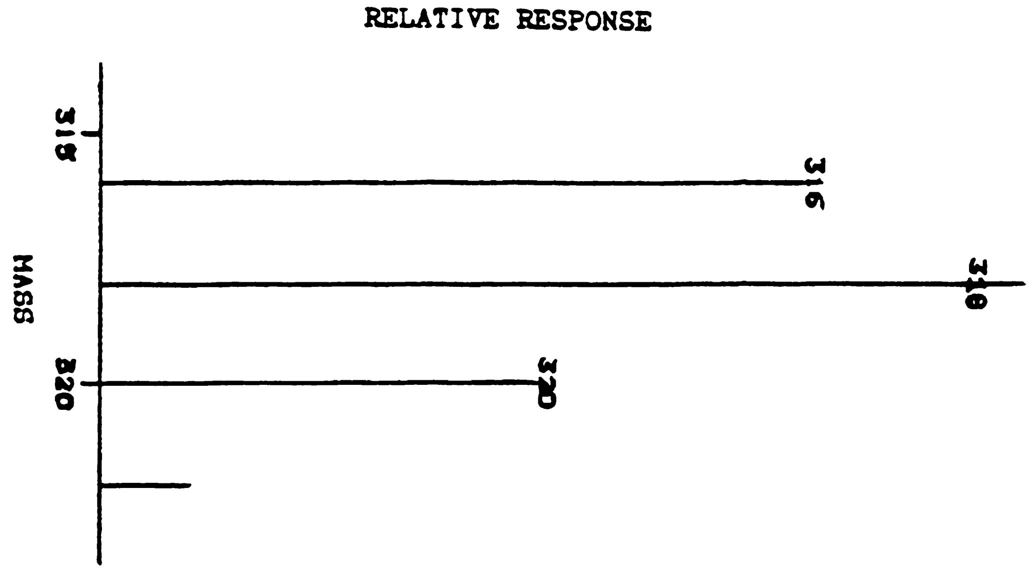

RELATIVE RESPONSE
2 :49
A
100% = 31104
28 45
B
100% = 27264
31 01
C
RIC=410
RIC=316
RIC=332
25:00
30:00
35:00
40:00
45:00
RETENTION TIME, MIN.

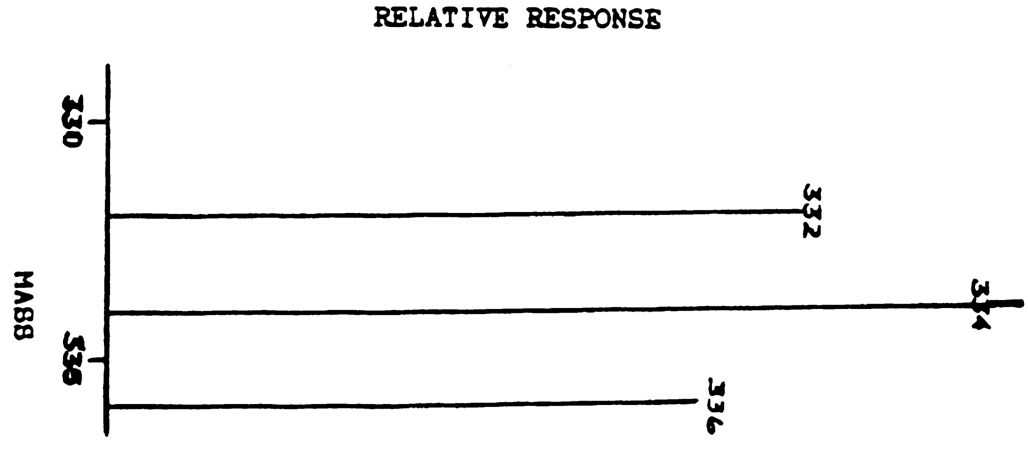
Figure 13.

Figure 14. Chlorine isotope profiles for $^{13}C_{12}$-2,3,7,8-TCDF (a) and $^{13}C_{12}$-2,3,7,8-TCDD (b).



a

^{13}C-2,3,7,8-TCDF



b

^{13}C-2,3,7,8-TCDD

Figure 14

Figure 15. a. A 3ul Carp 71 sample containing 2,3,7,8-tetrachlorodibenzo-p-dioxin, selected ion current profile. b. Selected ion monitoring for carp 71 sample. Transchlordane (410), $^{13}C_{12}$-2,3,7,8-TCDF (316), and $^{13}C_{12}$-2,3,7,8-TCDD (332). c. Carp 71 sample peaks D and C are 2,3,7,8-TCDD (322) and $^{13}C_{12}$-2,3,7,8-TCDD (332).

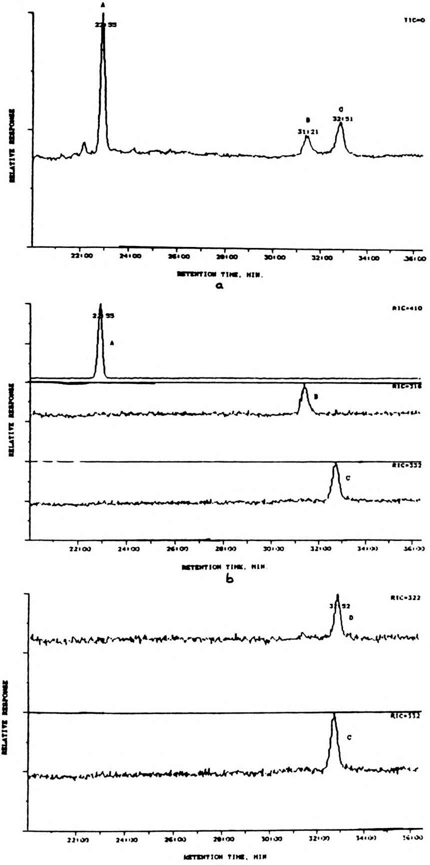

A
2 55
TIC=0
B
31:21
C
32:51
RELATIVE RESPONSE
22:00
24:00
26:00
28:00
30:00
32:00
34:00
36:00
RETENTION TIME, MIN.
a
2 55
A
RIC=410
B
RIC=318
C
RIC=332
RELATIVE RESPONSE
RETENTION TIME, MIN.
b
3 52
D
RIC=322
C
RIC=332
RELATIVE RESPONSE
RETENTION TIME, MIN
c

Figure 15.

C. Fish Egg Samples

Chinook salmon eggs were analyzed for 2,3,7,8-tetrachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzo-p-dioxin (Table 7). Standard curves for 2,3,7,8-TCDF and 2,3,7,8-TCDD have been shown in Figures 16 and 17. Typical GC/MS conditions for these analyses have been shown in Appendix C. No detectable levels of these compounds were found. Further investigations into improving recoveries must be performed before concluding that there are no levels of these toxic compounds present in fish and fish eggs of this area (Lake Manistee).

Table 7. Levels of 2,3,7,8-tetrachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzo-p-dioxin found in Chinook salmon eggs from the Manistee Wier, Lake Michigan. Recoveries are based on amount of ^{13}C-labeled compounds recovered.

Sample	2,3,7,8-TCDF	2,3,7,8-TCDF	% Recovery
LM-186	N.D.	N.D.	12
LM-187	N.D.	N.D.	33
LM-188	N.D.	N.D.	38
LM-191	N.D.	N.D.	49
LM-192	N.D.	N.D.	45
LM-195	N.D.	N.D.	36

Figure 16. Standard curve for 2,3,7,8-tetrachlorodibenzofuran (injected standards) for fish egg analysis using methane negative chemical ionization with selected ion monitoring gas chromatography mass spectrometry. The slope of the line is 7500 and the correlation coefficient is 0.89 (see Table 7).

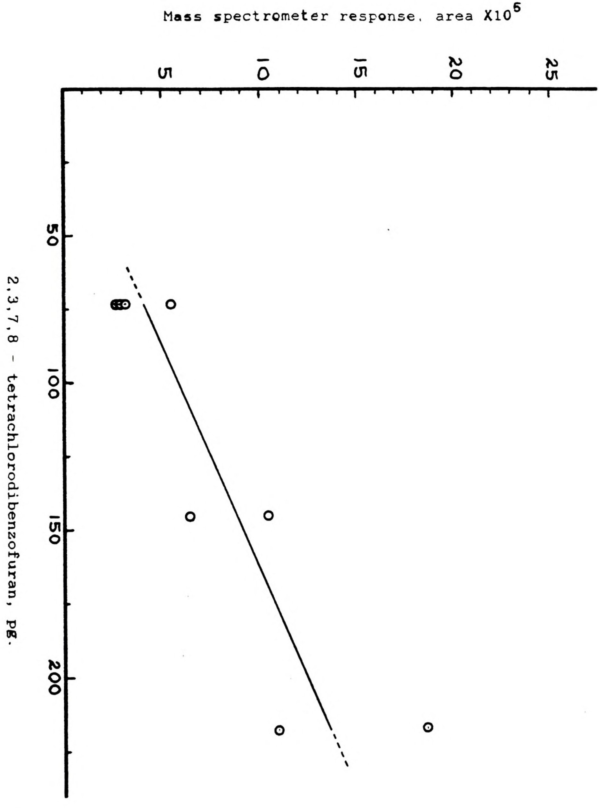

Mass spectrometer response, area X10^5
5
10
15
20
25
50
100
150
200
2,3,7,8 - tetrachlorodibenzofuran, pg.

Figure 16.

Figure 17. Standard curve for 2,3,7,8-tetrachlorodibenzo-p-dioxin (injected standards) for fish egg analysis using methane negative chemical ionization selected ion monitoring gas chromatography mass spectrometry. The slope of the line is 140000 and the correlation coefficient is 0.92 (see Table 7).

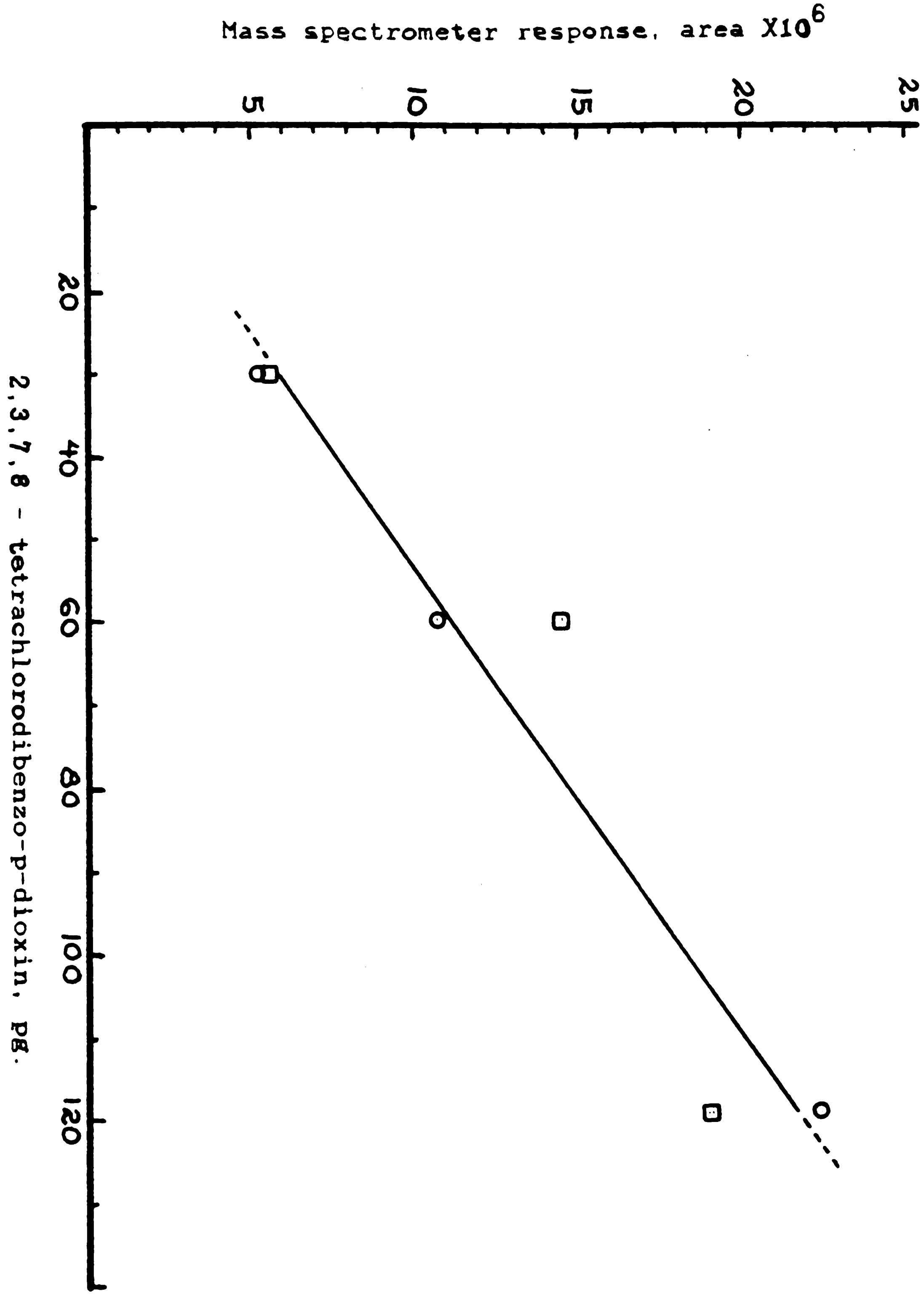

Mass spectrometer response, area X10^6
5
10
15
20
25
20
40
60
80
100
120
2,3,7,8 - tetrachlorodibenzo-p-dioxin, pg.

Figure 17.

VII. SUMMARY AND CONCLUSION

The goal of this research project was to adapt methodology for the determination of 2,3,7,8-tetrachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzo-p-dioxin in fish fillets to existing equipment in our laboratory in order that these compounds may also be determined in fish eggs. Another goal was to determine if there were trace levels of these toxic compounds out in the environment.

By taking a method that was originally developed for isomer-specific analysis of fish fillets, and altering it, fish eggs were analyzed for 2,3,7,8-tetrachlorodibenzofuran and 2,3,7,8-tetrachlorodibenzo-p-dioxin. The amount of anhydrous sodium sulfate in the original procedure was altered from a 4:1 ratio to a 3:1 ratio to sample. Also changed was the carbon column from a carbon dispersed on glass fibers to a mixture of carbon/celite. All columns were studied using a labelled spike to trace the recovery from the column. The resulting percent recovery was 10-50%.

The final results for egg samples analyzed were no detectable levels of 2,3,7,8-TCDF and 2,3,7,8-TCDD in all samples. Since the analytical methodology separated the chlorinated dibenzofurans and dibenzo-p-dioxins from other potential environmental interferences, such as PCB's, no analyses were done for these compounds. No interferences appeared in the selected ion monitoring. More data would have to be obtained and improvements in recovery would have to be developed in order to assess whether there are environmental levels of these toxic compounds in fish eggs.

VIII. FUTURE WORK

Analyses that need to be further investigated include using larger sample sizes, improvements on the carbon/celite recovery through examining effects of changing the elution solvents and of using a pressurized column system. In addition, a method to separate congener groups from each other prior to analysis by GC/MS should be used to prevent overlapping isotope ratios. The use of HPLC to separate congeners would be beneficial although it would result in longer analysis times. One possibility would be to set a carbon column in tandem with the HPLC column in a pressurized apparatus to facilitate clean-up and separation. There are also improvements to be made in the reproducibility of using negative chemical ionization gas chromatography mass spectrometry. Better pressure control would have to be incorporated into the mass spectrometer ion source through the use of improved gauges and gas lines. Using a mass spectrometer with better control of source temperature would also prove beneficial. Use of an on-column injector has already been incorporated into the method to reduce losses on transfer to the GC/MS. Without reproducible results, it is very difficult to interpret the data.

APPENDICES

APPENDIX A

Typical GC/MS conditions for fish egg analysis using methane negative chemical ionization gas chromatography mass spectrometry with selected ion monitoring.

GC program:

100 to 200 °C 30° C/min
200 to 270 °C 10° C/min

Column:

On-column injector
DB-5, 30 meter capillary
0.25u phase thickness
o.25 mm inner dia.

Pressures:

methane	10^{-1} torr
primary	6.1×10^{-2} torr
secondary	2.2×10^{-4} torr
J-1 source	2.0×10^{-4} torr
J-2 analyzer	1.8×10^{-5} torr

Electronics:

IE	0.100	1	-013
e-	+81.1	2	+079
Focal	+20.0	3	-012
ions	+6.5	4	+052
ext	-30.1	5	+012
mult	-2.46	6	+009
pol	+4.89	7	+116
Res	390	8	+071
Pol	575		

APPENDIX B

Typical GC/MS conditions for analysis of fish samples using methane negative chemical ionization gas chromatography mass spectrometry with selected ion monitoring.

GC program:

100 to 260 °C 30°C/min and hold

Column:

On-column injector
DB-5, 60 meter capillary
0.25u phase thickness
0.25mm inner dia.

Pressures:

methane	10^{-1} torr
primary	4.7×10^{-2} torr
secondary	2.0×10^{-4} torr
J-1 source	2.0×10^{-4} torr
J-2 analyzer	1.6×10^{-5} torr

Electronics:

IE	0.100	1	-015
e-	72.6	2	+161
Focal	96.2	3	-011
ions	+5.4	4	+064
ext	-18.9	5	+010
mult	-2.48	6	+027
pol	+4.85	7	+125
Res	394	8	+075
Pol	030		

APPENDIX C

Typical GC/MS conditions for fish egg analysis using methane negative chemical ionization gas chromatography mass spectrometry with selected ion monitoring.

GC program:

100 to 260°C 30°C/min and hold

Column:

On-column injector
DB-5, 60 meter capillary
0.25u phase thickness
0.25 mm inner dia.

Pressures:

methane	10^{-1} torr
primary	4.4×10^{-2} torr
secondary	1.9×10^{-4} torr
J-1 source	1.4×10^{-4} torr
J-2 analyzer	1.6×10^{-5} torr

Electronics:

IE	0.100	1	-016
e-	70.4	2	+162
Focal	59.6	3	-012
ions	+14.0	4	+057
ext	-25.7	5	+014
mult	-2.52	6	-005
pol	+4.88	7	+125
Res	392	8	+075
Pol	048		

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