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PART PER TRILLION DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO - PARA DIOXIN IN MICHIGAN FISH

Michigan State University PH.D. 1983

PART PER TRILLION DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN MICHIGAN FISH

By

Swiatoslav Wolodymyr Kaczmar

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ABSTRACT

PART PER TRILLION DETERMINATION OF
2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN
IN MICHIGAN FISH

By

Swiatoslav Wolodymyr Kaczmar

Samples of Carp (Cyprinus carpio) and Sucker (Catostomus commersoni) were collected from major rivers in Michigan and analyzed for the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by a procedure optimized for the isomer-specific quantification of 2,3,7,8-TCDD at the ng/kg level. Reference standards of 21 TCDD isomers were synthesized by controlled pyrolysis of selected chlorophenols. Residues of 2,3,7,8-TCDD ranging from 17 to 586 ng/kg were detected in fish from various portions of the State, indicating that 2,3,7,8-TCDD is more widely distributed in Michigan than previously thought.

To my parents

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I. INTRODUCTION

Chlorinated dibenzo-p-dioxins are a group of chemical compounds which are among the most hazardous pollutants in the environment. These compounds, collectively referred to as dioxins or PCDDs, are tricyclic aromatic compounds which can occur as 75 unique congeners differing by extent and sites of chlorination. The basic dibenzo-p-dioxin molecule is nearly planar and has eight possible sites of chemical substitution (Figure 1).

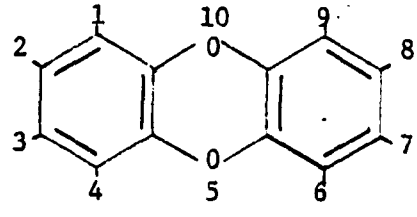


Fig. 1. Dibenzo Dioxin.

It can be chlorinated at any of the ring positions with the exception of the oxygens at points 5 and 10. There are two possible monochlorinated isomers (MCDD), 10 dichlorinated (DCDD), 14 trichlorinated (T_3CDD), 22 tetrachlorinated (TCDD), 14 pentachlorinated (P_5CDD), 10 hexachlorinated (H_6CDD), two heptachlorinated (H_7CDD) and a single octachlorinated isomer (OCDD).

Chlorinated dioxins are extremely stable, non-reactive compounds. The 2,3,7,8-TCDD isomer is resistant to the action

of concentrated acids and bases and maintains its chemical stability at temperatures as high as 700° C. In contrast to the chemical inertness of the dioxins, they are relatively photolabile and have been demonstrated to photolyze in the environment with typical half-lives of 2-3 hours. TCDD does not undergo hydrolysis in water and is resistant to microbial attack. The 2,3,7,8-TCDD isomer exhibits an extremely low vapor pressure (1.5×10^{-5} mm Hg), is soluble in water to only 0.2 ug/l and has an estimated octanol/water partition coefficient of 1×10^{6}. The environmental dynamics of chlorinated dioxins are similar to some of the longer lived chlorinated hydrocarbons.

PCDDs can be synthesized by direct halogenation of the unsubstituted dioxin using chlorine gas and a suitable catalyst. They can also be prepared by pyrolysis of ortho substituted chlorinated phenol salts. Both methods produce dioxins in high yield, but the latter reaction allows a greater degree of control over the specific end products. Although a few of these compounds have been synthesized in the laboratory, they are not intentionally produced for any commercial purpose, but arise as impurities in materials synthesized by treating chlorinated benzene at elevated temperature and pressure under alkaline conditions (Figure 2).

This synthetic step is utilized in the production of several commercially important products, notably; the fungicide pentachlorophenol (PCP), the germicide hexachlorophene and the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). PCDDs have been detected in significant amounts in all of these products.

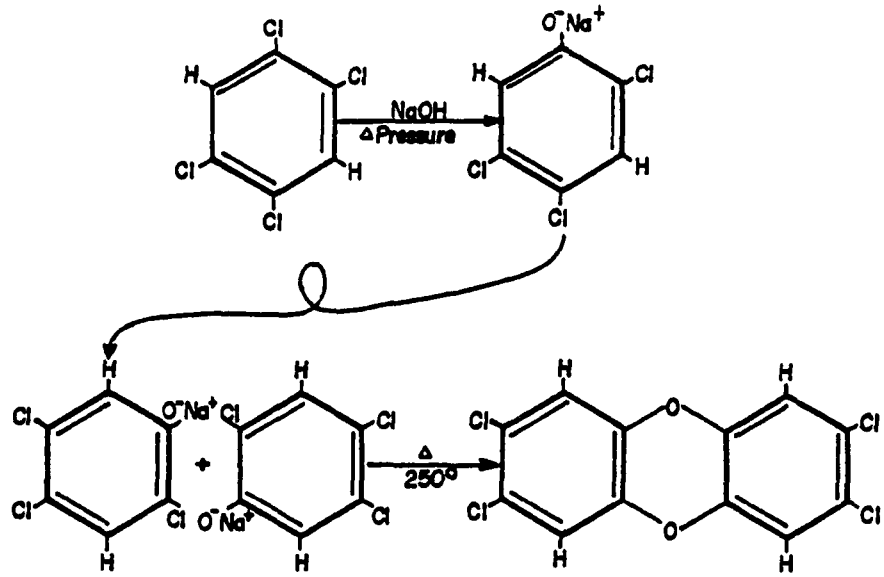


Fig. 2. Formation of 2,3,7,8 TCDD during commercial synthesis of chlorophenols.

PCDDs have also been detected in fly ash and extracts from a number of combusted materials. It is theorized that their presence is the result of reactions occurring at trace levels during combustion. Because chlorophenols are very important chemical intermediates and combustion is relied upon as a means of waste disposal and energy conversion, PCDDs could be widely distributed in the environment. However, due to the extreme analytical sophistication required for trace dioxin analysis, very few residue data are available with which to evaluate the environmental implications of dioxin-yielding processes.

In this study an accepted technique for dioxin analysis is verified and its chromatographic resolution is extended to specifically detect the 2,3,7,8-TCDD isomer. Samples of fish from 19 major rivers in Michigan are analyzed for 2,3,7,8-TCDD, providing data on the residue levels and geographical distribution of 2,3,7,8-TCDD in the state.

II. TOXICOLOGY OF THE DIOXINS

A. Acute Effects

PCDDs, particularly the 2,3,7,8-TCDD isomer, are of special concern due to their extremely high toxicity. The 2,3,7,8-TCDD isomer is perhaps the most toxic small molecule known to man. Its single-dose LD_{50} to various mammals is on the order of a few micrograms per kilogram of body weight. There is a marked variation in species sensitivity to 2,3,7,8-TCDD, whose LD_{50} ranges from 0.6 ug/kg in the guinea pig to over 3,000 ug/kg in hamsters (Table 1). Administration of 2,3,7,8-TCDD does not cause immediate death; but acts slowly, affecting progressive weight loss and debilitation. Death usually occurs more than 3 weeks following administration of a fatal dose. Histologic examination at necropsy reveals multiple organ damage.

Although the exact mode of action of TCDD has not been delineated, its activity appears to be linked to its affinity towards a specific cytosolic receptor (1). The receptor is a protein that binds with TCDD, then travels to the nucleus and to a structural gene (which has in mice been termed the "Ah locus") initiating a pleiotropic response resulting in the induction of a number of coordinately expressed and possibly repressed critical proteins or enzymes.

Interestingly, this receptor has been demonstrated to exert varying degrees of affinity towards a number of halogenated aromatic hydrocarbons which include the chlorinated dibenzo-p-dioxins, dibenzofurans, azo(xy)benzene, naphthalenes and biphenyls and

Table 1.

Single-Dose LD_{50} Values of 2,3,7,8 TCDD in Various Mammalian Species.

Animal and Treatment	Observations	Reference
Guinea pig, oral single dose	LD_{50} (male) = 0.6 ug/kg LD_{50} (female) = 2.2 ug/kg	(2)
Guinea pig (female) 1 ug/kg once a week, orally:	LD_{100} after 24-32 days	(3)
Guinea pig, oral single dose:	LD_{90} = 3 ug/kg	(4)
Guinea pig (female) single oral dose:	LD_{50} = 2 ug/kg	(5)
Monkey, oral:	LD_{50} = 70 ug/kg	(6)
Rat, intraperitoneal		(7)
adult male:	LD_{50} = 60 ug/kg	
weanling male:	LD_{50} = 25 ug/kg	
3-methylcholanthrene pre-treated weanling male:	LD_{50} = 44 ug/kg	
adult female:	LD_{50} = 25 ug/kg	
Rat, oral:	LD_{50} = 50 ug/kg	(8)
Rat, oral:		(2)
male	LD_{50} = 22 ug/kg	
female	LD_{50} = 45 ug/kg	
Rabbit,		(2)
oral:	LD_{50} = 115 ug/kg	
dermal:	LD_{50} = 275 ug/kg	
Mouse, intraperitoneal		(9)
C57BL/6J:	LD_{50} = 132 ug/kg	
DBA/2J:	LD_{50} = 620 ug/kg	
$B6D2F_1/J$:	LD_{50} = 300 ug/kg	
Mouse, C57/Bl, oral:	LD_{50} = 126 ug/kg	(10)
Mouse, oral:	LD_{50} = 114 ug/kg	(11)
Hamster, oral:	LD_{50} = 5,051 ug/kg	(12)
Hamster,		(13)
intraperitoneal:	LD_{50} = 3,000 ug/kg	
oral:	LD_{50} = 1,157 ug/kg	

the brominated biphenyls. Whereas the patterns of toxic response and species sensitivity are common amongst certain halogenated aromatic hydrocarbons, the dose required to achieve a particular degree of toxicity is related to the material's measured affinity for the receptor. The 2,3,7,8-TCDD congener has been shown to have the highest affinity for the receptor and the lowest ED_{50}. The degree of affinity of the other active congeners appears to be a function of their ability to act as a planar stereoisomer of 2,3,7,8-TCDD.

B. Sub-acute Effects

Administration of 2,3,7,8-TCDD elicits a wide range of toxic responses, none of which is thought to be independantly responsible for the ultimate death of the test animal. TCDD poisoning can be described as a debilitating process induced by pathological changes in a number of organs and systems. There are marked species, strain and sex related differences in observed LD_{50}'s and patterns of toxic response to TCDD. Table 2 lists the observations of a number of investigators conducting research into the subacute effects of 2,3,7,8-TCDD. Neglecting species differences, the most common responses include:

1) a wasting syndrome: a progressive weight loss which may not be related simply to decreased food consumption;

2) skin disorders: acneiform eruptions or chloracne, alopecia, edema, hyperkeratosis and blepharitis due to hypertrophy of the Meibomian glands;

3) lymphoid evolution: atrophy of the thymus and spleen causing humoral and cell-mediated immunosuppression and/or bone marrow and hematological dyscrasias;

4) hepatotoxicity: a) hepatomegaly due to hyperplasia and hypertrophy of parenchymal cells with a proliferation of the smooth endoplasmic reticulum, b) general or localized necrosis, c) lipid accumulation.

5) disordered porphyrin metabolism: porphyria cutanea tarda;

6) endocrine and reproductive dysfunction: altered plasma levels of steroid and thyroid hormones with a) menstrual irregularities, reduced conception rate, early abortion, excessive menstrual and post-conceptional hemorrhage and anovolution in females and b) testicular atrophy and decreased spermatogenesis in males;

7) teratogenesis: kidney malformations and cleft palate

8) carcinogenesis.

In addition to 2,3,7,8-TCDD, most of these effects have been elicited by other dioxin congeners as well as by certain polychlorinated dibenzofurans, polychlorinated biphenyls and polybrominated biphenyls. The commonality of responses further suggests that these materials are acting by the same mechanism.

Table 2.

Sub-acute effects of 2,3,7,8 TCDD

Animal and Treatment	Observations	Reference
Rat, oral, 50 ug/kg	Hypertrophy of liver and regression of thymus.	(8)
Rat, oral, 0.1-10 ug/kg	A single dose of 0.1 ug/kg increased liver weights. Daily doses at 10 ug/kg caused death of all animals within 2-4 days. there was an accumulation of large amounts of lipid in the liver, accompanied by an increase in incorporation of ^{14}C leucine into liver protein.	(14)
Rat, oral, single dose, 5 and 25 ug/kg	Three days after dosing, changes in endoplasmic reticulum, especially of the bile duct. Regeneration after 20 days.	(15)
Rats, oral, single dose 200 ug/kg	Depressed food intake and weight loss. Mean time to death was 20 days. Gastric hemorrhagy and jaundice. Pathologic changes in polyneucleated liver parenchymal cells.	(16)
Rats, 0.1-25 ug/kg Mice, 1.0-50 ug/kg Guinea pigs, 0.0008-3.0 ug/kg. All orally	Influence on the lymphoid organs, especially thymus. Depletion of lymphoid cells in spleen and lymph nodes. Liver changes less in mice and guinea pigs than rats. At sub-lethal doses, degenerative livers, megalocytosis and unusual numbers of multinucleated giant hepatocytes. Changes in cells of renal collecting tubule and in thyroid follicles.	(17)
Rats, oral, single dose.	Three days after dosing, lesions of plasma membrane associated enzymes. Complete loss of ATPase reaction in parenchymal cells of the centri-lobular zone. Changes in 6-nucleo-tidase and acid phosphatases were secondary to the parenchymal cell damage.	(18)

Table 2. cont'd.

Rats, oral; 0.001, 0.01, 0.1 and 1.0 ug/kg 5 days a week for 13 weeks	1.0 ug/kg: mortality, icterus, increased serum bilirubin and alkaline phosphatase. Porphyria, liver damage, thymic atrophy and functional suppression of reproductive organs. 0.1 ug/kg: Decreased appetite and body weight. Liver degeneration and lymphoid depletion. Porphyria and increases urinary Δ-ALA in females. Depression of packed cell volume, RBC and hemoglobin in males. 0.01 and 0.01 ug/kg: increased liver weights.	(19)
Rats, dietary, 0.001, 0.01, 0.1 ug/kg daily for 2 years.	0.1 ug/kg/day: increased incidence of carcinomas and increased mortality, decreased weight gain, porphyria, increased Δ-ALA and serum alkaline phosphatase, α-glutamyl transferase and SGPT. Morphologic changes in hepatic, lymphoid respiratory and vascular tissues. 0.01 ug/kg/day: same as higher dose, but to a lesser degree. 0.001 ug/kg/day: No observable effects in males. Increased incidence of swollen hepatocytes in females.	(20)
Rats, dietary, 0.001-0.1 ug/kg/day for 90 days prior to mating:	Three-generation reproductive study. 0.1 ug/kg/day: significant decreases in fertility and neonatal survival in F_0 generation. 0.01 ug/kg/day: significant decreases in fertility in F_1 and F_2 generations but not in F_0. Decreases in litter size, gestation survival and neonatal survival and growth. 0.001 ug/kg/day: no effects on reproductive capacity, survival or litter size and weight in any generation.	(21)
Rats, dietary, 50-1,000 ppb in feed	Atrophy of the thymus and spleen, dilation of common bile ducts, gastrointestinal hemorrhage, microscopically observed severe liver necrosis, cellular proliferation in the bile ducts, cellular proliferation in the bile ducts, cellular hyperplasia of the mucosa and edema in the common bile ducts; decreased spermatogenesis.	(22)

Table 2. cont'd.

Rats, Pre- and postnatal maternal exposure at 5 mg/kg	Suppression of thymus-dependant functions for 6 months.	(23)
Mice, oral 125 ug/kg single dose	Weight loss and death with fatty liver after 21 days. Progressive necrotic centrilobular liver lesions.	(10)
Mice, oral 0.5-20 ug/kg/week 4 weeks.	Dose schedules of 1 ug/kg or more, followed by Salmonella infection, resulted in significant increases in mortality and decrease in the time from infection to death. The most notable finding is that low levels of TCDD, which do not produce clinical pathological changes, have the capacity to affect host defense.	(24)
Mice, oral, female, 10 ug/kg/day	10-14 days after application; depression of the blood platelets by antiboldy reaction. Diminished clot retraction is consistent with the observed thrombocytopenia. The number of erythrocytes, their cell volume and the number of neutrophils was increased.	(25)
Mice, oral, 0.2-25 ug/kg/wk 2 or 6 weeks.	The application of 1, 5, or 25 ug/kg caused an increase of liver and thymus weights. Total neutrophils were increased. Hemoglobin, serum protein and globulins were significantly decreased after 6 doses of 25 ug/kg/wk. TCDD was porphyrogenic.	(11)
Mice, oral 1 and 10 ug/kg/day	Increase of SGOT and serum cholesterol, decrease of blood glucose, increase of SGOT, SGPT, lactate dehydrogenase and bilirubin in serum.	(26)
Mice, oral 10 and 100 ppb in feed for 5 weeks.	100 ppb: marked suppression of total serum protein, gamma globulin and albumin; increase in beta globulins. 10 ppb: reduction in the primary and secondary antibody response to tetanus toxin, sheep erythrocytes and lowered resistance to challenge with Salmonella typhimurium or Listeria monocytogenes.	(27)

Table 2. cont'd.

Mice and rats, 500 ug/kg	Tubule damage in kidneys, fragmentation of cell membranes, fatty degeneration of the liver.	(28)
Monkeys, rats and chickens, oral.	In all species, hypoplasia of the lymphatic tissue and bone marrow. Ascites, hydrothorax and hydropericard. In addition, rats developed gastric ulcers and enlargement of liver. Monkeys additionally showed alopecia, hyperkeratosis and a decrease of the number of spermatocytes and spermatides.	(29)
Male rats, 25 ug/kg Rabbits, 25 ug/kg Guinea pigs, 5 ug/kg intraperitoneally.	Liver function was assessed 10 days following administration. Rats: decreased ouabain clearance unaltered indocyanine green clearance elevated serum SDH, unaltered SGPT, GGPT; reduced bile flow, salt excretion and erythritol clearance. An 8% reduction in body weight. Rabbits: unaltered ouabain clearance; greatly reduced ICG clearance; elevated SDH and SGPT; unchanged GGTP; 2% reduction in body weight. Guinea pigs: unaltered ICG clearance; unchanged SDH, SGPT, and GGPT; 35% reduction in body weight.	(30)
Guinea pigs, oral, 0.008 to 1.0 ug/kg/wk for 8 weeks. Mouse, oral, 0.2-25 ug/kg Rats, dermal, 0.2-5.0 ug/kg	Guinea pigs: up to 0.2 ug/kg caused loss in weight, lymphopenia and atrophy of the lymphatic organs. All higher doses were lethal. Mice: Weight loss and thymic atrophy. Rats: Weight loss and reduction of weights of thymus and adrenal gland. No skin sensitivity.	(3)
Guinea Pig, i.p. 2 ug/kg single dose	Marked hyperlipidemia; 19-fold increase in VLDL; 4-fold increase in LDL.	(31)
Female Rhesus Monkey, 50 ppt in diet for 20 months.	Four abortions and 1 stilbirth following breeding attempts at 7 months with a cumulative dose of 0.36 ug/kg. Inability to conceive. Hair loss, hyperkeratosis, weight loss. Decrease in serum cholesterol, hematocrits and white blood cells. Increased SGPT.	(33)

Table 2. cont'd.

Female Rhesus Monkeys 500 ppt in diet for 9 months.	Anemia within 6 months. Pancytopenia after 9 months. Death in 5 of 8 between months 7 and 12 at a total exposure of 2-3 ug/kg. Extensive hemorrhage, hypocellularity of the bone marrow and lymph nodes. Hypertrophy, hyperplasia and metaplasia of the epithelium in the bronchial tree, bile ducts, pancreatic ducts, salivary gland ducts, palpebral conjunctivae. Squamous cell metaplasia and keratinization of the sebaceous glands and hair follicles in the skin.	(32)
Female Rhesus Monkeys Single oral dose 70 ug/kg	Significant weight loss, blepharitis, loss of fingernails and eyelashes; facial alopecia with acneiform eruptions: mild anemia, neutrophilia, lymphopenia; decreased serum cholesterol; increased serum triglycerides. Hyperplastic and metaplastic changes in sebaceous glands of eyelid, ear canal and epithelial hyperplasia in the renal pelvis, stomach, gall bladder and bile duct. Liver normal.	(6)
Rabbit, oral single dose, 1 ug/kg	Serious liver damage and chloracne.	(34)
Fish: Silver salmon and guppy ca. 23 mg/g for 24 hr. Rainbow trout, 6.3 ug/wk/10 animals	Irreversible toxic effects and death within 10-80 days of exposure. Inhibited growth and caused death of the trout after 33 days exposure.	(35)
Fish: Pike and rainbow trout, Freshly fertilized eggs, yolk sac fry and juveniles 0.1 to 100 ppt in tap water	Induced retardation of embryonic development and growth. Dose-related incidences of hemorrhages, edema and hepatic injury, followed by death. Survivors showed skeletal malformations, inclusion bodies in stomach, pancreas and liver.	(36)
Fish; guppies, 0.1, 1.0 and 10.0 ppb for 120 hrs	Caused 100% mortality within 37 days post-exposure. Toxicity delayed at least 5 days. Mean survival time = 21 days.	(38)

Table 2. cont'd.

Fish: Coho Salmon 96 hrs. at 0.54 ng/g and 5.4 ng/g. Guppies, 96 hrs. at 0.08 ng/g and 0.8 ng/g.	Coho Salmon: 0.54 ng/g; no observable effects up to 60 days postexposure. 5.4 ng/g: growth and survival reduced over 114 days postexposure. Guppies: 0.08 ng/g; no observable effects. 0.8 ng/g; developed fin disease 42 days postexposure.	(37)
Fish: model ecosystem 0.1, 1.0, 10.0 mg/kg applied to sediment. water concentration was 1.0, 10.0, and 100.0 ng/l at 3 days.	Mosquito fish died at all exposure levels. Death accompanied by nasal hemorrhagy. Snails, algae and water fleas were unaffected.	(39)
Hens, oral 9-60 ug in "toxic fatty material"	"Chick edema disease " (hydropericardium, hydroperitoneum) with high mortality.	(40)

ABBREVIATIONS:

Δ-ALA = Δ-aminolevulinic acid

RBC = red blood cells

SGPT = serum glutamic pyruvic transaminase

SGOT = serum glutamic oxaloacetic transaminase

SDH = sorbitol degydrogenase

ICG = indocyanin green

VLDL = very low density lippoprotein

LDL = low density lippoprotein

C. Metabolism of dioxins

An important aspect of the toxicology of 2,3,7,8-TCDD is its metabolic disposition in the host. Table 3. lists the results of a number of investigations conducted into the pharmacodynamics of TCDD. Two types of metabolism studies are considered: those dealing with the tissue levels and excretion rates of TCDD, and those concerned with the extent and nature of its metabolites.

Studies into the tissue distribution and excretion of 2,3,7,8-TCDD indicate that most of the TCDD administered to a test animal partitions into the liver, with lesser amounts associated with adipose tissue. The lowest levels are found in skeletal muscle, heart, testes, blood and the brain. A notable exception is the rhesus monkey, which absorbs most of the administered TCDD into skin and skeletal muscle, with less than 10% of the dose retained in the liver (42).

Measured whole-body half-lives appear to be species dependant, and range from 11 to 30 days. Excretion occurs primarily via feces, with smaller amounts removed with urine. Metabolism of 2,3,7,8-TCDD was initially thought to play a minor role in the excretion of TCDD, since a large amount of the administered dose was observed to be excreted in the feces in an unmetabolized form.

Tulp and Hutzinger (47) isolated mono- and dihydroxy metabolites of dibenzo-p-dioxin, 1-MCDD, 2-MCDD, 2,3-DCDD, 2,7-DCDD, 1,2,4-TrCDD and 1,2,3,4-TCDD from feces and urine of rats. Sulfur-containing metabolites, possibly glutathione or mercapturic acid conjugates, were also detected. They demonstrated

Table 3.

Metabolism and Pharmacodynamics of TCDD.

Animals and Treatment	Observations	Reference
Rats; ^{14}C-TCDD oral, 50 ug/kg	At 21 days: 56% of activity excreted in feces; 4.5% in urine; remainder present mainly in the liver, associated with microsomes.	(8)
Rats: oral in feed 7 and 20 ppb for 42 days	Excretion $T_{\frac{1}{2}}$ = 12 days in males and 15 days in females. At day 42, 85% and 70% of the total dose was still retained in the livers of males and females, respectively.	(41)
^{3}H-TCDD Rats and monkeys 400 ug/kg	Liver retention was 40% in rats and 5% in monkeys, who partitioned the TCDD into skin, muscle and fat while rats partitioned the TCDD mainly into liver.	(42)
Rats: oral	A large portion of the activity was associated with the microsomal fraction of the liver.	(29)
^{14}C-TCDD Rats: oral 50 ug/kg	Two days following treatment: 30% excreted in feces; 45% localized in liver; 10% localized in fat. Over 21 days: 53, 13 and 3% of the dose was excreted via feces, urine and expired air.	(43)
Rats: oral dietary as OCDD	Octachlorodibenzo-p-dioxin (OCDD) present unmetabolized in feces, liver and adipose tissue. None detected in other tissues or urine.	(44)
^{14}C-TCDD Pregnant rats: intraperitoneal	Minor (<1%) quantites found in pups following maternal exposure.	(45)
Rats: oral various congeners 250 mg/kg	Demonstrated that primary metabolism takes place as hydroxylation at the 2-, 3-, 7-, or 8- position exclusively via an arene oxide intermediate. Demonstrated the presence of sulfur-containing conjugates.	(47)
^{14}C-TCDD Male rats: oral 15 ug/kg 2, 4, or 6 doses.	Bile contained at least 5 distinct polar metabolites. ß-glucuronidase treatment verified metabolites as glucuronide conjugates.	(48)

Table 3. cont'd.

^{14}C-TCDD Rats: oral	$T_{\frac{1}{2}}$ = 5.6 to 17.4 days. 53.2% eliminated via feces 13.2% via urine 3.2% via respired air liver and fat contained 10 times more activity than other tissues.	(46)
^{14}C-TCDD Rats: oral single or repeated doses of 1 ug/kg 5 days/wk.	22 days following single application, liver and fat contained levels 50 and 10 times higher than other tissues. Excretion $T_{\frac{1}{2}}$ = 30 days. 99% of the activity excreted via feces. A body burden of 74% of the steady-state reached within 7 weeks with repeated dosing.	(49)
Mouse, rat and rabbit: ^{14}C-TCDD	Excretion via feces. TCDD persists unmetabolized in liver, concentrated in the microsomal fraction. No metabolites observed in treated microsome preparations from mouse, rat or rabbit liver.	(50)
^{14}C-TCDD Guinea pig: gavage single dose	Excretion $T_{\frac{1}{2}}$ = 22 to 43 days. 22 days following dosing; fat, liver, adrenal and thymus contained 0.75, 0.40, 0.33 and 0.72 %dose/g respectively. Plasma, urine and bile contained no activity.	(51)
^{14}C-TCDD Guinea pig: i.p. 20 ug/kg	Partitioned into liver microsomes and fat. Also found in skin. $T_{\frac{1}{2}}$ = 30 days. 94% in feces, 6% in urine.	(52)
^{14}C-TCDD Fish: 0.54 ng/kg 96 hrs	Excretion $T_{\frac{1}{2}}$ = 107 days.	(37)
Beef Cattle 24 ppt TCDD in feed 28 days	At day 28: 85 ng/kg in fat, 8 ng/kg in liver, 7 ng/kg in kidney, 2 ng/kg in muscle. Excretion $T_{\frac{1}{2}}$ from fat = 16.5 weeks. 95% steady-state reached in 499 days with a maximum steady-sate level of 594 ng/kg when fed 24 ppt in feed daily.	(68)

Table 3. cont'd.

^{14}C-TCDD Hamster: i.p. 650 ug/kg	TCDD in liver and adipose tissue exclusively in unmetabolized form. $T_{\frac{1}{2}}$ = 11 days 59% excreted in feces 41% in urine Activity in urine and feces due to metabolites, possibly glucuronide conjugates. No parent TCDD found in urine or bile.	(53)
^{14}C-TCDD Mouse: i.p. 10 ug/kg	C57Bl/6J: $T_{\frac{1}{2}}$ = 17 days 74% in feces 26% in urine DBA/2J $T_{\frac{1}{2}}$ = 37 days 70% in feces 30% in urine B6D2F$_1$/J: $T_{\frac{1}{2}}$ =17 days 73% in feces 27% in urine Both parent (25-45%) and metabolites extracted from feces.	(54)
^{14}C-TCDD Rats: i.p. 500 ug/kg	Urinary and biliary activity strictly due to metabolized TCDD. Metabolites unique from those found in guinea pigs or mice.	(54)
^{14}C-TCDD Guinea pigs: i.p. 500 ug/kg	Urinary and biliary excretion activity strictly due to metabolites of TCDD. Observed metabolites different from those extracted from mouse or rat urine.	(54)
^{3}H-TCDD Isolated hamster hepatocytes.	Observed at least 4 polar metabolites corresponding to those previously isolated from hamster urine and bile. Some of the metabolites were glucuronide conjugates.	(55)
Isolated rat hepatocytes from rats pretreated with: TCDD (5 ug/kg i.p.) phenobarbital (PB) $CoCl_2$ (60 mg/kg s.c.) Some hepatocytes were also treated with: SKF-525A (0.1mM) metapyrone (0.5 mM)	Hepatocyte preparations from TCDD or PB- treated animals showed increased metabolite formation while $CoCl_2$, SKS-525A or metapyrone treatment reduced metabolism, indicating that TCDD is metabolized by cytochrome p450 monooxygenases.	(55)

Table 3. cont'd.

Rats:	Naturally ocurring age and sex related and TCDD-induced differences in MFO activity were inversely correlated with the 20-day LD_{50} of TCDD. Suggests that TCDD induces its own metabolism.	(7)
^{3}H-TCDD Rats: female Sprague-Dawley Various formulations	Monitored uptake by liver as a function of formulation. Oral: TCDD in 50% ethanol = 36.7% Aqueous suspension of soil and TCDD = 16% Aqueous suspension of activated carbon + TCDD = 0.07% Dermal: Methanol + TCDD = 14.8% Vaseline + TCDD = 1.4% Polyethylene glycol + TCDD = 9.3% Soil/water paste + TCDD = 1.7% Activated carbon/water + TCDD paste = 0.05%	(56)
^{3}H-TCDD Beagle: 1 year old Four 1.4 mg doses Oral intubation	Collected bile. no glucuronide conjugates bile extract contained 5 identifiable metabolites: 2,3,6,8-tetrachloro-7-hydroxydibenzo-p-dioxin: 2,3,8-trichloro-7-hydroxydibenzo-p-dioxin; tetrachlorodimethoxydiphenylether; 1,2,-dichloro-4,5,-dimethoxybenzene.	(58)
Guinea pigs: oral TCDD metabolites isolated from dog bile administered at 0.6, 6.0 and 60 ug/kg to the guinea pigs	The bile extract TCDD-metabolites were 100 times less toxic than 2,3,7,8 TCDD.	(59)
^{3}H-TCDD Isolated rat hepatocytes:	Incubated isolated hepatocytes with TCDD. Identified metabolites as glucuronide conjugates. Primary metabolites extracted following treatment with -glucuronidase identified as 1-hydroxy-2,3,7,8-tetrachlorodibenzo-p-dioxin and 8-hydroxy-2,3,7-trichlorodibenzo-p-dioxin.	(57)

MFO = mixed function oxidase

that primary metabolism of dioxins takes place exclusively at the 2-, 3-, 7-, or 8- positions via 2,3 epoxides. Since these positions are blocked by chlorine in 2,3,7,8-TCDD or OCDD, they concluded that metabolism of these two congeners by this route should not readily take place.

However, significant amounts of polar metabolites of 2,3,7,8-TCDD have since been detected in urine and bile of treated animals (48, 53, 54, 58, 59) and in extracts from treated cultured rat hepatocytes (55, 57). Incubation of the metabolites with -glucuronidase indicated that some of the metabolites may be glucuronide conjugates (48, 53, 55, 57). Metabolites isolated from rat hepatocyte cultures treated with α-glucuronidase were identified as 1-hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-TrCDD (57), while extracts from bile of a 2,3,7,8-TCDD treated dog were found to contain 5 major identifiable non-conjugated primary metabolites: 2,3,6,8-tetrachloro-7-hydroxydibenzo-p-dioxin; 2,3,8-trichloro-7-hydroxydibenzo-p-dioxin; an unassigned trichloro-dihydroxydibenzo-p-dioxin; a tetrachlorodihydroxydiphenylether and 4,5-dichlorocatechol (58). The major metabolite from the dog, 2,3,6,8-tetrachloro-7-hydroxydibenzo-p-dioxin suggests metabolism through an arene oxide intermediate via an NIH shift.

Induction of mixed function oxidase (MFO) enzyme systems increased metabolite formation, suggesting that 2,3,7,8-TCDD is metabolized by cytochrome p-450 containing monooxygenases (55). Animals with lower basal MFO activity exhibited lower LD_{50} values while LD_{50}s were increased in TCDD or 3-methylcholanthrene (3-MC)

induced individuals (7). There is also some correlation observed between species sensitivity towards TCDD poisoning and rates of elimination and metabolism of 2,3,7,8-TCDD. For example the guinea pig, which exhibits an LD_{50} of 2 ug/kg and a measured excretion half-life of 30 days, excretes 94% of the applied dose in the feces in the unmetabolized form (52); while the hamster, which exhibits an LD_{50} of greater than 3,000 ug/kg and an excretion half-life of 11 days, excretes 40% of the applied dose as a metabolite in urine (53). The dog is another example of an animal which has a high 2,3,7,8-TCDD LD_{50} and considerable ability to metabolize 2,3,7,8-TCDD (58). Although the relationship is not clearly consistent among all of the species studied, the capacity to metabolize and rapidly excrete 2,3,7,8-TCDD may in part contribute to and partially explain differences in species susceptibility towards TCDD toxicity.

Studies conducted into the metabolism and biochemical responses elicited by TCDD have also shown that 2,3,7,8-TCDD is an extremely potent stimulator of enzymes associated with the metabolism of xenobiotics. TCDD has been demonstrated to induce aryl hydrocarbon hydroxylase (AHH), 7-ethoxycoumarin-O-deethylase, hepatic p-nitroanisole-o-demethylase and 3-methyl-4-methylaminoazobenzene-N-demethylase. TCDD has the ability to induce cytochrome p-450 in liver, bowel, lung, kidney and skin (60). The 2,3,7,8-TCDD isomer is the most potent inducer of AHH and cytochrome p-450 known. It is 30,000 times more potent than 3-MC as as inducer of hepatic hydroxylase activity in the rat (60).

The implications of the induction of these enzyme systems

are varied. On the one hand, an induced individual is capable of metabolizing and excreting xenobiotics at a faster rate, thereby reducing the time and extent of exposure and concomitant manifestations of toxicity. However, a number of toxic compounds and carcinogens require metabolic activation. An individual in an induced state can activate these materials faster and to a greater extent, thereby increasing their toxic potential. Another implication of the induction of metabolizing enzymes is their increased ability to degrade or produce endogenous materials, causing severe metabolic imbalance. For example, Δ-aminolevulinic acid synthetase activity is increased following TCDD administration, causing severe disruption of heme biosynthesis resulting in porphyria cutanea tarda. The toxicity of 2,3,7,8 TCDD may be directly linked to the induction of enzymes which it instigates. However, this relationship is not clear, since studies into the effects of enzyme induction by 3-MC have failed to reproduce the toxic manifestations elicited by TCDD.

Due to the similarities between enzyme systems induced by TCDD and those induced by polynuclear aromatic hydrocarbons such as benzo-a-pyrene and 3-methylcholanthrene (3-MC), induction by 2,3,7,8-TCDD has been classified as 3-MC type induction. Studies by Poland (61) have shown that 3-MC type induction is a receptor-mediated process which is initiated by binding to a specific cytosolic receptor. This single receptor is believed to be common for most materials eliciting 3-MC type induction. Binding to this receptor is a competitive process and the relative

potency of various PCDD congeners for lethal, teratogenic and acneigenic effects has been demonstrated (62). Those congeners which do not evoke a response in test animals have a very mild or nonexistent affinity for the receptor while those of intermediate to high binding affinity, such as 1,2,3,4,7,8-H_6CDD and 2,3,7,8-TCDD are of medium and high toxicity, respectively.

Figure 3 presents data of Poland (61) which demonstrates the correlation between binding affinity of various PCDD congeners to mouse hepatic cytosol and their AHH-inducing ability in the chick embryo. The observed trends are supported by observations made by McConnel (5), who reported that the 2-, 3-, 7-, and 8- positions must be chlorinated to achieve the greatest degree of toxicity. Addition of chlorine atoms at the ortho position reduces toxicity, but not as severely as the deletion of a chlorine at one of the lateral positions.

A study conducted by Bradlaw (63) investigated the comparative induction of AHH activity in rat hepatoma cell cultures by various analogues of dibenzo-p-dioxin. The trends they observed are consistent with the requirements outlined by McConnel. They found the following congeners, listed in order of decreasing potency towards AHH induction, to have greater than 0.2% of the activity of 2,3,7,8-TCDD: 2,3,7,8-TCDD, 2,3,7,8-TBrCDD, 1,2,3,4,7,8-H_6CDD, 1,2,3,7,8-P_5CDD, 1,2,3,4,7 P_5CDD, 1,3,7,8-TCDD, 1,2,3,6,7,8-H_6CDD, 1,2,3,7,8,9-H_6CDD, 1,2,3,4,6,7,8-H_7CDD. Congeners which induced less than 0.2% activity were: 2,3,7-TrCDD, 1,2,3,8-TCDD, 1,2,3,4,6,7,9-H_7CDD, 1,2,3,6,7,9-H_6CDD. Inactive congeners tested were dibenzo-p-dioxin, 1,3,-; 1,6-;

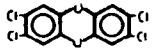
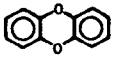
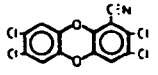
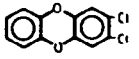
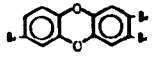
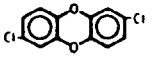
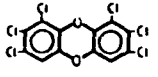
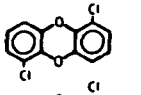
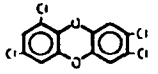
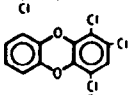
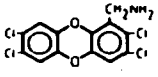
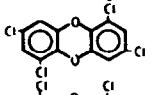
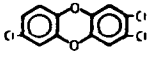
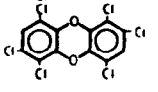
	RELATIVE BINDING AFFINITY	RELATIVE BIOLOGICAL POTENCY		RELATIVE BINDING AFFINITY	RELATIVE BIOLOGICAL POTENCY
	100†	100††		inactive (5.4×10^{-7})*	inactive (9.4×10^{-8})**
	167	100		inactive (2.7×10^{-6})	inactive (9.4×10^{-8})
	43	43		inactive (5.4×10^{-6})	inactive (9.4×10^{-8})
	20	22		inactive (5.4×10^{-6})	inactive (4.7×10^{-7})
	16	8		inactive (5.4×10^{-6})	inactive (9.4×10^{-8})
	13	3		inactive (2.7×10^{-6})	inactive (9.4×10^{-8})
	14	006		inactive (1.1×10^{-6})	inactive (9.4×10^{-6})

Figure 3.

The Cytosolic Binding Affinity and Biological Potency of Dibenzo-p-Dioxin Congeners Relative to 2,3,7,8-TCDD. (From Poland et al. Ref. 61)

2,3-; 2,7-; and 2,8-DCDD, 1,2,3,4-TCDD and 1,3,6,8-TCDD and 1,2,4,6,7,9-H_6CDD.

Table 4 lists a number of halogenated aromatic hydrocarbons, including dioxins, which evoke identical patterns of toxic response in the guinea pig but differ in potency. They are all stereoisomers of 2,3,7,8-TCDD. The observed order of reactivity in this table are consistent with the theory that the molecule must be nearly planar and have molecular structures that roughly fit into a rectangle 3 x 10 Å with halogen atoms in the four corners of the rectangle (65). Additional halogens at the ortho positions fall outside the rectangle and may, in some cases, not seriously effect receptor binding. However, if they destabilize the overall polarizability of the carbon-halogen bonds in the molecule, they can effect its attraction towards the polar receptor matrix and decrease its binding affinity and ultimate biological potency.

A very interesting observation was made by Cheney (66) on the basis of regression analysis of the affinity of a number of 2,3,7,8-TCDD congeners and their potency for AHH induction. It was determined that lateral substituents do not always affect binding and induction of AHH to the same degree. Examples of this can be seen in Figure 3. Cheney reasoned that the discrepancy could be the result of a decrease in the effective dose caused by metabolism or excretion prior to reaching the receptor, but he theorized that the receptor could also require activation subsequent to binding. It was observed that the lateral substituents effect binding, but also must function to "turn on" the receptor to an active state which proceeds to

Table 4.

Toxicity of Various Stereoisomers of 2,3,7,8-TCDD in the Guinea Pig[b]

Name of Compound	LD_{50} (ug/kg) a
2,3,7,8-Tetrachlorodibenzo-p-dioxin	2
1,2,3,7,8-Penatachlorodibenzo-p-dioxin	3
2,3,7,8-Tetrachlorodibenzofuran	7
2,3,4,7,8-Pentachlorodibenzofuran	<10
2,3,7,8-Tetrabromodibenzofuran	<15
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	73
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	60-100
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	70-100
2,3,4,6,7,8-Hexachlorodibenzofuran	120
2,3,7,8-Tetrachlorofluorenone	>100
1,2,4,6,7-Pentabromonaphthalene	200
2,3,6,7-Tetrabromonaphthalene	242
1,2,3,4,6,7-Hexabromonaphthalene	361
3,3',4,4'-Tetrachlorobiphenyl	<552
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	>600
1,2,4,7,8-Pentachlorodibenzo-p-dioxin	1125
2,3,3',4,4',5,5'-Heptachlorobiphenyl	>3000
3,3',5,5'-Tetrafluoro-4,4'-dichlorobiphenyl	>3000
2,3,6,7-Tetrachloronaphthalene	>3000
1,2,3,5,6,7-Hexabromonaphthalene	>3610
3,3',4,4',5,5'-Hexachlorobiphenyl	223
2,3,7-Trichlorodibenzo-p-dioxin	29,444

[a]Hartley strain guinea pig given a single oral dose (gavage) and observed for 30 days

[b]From Mckinney and McConnell,(Ref.64)

initiate a series of events resulting in AHH induction and numerous other responses. There are stereoisomers which can be shown to bind, but do not appear to "turn on" the receptor very efficiently. A lateral substituent that was observed to have this capacity is the NO_2 group which, when in a configuration out of the lateral plane of the dioxin molecule, functions to facilitate binding to the receptor but does not activate it. According to this theory, a compound could be devised which would bind to the receptor with a very high affinity, but not turn it on, thereby acting as an antidote for chlorinated hydrocarbon poisoning of the 2,3,7,8-TCDD type. Blocking the activity of the receptor would also prove very valuable in determining its function in the normal activities of the cell.

D. Teratogenicity of TCDD

In addtition to highly fetotoxic and reproductive effects, 2,3,7,8-TCDD has been shown to be a very potent teratogen. Table 5 lists the results of a number of studies performed which examined the teratogenic potential of 2,3,7,8-TCDD. Administration of 2,3,7,8-TCDD to mice, rats, hamsters, rabbits and monkeys during gestation induced various malformations, including: Fetal cleft palate, hydronephrosis, marked hemorhage, edema, extra ribs and the absence of eyelids. The teratogenic effect most commonly observed was fetal cleft palate in mice. It appears that 2,3,7,8-TCDD is the most potent teratogen observed to date. Doses as low as 1 ug/kg cause teratogenic effects in mice. Poland has demonstrated that the

Table 5.

Teratogenicity Studies With 2,3,7,8 TCDD.

Animal and Treatment	Observation	Reference
Chick embryo: injection of 0.9 ug into yolk sack	Toxic effects to the embryos, resulting in 40% hatchability and pronounced embryopathies.	(40)
Hamster, oral 2,4,5-T with 0.1-45 ug/kg TCDD	Absence of eyelids; hemorrhages and marked edema.	(67)
Rats: oral 500 mg/kg p.c. days 13 to 15 of gestation	Fatty inclusions in embryonic livers.	(68)
Mouse: oral 1-3 ug/kg Strains: CD-1, DBA/2J and C57BL/6J	All three strains developed cystinephrotic kidneys and cleft palate in offspring.	(69)
Rats: oral 0.5 ug/kg	Cystinephrotic kidneys in pups.	(69)
Mice: oral 3 ug/kg C57BL/6J	Application during pregnancy caused fetal cleft palate and cystinephrotic kidneys. Postnatal maternal exposure caused hydronephrosis in foster pups.	(70)
Rats: oral single dose of ^{14}C-TCDD (5 ug)	A very high activity was present in the fetuses, especially in liver.	(71)
Mice: oral 1 ug/kg/day	Application from day 6 to 15 of pregnancy caused an increase in fetal mortality and cleft palate.	(72)
Mice: oral 20-50 ug/kg in the interval 7-13 days of pregnancy	A single dose in this interval caused the same teratogenic effect without fetocidal influence. The teratogenic maximum was by dosing at day 11.	(72)
Rats: oral Sprague-Dawley 0.03-8.0 ug/kg/day days 6-15 of gestation	Teratogenic no-effect level 0.03 ug/kg After application of 0.125-2.0 ug/kg, there was a increase in the number of resorptions and dead fetuses, intestinal hemorrhages and anomalies of the renal pelvis. All higher doses caused maternal death.	(73)

Table 5. cont'd.

Rats: 2,3,7,8-TCDD Mice: (i.p.)	Application during the latter half of gestation and in the postnatal period resulted in severe depletion of lymphocytes in the thymic cortex of the offspring.	(74)
Monkeys: oral 0.05-10 mg/kg 2,4,5-T with 0.05 mg/kg TCDD	No observed teratogenic effects.	
Mice: CF-1 0.001-3 ug/kg/day	Treatment on days 6-15 by oral gavage. 3.0 ug/kg/day: fetal cleft palate and dilated renal pelvis. 1.0 ug/kg/day: cleft palate No effects at lower doses. No-effect dosage estimated at 0.1 ug/kg/day.	(76)
Mice: CD-1 Various dioxins 1-1000 ug/kg/day	Dibenzo; 2,7-dichloro-; 2,3,7-trichloro-; 1,2,3,4-tetrachloro-; 2,3,7,8-tetrachloro- and octachlorodibenzo-p-dioxin administered on days 7-16 of gestation. Only 2,3,7,8 TCDD was teratogenic.	(77)
Mice: NMR-1 Subcutaneous 5-40 ug/kg/day	Cleft palate at all dose levels.	(79)
Rats: Wistar oral 1-16 ug/kg/day	Treatment during days 6-15 of gestation caused visceral anomalies characterized by edema and hemorrhage. Postnataly, the survivability, weight gain and reproductive ability of the progeny were affected.	(78)
Rhesus Monkeys: 9 days by gavage total dose was 0.2 ug/kg and 1.0 ug/kg Days 20-40	All fetuses exhibited abnormalities of the soft palate.	(80)
Rabbits: New Zealand White 0.1, 0.25, 0.5 and 1.0 ug/kg/day at days 6 to 15	Severe maternal and embryonal toxicity at 0.25 ug/kg/day and above. Extra skeletal ribs and kidney anomalies at all dose levels.	(81)

Table 5. cont'd.

Human fetuses: collected from early abortions in Seveso, Italy.	In 7 of 22 fetuses, microprecipitates observed to be regularly present beneath the trophoblastic basement membrane. No additional anomalies observed.	(82)

teratogenic potency of 2,3,7,8-TCDD is related to its binding affinity towards the TCDD receptor (83). The observed no-effect level in mice and rats is about 0.1 ug/kg. Aside from inconclusive data available from studies conducted on women exposed to 2,3,7,8-TCDD at Seveso, Italy following an industrial accident (84), which suggests an increase in birth defects, there is no direct evidence of teratogenicity induced by TCDD in human fetuses.

E. Mutagenicity and Carcinogenicity

A number of studies have been conducted which investigated the mutagenic potential of 2,3,7,8-TCDD. Indications of mutagenicity were observed in tests using the African Blood Lily, which revealed chromosomal abnormalities and the inhibition of mitosis (89). Induction of prophage in *E. coli* K-39 and streptomycin independance in *E. coli* Sd-4 also suggest that 2,3,7,8-TCDD may be a mutagen (88). However, the majority of the available data (Table 6) indicates 2,3,7,8-TCDD to be non-mutagenic. Additionally, Poland and Glover (103) have demonstrated that 2,3,7,8-TCDD does not significantly bind to rat liver DNA *in vivo*, nor that it is metabolized to an electrophile which subsequently binds to DNA.

However, the results presented in Table 7 indicate that 2,3,7,8-TCDD is extremely active in carcinogenicity bioassays. In long-term rat and mouse feeding studies, 2,3,7,8-TCDD has been shown to cause an increase in the incidence of a wide variety of neoplasms, including hepatocellular and squamous

cell carcinomas of the lung, hard palate/nasal turbinates or tongue; neoplastic changes in the kidney and bile duct; liver and skin carcinoma; angiosarcomas and thyroid follicular cell adenomas. The observed effects occurred following administration of extremely low doses of TCDD, suggesting that 2,3,7,8-TCDD is a very powerful "complete" carcinogen.

The diversity of observed neoplastic changes is uncommon to most carcinogens. In light of this diversity and the lack of evidence of DNA-binding by TCDD, it could be theorized that TCDD is actually acting as a powerful promoter. But data from two-stage mouse skin carcinogenesis bioassays does not support this conclusion. The 2,3,7,8-TCDD isomer was not shown to promote the incidence of skin tumors following initiation with 7,12-dimethylbenz(a)anthracene (DMBA) or benzo(a)pyrene (BAP) (99,100), Additionally, it was shown to inhibit skin tumors when applied prior to or simultaneously with DMBA or BAP (100). However, when administered orally to partially hepatectomized rats treated with diethylnitrosamine (DEN), 2,3,7,8-TCDD acted as a promoter and increased the incidence of liver tumors (102).

Regardless of the contradictory results, 2,3,7,8-TCDD clearly causes an increase in the incidence of neoplasms at very low doses. Because 2,3,7,8-TCDD also induces a range of enzyme systems and causes immunosuppressive effects at or below these levels, it is possible that the carcinogenicity arises via an indirect mechanism where TCDD is neither the material actually interacting with DNA nor the substance promoting the proliferation of cancerous cells.

Table 6.

TCDD-induced Mutagenicity Studies.

Animal and Treatment	Observations	Reference
Cell cultures: 10^{-6} M TCDD	No significant inhibition of growth in following mammalian cells: HeLa, Balb 3T3, virus transformed mouse fibroblasts, human fibroblasts and lymphocytes when observed for four days. No morphological changes observed by electron microscopy.	(85)
Rats: oral 10-20 ug/kg intraperitoneal 5-15 ug/kg	Investigation of choromosomes in bone marrow: no increase in abberation rate in all cases	(86)
Rats: oral 10 ug/kg. observed 0, 24 or 72 hours following partial hepatectomy	No effect on DNA synthesis in the remaining liver lobes.	(87)
Salmonella typhimurium TA 1532 and TA 1530 E. coli K-39	High mutagenic frequency in Salmonella TA 1532, which reverts by frame-shift mutation; Negative for base-pair substitution by TA 1530; Positive for prophage induction in E. coli K-39.	(88)
African Blood Lily:	Induction of mutations; inhibition of mitosis.	(89)
Salmonella typhimurium Strains G46, TA 1530, TA 1531, TA 1532 and TA 1534.	Induction of mutation in TA 1532 only. Negative in other strains	(90)
Wistar Rats	Negative in dominant lethal test.	(78)
Rats: bone marrow cells	Weakly positive mutagenicity. Observation in absence of control.	(91)
Salmonella typhimurium histidine auxotrophs. 0-20 ug TCDD per plate	TA 1535, TA 100, TA 1538, TA 98 and TA 1537 strains. No strain showed mutagenicity in presence of mammalian metabolic activating systems from livers of Arochlor 1254 treated rats and hamsters or TCDD treated hamsters.	

Table 6. cont'd.

Maternal peripheral blood and fetal tissues from TCDD-exposed humans at Seveso, Italy.	No mutagenic effects observed.	(93)

Table 7.

2,3,7,8-TCDD Carcinogenesis Studies.

Animal and Treatment	Observations	Reference
Swiss Mice: TCPE + TCDD gastric intubation once weekly for one year	Treatment with TCPE containing TCDD as an impurity and TCDD alone at TCDD dose levels of 0.007 to 0.112 ug/kg resulted in an increase in the incidence of hepatic tumors.	(94)
Mice: CD-1 Two-stage skin carcinogenesis bioassay. 2 ug TCDD/mouse followed by 5 ug TPA weekly for 32 weeks.	TCDD was shown to be a weak tumor initiating agent and an inactive cocarcinogen.	(95)
Rats: dietary 0.001-0.10 ug/kg/day for 2 years.	A two year rat feeding study at 0.01 and 0.1 ug/kg/day: increase in hepatocellular and squamous cell carcinomas of the lung, hard-palate/nasal turbinates or tongue. Decreased incidence of tumors of the pituitary, uterus, mammary glands, pancreas and adrenal gland. At 0.001 ug/kg/day: no discernable effects.	(20)
Rats: dietary 5 ppb in feed	Death within 40 weeks. Neoplastic changes observed were kidney, bile duct, liver and skin carcinoma and an angiosarcoma.	(96)
Rats: dietary 1 ppt to 1 ppm in feed	Increased incidence of neoplasms, notably tumors of ductal epithelium and cholangiosarcomas at or above 5 ppt for 78 weeks. Suggested that TCDD is a potent promoter.	(97)
Rats: oral and dermal	Both TCDD and a mixture of isomers of hexachlorodibenzo-p-dioxin are weak carcinogens if administered orally, but when applied dermally they are complete carcinogens.	(98)

Table 7 cont'd.

Mice: Two-stage skin promoter assay. TCDD and 7,12-dimethyl-benz(a)anthracene (DMBA)	A) TCDD + DMBA: effects were additive. B) TCDD (1 ug) 3 days prior to initiation by DMBA: DMBA activity decreased by 93%. C) Dioxin applied at 0.1 ug twice weekly for 30 weeks did not act as a promotor following 200 nmol DMBA.	(100)
TCDD + DMBA or TCDD + BAP on mouse skin with TPA as a promoter.	TCDD inhibited tumor initiation by both BAP and DMBA.	(101)
Rats: Osborne-Mendel gavage 0.01, 0.05 or 0.5 ug/kg/wk in two doses for 104 weeks.	Effects significant in high dose group only. Male Rats: increased incidence of thyroid follicular-cell adenomas. Female Rats: increase in hepatic neoplastic nodules.	(101)
Mice: $B6C3F_1$ males dosed at 0.01, 0.05 or 0.50 ug/kg wk. females at 0.04, 0.2 or 2.0 ug/kg/wk.	Male and female mice: increase in hepatocellular carcinomas. Female mice: increase in thyroid follicular cell adenomas.	(101)
Rats: orally after diethyl nitrosoamine (DEN) and partial hepatectomy	TCDD determined to be a promoter of liver tumors.	(102)

TCPE = tetrachlorophenyl ether

TPA = t-phorbol acetate

BAP = benzo(a)pyrene

F. Human Effects of TCDD

The effects of exposure of humans to 2,3,7,8-TCDD are not extensively documented. This is presumably due to the limited availability of TCDD-exposed human subjects and variations in estimates of exposure. Most information available today is based on studies of victims of occupational exposure and industrial accidents. In many cases, the subjects underwent routine medical examinations following the appearance of acute symptoms, but long-term monitoring of chronic exposures was seldom carried out. Contamination of work areas as high as 2,420 mg/m^2 have been reported, yet few human deaths due to acute TCDD poisoning have been recorded (104).

The most common symptom of human TCDD exposure is contact chloracne, along with some cases of neurological disorders and porphyria cutanea tarda. Table 8 lists the generalized symptoms of TCDD exposure in human beings. The range of toxic responses is consistent with animal data, along with subjective observations of sensory and psychological impairment. The scarcity of mortality data suggests that human beings are not as sensitive towards TCDD poisoning as are test animals such as the guinea pig and the rat. On this basis, it would appear that the TCDD receptor has a lower binding affinity or that human beings are capable of excreting and metabolizing TCDD more rapidly than most species tested.

A number of epidemiological investigations have been conducted on populations of occupationally exposed workers, which attempted to assess the relationship between TCDD and occupational

Table 8.

Toxic Effects of TCDD Reported in Man.

Dermatological:	chloracne porphyria cutanea tarda hyperpigmentation and hirsutism
Internal:	liver damage (mild fibrosis, fatty changes, haemofuscin deposition and parenchymal-cell degeneration) raised serum hepatic enzyme levels disorders of fat metabolism cardiovascular disorders respiratory tract disorders pancreatic disorders
Neurological:	A) peripheral: polyneuropathies sensorial impairments (sight, hearing, smell, taste) B) central: lassitude, weakness, impotence, loss of libido

from Reggiani (104)

cancer. These studies have been reviewed by Tognoni (105), Reggiani (104) and Hay (106). Minor increases in the incidence of stomach cancers and a possible increase in soft tissue sarcomas were observed in 3 out of 10 groups studied (104). The observed increases did not correspond to incidents where the exposure had been the highest, but were observed in groups which were exposed to low levels of TCDD associated with the application of phenoxy acid herbicides. Regardless of the weaknesses inherent to epidemiological assessments, one would have to conclude that 2,3,7,8-TCDD is not as potent a carcinogen in human beings as would be expected on the basis of extrapolations from laboratory animal carcinogenesis bioassays.

III. ENVIRONMENTAL INPUTS AND FATE OF TCDD

A. Chlorophenol Manufacturing

Dioxins are not produced for any commercial purpose, but can arise as by-products of the synthesis of chlorophenols from chlorobenzene by the alkaline hydrolysis method. The formation of dioxins occurs as a condensation reaction between two ortho-chlorinated phenol salts, as outlined in Figure 2. The 2,3,7,8-TCDD isomer can only be produced from 2,4,5-trichlorophenol. Other o-chlorophenols yield different dioxin congeners. The condensation reaction is favored at elevated reactor temperatures and pressure and becomes a major spontaneous exothermic process at reactor temperatures above 250° C.

Although its formation can be minimized by maintaining a low reactor temperature, no 2,4,5-trichlorophenol produced by

the alkaline hydrolysis process is free from contamination by 2,3,7,8-TCDD. Levels of TCDD associated with the operation of a modern trichlorophenol manufacturing facility have been reported by Van Ness (107). Samples of aqueous effluent from the facility contained up to 100 ng/kg (ppt) TCDD and a soil sample at the site contained 559 ug/kg (ppb) TCDD. Samples of solid and liquid sludge and sump fluid contained 374, 445 and 676 ug/kg TCDD, respectively, while trichlorophenol still bottom contained 111 mg/kg (ppm) of TCDD. These relatively high levels demonstrate that significant amounts of TCDD can be associated with the production of trichlorophenol, posing especially arduous problems of occupational hygeine and waste disposal.

The 2,4,5-trichlorophenol is a very important chemical intermediate which has been produced in large quantities for over 40 years. During this period, standard practices of quality control and waste disposal have varied, The health hazards associated with 2,3,7,8-TCDD have not been fully realized until very recently. In fact, during the last major TCDD accident, which occurred in Seveso, Italy in 1976, the officers of the plant were reportedly unaware of the hazards of dioxins until well after the explosion (108). Recently, severe contamination by 2,3,7,8-TCDD has been reported in Missouri as a result of disposal of chlorophenol production wastes by application to roads as a means of dust control (109). It is quite likely that a number of similar instances have taken place unreported and are contibuting to the presence of environmental residues of 2,3,7,8-TCDD.

B. TCDD release during industrial accidents

There have been a number of serious accidents which occurred during the manufacture of 2,4,5-trichlorophenol from chlorinated benzene in which a chlorophenol reactor overheated, proceeded to form large amounts of dioxins and exploded (108). A large number of serious cases of occupational exposure to 2,3,7,8-TCDD have also been reported, presumably the result of handling TCDD-contaminated chlorophenols; products made from 2,4,5-trichlorophenol and during routine operations of plant maintenance and waste disposal.

The most recent accident took place in Seveso, Italy in July of 1976 when a chemical reactor in a factory producing 2,4,5-trichlorophenol for use in hexachlorophene synthesis overheated, went exothermic and blew its contents out a safety valve which was vented to the outside of the plant. The exploded contents of the reactor formed a cloud which was carried away from the factory, heavily contaminating a 700 meter by 5 km section of the city with 2,3,7,8-TCDD. A week later, the inhabitants of the town were told what had happened, and two weeks later, persons living in the area most heavily contaminated were evacuated from their homes. The amount of TCDD released has not been determined, but estimates range between 300 grams and 130 kg (110). There was an estimated total of 49,500 kg of soil into which TCDD had penetrated and lodged, and many homes and schools were severely contaminated. Removal was judged impossible and burning was ruled out due to the

thermal stability of TCDD and the possibility of recontamination.

Dogs, cats, rodents and birds present in the effected area died within a short time but interestingly, the only human effects reported were a large number of serious cases of contact chloracne. To date, there have been no fatalities recorded as a result of this incident. Women who were pregnant and exposed were urged to undergo abortions, while those of childbearing age were warned not to attempt to conceive children. There have been two children with deformities born to exposed women. The first abnormality observed was a slight intestinal obstruction in an infant born to a woman who had reportedly eaten contaminated garden produce; while a woman living in an area not officially considered contaminated gave birth to a child with a minor genital malformation (11). Both children were born at a date which indicated that they were beyond the teratogen-sensitive period of organogenesis at the time of the exposure, and it has not been determined whether they represent an increase over normally expected background levels.

Currently, a one square kilometer area of Seveso has been sealed off from access and will probably remain unusable for at least 15 years. Much of the decontamination of other exposed parts of the city has been carried out by removal of ground cover and by the ultraviolet photolysis of the interiors of homes.

C. Product Contamination

1. Phenoxy acid herbicides

Most products using 2,4,5-trichlorophenol as an intermediate have been shown to contain varying amounts of 2,3,7,8-TCDD as an impurity. The germicide hexachlorophene; an herbicide of major importance, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and its propionic acid analog, Silvex, are all synthesized from 2,4,5-trichlorophenol. Prior to 1971, these products commonly contained up to 50 mg/kg (ppm) of 2,3,7,8-TCDD. However, following the recognition of the toxic potential of TCDD, steps were taken to reduce the contamination by closely monitoring conditions during the production of the trichlorophenol intermediate. An industry standard for TCDD in 2,4,5-T has been set at 0.1 mg/kg, which is the typical TCDD level found in much of the 2,4,5-T produced today. Nevertheless, the United Sates Environmental Protection Agency has suspended the registration of 2,4,5-T and Silvex in the U.S.

A number of studies have been conducted to determine whether residues of TCDD could be found in areas sprayed with 2,4,5-T (112-117). However, due to the low levels of application of TCDD and the analytical sensitivity required to detect the TCDD residues, most investigators reported negative findings. A study conducted by Woolson (116) analyzed soil core samples taken from a sandy area in Florida which had been sprayed with 2,4,5-T at application rates up to 11 g/m^2 (947 lbs/acre). Application was conducted during the period 1962-1969, when

TCDD levels present in 2,4,5-T ranged between 30-50 mg/kg. This study detected no traces of TCDD in the soil at a minimum detectable level of 1 ug/kg. Another series of experiments was conducted to determine whether residues of TCDD were present in environments routinely treated with 2,4,5-T (112-114). The studies were conducted with purified 2,4,5-T. Samples of fish, water, mud, human milk, cow milk and beef fat were analyzed by an analytical procedure sensitive to part per trillion (ng/kg) levels of TCDD. None of the samples analyzed were found to contain TCDD, with the exception of samples of beef fat which were positive at 3 ng/kg. A study conducted by Newton (115) demonstrated that 2,4,5-T containing 20 ug/kg or less applied to a forest ecosystem did not significantly effect the health of the forest herbivore, the mountain beaver. There were no residues of TCDD detected in the livers of trapped beaver at a detection limit of 5 to 10 ng/kg. However, other studies have shown that cattle grazing in rangeland sprayed with 2,4,5-T contained residues of TCDD at levels ranging from 5 to 143 ng/kg (117). The inconsistencies in the residue data most likely reflect the variability of residues of 2,3,7,8-TCDD in the 2,4,5-T used for application.

2. Agent Orange

During the Vietnam War, a 1:1 mixture of 2,4,5-T and 2,4,-D (2,4,-dichlorophenoxyacetic acid) known as "Agent Orange" was sprayed over a very large portion of Vietnam by the military to defoliate the terrain. More than 10% of the inland forests, 36% of the mangrove forests, 3% of the cultivated and 5% of "other"

land was sprayed one or more times (118). A total estimated volume of 45 million liters of Agent Orange, which contained 2,3,7,8-TCDD at levels as high as 47 mg/l was applied. There have been many claims made by veterans of the war in Vietnam as well as by the Vietnamese natives that Agent Orange was the cause of various cancers and birth defects. At the present time, an epidemiological survey is being conducted on veterans and their families to determine whether such a relationship exists.

A study conducted by Baughman (120) analyzed fish and crustaceans collected from Vietnam for 2,3,7,8-TCDD. TCDD was detected in all of the samples at levels ranging from 18 to 810 ng/kg. The highest concentrations of TCDD were measured in catfish taken from the Dong Nai river in the interior, where spraying was reportedly the heaviest. A study conducted by Young (121) on a 2.6 square kilometer military test site which had been sprayed with a total of 73,000 kg of 2,4,5-T and 76,000 kg of 2,4-D detected 10 ng/kg to 710 ng/kg 2,3,7,8-TCDD in the top 15 cm of soil at the site. In some instances, ten years had elapsed since the last aerial application. Liver tissues of rodents inhabiting the test site contained 210 to 1,300 ng/kg TCDD, but no gross or histological evidence of carcinogenesis, teratogenesis or toxicity was observed in 112 adults and 87 fetuses. Concentrations of TCDD (12 ng/kg) were detected in two species of fish in a stream running through the site. Levels of 2,3,7,8-TCDD in skin, muscle, gonads and gut of a spotted sunfish from a test pond at the site were 4, 4, 18 and 85 ng/kg, respectively.

3. Pentachlorophenol

Significant levels of dioxins have also been detected in technical grade pentachlorophenol (122-125). A study conducted in our laboratory on an industry composite sample of technical pentachlorophenol (PCP) measured over 1,000 mg/kg each of hexa- and heptachlorinated dioxins and 900 mg/kg OCDD (126). The 2,3,7,8 isomer of TCDD was not detected at a limit of detection of 0.1 mg/kg, but other tetrachlorinated isomers were detected at 1 mg/kg.

Technical grade PCP has been demonstrated to induce the type of toxicity characteristic to dioxin poisoning while purified PCP has not. PCP is widely used in the preservation of wood, and is probably one of the most versatile pesticides in use today. Dioxins have been shown to be associated with the lumber and pulp industry, where PCP is used as a slimicide and preservative (127). Shavings, sawdust and wastes are routinely burned, resulting in the formation and release of dioxins. Due to its wide use and its relatively high dioxin content and potential for formation during burning, PCP should be considered a dangerous material.

A study by Firestone (128) investigated the accumulation of dioxins in cows fed technical grade PCP. Cows were fed PCP containing 20, 280, and 700 mg/kg hexa, hepta and octachlorodioxin at PCP dose levels of 20 mg/kg/day for 10 days and 10 mg/kg/day for the next 60 days. Composite milk fat contained 85 ug/kg total dioxins at the end of the treatment period, whereas total dioxins in the blood were 0.25 ug/kg.

D. Formation of Dioxins During Combustion

In 1977, Olie (129) conducted a survey of the organochlorine residues present in fly ash and made an observation which suggested that chlorinated dioxins are produced during combustion. He detected varying amounts of chlorinated benzenes, phenols, dioxins and dibenzofurans in samples of fly ash and flue gases from three municipal incinerators in Holland. These materials could not be accounted for on the basis of their presence in the waste prior to incineration. It was theorized that the dioxins were formed as a result of the condensation of chlorinated phenols produced de novo by reaction of organic constituents of the waste such as polyethylene with inorganic chlorides under the pyrolytic conditions in the incinerator.

Since that time, a number of investigators have consistently detected dioxins in fly ash and exhaust gases from municipal and chemical waste incinerators in Europe, the United States, Canada and Japan (130-139). The results of some of these studies are listed in Table 9. The dioxins present at the highest concentrations are the higher chlorinated, tetra through octachlorinated congeners. Of these, the hexachlorinated isomers are the most abundant. Reported total dioxin levels in fly ash range from 200 to over 5,000 ug/kg, with TCDD levels ranging from 2 to over 300 ug/kg (138).

Few studies have specifically measured the 2,3,7,8-TCDD isomer. This is mostly due to the difficulty of isomer-specific dioxin analysis. Most recently, Tierman (140) identified 20 of

the 22 tetrachlorinated isomers in a sample of municipal waste fly ash from the U.S. The 2,3,7,8-TCDD isomer was present in the greatest concentration (2.1 ug/kg), representing 15% of the total TCDD content of the fly ash. Researchers at the Dow Chemical Company in Midland, Michigan estimate flue gas from municipal incinerators to contain an average of 2 ng 2,3,7,8-TCDD per Normal cubic meter (ng/Nm^3) (141). On the basis of a ton of waste producing 30 kg of fly ash and 7000 Nm^3 of flue gas; a ton of waste should yield an estimated 63 and 14 ug of 2,3,7,8-TCDD in fly ash and flue gas, respectively.

A comprehensive survey of PCDDs and PCDFs and related compounds present in incinerator effluents was conducted by Olie (138). Average and peak values for PCDDs, PCDFs, chlorinated benzenes and phenols were obtained by sampling the major municipal incinerators in Holland over a two year period. On the basis of data collected, they estimated the total amount of PCDDs and PCDFs produced by incineration of municipal waste on a yearly basis to be 145 kg of total dioxins and 97 kg total chlorinated dibenzofurans (Table 9h). They further estimated the toxic potential of the PCDDs and PCDFs produced to be equivalent to 10 kg of 2,3,7,8-TCDD/yr in fly ash and 2 kg/yr in flue gas. These figures were arrived at by assigning a toxicity factor of 1 to 2,3,7,8-TCDD and a factor of 0.1 to the other compounds with at least three chlorine atoms in the lateral positions and at least one hydrogen at the peri positions.

When estimated in this manner, 2,3,7,8-TCDD represents only about 2% of the toxic potential of fly ash and incinerator

Table 9.

Dioxin Levels Associated with Incineration of Municipal Wastes.

a. Municipal and industrial waste: (Ref. 132)

	TCDD	P_5CDD	H_6CDD	H_7CDD	OCDD
Municipal waste: (ug/kg)					
ash collected from oven at 900°C.	N.D.	N.D.	N.D.	N.D.	N.D.
ash collected from stack at 260°C.	2	8	30	60	120
Industrial waste incinerator:	100	160	180	130	140

N.D. = not detectable

b. Municipal incinerator fly ash collected from electrostatic precipitator (E.S.P.): 3 samples. (Ref. 133)

TCDD: 6, 1.1 and 2.7 ug/kg

OCDD: 150, 30 and 0.7 ug/kg

c. Municipal waste incinerator fly ash (E.S.P.): (Ref. 134)
5 samples:

TCDD: 12, 10, 9, 5, and 2.5 ug/kg

Table 9. cont'd.

d. Municipal waste: results of 80 analyses from 25 different incinerators (ug/kg) (Ref.135)

	TCDD	P_5CDD	H_6CDD	H_7CDD	OCDD
low value (E.S.P.)	5	31	80	190	266
median value (E.S.P.)	54	182	326	288	106
high value (E.S.P.)	110	488	1200	902	110
Stack particulates	100	800	1370	1370	310

e. Variation of PCDD content of fly ash (E.S.P.) from one incinerator over a 4-week period (ug/kg) (Ref.134.)

	TCDD	P_5CDD	H_6CDD	H_7CDD	OCDD
week one	81	252	406	321	82
week two	56	173	388	385	124
week three	54	182	326	288	106
week four	41	159	381	458	226

f. Variation of PCDD content of flue gases collected over a 9-month period from one municipal incinerator. nanograms/Normal cubic centimeter ($ng/N\ cm^3$) (Ref. 136)

	TCDD	P_5CDD	H_6CDD	H_7CDD	OCDD
high value	1127	2089	3805	2884	631
mean value	128	260	366	286	126
low value	7	19	15	13	19

Table 9 cont'd.

g. Dioxin levels measured at 6 different municipal incinerators: (Ref. 137)

		TCDD	P_5CDD	H_6CDD	H_7CDD	OCDD
1:	E.S.P. fly ash (ug/kg)	--	--	--	--	--
	stack particulates *	1.1	2.7	11.5	1.0	8.0
	flue gas *	19.6	27.9	178.2	159.6	63.9
2:	E.S.P. fly ash (ug/kg)	0.25	1.7	294.0	8.9	295.0
	stack particulates	172.2	172.3	12,015	575.0	7,312
	flue gas	17.0	107.0	26,620	828.0	1,179
3:	E.S.P. fly ash (ug/kg)	N.D.	0.9	1.8	3.1	1.5
	stack particulates	0.037	0.3	6.7	0.2	1.7
	flue gas	19.0	40.0	65,420	124.0	776.0
4:	E.S.P. fly ash (ug/kg)	46.4	65.4	2,496	87.9	841.5
	stack particulates	10.9	2.8	0.5	3.2	39.0
	flue gas	60.0	33.0	1,390	167.0	2,703
5:	E.S.P. fly ash (ug/kg)	0.7	0.05	0.02	0.01	0.1
	stack particulates	0.34	2.4	196.0	9.9	173.0
	flue gas	9.6	21.0	328.0	46.0	244.0
6:	E.S.P. fly ash (ug/kg)	N.D.	N.D.	N.D.	0.001	5.9
	stack particulates	N.D.	0.01	0.28	N.D.	0.5
	flue gas	19.0	11.0	480.0	6.0	71.0

* Stack particulates and flue gas levels reported as nanograms dioxin per Normal cubic meter of flue gas ($ng/N\ m^3$)

Table 9. cont'd.

h. Average concentration and estimated yearly output of PCDD and PCDF in fly ash and flue gas of the major municipal incinerators in the Netherlands (reported as the total of all incinerators sampled.) (Ref. 138)

	fly ash		flue gas	
	average conc. (ug/kg)	total amount per year (kg)	average conc. (ng/Nm^3)	total amount per year (kg)
TCDD	93	5.6	57	0.8
P_5CDD	254	15.2	244	3.4
H_6CDD	604	36.2	440	6.2
H_7CDD	760	45.6	347	4.9
OCDD	345	20.7	452	6.3
total	2056	123.3	1540	21.6
(Furans)				
TCDF	173	10.4	161	2.3
P_5CDF	312	18.7	272	3.8
H_6CDF	459	27.5	528	7.4
H_7CDF	314	18.8	293	4.1
OCDF	51	3.1	68	1.0
total	1309	78.5	1322	18.6

gas. An early life stage fish bioassay using extracts from municipal fly ash supports this estimate (142). Fly ash extracts were determined to be more than 10 times as toxic as would be expected on the basis of 2,3,7,8-TCDD present in the extract. Therefore, the majority of the toxic potential of the dioxin type associated with fly ash is the result of the less toxic dioxin and dibenzofuran congeners which are present in fly ash at much higher concentrations than 2,3,7,8-TCDD.

Olie (138) also noted that the amounts of dioxins produced and the ratios of the numerical isomers vary with temperature, the point in the incinerator where they are collected and among the various incinerators. No dioxins were detected in waste slag, while the concentrations and congener ratios associated with fly ash collected from electrostatic precipitators are different from those escaping with flue gas. Flue gases contain less PCDD than either type of particulate. This indicates that the formation of PCDD is a dynamic process taking place on the surface of particulates, which may be acting as catalytic sites.

Eiceman (143) investigated the behavior of 1,2,3,4-TCDD adsorbed onto fly ash by simulating emission conditions in a municipal incinerator. He demonstrated gas-stream conversion of adsorbed 1,2,3,4-TCDD to P_5CD, H_6CDD, H_7CDD and OCDD in the presence of as little as 1% HCl gas in air. The value of 1% HCl is within the range of HCl levels present in modern municipal incinerators. Reaction temperatures from 50°C to 250°C were monitored. The highest conversion was observed at 150°C. Below this temperature, the heat of reaction for chlorination

of dioxin (an endothermic process, ΔH=+71 kJ/mole/chlorine) was not efficiently achieved. The reduction in conversion at the higher temperature was believed to be due to stronger adsorption of the dioxin molecule to the particle surface, which hindered interaction with the gas stream. These results partially explain the observed variations in isomer ratios and the predominance of the higher chlorinated congeners.

There are a number of possible explanations for the presence of dioxins in combusted materials. The most straightforward is the assumption that dioxins are already present in the materials being combusted. They may be present as a result of dioxin contamination in herbicide formulations, in polychlorinated biphenyls or as components of halogenated phenols such as pentachlorophenol. However, this would require a much higher level of initial contamination than can be explained by a mass balance. Analyses of compost (144) and municipal waste prior to incineration (145) have not revealed the presence of any dioxins.

An explanation which reflects a good deal of experimental evidence is that the dioxins arise from chlorinated precursors of anthropogenic origin present in the waste being burned. Laboratory scale combustion studies have shown that 2,4,5-T (146), chlorophenols (147-149), PCBs (150,151), chlorinated benzenes (152), chlorinated aliphatics (155,156) and chlorinated polymers, such as polyvinyl chloride (153) can yield chlorinated dioxins and dibenzofurans or their precursors. Mechansisms of their formation are presented in a review by Choudry (154). Figure 4 outlines the reactions which various organic materials

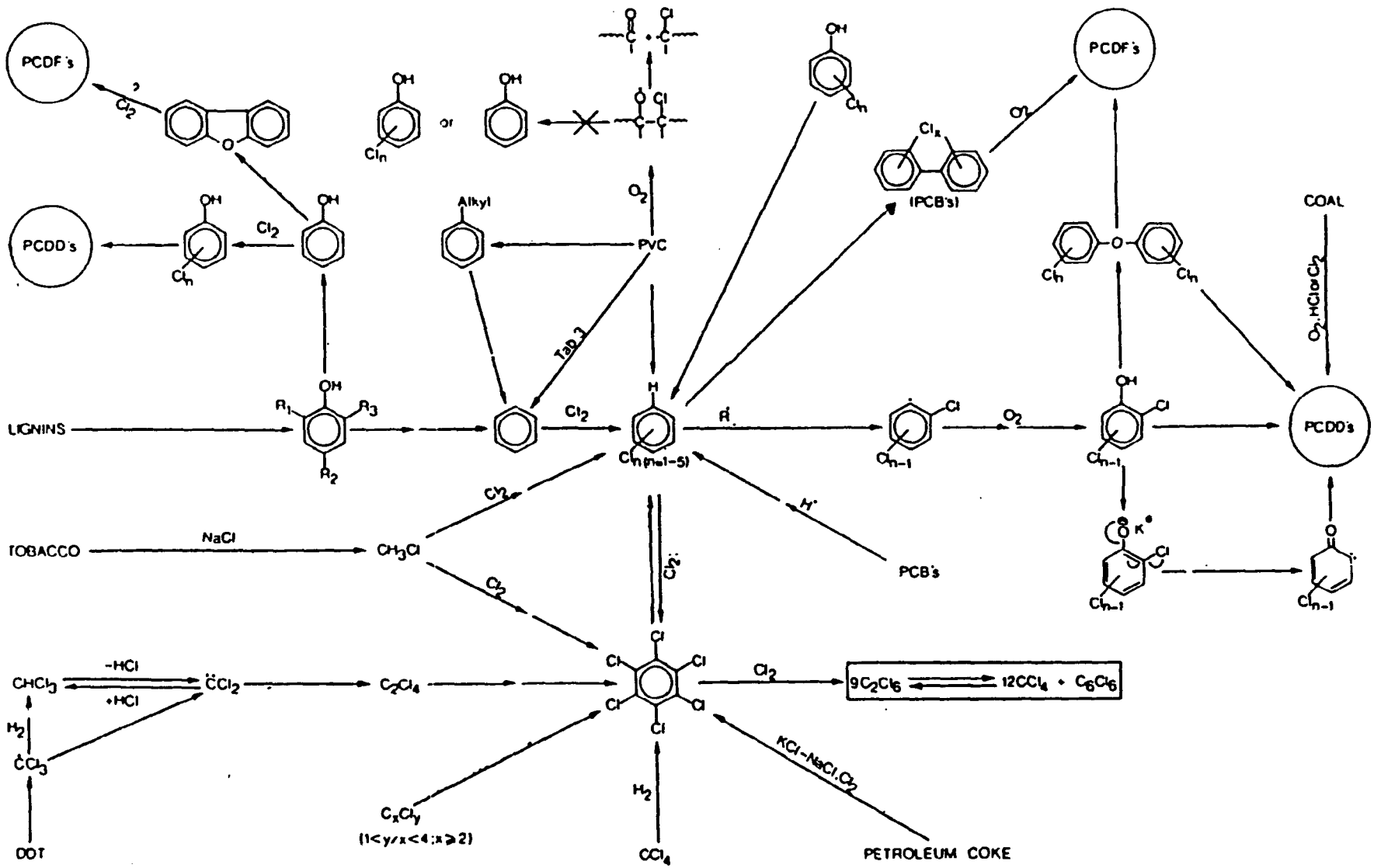


Figure 4.

Routes of Formation of Chlorinated Dioxins During Combustion. (From Choudry et al. Ref. 154)

undergo to form PCDD and PCDF during combustion.

Chlorinated phenols yield PCDD via the previously outlined condensation reaction (Fig. 2). The herbicide 2,4,5-T and similar chlorinated phenoxy acid compounds should also form dioxins by condensation following cleavage of the ether linkage. Ortho chlorinated phenols are the ultimate precursors required for formation of PCDD from chlorinated waste constituents. Any material which can react to produce an ortho chlorinated phenol during combustion will yield dioxins, which can be further chlorinated via gas-phase reactions with HCl or Cl_2 in the incinerator stack at the appropriate temperature and O_2 level.

Chlorobenzene is a key intermediate in the thermal formation of chlorophenols. It reacts via a free radical mechanism in which a hydrogen atom on chlorobenzene is abstracted by a chlorine or hydrogen free radical to yield a chlorobenzene free radical and HCl or H_2. In the presence of oxygen, the chlorobenzene free radical reacts to produce the chlorophenol (152).

Hexachlorobenzene (HCB) and pentachlorobenzene are usually the major chloroaromatic constituents present in combustion train exhaust gases containing chlorinated dioxins. It is believed that hexachlorobenzene undergoes reductive dechlorination to pentachlorobenzene which can further react via free radical mechanisms to form chlorophenols and ultimately PCDD and PCDF. The source of the HCB can be directly anthropogenic, it can arise via gas phase chlorination of lower chlorinated benzenes or it can be formed from chlorinated aliphatics. Chlorinated aliphatics which satisfy the relation: C_xCl_y; $1<y/x<4$ $x\geq 2$ such as trichloroethane can cyclize to produce HCB (156). HCB

has been shown to exist in equilibrium with hexachloroethane at 405°C according to the relation (155):

$$9(C_2Cl_6) \rightleftarrows 12(CCl_4) + C_6Cl_6 \quad K_1=2.7x10^{11}$$

One of the major chlorinated organic materials present in municipal waste is polyvinyl chloride (PVC). Combustion experiments have demonstrated that hexachlorobenzene is produced during the thermal degradation of PVC (153). However, the mechanism by which PVC degrades is dehydrochlorination, which precludes the formation of HCB by cyclization. Since major products of PVC pyrolysis are non-halogenated aromatics, such as benzene and phenol, it is likely that the chlorinated analogs, chlorobenzene and chlorophenol, are produced by chlorination reactions of benzene and phenol. This behavior indicates that non-halogenated aromatics of natural origin can assume a major role in the formation of PCDD and PCDF.

A series of investigations entitled "the trace chemistries of fire" reported the presence of chlorinated dioxins in a variety of combusted materials which contained no anthropogenic chlorinated precursors (157). Samples of char-broiled steak, cigarette smoke, home fireplace soot, automobile mufflers and fly ash from a coal-fired powerhouse all contained detectable levels of chlorinated dioxins. It was theorized that chlorinated dioxins arise via a series of trace level reactions during the normal combustion of most organic materials, and that these reactions have been taking place since the advent of fire.

There are a number of pieces of evidence which substantiate this theory. Chlorinated dioxins have been detected during the laboratory combustion of coal in the presence of air; with or

without the addition of HCl or Cl_2 gas (158). This would suggest that dioxins should be present in fly ash from most coal fired power installations.

The thermal degradation of lignin, the second most abundant organic compound found in nature, yields benzaldhyde, phenol, cresols, catechol and other low molecular weight aromatics which can conceivably undergo high temperature chlorination and produce chlorinated benzenes, phenols and other chlorinated aromatics, including dioxins (159). Black liquor from the pulp industry contains an appreciable amount of lignin and is routinely incinerated as a means of waste disposal. Combustion of this material yielded over 600 mg HCB per ton of dewatered liquor (160). Highest yields were obtained at temperatures between 400° and 500° C. Following bleaching, which raises the organic chlorine content but destroys the polymeric nature of the lignin, drastic reductions in the yield of HCB were observed. It is difficult to speculate as to the cause for this reduction.

HCB and chlorophenols have also been found to arise during the combustion of wood in an open fire (160). It was estimated that one kilogram of wood yields 40 ug of HCB and 4.2 mg of chlorinated phenols. It is likely that these same reactions take place during forest fires, which have been occuring for millions of years.

Petroleum coke has been shown to yield mg/g levels of CCl_4 and HCB as well as phosgene ($COCl_2$) following heating at 700° C with Cl_2, KCl or NaCl in the absence of oxygen (161). The high yields observed are partly due to an 80:1 ratio of chloride salts to petroleum coke. Normal HCl levels associated with

combustion in municipal incinerators are 3.25 kg per ton of waste incinerated. Although the experiments listed for lignin, wood and coke have not demonstrated the formation of dioxins, the potential for formation of PCDD from them during incineration can be inferred, since hexachlorophene and chlorophenols do indeed react at elevated temperatures to produce dioxins. An incinerator is a unique reactor since it allows for different oxidation and temperature condiditons to exist during a particle residence time which is significantly longer than encountered during open combustion. For this reason, the yields and isomer distributions would certainly differ during open burning of wood and wastes.

The formation of chlorinated organics during combustion, as presented in the foregoing discussion, indicates that notable amounts of chlorinated dioxins and related compounds are released to the environment as a result of these processes. They may represent a source of greater magnitude than the amount released during chlorophenol synthesis or as associated with the use of dioxin containing products. This could be especially true if the amounts of the toxic non-2,3,7,8-TCDD, PCDDs and PCDFs released during combustion are considered.

E. Environmental dynamics and fate

Chlorinated dioxins are extremely stable, non-reactive compounds. The 2,3,7,8-TCDD isomer is resistant to the action of concentrated acids and bases and maintains its chemical stability at temperatures as high as 700°C. TCDD does not undergo hydrolysis in water and is resistant to microbial attack. The 2,3,7,8-TCDD isomer exhibits an extremely low vapor pressure (1.5×10^{-5} mm Hg), is soluble in water to only 0.2 ug/l and has an estimated octanol/water partition coefficient of 1×10^{6}.

The environmental dynamics of chlorinated dioxins are similar to some of the longer lived chlorinated hydrocarbons. Upon entering the environment, 2,3,7,8-TCDD becomes strongly bound to soil. A soil adsorption coefficient of 10^{4} to Lakeland sandy loam can be estimated on the basis of data of Isensee (168). Studies by Kearney (162) evaluated the mobility of 2,3,7,8-TCDD by soil thin-layer chromatography using five different soil types. TCDD was determined to be immobile in all of the soils tested. Similar conclusions were drawn by Matsumura and Benezet (161), using a column of sandy loam.

Analyses of soil near the Icmesa chemical plant at Seveso, Italy revealed that 2,3,7,8-TCDD disappeared at a rapid rate during the first six months following the explosion and then maintained an almost steady level over the next year (163). The initial reduction was probably due to photolysis or volatization of TCDD from the exposed surfaces. The majority of the remaining residues were initially contained in the top 4 cm of soil. Six months later, most of the residues were still in the

top 4 cm of soil, but significant amounts had migrated as deep as 24 cm. No further movement or loss was observed in later analyses, indicating that the TCDD had now become firmly bound.

The minimal reduction in the soil content of TCDD following the initial losses suggests that 2,3,7,8-TCDD is not readily degraded by soil microorganisms. A number of investigations have been conducted into the microbial degradation of 2,3,7,8-TCDD, using ^{14}C-labelled material (161,165-167). Although reductions in ^{14}C activity were observed, with estimated half-lives ranging from one year to 600 days, the reductions could not be accounted for on the basis of the appearance of microbial metabolites of ^{14}C-2,3,7,8-TCDD. The disappearance of TCDD was presumed to be mostly due to the volatization of the parent dioxin.

Volatization of TCDD is not an unreasonable explanation, because its low water solubility should promote partitioning out of the aqueous phase. Volatization may have contributed to the rapid losses of TCDD observed during the first six months at Seveso. As the residues migrated into the soil, processes of mass transfer which usually function to supply solute to the surface could not take place effectively (due to the low water solubility of TCDD and its high soil affinity), and removal of residues by volatization became minimal.

A hydroxylated metabolite of 2,3,7,8-TCDD (hydroxy 2,3,7,8-TCDD) was recently isolated and identified in extracts of TCDD-treated soil microbial cultures (164). Microorganisms collected at Seveso and Pseudomonas testosteroni strain G1036

metabolized 1% of the applied ^{14}C-2,3,7,8-TCDD following 12 weeks of incubation, indicating that microbial transformation represents only a minor route for degradation of 2,3,7,8-TCDD.

A study by Isensee (168) measured the bioaccumulation of 2,3,7,8-TCDD by aquatic organisms in a model ecosystem. Bioaccumulation of TCDD applied to sediment at a concentration of 0.1 ug/kg was monitored in algae, duckweed, snails, daphnids, Gambusia and catfish. The bioaccumulation factor was found to be directly related to the water concentration of TCDD, and ranged from $4\text{-}9 \times 10^3$ in duckweed, algae and catfish to 2×10^6 in snails, Gambusia and daphnids, calculated on a dry weight basis. A similar study was conducted by Matsumura and Benezet (161) who reported bioaccumulation factors of 1.5×10^3 for brine shrimp and 9×10^3 for mosquito larvae, on a wet weight basis. DDT was also evaluated in the same model ecosystem and was determined to be accumulated at a level almost an order of magnitude greater than TCDD. This could be explained by DDT's greater water solubility (1.2 ug/kg) and similar partition coefficient (10^6), and would indicate that TCDD is not likely to accumulate in as many biological systems and to as great an extent as DDT.

Studies at Seveso conducted during the growing season one year after the accident indicate that although soil concentrations of TCDD remain at about 10 ug/kg, major amounts of TCDD are not translocated into vegetation (169). Samples of fruit contained about 30 ng/kg 2,3,7,8-TCDD, primarily in the peels (100 ng/kg). Samples of corn kernels or cobs did not contain any TCDD (1 ng/kg) while the corn sheath contained 8 ng/kg TCDD.

Carrots grown in the contaminated soil contained 224 ng/kg and 5.7 ng/kg in the peels and edible portions, respectively. These results indicate that the TCDD residues are not the result of plant uptake and translocation, but arise as a result of contamination of exposed surfaces. Similar behavior was observed by Isensee (170) in soybeans and oats. In contrast to the negligible amounts of TCDD taken up by plants, liver samples of farm animals and wildlife from the affected area have revealed that they picked up TCDD by grazing. Residues ranging from 200 ng/kg in frogs to 633 ug/kg in rabbits were recorded (171) in animals which entered the area after the accident had occured. Samples of cow's milk contained TCDD residues as high as 8 ug/kg.

The major route by which 2,3,7,8-TCDD degrades in the environment is through photolysis. Crosby and Wong (172) reported that thin films of 2,3,7,8-TCDD on plant surfaces and soil were rapidly photodegraded with typical half-lives of six hours. Although the exact mechanism has not been established, it is believed that photolysis of TCDD takes place through the loss of chlorine atoms by the dissociation of excited molecules to free radicals or through nucleophilic displacement. This reaction occurs only in the presence of a system which can act as a hydrogen donor. Such donors may be represented in the environment by the waxy cuticles on vegetation or by humic materials present in soil.

Studies performed by Kawaguchi (173) investigated the photolysis of chlorinated dioxins in methanol, isooctane and water, and as effected by the presence of O_2 or the triplet

quencher, 9,10 anthroquinone. Photolysis took place preferentially at the lateral positions of chlorinated dioxins and was determined to be taking place via reductive dechlorination. Decomposition was rapid ($t_{½}$=6 min for 2,3,7,8-TCDD) in isooctane and methanol, but was very slow in suspension in organic-free distilled deionized water. The quenching effects of O_2 or 9,10 anthroquinone indicated that degradation occured via triplet-state excited intermediates.

Nestrick (174) measured the photolysis rates of all 22 TCDD isomers, and determined that photolytic dechlorination is favored at the lateral positions of the dioxin molecule. Laboratory photolysis of isooctane solutions of the TCDD isomers resulted in empirically calculated photolysis half-lives ranging from 56 min for 2,3,7,8-TCDD and 632 min for 1,2,6,7-TCDD to 8400 min for 1,4,6,9-TCDD. Similar trends were observed in photolysis experiments of TCDD isomers as thin films on glass, but 1,4,6,9-TCDD and 2,3,7,8-TCDD were both observed to have the longest half-lives. The dramatic reduction in the photolysis rate of 2,3,7,8-TCDD was explained as possibly caused by adsorptive effects induced by the symmetry of the molecules. This would suggest that photodegradation of 2,3,7,8-TCDD may be hindered following adsorption onto soil particles.

Although studies of the photodegradation of chlorinated dioxins in water solution suggest that photolysis should not occur in the aquatic environment, these studies fail to take into account the sensitizing effects of dissolved materials present in nature. Lakes and streams, as well as moist surfaces, are a dilute mixture of dissolved materials and dispersed

particulates which can shuttle radiant energy and provide catalytic surfaces for photolysis and thus effect the rates, mechanisms and products of photolytic reactions to an extent which cannot be modeled in laboratory systems.

Humic acids absorb radiant energy and have been reported to chemiluminesce, which could generate free radicals and initiate electronic and energy transfer processes and degrade materials with which they complex (175). Photolysis of Atrazine in distilled water is increased in the presence of fulvic acid (176) and rice paddy water exerts a marked photosensitizing effect on the photolysis of isoprothiolane (177), which photolyzes extremely slowly in deionized water. The presence of surfactants (178) and suspended sediments (179) enhance the photodegradation of hydrophobic chlorinated compounds in water and favors photolysis by reductive dechlorination.

Since chlorinated dioxins and furans (180) rapidy photolyze under laboratory condiditons, it can be assumed that, with the aid of substrates and sensitizers present in natural waters, they should photodegrade in the aquatic as well as terrestrial environment.

F. The dioxin controversy in Michigan

In 1977, the Dow Chemical, Michigan Division at Midland, Michigan was implicated in polluting the Tittabawassee and Saginaw Rivers with 2,3,7,8-TCDD as a result of their chlorophenol and 2,4,5-T production activities. Significant levels of 2,3,7,8-TCDD were detected in fish sampled downstream to Dow's Midland facility. Dow claimed that it was not responsible

for the TCDD residues in the fish and conducted its study into the trace chemisitries of fire (157) in response to the allegations. Dow reasoned that the TCDD contamination of the fish was the result of formation and release of dioxins during combustion and not due to their manufacturing process. However, the E.P.A. did not agree with Dow. They reviewed the data presented in the trace chemistries of fire argument and noted that much higher levels of dioxins were present in Midland than in other metropolitan areas. TCDDs, especially the 2,3,7,8 isomer, were found mainly in the Midland area whereas most other cities contained the higher chlorinated congeners and essentially no 2,3,7,8-TCDD.

A survey of 2,3,7,8-TCDD levels in fish from the Midland area conducted by the E.P.A. in 1978 showed that TCDD was not present in three fish taken upstream to the Dow facility, but high levels were present in almost all fish sampled downstream to the facility and in Saginaw Bay. The results of this survey are listed in Table 10. These results and the data presented by Dow in the trace chemistry report (Table 11) led the E.P.A. to conclude that the plant "represents the major, if not the only source of the TCDD contamination found in the Tittabawassee and Saginaw Rivers and Saginaw Bay in Michigan" (181).

An interesting result of the E.P.A. survey was the detection of TCDD in fish samples taken from the Grand River at Eagle, Michigan. These samples were collected from an entirely different watershed, and should have contained no TCDDs; yet levels as high as those found in fish from the Saginaw River were measured. Later studies conducted by Dow also confirmed the

TABLE 10
RESULTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) FISH MONITORING PROGRAM CONDUCTED BY THE U.S. ENVIRONMENTAL PROTECTION AGENCY IN MICHIGAN DURING 1978

Location	Fish Species	Fish Length (cm)	TCDD Level (ppt)[a]	TCDD Detection Limits (ppt)
Tittabawassee River				
Tittabawassee Road	Carp	42.2	52 100	8 (Dow Study)
State Road	Yellow Perch	16.2[b] 11.0	20	8
	Carp	39.7	93	6
Freeland Road	Carp	40.0	32 70	4 (Dow Study)
	Yellow Perch	16.8 12.3	10	5
Smith's Crossing Road	Channel Catfish	50.3	273 230	6 (Dow Study)
	Carp	38.0	22	11
	Channel Catfish	38.2	695	60
	Carp	23.1	49	1
	Sucker	35.6	8	4
Above Dow Dam	Channel Catfish	30.9	42	9
	Carp	34.6	ND[c]	5
	Carp	33.0	ND	9
	Channel Catfish	38.1	28	2
	Yellow Perch	19.3	ND	4
Dublin Road	Carp	52.2	ND	9
Saginaw River				
Wickes Park	Carp	46.0	62	13
	Yellow Perch	11.9 11.4	ND	11

Location	Fish Species	Fish Length (cm)	TCDD Level[a] (ppt)	TCDD Detection Limits (ppt)
Block's Marina	Channel Catfish	69.0	105	7
	Channel Catfish	48.5	52	8
	Carp	51.8	28	4
Mouth	Channel Catfish	46.0	30	6
	Carp	45.0	153	13
	Yellow Perch	16.0 16.0	11	4
Saginaw Twp. Park			70	(Dow Study)
Grand River				
Jones Road near Eagle	Carp	39.0	20	4
	Carp	39.0	41	8
	Channel Catfish	--	29	6
	Smallmouth Bass	35	7	6
	Smallmouth Bass	38.0	8	6
Near Ionia	Carp	50.8	ND	2
	Sucker	48.3	ND	2
Saginaw Bay				
Lat. 43° 40' 16"	Sucker	28.2 31.0	4	2
Long. 83° 50' 21"	Yellow Perch	24.2 26.8	ND	3
Lake Michigan				
Off Saugatuck	Lake Trout	50.8	ND	7

a ppt indicates parts per trillion which is equivalent to ng/kg.

b signifies composite fish sample.

c ND indicates that TCDD was not detected in the fish sample at the corresponding detection limit.

Table 11.

Excerpts of Data from the
Trace Chemistries of Fire Report (Ref. 157)

a) Chlorinated Dioxins in Soil (ug/kg)

Source	2,3,7,8 TCDD	other TCDD	H_6CDD	H_7CDD	OCDD
Gaylord, MI	N.D.	N.D.	N.D.	N.D.-.05	N.D.-0.02
Lansing, MI	N.D.	N.D.	.03-1.2	.03-2.0	.05-2.0
Chicago, IL	N.D.	.005-.003	.03-0.3	0.1-3.0	0.4-22.0
Dow-Midland	0.3-100	0.8-18.0	7.0-280	70-3200	490-20,000

b) Chlorinated Dioxins in Dust (ppb)

Source	2,3,7,8 TCDD	other TCDD	H_6CDD	H_7CDD	OCDD
Dow-Lab	0.7-3.0	0.5-2.0	9.0-35.0	140-1200	650-7500
Midland, MI	*	0.03-0.04	0.2-0.4	2.0-4.0	20.0-30.0
Detroit, MI	*	N.D.-0.03	N.D.-0.03	0.3-4.0	0.10-4.0
St. Louis, MO	0.12	0.16	2.0	34.0	210.0
Chicago, IL	*	0.04	N.D.-0.3	0.6-3.0	3.0-8.0

* analyzed by procedure which was not selective for 2,3,7,8-TCDD

presence of 2,3,7,8-TCDD in the Flint, Cass and Shiawassee Rivers in Michigan (182). These data suggest that TCDD residues are not confined to the Saginaw River watershed and may not be present in other portions on the State.

Due to the large amount of time and effort required and the high cost associated with dioxin analyses, no further studies into the dioxin levels present in Michigan have been conducted and the question of the source of the dioxin residues in the Tittabawassee and Saginaw Rivers remains unanswered. Another factor preventing the resolution of the dioxin controversy is the high level of sophistication involved in the instrumentation required for dioxin analysis. There are very few laboratories in the country equipped with the proper instruments and staff necessary to perform these analyses. The analytical procedure is generally accepted by the scientific community as state-of-the-art, and before the Dow or E.P.A. claims can be substantiated or disproved, a more comprehensive survey needs to be conducted.

IV. EXPERIMENTAL

A) Purpose of Research

During the summer of 1979, the analytical laboratory at the M.S.U. Pesticide Research Center, under the direction of Professor Matthew Zabik, conducted an evaluation of current methodology for the analysis of environmental residues of 2,3,7,8-TCDD. An analytical method developed by the Dow Chemical Company Michigan Division, known as method ML-AM-78-63 (183), was chosen as representing the state of the art in dioxin residue analysis. It was anticipated that, with the addition to and updating of existing instrumentation, the Pesticide Research Center would soon possess the analytical capability to perform analyses of environmental samples for TCDD residues at the part per trillion level.

Upon the urging of the Michigan Department of Natural Resources and researchers at Dow Chemical, both eager to resolve the dioxin controversy in Michigan, a project to quantify TCDD residues in Michigan fish was considered. A proposal to investigate the routes of entry of chlorinated dioxins to the environment and new analytical methodologies for TCDD analysis was drafted and awarded limited renewable funding through the Michigan Agricultural Experiment Station. The major goals of the research were:

1) To evaluate a state of the art procedure for the quantitation of dioxin residues at the part per trillion level.

2) To maximize chromatographic resolution of the TCDD isomers to allow specific detection of 2,3,7,8-TCDD.

3) To determine the extent and distribution of residues of 2,3,7,8-TCDD in the State of Michigan.

4) To provide a data base with which to evaluate the sources and possible impact of residues of 2,3,7,8-TCDD on the environment and public health.

The project to analyze samples of fish for TCDD was initiated before the techniques were verified and all necessary instrumentation was available. In order to utilize time most efficiently and deal with limitations and availability of instrumentation, work was carried out in the following phases, concurrent with efforts to computerize a DuPont 321 electric sector mass spectrometer:

Phase I. Collection of environmental samples: Approximately 500 samples of fish were collected from the major rivers in Michigan.

Phase II Aquisition of specialized materials: Glassware, reagents, adsorbents and gases were prepared and purified as required.

Phase III Method validation: All steps in the analytical procedure were verified and maximized for recovery of TCDD using ^{14}C-2,3,7,8-TCDD. Quality control measures were developed and utilized.

Phase IV Sample preparation: Samples were extracted and processed through column clean up. Approximately 250 samples were processed. Extracts were stored dry at -15° C.

Phase V HPLC clean up: 150 samples were suitable for HPLC purification. Extracts were stored in hexane at -15° C awaiting GC/MS quantification.

Phase VI TCDD synthesis: Primary standards of the 22 isomers of TCDD were synthesized and characterized by HPLC, EC-GC and GC/MS.

Phase VII Resolution and sensitivity maximization: Capillary GC/MS resolution of 2,3,7,8-TCDD from the other TCDD isomers was maximized. GC/MS sensitivity towards 2,3,7,8-TCDD was maximized to 20 picograms.

Phase VIII GC/MS quantification: Sample extracts were quantified for 2,3,7,8-TCDD. Approximately 75 samples of the initial 250 samples processed had recoveries and clean up suitable for quantification. The first sample was quantified three years following initiation of research.

B) Introduction to Analytical Problem and Methodology

The analytical technique employed in this study was designed to isolate and quantify as little as 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 clean up 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 minimum amount which can be routinely detected by most GC/MS systems using the specific ion detection mode is 20 picograms. Thus 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 millionfold 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.

The analytical technique (outlined in Figure 5) is based on the inertness of chlorinated dioxins towards the action of concentrated acids and bases and the differential elution behavior of TCDD on modified liquid chromatographic supports, high

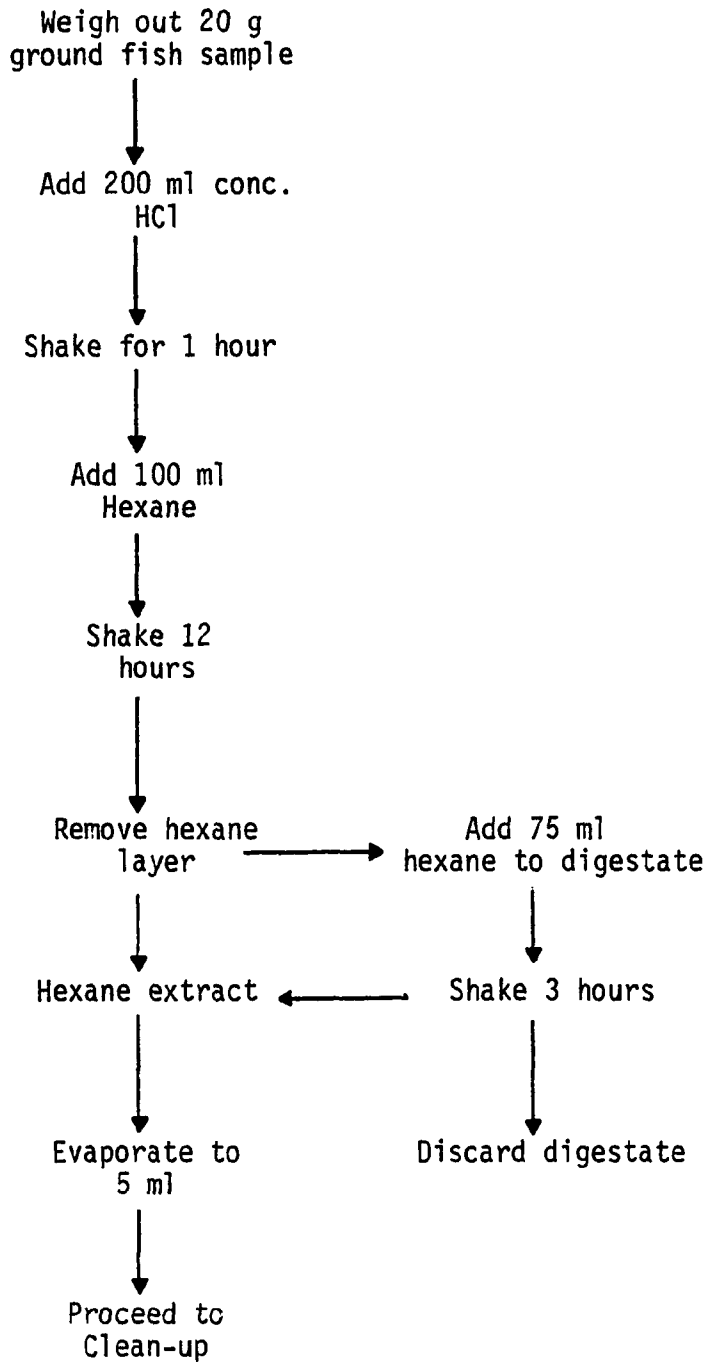
performance liquid chromatography (HPLC) and during capillary gas chromatography. The sample preparation and clean up procedure consists of four basic parts: 1) Sample digestion and extraction to remove the fish tissue matrix and transfer the extractable residue into hexane; 2) Removal of lipids through the action of oxidizing reagents; 3) Separation of TCDD from interfering compounds; 4) Final HPLC cleanup to remove residual trace contaminants and provide separation of TCDD from the other chlorinated dioxins. Five nanograms of ^{13}C-2,3,7,8-TCDD is used as a carrier and internal standard to correct for indeterminate losses of TCDD during extraction, cleanup and volume reduction steps.

Figure 5 is a flow diagram of the procedure. A 20 gram aliquot of homogenized fish fortified with 5.0 ng of ^{13}C-2,3,7,8-TCDD is digested in concentrated HCL. The HCl treatment dissolves the fish matrix and hydrolyzes its acid-reactive components. The digested sample is extracted with hexane and the extract is treated to one or more washes in concentrated H_2SO_4 and eluted through a column of silicic acid coated with H_2SO_4 followed by elution through a column of NaOH-treated silicic acid. The acid wash and column treatments function to further hydrolyze and saponify fats, oils and other acid or base-reactive components of the extract. The extract now contains chlorinated dioxins and other non-reactive materials such as DDT, DDE, PCBs or PBBs. The extract is concentrated and eluted through a $AgNO_3$/silica column which retains DDE, sulfides, aliphatic halides, weak acids, phosphates and a variety of unknown color bodies. Additionally, it separates chlorinated dioxins

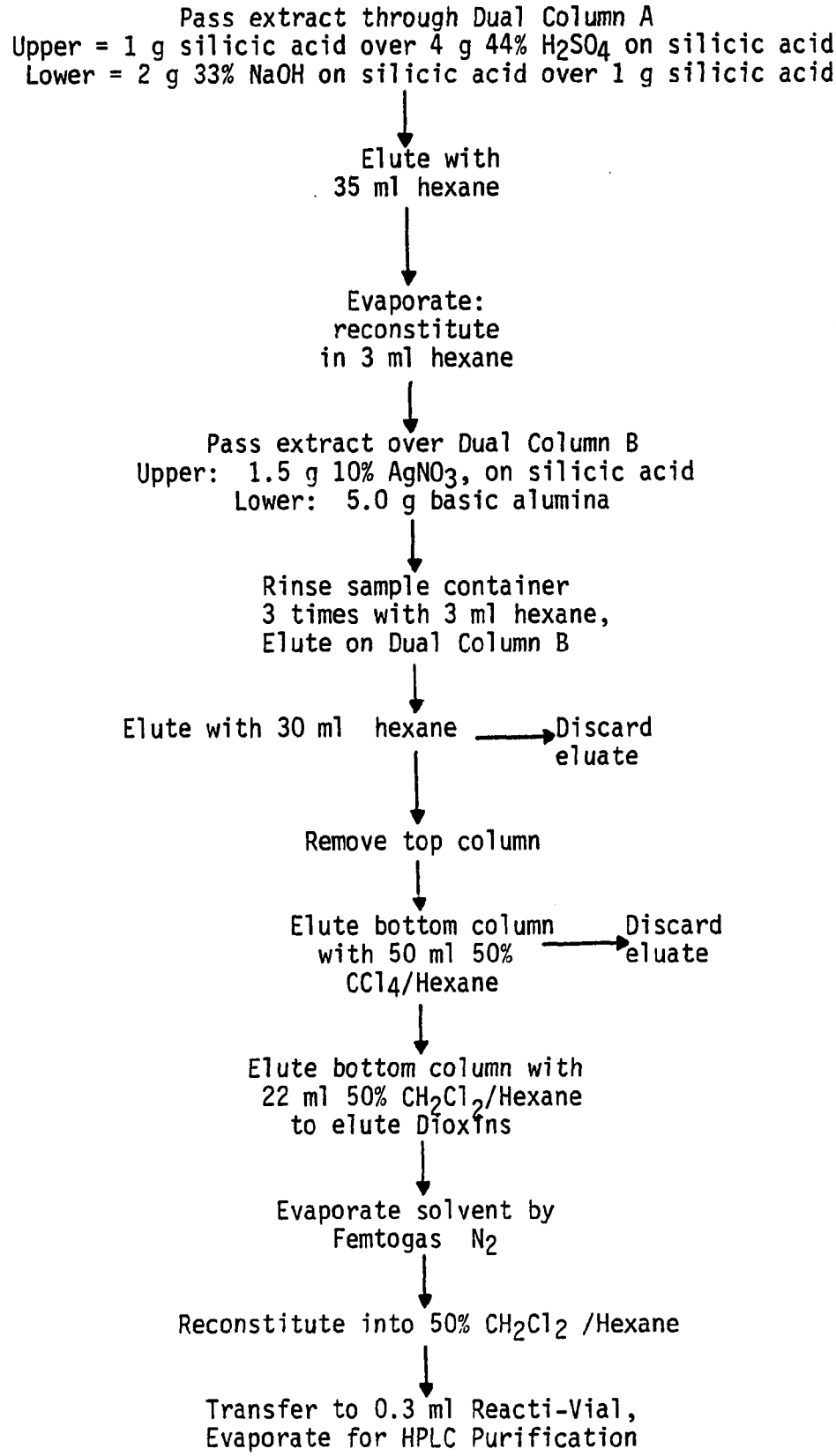
Figure 5

Flow diagram of analytical procedure utilized for determination of 2,3,7,8-TCDD in fish

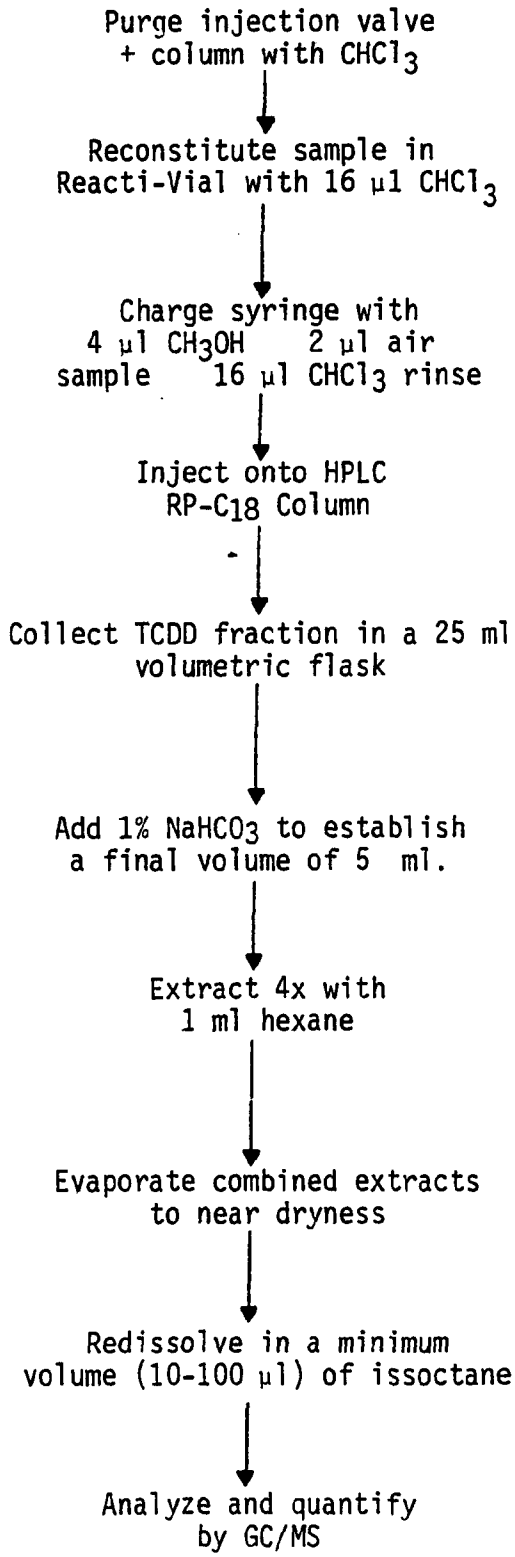
I. Fish Digestion Procedure



II. Dual Column Cleanup Procedure



III. HPLC Purification



from chlorinated benzyl phenyl ethers. Eluent from the $AgNO_3$/ silica column elutes directly onto an activated basic alumina column. Following elution of the extract to bed volume, the alumina column is eluted with 50 ml 1:1 CCl_4:hexane. The PCBs, PCTs (polychlorinated terphenyls) and any previously non-adsorbed DDE are eluted, while the dioxins are retained. The dioxins are then eluted from the column in 22 ml of 1:1 CH_2Cl_2:hexane. The extract is evaporated to dryness and reconstituted into about 10 ul of $CHCl_3$ and injected onto a reversed-phase HPLC column. The HPLC step functions to further clean up the extract and separate the numerical PCDD congeners. It also resolves some of the TCDD isomers. The fraction corresponding to 2,3,7,8-TCDD is collected and extracted into hexane. This extract is reduced to dryness, reconstituted into 5 ul of isooctane and injected onto a capillary GC/MS for final quantification. The ion masses 319.9, 321.9 and 323.9 corresponding to the molecular ion cluster of native 2,3,7,8-TCDD and 331.9 as the most intense ion of the ^{13}C-2,3,7,8-TCDD are monitored and quantified relative to a standard curve.

C. Detail of Procedure for TCDD analysis*

*adapted from procedure ML-AM-78-63; The Dow Chemical Company, Michigan Division.

Sampling

Samples of Carp (Cyprinus carpio) and Sucker (Catostomus commersoni), approximately 40 cm in length, were collected from each of the major rivers in Michigan by electroshock technique by the Michigan Department of Natural Resources, Fisheries Division. Samples were transported to the laboratory on ice, where they were weighed, measured and about 20 scales removed for age determination. The samples were covered with aluminum foil, labelled, placed into individual polyethylene bags and stored frozen at -15°C until used.

Sample Preparation

Skinless, boneless fillets of the edible portions of each fish are ground and blended in a Hobart food chopper. A 20 gram subsample of each fish is used for TCDD analysis. The remainder of the fish sample is placed in a polyethylene bag, tagged and stored frozen at -15°C.

Fish Sample Extraction

1. Samples are processed in batches of twenty. Each batch is comprised of 18 fish samples and 2 procedural blanks.

2. To a 20 gram sample of fish or a blank consisting of an empty flask, 5.0 ng of the ^{13}C-2,3,7,8-TCDD tracer is added followed by 200 ml of concentrated HCl. The mixture is contained in a 500 ml Erlenmyer flask with a ground glass top.

3. Shake for one hour, or until the fish is completely dissolved.

4. Add 100 ml of hexane and repeat shaking for 12 hours or overnight.

5. Allow the layers to separate and withdraw as much hexane as possible. Transfer hexane to a 250 ml separatory funnel.

6. Add 75 ml fresh hexane to fish digestate and repeat mechanical shaking for 3 hours.

7. Allow layers to separate, withdraw the hexane and add it to the first hexane extract.

8. Discard digestate. Use caution with disposal. Treat as concentrated HCl.

Bulk Matrix Removal

1. Hexane extracts are given from one to four concentrated sulfuric acid washes in a separatory funnel to reduce matrix concentration.

2. The extract is reduced to a volume of 10 ml by rotary vacuum evaporation.

3. The extract is now passed through a dual column system. The top column is a Macro (see specialized glassware) which contains two layers: 1 g silica over 4 g 44% H_2SO_4 on silica (see adsorbent section). The bottom column is a High Aspect (see specialized glassware) and contains two layers: 2 g 33% 1N NaOH on silica over 1 g silica. Eluent from the top column passes onto the bottom column and is collected in a 125 ml Erlenmeyer flask with a 24/40 ground glass top. The sample container and columns are rinsed with two 10 ml portions of hexane and the total eluent is reduced to a volume of 5 ml by rotary vacuum evaporation and transferred, with two 5 ml rinses of hexane, to a 25 ml screw capped vial with a teflon lined cap and stored at -15°C until ready for adsorbant clean up.

Adsorbent Cleanup

1. The extract is put on the following dual column system: The top column is a Macro containing 1.5 g 10% $AgNO_3$ on silica and the bottom column is a High Aspect containing 5 g basic alumina. When drained to bed volume, the vial is rinsed with 3 ml hexane and this is placed on the column system. Rinsing is repeated two additional times. The system is now eluted with 30 ml hexane. When drained to bed level the top column is removed. Allow bottom column to drain to bed level, then elute with 50 ml of a 1:1 mixture of CCl_4 and hexane. The hexane and CCl_4:hexand effluents are discarded. Chlorinated dioxins are eluted with 22 ml of a 1:1 mixture of $CHCl_2$ and hexane. The effluent is collected in a 25 ml well-bottomed pyrex vial (see special glassware) and evaporated to 2 ml under Femtogas nitrogen and stored at -15°C until it is purified by HPLC.

Reverse Phase High Performance Liquid Chromatographic Cleanup

1. The HPLC is calibrated using chlorinated dioxin standards and appropriate collection zones are determined.

2. The cleaned up sample is evaporated to dryness under a gentle stream of Femtogas nitrogen.

3. The injection valve and column/detector system is purged with $CHCl_3$ to prevent sample contamination from the standard or the previous sample.

4. The residue is quantitatively transferred to the HPLC in the following manner: Place 16 ul of $CHCl_3$ in the well-bottomed vial containing the sample and allow to stand for approximately 10 minutes. While standing, the vial is periodically rotated to rinse the walls of the vial (only the well) with solvent. This is accomplished in such a manner so as to permit no contact of the solvent with the upper portions of the vial. A 50 ul Hamilton syringe, equipped with a non-tapered fixed needle for use with Rheodyne valves, is charged with 4 ul methanol followed by a 2 ul plug of air. The residue solution is carefully drawn into the syringe until an air bubble appears in the barrel. A second 16 ul aliquot of $CHCl_3$ is placed in the vial, rotated for rinsing and drawn into the 50 ul syringe. The total volume to be injected into the HPLC should not exceed 42 ul.

5. Chlorinated dioxin fractions are collected from the HPLC by allowing the methanol effluent to drain directly into a 25 ml volumetric flask charged with approximately 1 ml hexane. After collection, the solvents are mixed by swirling and then sufficient 1% aqueous $NaHCO_3$ is added to raise the organic layer into the neck of the volumetric flask. The hexane layer is transferred to a 7 ml well-bottomed vial. The remaining aqueous layer is thoroughly extracted 3 additional times with approximately 1 ml hexane. The hexane extracts are combined in the well-bottomed vial and stored frozen at -15 ^{o}C until analyzed by GC/MS.

GC/MS Quantification

1. The sample residue from the HPLC purification is thawed and gently evaporated to dryness in the vial

2. At least 10 minutes prior to analysis, the residue is redissolved in 10 ul of isooctane. If a high level of dioxin is anticipated, as evidenced by the presence of a crystalline solid, a larger volume of isooctane may be used.

3. A 1 to 2 ul aliquot of the sample is injected into the calibrated GC/MS system for final quantification. At least every sixth injection is a standard containing ^{12}C- and ^{13}C-2,3,7,8-TCDD at a concentration in the range of the samples.

Calculations

The concentration of 2,3,7,8-TCDD present in the fish sample is calculated by the following formula:

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

where:

A = peak area of native 2,3,7,8-TCDD in the sample

B = peak area of added ^{13}C-2,3,7,8-TCDD in the sample

C = peak area of ^{12}C-2,3,7,8-TCDD in the standard

D = peak area of ^{13}C-2,3,7,8-TCDD in the standard

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

F = mass of ^{13}C-2,3,7,8-TCDD in the standard (ng)

G = mass of ^{13}C-2,3,7,8-TCDD added to the sample (5 ng)

H = mass of the fish sample

The first term calculates the concentration of native 2,3,7,8-TCDD in the injection aliquot, while the second term corrects for recovery of the internal standard. In samples which do not contain any native 2,3,7,8-TCDD, the limit of detection for that sample is calculated by multiplying the second term by the minimum mass of 2,3,7,8-TCDD which can be detected by the GC/MS system.

Notes

A. Severe and transient interference problems associated with ultra-trace level determination of chlorinated dioxins have been observed. At least a part of this problem is related to adsorbent contaminations. Resulting from a great deal of experimentation, it has been determined that the described adsorbent cleanup procedures are the best currently available. All steps described herein are conducted using adsorbents prepared according to these procedures.

B. Another source of interferences for ultra-trace level chlorinated dioxin determinations had been shown to be related to contaminants present in cylinder gases used for evaporation of solvents. Attached procedures describe the Femtogas purification system. All evaporations in the method are accomplished with Matheson prepurified nitrogen passed through the Femtogas system.

C. All chromatography columns described used glass wool as a bed support. Glass wool is Soxhlet extracted in benzene for 24 hours, CH_2Cl_2 rinsed and air dried before use. All silica and modified silica columns are prewashed with 15 ml hexane just prior to use.

D. The $AgNO_3$/silica removes DDE chemically and provides marginal fractionation of dioxins from their benzyl phenyl ether counterparts with the dioxins eluting ahead of the ethers. Also, this column chemically removes sulfides, aliphatic halides, weak acids, phosphates and a variety of unknown color bodies.

E. The caustic silica column removes water soluble species, acids, phenols and other base-sensitive compounds.

F. The high aspect basic alumina column removes any remaining PCBs, PCTs, DDE and gives some separation of benzyl phenyl ethers.

G. Final reverse phase HPLC provides separation of components present in the residue. TCDDs are well separated from Cl_5-benzyl phenyl ethers. This step also allows each dioxin class to be collected in separate fractions (Cl_4 through Cl_8). In this manner, high levels of OCDD in the sample will not cause an interference when determining very low TCDD levels.

Equipment

A. Gas Chromatograph/Mass Spectrometer (GC/MS) Hewlett-Packard model 5985 equipped with manual override accessory.

B. Column for GC/MS: 60 m x 0.32 mm i.d. wide bore fused silica capillary column coated with a bonded stationary phase of 5% phenyl, methyl silicone (equivalent to OV-101) of 0.25 um film thickness, available from J + W Scientific Cordova, CA 95670.

C. Liquid Chromatograph (HPLC): Altex microprocessor controlled system model 420 equipped with a Hitachi ultra-violet detector and flow cell (model 100-40). Available from Altex Scientific, Berkely, CA 94710.

D. Liquid chromatographic pump: Altex model 110 pump. Available from Altex Scientific, Berkeley, CA 94710.

E. Column oven: DuPont Column compartment. Available from DuPont Instruments, Wilmington, DE 19899.

F. Liquid chromatographic column: Two DuPont Zorbax-ODS RP-18 columns, 6.2 x 250 mm, connected in series. Available from DuPont Instruments, Wilmington, DE 19899.

G. Liquid chromatographic injector: Rheodyne model 7120 high pressure injector, equipped with a 50 ul sample loop. Available from Rheodyne Inc., Berkeley, CA 94710.

H. Glass chromatography columns: Macro and High Aspect columns. See specialized glassware section for dimensions.

I. Temperature controlled clam-shell tube furnace equipped with a Pyrex glass tube having appropriate inlet and outlet joints and fittings. Available as a "Hevi-Duty" tube furnace from Lindberg Furnace, Milwaukee, WI.

J. Well-bottomed vials: 7 ml and 25 ml sizes. See specialized glassware section.

K. Femtogas purification train: See specialized glassware section.

Reagents

A. Solvents: All solvents are distilled-in-glass quality available from Burdick and Jackson, Muskegon, MI.

1. Benzene
2. Hexane
3. Methylene Chloride
4. Carbon Tetrachloride
5. Methanol
6. Chloroform
7. Isooctane

B. Carbon tetrachloride in hexane, 50% by volume

C. Methylene chloride in hexane, 50%by volume

D. Silica gel: Chromatographic grade silica acid as 100/120 mesh Bio-Sil A, available from Bio-Rad Laboratories, Richmond, CA 94804.

E. Basic alumina: Chromatographic grade aluminum oxide as 100/200 mesh Bio-Rad Basic alumina AG-10, available from Bio-Rad Laboratories, Richmond, CA 94804.

F. Silver Nitrate, $AgNO_3$ crystal: Baker Analyzed Reagent, available from J.T. Baker Chemical Company, Phillipsburg, NJ.

G. Sodium Hydroxide, Available from J.T. Baker Chemical Co. Made to 1N aqueous solution in organic-free distilled water.

H. Sodium bicarbonate $NaHCO_3$: Baker Analyzed Reagent, available from J.T. Baker Chemical Co. Made to a 1% by weight aqueous solution in organic-free distilled deionized water.

I. Phosphorus pentoxide: Baker Analyzed Reagent available from J.T. Baker Chemical Co.

J. Purified water: Distilled water is passed through the Millipore-Q water purifier (mixed ion-exchange bed, carbon filter and millipore filter) for use in this method, available from Millipore Corp., Bedford, MA 01730.

K. Femtogas nitrogen: Cylinder nitrogen gas (Matheson, 99.9% pure) is further purified by passing through the purification train described in the specialized glass section.

L. Chlorinated dioxin standards: Available from Analabs, North Haven, CT 06473. Samples of ^{12}C and ^{13}C-2,3,7,8-TCDD are obtained from the Dow Chemical Company, Midland, MI.

Preparation of Adsorbents

A. Silica

1. Approximately 35 g silicic acid is gently packed into the tube furnace in a pyrex glass tube using a glass wool plug as the bed support. Appropriate connections are made to permit a continuous dry nitrogen purge, and the system is placed in a clam-shell tube furnace adjusted to 180°C. The adsorbent is maintained at these conditions for a period of 30 minutes.

2. The glass tube is removed from the furnace and cooled to ambient temperature.

3. Nitrogen purging is discontinued and consecutive approximately 75 ml aliquots of methanol then methylene chloride are passed through the adsorbent bed.

4. The methylene chloride saturated adsorbent is returned to the tube furnace which has been adjusted to approximately 50°C and the dry nitrogen purge is reestablished. Over a period of approximately 25 minutes the furnace temperature is increased in a stepwise manner to 180°C. The adsorbent is maintained at 180°C for 90 minutes. Caution: the effluent gases from this operation must be vented to a fume hood.

5. The dried and activated silica is cooled, transferred to a capped bottle and stored in a desiccator over phosphorus pentoxide until used.

B. Sodium Hydroxide 33% 1N/Silica

1. The silica support for this reagent is solvent rinsed and activated by the procedure given in part A.

2. Approximately 35 g silica is placed in an 500 ml bottle. Sufficient 1N aqueous sodium hydroxide is added directly to the silica so as to yield a concentration of 33% based upon total weight.

3. The adsorbent is shaken manually until no clumping can be observed and is then transferred to a capped glass bottle for storage until use. The reagent should not be stored in a dessicator.

C. Silver Nitrate 10%/Silica

1. The silica support for this reagent is solvent rinsed and activated by the procedure given in part A.

2 Approximately 35 g silica is placed in an amber glass bottle and its weight is determined.

3. Using the support weight, calculate the amount of silver nitrate required to yield 10% by weight based on the resulted total weight. Similarly, a second calculation is made to determine the amount of de-ionized water required to yield 30% by weight weight based upon the total of silica and the water only. In accordance with the determined quantities, the silver nitrate is dissolved in de-ionized water.

4. The aqueous silver nitrate solution is added to the silica support in a stepwise fashion with gentle shaking to produce a uniformly coated, free flowing powder. The finished product is allowed to stand for a minimum of period of 30 minutes prior to drying.

5. The adsorbent is gently packed into the pyrex glass tube using a glass wool plug as the bed support. Appropriate connections are made to permit a continuous dry nitrogen purge, and the system is placed in the tube furnace adjusted to approximately 70 °C. In a stepwise manner, over a period of approximately 5 hours the temperature is increased to 125° C. Provisions should be made to permit condensate to drain from the exit port during this phase of the preparation.

6. From the point that condensation ceases, the adsorbent is activated for an additional period of approximately 15 hours at 125°C.

7. The finished adsorbent is cooled and stored in a capped amber glass bottle in a desiccator over phosphorus pentoxide until used.

D. Basic Alumina

1. Approximately 50 g aluminum oxide is gently packed into the pyrex glass tube using a glass wool plug as the bed support. Appropriate connections are made to permit a continuous dry nitrogen purge, and the system is placed in the tube furmace. The temperature is increased in a stepwise fashion to 300 °C where it is maintained for a period of approximately 60 minutes.

2. The glass tube is removed from the furnace and cooled to ambient temperature.

3. Nitrogen purging is discontinued, and approximately 150 ml of methylene chloride is passed through the adsorbent bed.

4. The methylene chloride saturated adsorbent is returned to the tube furnace which has been adjusted to approximately

50°C and the dry nitrogen purge reestablished. Over a period of approximately 25 minutes the furnace temperature is increased to approximately 180°C. This temperature is maintained until solvent condensation at the exit port ceases. The basic alumina is then activated for an additional period of 90 minutes at 300°C. Caution: the effluent gases from this operation must be vented to a fume hood.

5. The finished adsorbent is cooled and stored in a capped amber glass bottle in a desiccator over phosphorus pentoxide until used.

E. Sulfuric Acid, 44%, on Silica

1. The silica support for this reagent is solvent rinsed and activated by the procedures described in part A.

2. Approximately 35 g silica are placed in a 500 ml bottle. Sufficient concentrated sulfuric acid is added directly to the silica to yield an acid concentration of 44% based on total weight.

3. The material is manually shaken until no clumping can be observed, and is then transferred to a glass bottle and stored in a desiccator over phosphorus pentoxide until used.

4. CAUTION: This reagent retains all the properties of concentrated sulfuric acid and should be handled accordingly.

F. Nitrogen for Evaporation

Another source of interferences for ultra-trace level dioxin determinations has been shown to be related to dioxin contaminants present in cylinder gases used for solvent evaporation. This contamination generally appears as an increased background during GC/MS analysis. This section describes the components of the Femtogas Purification System. Basically, the Femtogas system consists of a series of adsorbent traps designed to remove a wide variety of impurities from the influent gas stream. The physical arrangement of the Femtogas evaporation train is presented in section G.

1. First and second columns: Adsorption/Partition system. This column packing is designed to be the primary "knock-down" trap for the blow-down gas cleanup system. Tank gas (Nitrogen) will pass into these columns first. Basically it is a composite system similar to a GC packing. 60/80 mesh Chromosorb W-AW serves as the porous support

matrix for the active adsorption and partition agents. The adsorbent is an equal mixture of "micronized" carbons: graphite (Carbopack B) and active carbon (Amoco Active Carbon, Grade PX-21), and Apiezon L. The adsorbent is prepared by grinding equal amounts of graphite Carbopack B and Amoco Active Carbon with a mortar and pestle. Chloroform is poured on the mixture in a beaker and sonified for 30 minutes. An amount of Apiezon L, equal to the mass of Carbopack B is dissolved into the mixture. Chromosorb W-AW (60/80 mesh) is added to the mixture to establish the adsorbents as 10% by weight of each on Chromosorb W-AW. The chloroform is evaporated by rotary vacuum evaporation to yield a free-flowing powder.

2. Third Column: Molecular Sieve
The 13X molecular sieve column cleanses the gas stream of water vapor and residual hydrocarbons. Gas chromatography grade 80/100 mesh material was pre-conditioned and activated at 400 °C with 100 cc/min nitrogen flow for approximately four hours in a tube furnace prior to use.

3. Fourth Column: 20% H_2SO_4 on silica
Exposure of the blow-down gas to concentrated sulfuric acid on a high surface area support provides an efficient chemical cleansing action in addition to the partition and adsorption mechanisms described. This packing will remove trace water vapor and a large variety of reactive organics (i.e., amines, alcohols, unsaturates, aromatics, esters and other oxidizable species) without significantly impeding the flow rate characteristics of the system.

4. Fifth Column: Carbosieve S (60/80 mesh)
Carbosieve S is a highly purified, spherical form of carbon molecular sieve available from Supelco for use as a gas chromatography adsorbent. Because of its high surface area (approximately 560 m^2/g) and small pore size (19-12A) this material demonstrates a very high degree of adsorptivity for most organics. This reagent is used as received from the supplier without further purification.

Specialized Glassware

A. Well-bottomed Vials

Standard Pyrex screw cap vials (7ml and 25ml) with teflon lined caps are modified to provide a 0.2 ml well in the bottom, similar to a reacti-vial. This obviates extra sample transfer steps and provides a convenient vessel for reconstitution of evaporated extracts with minimal solvent volumes. The vials are heated at the bottom by an oxygen-propane torch until the glass becomes red-hot and begins to melt. A 5 mm wide hardened steel Phillips-head screwdriver is inserted into the vial and gently

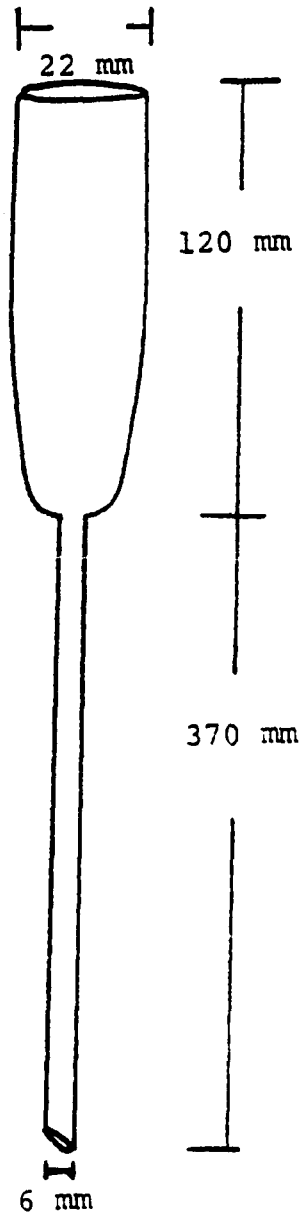
pressed into the vial bottom, forming a well and quickly removed. The vial is annealed by heating, allowed to cool slowly and used.

B. Macro Column

The Macro column is a modified 10 ml disposable glass pipette, 10 mm i.d. x 25 cm long. The pipette is scored approximately 15 mm below the top and broken off to remove the narrow lip.

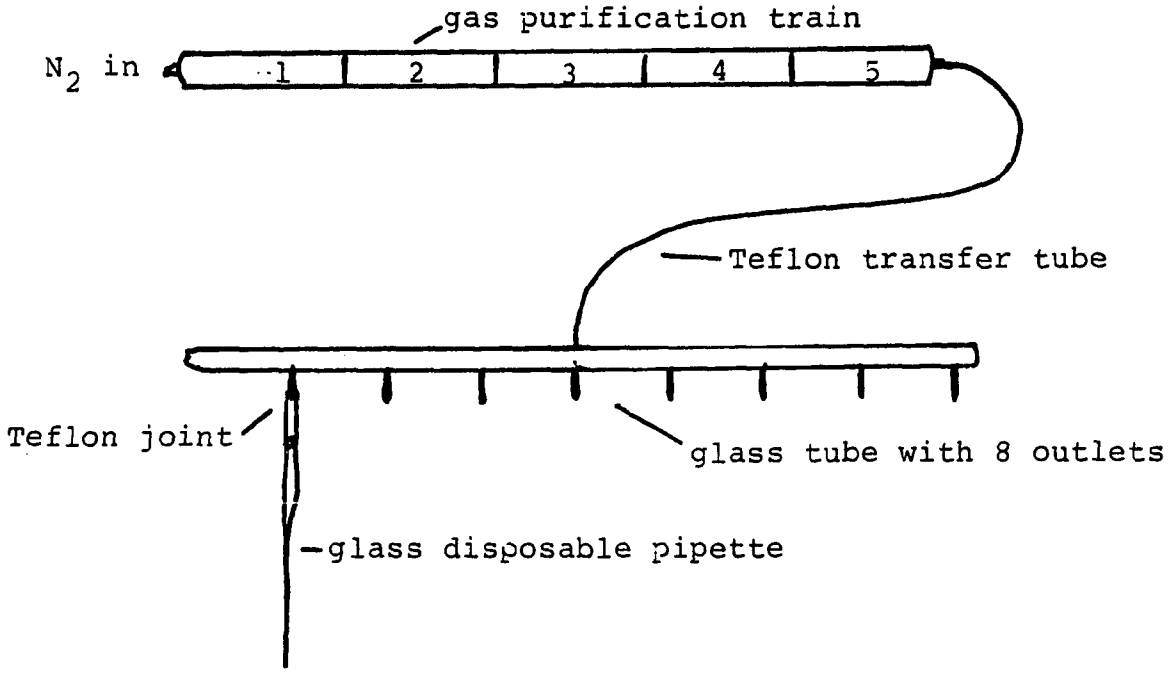
C. High Aspect Column

The High Aspect column is fabricated by a professional glassblower to the following specifications:



D. Femtogas Purification Train

The Femtogas adsorbents described in the previous section are packed over extracted glass wool into a series of 10 cm x 1 cm i.d. ground glass jointed glass tubes. All transfer lines are of teflon tubing. The effluent from the train is plumbed to a glass tube with eight outlets (pictured below). A disposable transfer pipette is attached to each outlet via a piece of teflon tubing, providing a disposable orifice for sample evaporation which is discarded after each use, preventing cross-contamination of samples.



Operating Conditions

A. Liquid Chromatographic Conditions

1. Temperature of HPLC column: 50°C
2. Eluent: 2% H_2O in methanol at 1.0 ml/min
3. UV detector Conditions:
   a. Wavelength: 235 nm
   b. Sensitivity: 0.01 absorbance units full scale
4. Injection volume: approximately 35 ul

B. GC/MS Conditions

1. Column arrangement: Positioned directly into the source area, bypassing the jet separator.
2. Temperatures:
   a. Column: Temperature programmed from 50°C to 250°C at 20°C/min. Isothermal at 250°C.
   b. Injection port: 250°C.
   c. Transfer line: 250°C.
   d. Source: 250°C.
3. Carrier conditions:
   a. Carrier gas: Helium
   b. Flow rate: 16.4 cc/min
   c. Column head pressure: 19.1 p.s.i.
   d. Split ratio: 10:1
4. Sample volume: 1-2 ul injected in the splitless mode.
5. Ions monitored: TCDD m/e⁻: 319.9, 321.9, 323.9
   ^{13}C-TCDD: 331.9
   masses offset tuned to maximize at 320.0, 322.0, 324.0, and 332.0
6. Ion dwell times: 50 msec at each of the four ions.
7. Elctron multiplier voltage: 2600 V.

Calibration

A. Liquid Chromatographic Calibration

1. The HPLC system is used to separate various chlorinated dioxin fractions. A mixed standard containing 1 mg/l each of 1Cl-DD, 2-Cl-DD, 2,3-Cl_2-DD, 2,7-Cl_2-DD, 1,2,3-Cl_3-DD, 1,2,3,4-TCDD, 2,3,7,8-TCDD, a H_6CDD mixture, H_7CDD and OCDD is injected to establish appropriate collection zones.

B. GC/MS Calibration

1. The GC/MS is calibrated by injecting 2 ul of a standard with 500 ng/ml 2,3,7,8-TCDD and 500 ng/ml of the ^{13}C-2,3,7,8-TCDD internal standard in isooctane. When constant response is achieved (within 10%), calibration is complete.

2. Response is 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 quantification.

D. SYNTHESIS AND DETERMINATION OF RESOLUTION OF TCDD ISOMERS

The determination of 2,3,7,8-TCDD residues is confounded by the existence of 22 different tetrachlorinated dioxin isomers. Although each isomer has unique chemical and physical properties, there is no way of differentiating between the 22 isomers on the basis of their electron impact (EI) mass spectrum. All of the isomers exhibit molecular ion clusters at m/e 319.9, 321.9 and 323.9 and could be erroneously interpreted as 2,3,7,8-TCDD if they were to co-elute with 2,3,7,8-TCDD during final capillary GC/MS quantification. A false positive assignment can be prevented by maximizing the GC resolution of 2,3,7,8-TCDD from the other 21 TCDD isomers. This requires the determination of the capillary GC retention time of each isomer using authentic TCDD standards.

Standards of the 22 TCDD isomers are not commercially available. Only a few laboratories possess TCDD standards in limited amounts and they are reluctant to give up any of this valuable material. Since complete resolution of 2,3,7,8-TCDD from the other TCDDs was a major goal of this work, the required dioxins were synthesized for use as standards.

Chlorinated dioxins can be synthesized by the condensation of ortho chlorinated phenol salts, by chlorination of dibenzodioxin or by regiospecific syntheses designed to yield isomerically pure chlorinated dioxin isomers. The synthetic scheme utilizing ortho chlorinated phenolates is favored over the others because it uses readily available starting materials whose pyrolysis products are predictable (149).

The major drawback of synthesis of TCDD by the condensation of chlorophenate salts is the formation of more than one TCDD product per chlorophenate pyrolyzed. Figure 6 outlines the reaction pathways of 2,3,6-trichlorophenate. Four different TCDDs are expected from the pyrolytic condensation: two representing direct condensation and two products arising via a Smiles rearrangement (149). Pyrolytic condensation of various chlorophenates yields from one to nine TCDD products which can be predicted on the basis of the starting materials. Although this technique is not suitable for synthesis of pure isomers of TCDD, it can be very effectively utilized to evaluate the chromatographic resolution of the TCDDs. Peak assignment is carried out on the basis of recognition of patterns of TCDD isomers common to certain pyrolyzates. If necessary, small quantities of pure isomers can be isolated by collection of HPLC fractions provided that complete resolution of the particular isomer is achieved.

1. Experimental

Mixtures of TCDD isomers are synthesized by a controlled flow pyrolysis technique (149). A solution of ortho chlorophenate salt is adsorbed onto purified silicic acid, packed into a reaction tube and heated in a tube furnace under a flow of nitrogen. The chlorophenol salt reacts to form chlorinated dioxins, polymeric tars and predioxins. The dioxins are eluted from the reaction tube with methylene chloride, leaving the more polar materials adsorbed on the silica matrix.

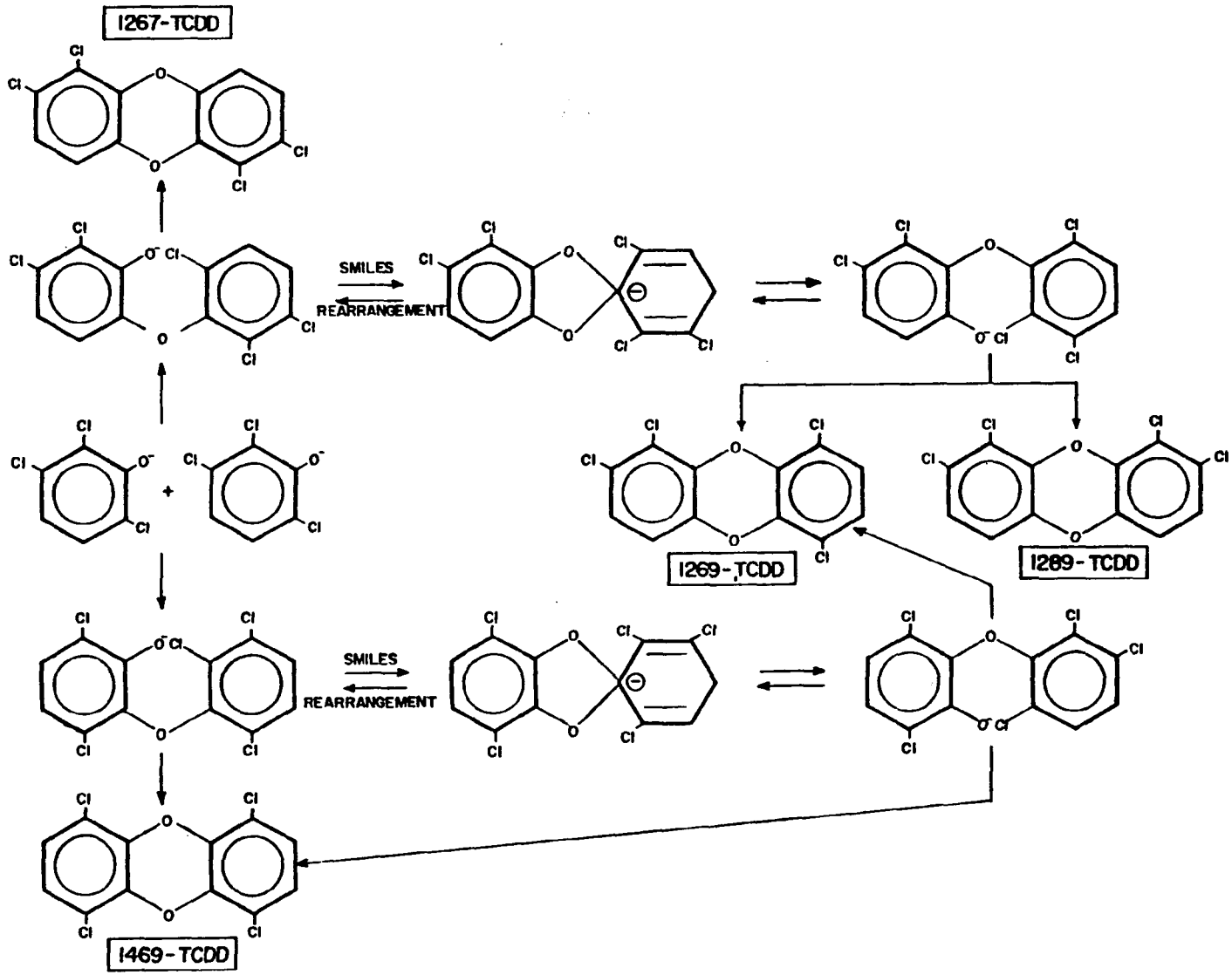


Figure 6.

Reaction Pathways of 2,3,6-trichlorophenate

A. Reagents

1. Chlorophenols obtained from Aldrich (Miwaukee, WI) are used as supplied. All were of reported purity of at least 98%.

2. Silicic acid (Bio-Sil 100/200 mesh) obtained from Bio-Rad Laboratories is purified by washing in concentrated HCl followed by numerous rinses in distilled, deionized water, methanol and finally methylene chloride. The silica is then activated in an oven at 190°C for a minimum of 48 hours, transferred to a capped bottle and stored in a desiccator over phosphorus pentoxide.

3. Caustic silica is prepared by adding 35 ml of 1M aqueous potassium hydroxide solution to 100 g of purified silica. the mixture is shaken until it becomes a free-flowing powder, transferred to a beaker and then activated in an oven at 190°C for at least 48 hours. The caustic silica is stored in the same manner as the purified silicic acid.

4. Purge gas for the pyrolysis reactor is pre-purified nitrogen (Matheson 99.99%) and used as supplied.

B. Procedure

2:2 TCDD Isomers

1. Trichlorophenol (200 mg or 100 mg of each for mixed reactions) is dissolved in 7 ml of 1M KOH in methanol.

2. The solution is extracted twice with 10 ml of hexane and the hexane is discarded.

3. The chlorophenate solution is combined with 12 g of purified silica and manually shaken until a free-flowing powder is produced.

4. The chlorophenate silica is poured into a 180 mm x 50 cm pyrex tube which contains 6 g of caustic silica over a glass wool plug.

5. The reaction tube is supported vertically and placed into a Lindbergh tube furnace.

6. The inlet (K-phenate) side of the tube is connected to a tank of nitrogen, the exhaust is piped through activated carbon and a 200 cc/min nitrogen flow is established.

7. The temperature of the tube furnace is adjusted to 130°C for 25 min or until no condensate is visible at the exit port . The nitrogen flow is reduced to 10 cc/min.

8. The furnace temperature is raised to 180°C for 10 min to remove trace residual water and then increased to 280°C, where it is maintained for 30 min.

9. The reaction tube is removed from the tube furnace to cool and the nitrogen flow is stopped.

10. The cooled reaction tube is eluted (K-phenate on top) with two 50 ml portions of methylene chloride.

11. The extract is reduced in volume to 3 ml by rotary evaporation and transferred to a 30 ml screw cap vial with three 5 ml rinses of methylene chloride.

12. The extract is evaporated to dryness under a stream of purified nitrogen.

13. Aqueous KOH (15 ml) is added to the residue. The mixture is shaken and then extracted three times with 10 ml portions of hexane.

14. The hexane extract is washed with three 10 ml portions of distilled, deionized water, filtered through Na_2SO_4, transferred into a tared 30 ml screw cap vial, evaporated to dryness and weighed for calculation of yield.

3:1 TCDD isomer syntheses

The TCDD isomers with three chlorines on one ring and one chlorine on the other are synthesized and purified in the same manner as the 2:2 isomers, but require a slight modification in the reaction train as a consequence of the volatiliy of dichlorophenols. The modification limits the hydrolysis of the dichlorophenate by assuring more rapid, thorough drying of the chlorophenate plug. A 5 cm plug of glass wool is used in place of the silica support. The chlorophenate solution is transferred onto it and the plug is dried at 120°C for 15 min under 200 cc/min nitrogen flow. After cooling, 6 g of caustic silica is packed in the reaction tube between two pieces of glass wool. The glass tube is slid up so that the chlorophenate plug is outside the heated zone and the oven temperature is raised to 280°C. A nitrogen flow of 40 cc/min is now established and the reactor tube is slid into the heated zone for 15 min. After cooling, the tube is eluted with two 50 ml portions of methylene chloride and the extract is processed as in the 2:2 syntheses.

2. GC/MS Evaluation of Pyrolysis products

One ul of pyrolysis extract (approximately 20 ng) is injected into a Hewlett-Packard GC/MS model 5985 fitted with a 60 met x 0.32 mm i.d. fused silica capillary GC column coated with a bonded phase (DB-5) of 0.25 um film thickness. The column is available from J & W Scientific, Rancho Cordova CA 95670. The column is routed directly into the source area, bypassing the jet separator. Injection is accomplished in the splitless mode. The GC/MS is operated under the following conditions:

Column head pressure: 19.1 p.s.i.

Injection port temperature: 250°C

Transfer line temperature: 250°C

Source temperature: 250°C

Column flow rate: 16.4 cc/min

Temperature programming: 50°C to 250°C at 20°C/min. then isothermal at 250°C

Specific ions monitored: 319.9, 321.9, 323.9, 325.9 offset tuned to maximize at 320.0, 322.0, 324.0, 325.0

Ion dwell times:
320.0 = 300 msec
322.0 = 400 msec
324.0 = 200 msec
326.0 = 100 msec

Electron multiplier voltage: 2600 V

Scan delay: 14.0 min.

Pyrolysis products are also evaluated by electron capture capillary GC and by reversed and normal (silica) phase HPLC.

3. Results

Table 12 lists the yields and expected TCDD products of the ortho chlorophenate pyrolyzates. The yields, calculated on the basis of the final mass of the crude pyrolyzates, ranged from 1.5 to 12.6%. Actual yields of TCDDs were difficult to estimate due to the presence of non-TCDD impurities present in the extracts. Electron-capture gas chromatography and UV-detection HPLC profiles of the crude pyrolyzates revealed a larger number of peaks than observed by TCDD-specific selected ion GC/MS chromatograms. The impurities were present at concentrations similar to the TCDDs, when evaluated on the basis of their peak heights. They eluted in the region representing compounds more polar than the TCDDs and were most likely TCDD precursors such as chlorophenoxyphenols, chlorinated diphenyl ethers and lower chlorinated dioxins such as DCDDs and T_3CDDs. No attempts were made to characterize them by GC/MS.

The Table of expected products (Table 12) indicates that certain isomers occur in more than one pyrolyzate. For example, 1,2,6,7 and 1,2,8,9-TCDD are produced from the pyrolysis of 2,3,6- as well as 2,3,4-trichlorophenate. The added products arise as a result of a Smiles rearrangement. Additionally, in the mixed reactions of two different chlorophenates, new products are produced, but condensation of the two identical chlorophenates also takes place, yielding previously observed TCDDs. These repeating patterns, along with the formation of unique isomers, are the basis for assigning identities to peaks observed by capillary GC/MS of the chlorophenol pyrolyzates.

Because most pyrolyzates yield pairs of TCDDs, the identity of many isomers cannot be definitely assigned by their GC/MS peak profiles. Nonetheless, this method allows assigning their identities as one of two possible isomers, ruling out the other 20. Table 13 lists the capillary GC/MS retention times and identities of the TCDD peaks determined on the basis of the chromatograms of their parent pyrolyzates, presented in Figure 7. Five peaks were assigned positive identities, 10 peaks were identified as each representing one of two possible isomers and three pairs of TCDD isomers were identified, but could not be chromatographically resolved from each other. The identity of the peak representing 2,3,7,8-TCDD was further confirmed by the use of an authentic standard. 1,2,3,4-TCDD was not evaluated in a pyrolyzate, but was later assigned a retention time by capillary GC, using an authentic standard.

Tentative identities for the 5 resolvable pairs of peaks are also presented in Table 13. The identities were assigned on the basis of observed structural trends extended by a prediction of their ability to undergo resonance polarization. It appears that elution time on the DB-5 column is effected by the ability of the pi electrons of the chlorine atoms to become resonance delocalized. Structural features, such as vicinal, peri and lateral substitution were rated as to their expected effects on resonance forms and used as a basis for determining the relative retention times of the pair members. The tentative identities were also confirmed by comparison to a chromatogram published by Buser (184). The elution order of authentic standards of TCDD on a glass capillary column coated with OV-1 (equivalent

to DB-5) observed by Buser were identical to those determined on our system.

Inspection of the retention times of the TCDD isomers listed in Table 13 and presented in Figure 7 reveals that complete resolution of all 22 isomers was not achieved. 1,3,6,9-TCDD (peak 5) was not resolved from the 1,2,4,7- + 1,2,4,8-TCDD peak (peak 6); 1,2,6,8-TCDD (peak 8) was not resolved from 1,4,7,8-TCDD (peak 9) and the 1,2,3,6-, 1,2,6,9-, 1,2,3,7- and 1,2,3,8-TCDD group (peaks 11, 12 and 13) could not be resolved. However, the isomer of interest, 2,3,7,8-TCDD, was resolved from all of the other TCDD isomers. Its nearest eluting peak is the one representing 1,2,3,7- + 1,2,3,8-TCDD (peak 13). A mixture of 1.0 ng of 2,3,7,8-TCDD and the parent pyrolyzate of 1,2,3,7- and 1,2,3,8-TCDD is presented in Figure 7 . Although there is some overlap, the resolution is great enough to prevent a false positive value during sample quantification. In the event of variations in retention time during quantification, the 1,2,3,7- plus 1,2,3,8-TCDD peak could be mistakenly interpreted as 2,3,7,8-TCDD. However, the ^{13}C-2,3,7,8-TCDD internal standard would prevent this misinterpretation because its retention time would also vary, corresponding to native 2,3,7,8-TCDD.

On the basis of the results of this section, it can be concluded that 2,3,7,8-TCDD is satisfactorily resolved from the other 21 TCDD isomers. The achievement of this high a chromatographic resolution assures the positive confirmation of 2,3,7,8-TCDD by GC/MS during the final quantification of the fish sample extracts.

Table 12.

Products and Yields of Chlorophenate Pyrolyses

Starting Phenol(s)	Expected Products	Crude Yield
2,3,4-TCP[a]	1,2,6,7-TCDD 1,2,8,9-TCDD (S)[d]	8.0%
2,3,5-TCP	1,3,6,8-TCDD 1,3,7,9-TCDD (S)	12.6%
2,3,6-TCP	1,4,6,9-TCDD 1,2,6,9-TCDD 1,2,6,7-TCDD 1,2,8,9-TCDD (S)	8.6%
2,3,4-TCP + 2,3,5-TCP	(2,3,4)[e] products (2,3,5) products 1,2,6,8-TCDD 1,2,7,9-TCDD (S)	10.9%
2,3,4-TCP + 2,3,6-TCP	(2,3,4) products (2,3,6) products	9.2%
2,3,4-TCP + 2,4,5-TCP	(2,3,4) products 2,3,7,8-TCDD 1,2,7,8-TCDD	7.0%
2,3,5-TCP + 2,3,6-TCP	(2,3,5) products (2,3,6) products 1,3,6,9-TCDD 1,2,7,9-TCDD 1,2,6,8-TCDD (S)	4.1%
2,3,6-TCP + 2,4,5-TCP	(2,3,6) products 2,3,7,8-TCDD 1,4,7,8-TCDD 1,2,7,8-TCDD	3.0%
2,3,4,5-TeCP[b]+ 2,4-DCP[c]	1,2,3,7-TCDD 1,2,3,8-TCDD (S)	1.5%
2,3,4,6-TeCP + 2,6-DCP	1,2,3,9-TCDD 1,2,3,6-TCDD	2.1%
2,3,5,6-TeCP + 2,4-DCP	1,2,4,8-TCDD 1,2,4,7-TCDD (S)	
2,3,5,6-TeCP + 2,6-DCP	1,2,4,6-TCDD 1,2,4,9-TCDD	2.5%

a) TCP = trichlorophenol
b) TeCP = tetrachlorophenol
c) DCP = dichlorophenol
d) (S) = Smiles rearrangement product
e) ( ) = TCDD products from previous pyrolysis

Table 13

Capillary GC Retention Times of the 22 TCDDs

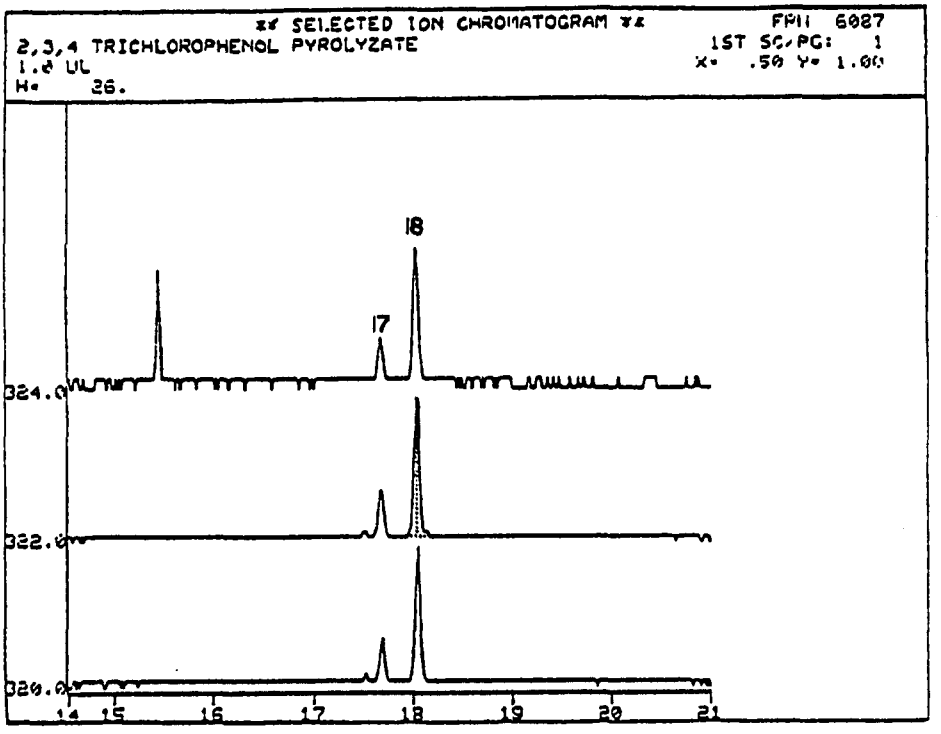
Peak[a]	Retention Time (min)	TCDD Isomer Pair	Assigned Identity
1	15.80	1,3,6,8/1,3,7,9[b]	1,3,6,8-TCDD
2	16.0	1,3,6,8/1,3,7,9	1,3,7,9-TCDD
3	16.10		1,3,6,9-TCDD
4	16.48	1,2,4,7 + 1,2,4,8[c]	1,2,4,7 + 1,2,4,8
5	16.53		1,3,7,8-TCDD
6	16.55	1,4,6,9/1,2,6,9	1,4,6,9-TCDD
7	16.62	1,2,4,6 + 1,2,4,9	1,2,4,6 + 1,2,4,9
8	16.70	1,2,6,8/1,2,7,9	1,2,6,8-TCDD
9	16.72		1,4,7,8-TCDD
10	16.95	1,2,6,8/1,2,7,9	1,2,7,9-TCDD
	17.05		1,2,3,4-TCDD
11	17.12	1,2,3,9/1,2,3,6	1,2,3,9-TCDD
12	17.15	1,4,6,9/1,2,6,9	1,2,6,9-TCDD
13	17.18	1,2,3,7 + 1,2,3,8	1,2,3,7 + 1,2,3,8
14	17.28		2,3,7,8-TCDD
15	17.42	1,2,3,9/1,2,3,6	1,2,3,9-TCDD
16	17.54		1,2,7,8-TCDD
17	17.65	1,2,6,7/1,2,8,9	1,2,6,7-TCDD
18	18.03	1,2,6,7/1,2,8,9	1,2,8,9-TCDD

a) peak numbers refer to peaks presented in figure 7

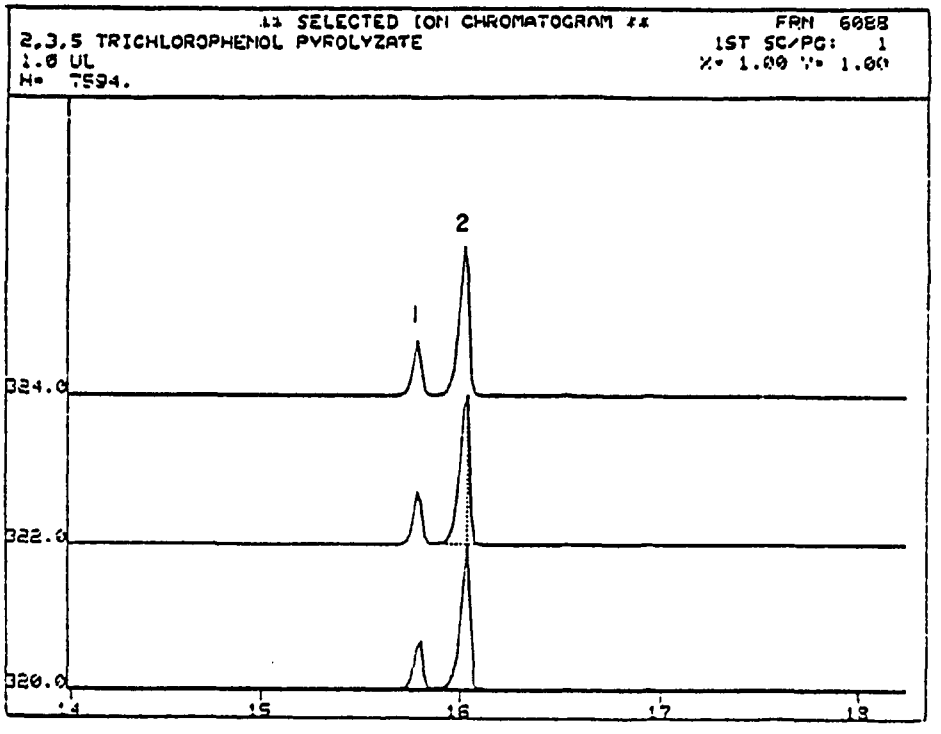
b) x/y = denotes a resovable product pair: x or y

c) x + y = denotes a non-resolvable pair: x and y

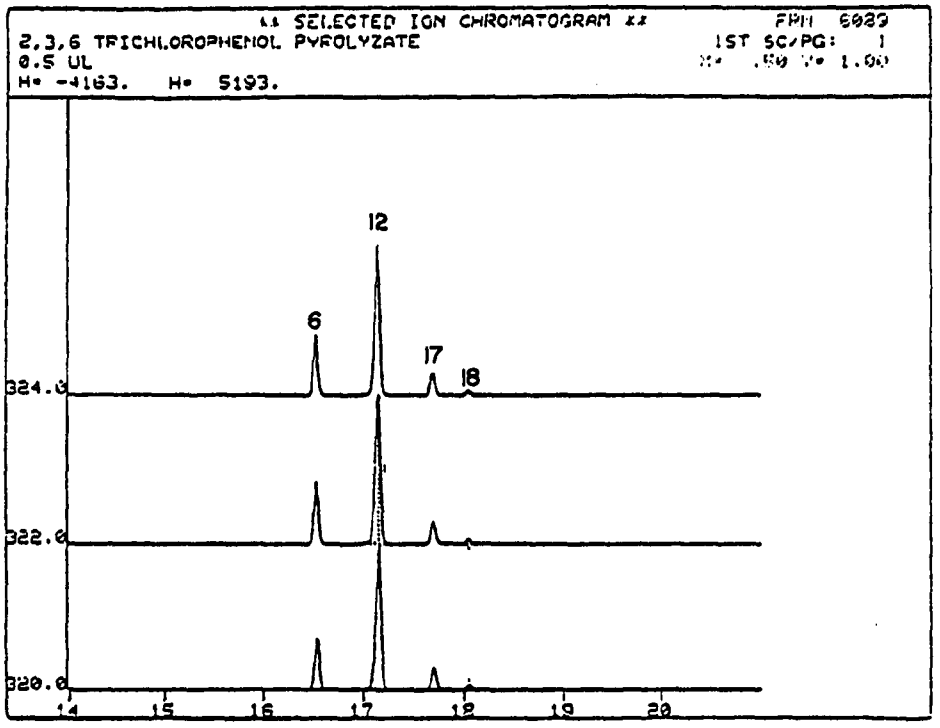
Figure 7. Specific ion GC/MS chromatograms of TCDD isomers by pyrolysis of selected trichlorophenol salts



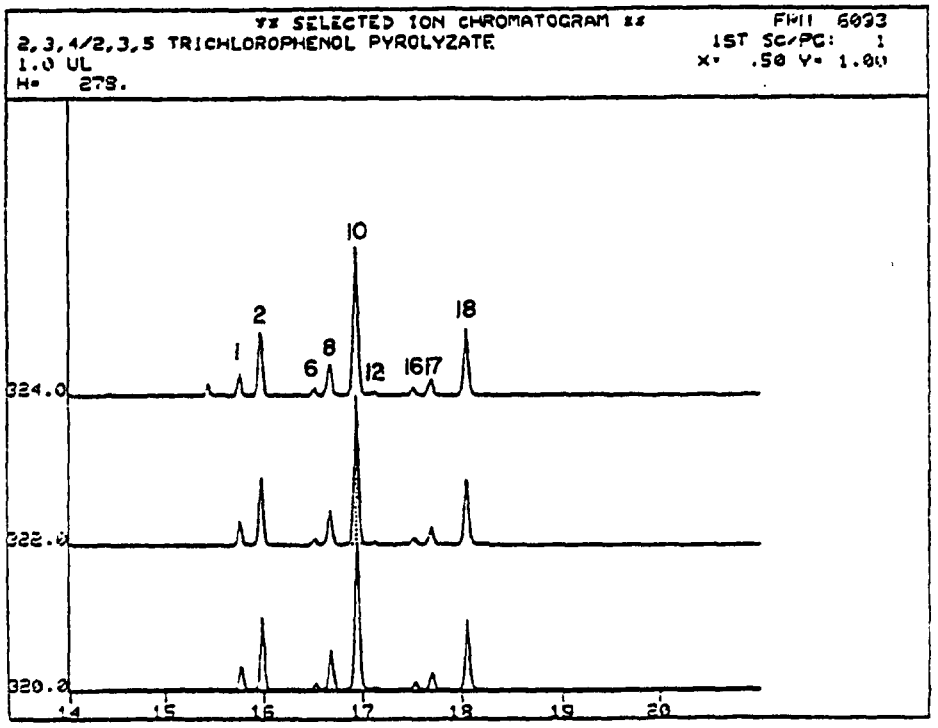
7a 2,3,4-trichlorophenate products



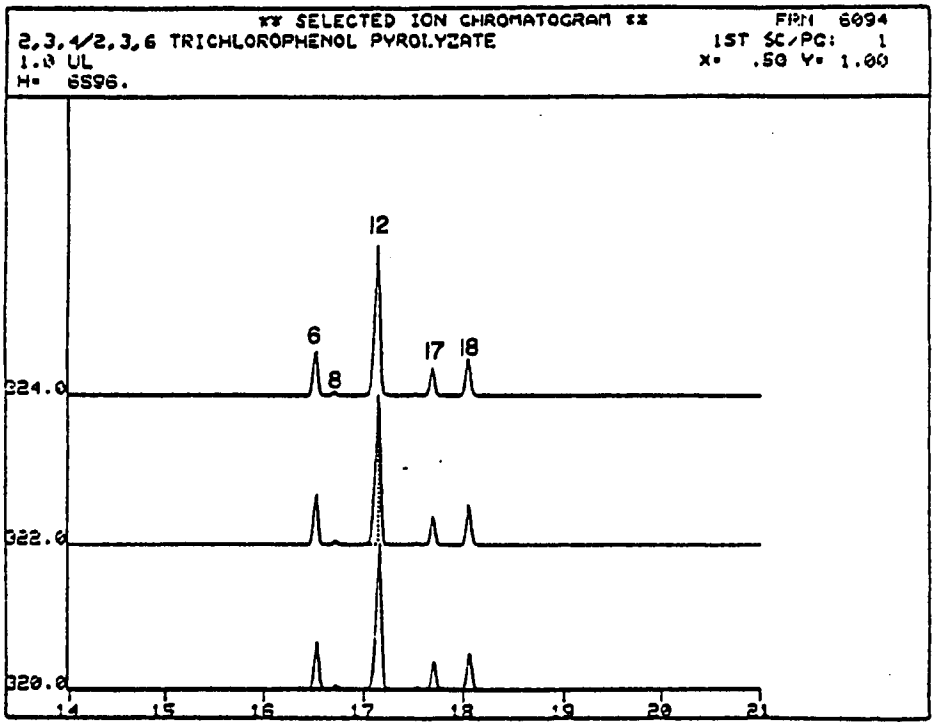
7b 2,3,5-trichlorophenate products



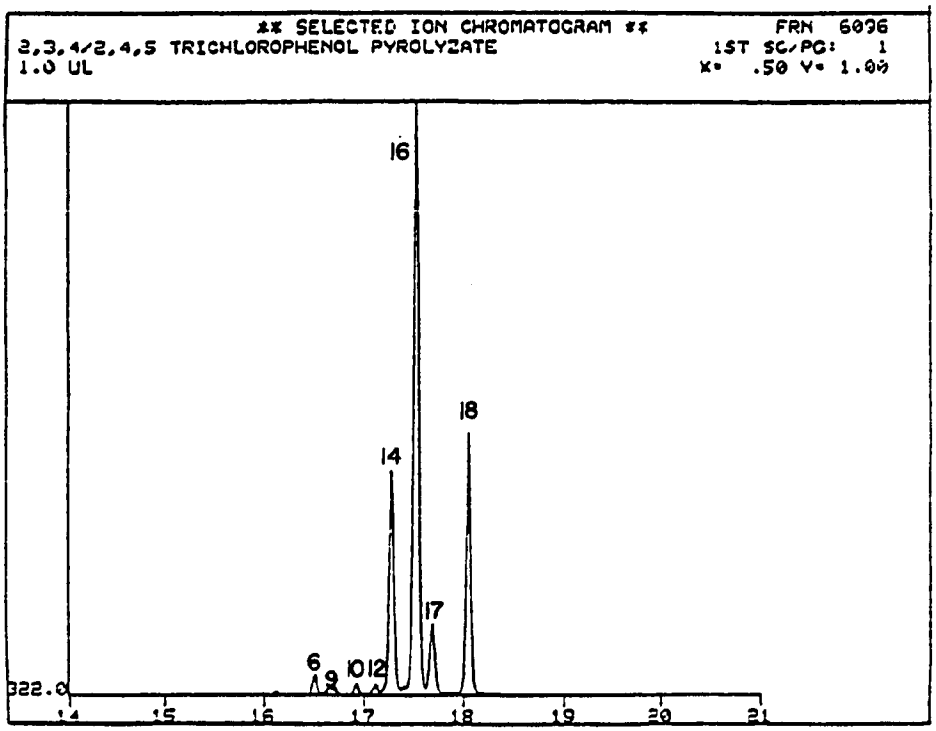
7c 2,3,6-trichlorophenate pyrolysis products



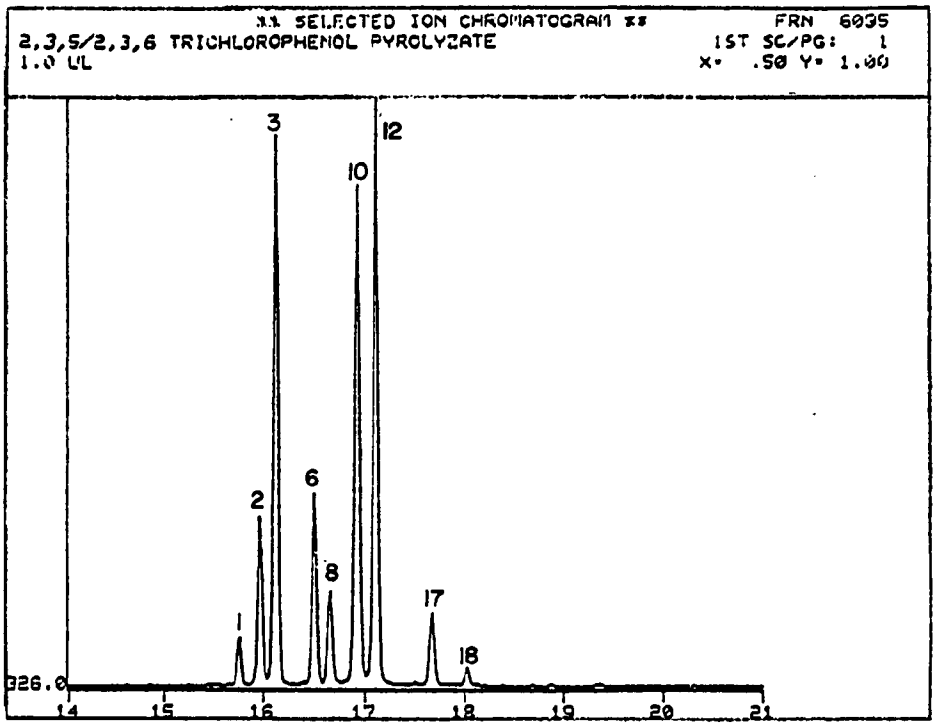
7d Products of 2,3,4- plus 2,3,5-trichlorophenate pyrolysis



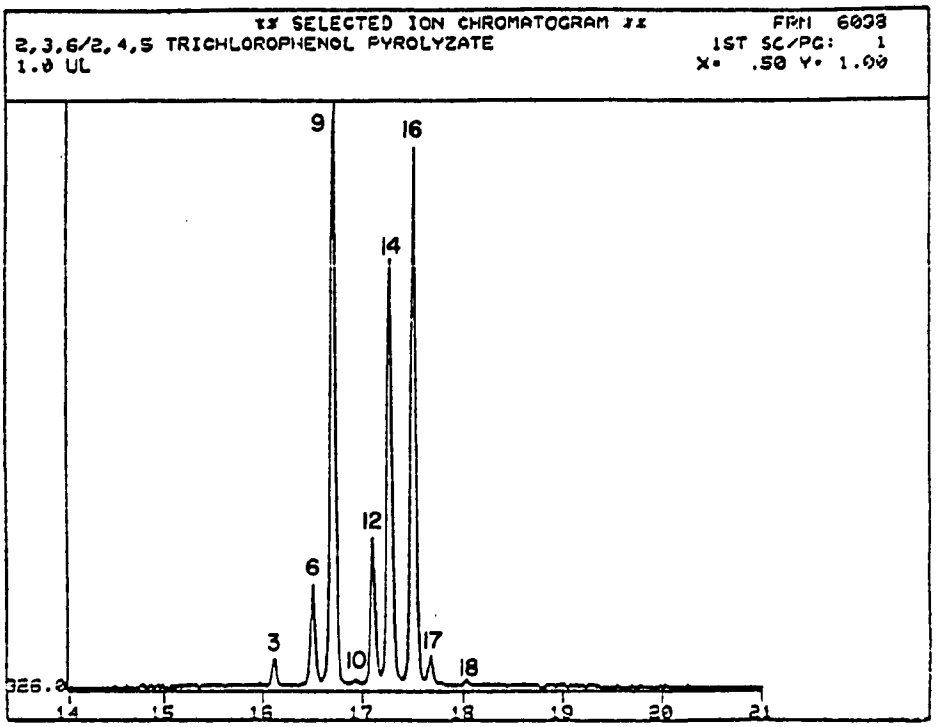
7e Products of 2,3,4- plus 2,3,6-trichlorophenate pyrolysis



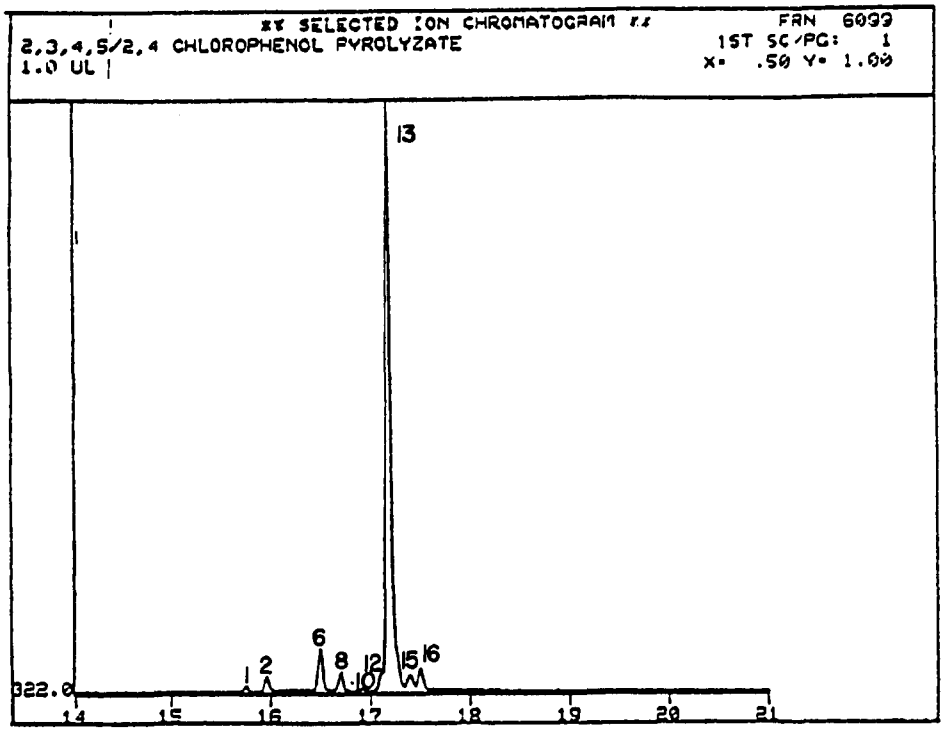
7f Products of 2,3,4- plus 2,4,5-trichlorophenate pyrolysis



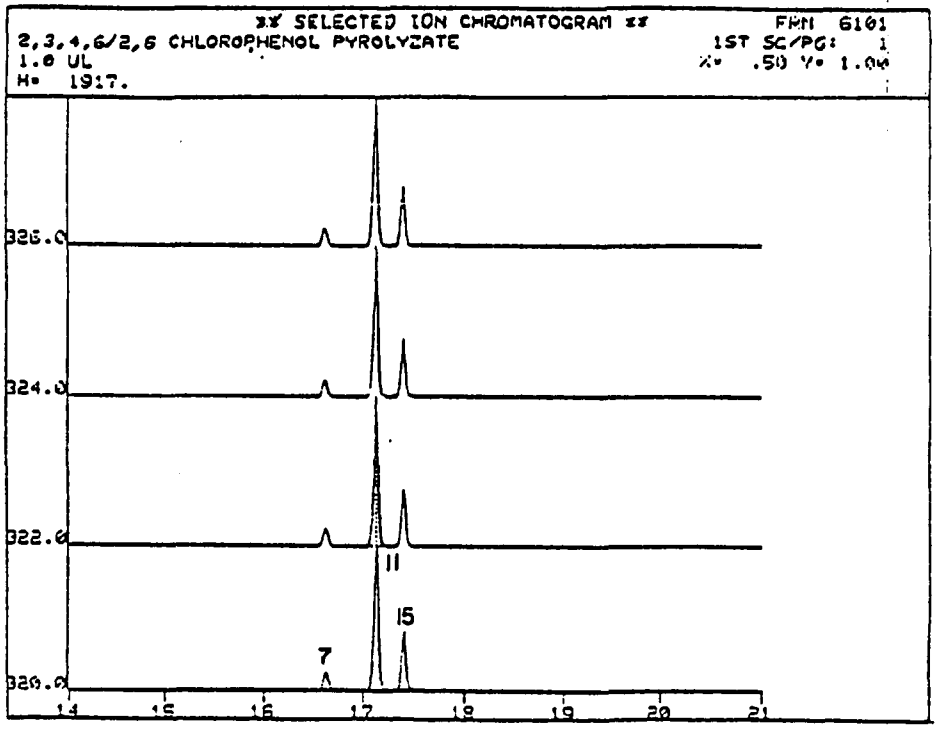
7g Products of 2,3,5- plus 2,3,6-trichlorophenate pyrolysis



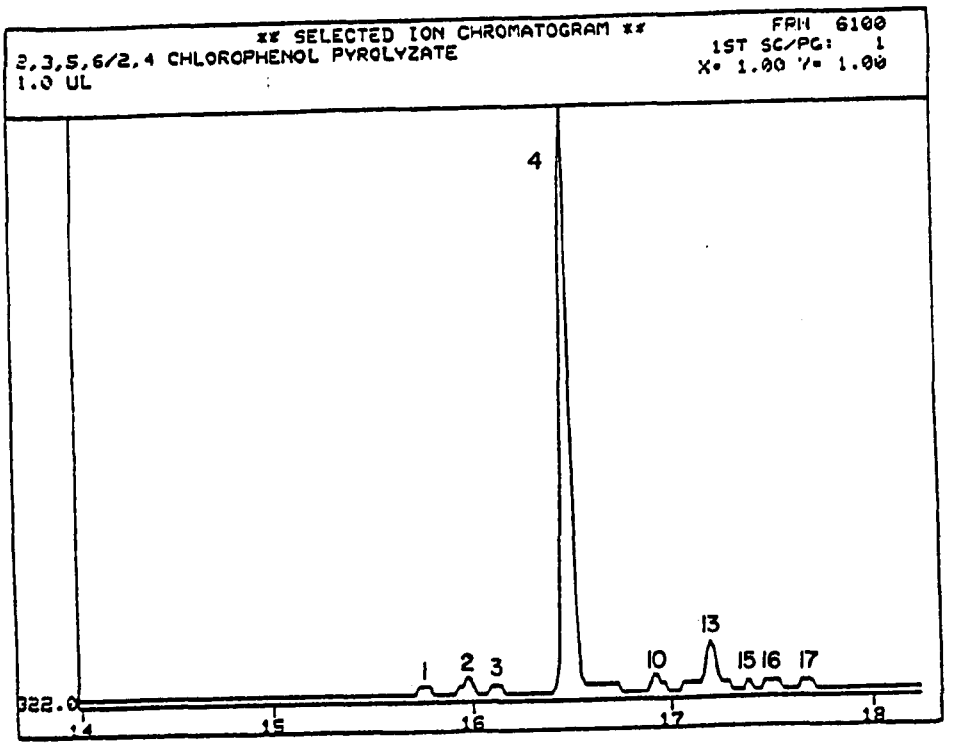
7h Products of 2,3,6- plus 2,4,5-trichlorophenate pyrolysis



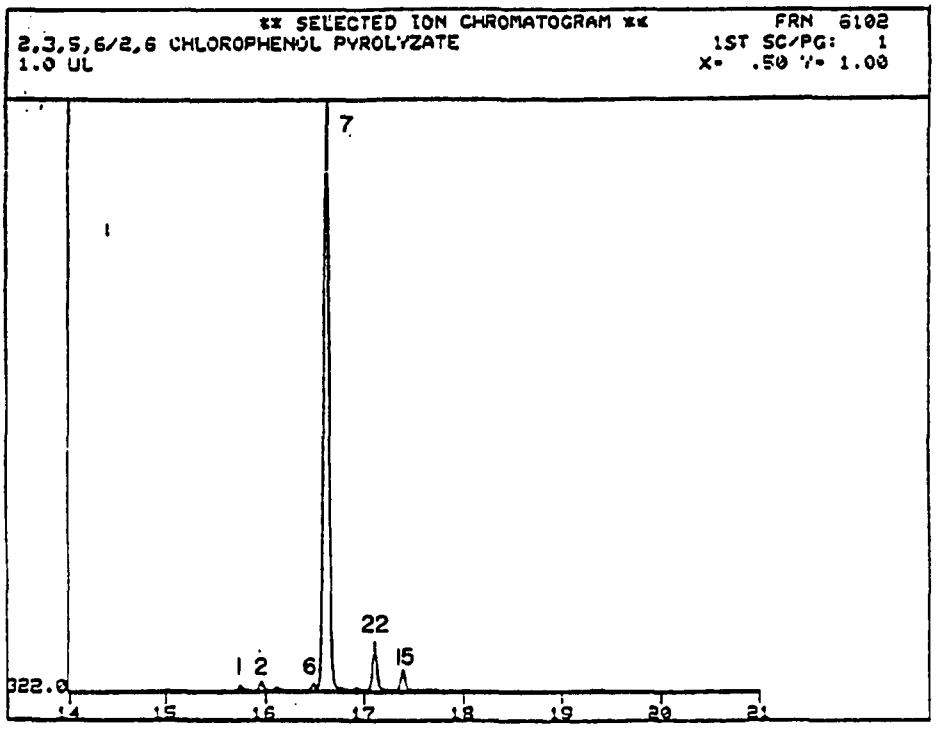
7i Products of 2,3,4- plus 2,4-dichlorophenate pyrolysis



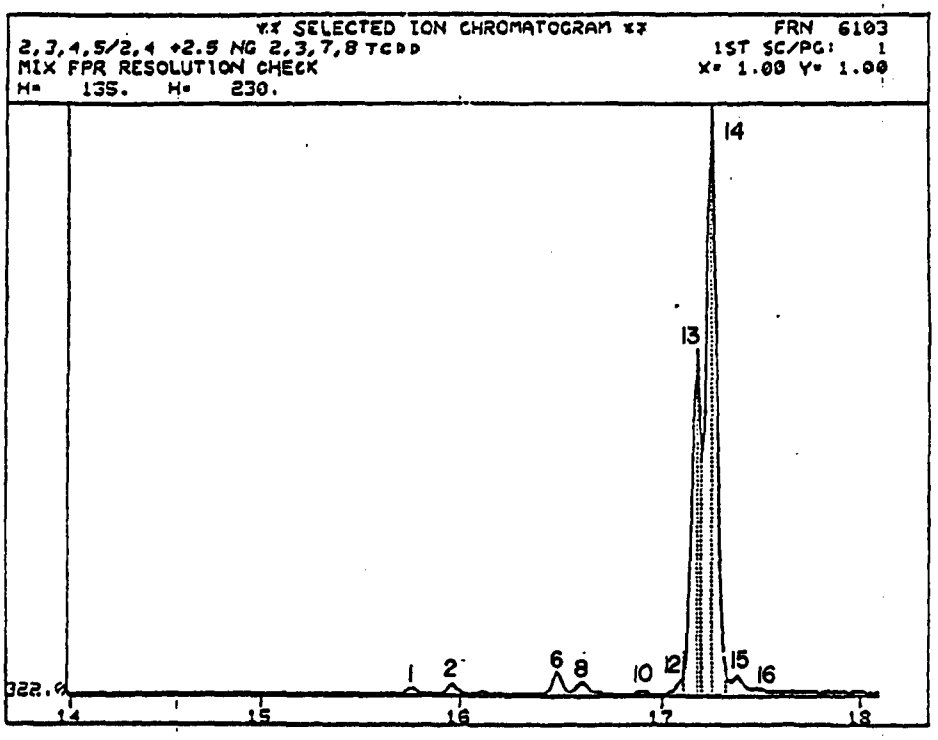
7j Pyrolysis products of 2,3,4- plus 2,6-dichlorophenate



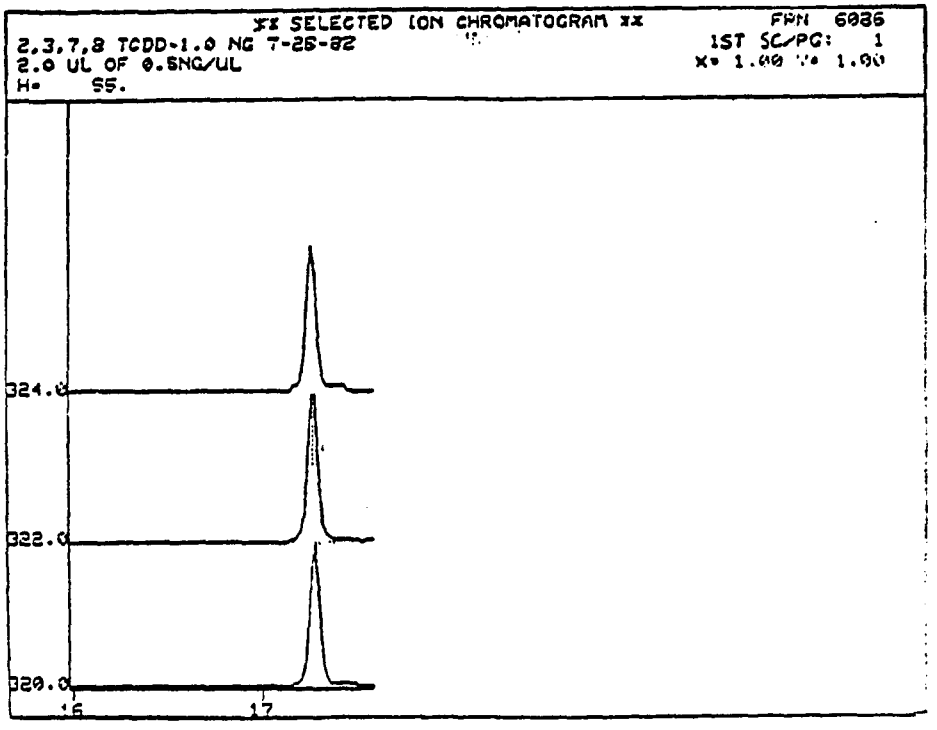
7k Pyrolysis products of 2,3,5- plus 2,4-dichlorophenate



71 Pyrolysis products of 2,3,5- plus 2,6-dichlorophenate



7m Resolution of 2,3,7,8-TCDD from nearest eluting TCDD isomers.



7n GC/MS chromatogram of 2,3,7,8-TCDD

E. Quality Control/Quality Assurance

1. Method validation

Each step in the analytical procedure was individually validated and maximized for recovery of 2,3,7,8-TCDD by use of a radioactive tracer. A 2.0 ng aliquot of ^{14}C-2,3,7,8-TCDD (specific activity: 126 mCi/mmole) in hexane was treated in the same manner as the sample. Quantification of recovery was accomplished by liquid scintillation counting. The extracts were reduced to a volume of approximately 3 ml, transferred into 15 ml of scintillation cocktail (Omnifluor; New England Nuclear), counted in a Searle, Isocap 300 liquid scintillation counter and quantified with respect to an external standard quench-corrected standard curve. The optimal observed recoveries are listed in Table 14.

Most steps in the procedure exhibited a greater than 90% recovery. However, the product of losses encountered at each step results in an expected overall recovery of between 10 and 60%. Although they may be a little low, the recoveries are satisfactory. They do not need to be consistent because the internal standard is used to correct for losses. However, a 10% recovery raises the theoretical lower limit of detection by a factor of 10, thereby lowering the sensitivity of the technique.

The highest losses were encountered during the HPLC and GC/MS transfer steps. The losses were caused by inefficient reconstitution and transfer of the sample extract. These steps require the entire sample extract to be dissolved and transferred with a minimum volume of solvent. Final GC/MS quantification

requires the total residue to be extracted into and transferred in only 2 ul of isooctane. Although any portion of the sample remaining in the vial can be used for subsequent quantification, the greatest sensitivity is achieved with an efficient transfer.

The recoveries listed in Table 14 are a range of maximum recoveries observed after the method was validated and maximized for recovery. There were many instances of extremely low recoveries encountered during initial method validation which identified potential quality control problems. Use of the ^{14}C-2,3,7,8-TCDD tracer was an integral part of the quality control program utilized in this work. It was especially useful because it could be applied at levels (1 to 2 ng) similar to those anticipated in the actual samples, making it an excellent tracer of low-level adsorptive losses; yet it could be easily detected and quantified using standard liquid scintillation technique.

A major quality control problem identified by use of the radioactive tracer was variation in adsorbant activity. The activity of adsorbents, especially the basic alumina, varied between batches resulting in elution of the dioxin fraction with the CCl_4:hexane fraction instead of eluting later with methylene chloride. An adsorbant quality control program was established to minimize such error. The activity of each batch of adsorbants was evaluated following purification and activation.

Table 14

Optimum Working Recoveries Determined Using ^{14}C-2,3,7,8-TCDD

	Treatment	% Recovery
A.	Acid digestion/hexane extraction	95.0
B.	H_2SO_4 wash	91.8
C.	Rotary evaporation	95.6 - 98.6
D.	H_2SO_4-Silica	78.2 - 94.8
E.	NaOH-Silica	84.8 - 89.0
F.	Rotary evaporation	95.6 - 98.6
G.	$AgNO_3$-Silica	99.8
H.	Activated Alumina	95.5
I.	N-Evaporation	92.1 - 96.5
J.	HPLC Purification	52.8 - 94.1
K.	Hexane extraction	91.0 - 99.8
L.	N-Evaporation	92.1 - 96.5
M.	GC/MS transfer	52.8 - 94.1

Total procedure recoveries were calculated as the product of the recoveries measured for each step.

% Recovery = (A x B x C...K x L x M)

Range of % Recovery = 10.8 - 56.1%

Significant losses were also observed during sample evaporation by Femtogas nitrogen. It was determined that losses could be minimized by using a very gentle stream of nitrogen and not heating the extracts to above 30 °C, thereby preventing volatization or aerosol formation. The extracts were removed from the gas stream just before going dry. If they were to be stored for any length of time, they were reconstituted into 5 ml of hexane and stored at -15 °C.

Another step at which major losses were encountered was during the loading of the HPLC injection loop with the sample extracts for HPLC purification. Due to non-laminar flow conditions in the sample loop, attempts to load more than 32 ul of extract into the 50 ul sample loop resulted in the establishment of a leading volume of extract which flowed out of the sample loop before the entire sample was loaded. This loss was minimized by maintaining an injection volume of less than 32 ul and by filling the loop with a slow, even motion. A 100 ul sample loop was later installed, further improving the efficiency of this step.

2. Reagent purity

In order to prevent the establishment of false positive values and reduce background noise, the solvents, adsorbents and gases used were of the highest possible purity. Nitrogen for evaporation and the clean-up adsorbents were purified by the methods reported in the previous section. Extracts of the

adsorbents or solvents were concentrated 1000:1 and evaluated by UV-HPLC. Entire batches of adsorbents or solvents were discarded if their extracts showed any UV-HPLC activity in the TCDD region. The extracts could not be evaluated by GC/MS due to limited availability of a functioning mass spectrometer. No wipe tests or blanks could be evaluated during the sample work-up period.

All glassware used was washed in hot, soapy water (Alconox), rinsed in distilled water, and then wash-bottle rinsed with distilled, deionized water, acetone and then glass-distilled hexane. The glassware was then placed in an oven and baked at 400°C for a minimum of 12 hours.

There was no quality control procedure which would have alerted the investigators to low-level contamination by 2,3,7,8-TCDD. This study relied solely on a set of procedural blanks for evaluating sample contamination. However, these were not analyzed until all of the fish samples had been processed and GC/MS conditions were optimized for detection of 2,3,7,8-TCDD. Fish samples were processed in batches of 20, with the inclusion of two procedural blanks per batch. Following GC/MS quantitation, all of the recoverable blanks were determined to be free of dioxin contamination at various limits of detection. A list of the procedural blanks and their lower limits of detection are presented in Table 15.

Table 15

Procedural Blanks

I.D. number	2,3,7,8-TCDD (ng/kg)	Limit of Detection (ng/kg)
1	N.D.	28
2	N.D.	9
3	N.D.	4
4	N.D.	10
5	N.D.	3
6	N.D.	5
7	N.D.	8
8	N.D.	20
9	N.D.	7
10	N.D.	53
11	N.D.	44
12	N.D.	36
13	N.D.	20

N.D. = no 2,3,7,8-TCDD detected at corresponding limit of detection.

Blanks contained no fish, but values calculated on the basis of a 20 g sample of fish with a minimum detectable mass of 20 pg of TCDD.

3. Maximization of selectivity towards 2,3,7,8-TCDD

The specificity of the technique for 2,3,7,8-TCDD was assured by isolating the 22 TCDD isomers and maximizing their capillary GC resolution. The previous section demonstrates that resolution of 2,3,7,8-TCDD from the other TCDDs was achieved. The satisfactory resolution of 2,3,7,8-TCDD, combined with use of the ^{13}C-2,3,7,8-TCDD internal standard and the simultaneous monitoring of the 319.9, 321.9 and 323.9 mass fragment cluster of TCDD assures that a peak with a GC retention time equivalent to 2,3,7,8-TCDD is indeed 2,3,7,8-TCDD. This protocol gave reasonable assurance that the compound being quantified was indeed 2,3,7,8-TCDD. A more positive confirmation could only have been achieved with a high resolution mass spectrometer.

F. Results of 2,3,7,8-TCDD Determinations

Listed in Table 17 are the levels of 2,3,7,8-TCDD detected in samples of fish collected from various rivers in Michigan and from the Great Lakes. A sample log listing the species, weights, lengths and age of the samples is contained in Table 16. A map of the lower peninsula of Michigan identifying the location of each sample site is presented in Figure 8.

The lower limit of detection of each sample was determined on the basis of a minimum detectable mass of 20 picograms of 2,3,7,8-TCDD corrected relative to the weight of sample aliquot of fish and the mass of the internal standard detected in the injection aliquot. A 20 g sample of fish with a 100% recovery

of internal standard would have a lower limit of detection of 1 ng/kg, or 1 part per trillion of 2,3,7,8-TCDD.

Due to variations in sample recoveries, the limit of detection of each sample is unique and should be considered when evaluating the significance of a sample which contained no detectable residue of 2,3,7,8-TCDD. In a number of cases, samples had detectable residues of 2,3,7,8-TCDD at levels much lower than the limit of detection for samples in which no TCDD was detected.

In addition to the fish residue survey, two short residue experiments were conducted, the results of which are listed in tables 18 and 19. The first experiment involved the analysis of liver samples from mink maintained on a diet of Saginaw Bay carp. The liver samples were analyzed in the same manner as the fish samples, with the exception that a sample size of about 12 g was used. The 12 g sample represented the entire mink liver.

The second short experiment involved the investigation of the effect of cooking on levels of TCDD present in the fish sample prior to treatment. Patties formed from the ground fish samples were cooked by broiling and analyzed for the presence of 2,3,7,8-TCDD. The residues were calculated on the basis of the final weight of the cooked fish and compared to the levels of 2,3,7,8-TCDD present in the fish prior to broiling.

Table 16

Fish Sample Log

Sample Number	Species	Length (cm)	Weight (kg)	Age (yr)
1	White Sucker	40.5	0.759	5
2	Carp	51.0	1.979	5
3	Redhorse Sucker	46.3	0.80	5
4	Carp	68.5	4.84	10
5	Carp	50.2	1.90	7
6	Carp	33.0	0.797	4
7	Carp	45.7	1.66	6
8	Carp	51.8	1.84	7
9	Carp	50.6	1.88	6
10	Carp	44.0	1.03	5
11	Carp	26.7	0.34	4
12	White Sucker	39.5	0.80	3
13	White Sucker	46.0	0.98	3
14	Carp	41.2	1.03	5
15	Carp	53.3	1.42	7
16	Carp	50.2	1.28	6
17	Carp	31.7	0.52	9
18	Carp	51.0	2.08	10
19	Carp	57.0	2.42	7
20	Carp	50.4	1.54	8
21	White Sucker	43.7	0.83	2
22	Carp	68.6	5.10	8
23	Carp	55.9	3.06	6
24	Carp	33.0	0.85	7
25	Carp	45.4	1.82	9
26	Carp	52.0	1.98	8
27	Carp	47.0	1.34	7
28	Carp	45.1	1.75	8
29	White Sucker	41.0	0.89	5
30	White Sucker	43.1	0.96	4

Table 16 (con't)

Sample Number	Species	Length (cm)	Weight (kg)	Age (yr)
31	Carp	51.0	1.91	4
32	White Sucker	39.7	0.65	3
33	Carp	52.1	1.76	6
34	Carp	33.6	1.07	8
35	Carp	88.9	5.30	8
36	White Sucker	43.2	0.89	5
37	Redhorse Sucker	32.7	0.43	5
38	Carp	45.7	1.37	10
39	Carp	43.6	1.45	6
40	White Sucker	34.5	0.45	3
41	White Sucker	31.7	0.32	2
42	White Sucker	33.6	0.41	2
43	Carp	38.1	0.77	5
44	Channel Catfish	44.4	1.23	-
45	Carp	53.5	2.22	8
46	Carp	52.0	2.00	8
47	White Sucker	43.1	0.96	4
48	Carp	49.4	1.73	6
49	White Sucker	43.6	1.02	4
50	Carp	49.4	1.73	4
51	Carp	50.0	1.78	5
52	Redhorse Sucker	43.3	1.00	5
53	Carp	38.1	0.63	7
54	Carp	53.5	2.40	4
55	Carp	60.5	2.65	6
56	White Sucker	48.3	0.73	4
57	White Sucker	51.2	1.565	4
58	Carp	44.0	1.31	3
59	Carp	41.6	1.12	3
60	Carp	38.4	1.21	3
61	Carp	44.6	1.42	4
62	Carp	35.5	0.78	3

Table 17

Results of Fish Analyzed for 2,3,7,8-TCDD

Sample Number	Map I.D.	Location	2,3,7,8-TCDD ng/kg
1	1	St. Joseph River at Benton Twp Park	125 (34)
2	2	Kalamazoo River at Saugatuck	214 (50)
47	2	Kalamazoo River at Saugatuck	n.d. (33)
48	2	Kalamazoo River at Saugatuck	n.d. (21)
49	2	Kalamazoo River at Saugatuck	n.d. (76)
50	2	Kalamazoo River at Saugatuck	n.d. (5)
51	2	Kalamazoo River at Saugatuck	n.d. (115)
5	3	Grand River at Spring Lake	324 (152)
52	3	Grand River at Spring Lake	n.d. (20)
53	3	Grand River at Spring Lake	n.d. (38)
54	3	Grand River at Spring Lake	n.d. (173)
6	4	Grand River at Grand Ledge	298 (158)
3	5	Muskegon River at Cobb Power Plant	123 (14)
55	5	Muskegon River at Cobb Power Plant	n.d. (51)
4	6	Muskegon River at Bridgeton	237 (141)
56	7	Muskegon River at US-131	n.d. (56)
57	8	White River at White Lake	n.d. (48)
58	8	White River at White Lake	n.d. (34)
29	9	Black River at Tower, MI.	n.d. (88)
30	9	Black River at Tower, MI.	n.d. (13)
31	10	AuSable River above Oscoda, MI.	n.d. (40)
32	11	AuSable River at Mouth	n.d. (35)
13	12	Tittabawassee R. 8 km upstream to Dow	287 (84)
14	12	Tittabawassee R. 8 km upstream to Dow	20 (14)
36	12	Tittabawassee R. 8 km upstream to Dow	n.d. (63)
10	13	Chippewa R. 16 km upstream to Dow	136 (36)
11	14	Pine River, below St. Louis	322 (34)
12	14	Pine River, below St. Louis	85 (67)

Table 17 cont'd

Sample Number	Map I.D.	Location	2,3,7,8-TCDD ng/kg
15	15	Tittabawassee, below Dow dam	17 (12)
16	15	Tittabawassee, below Dow dam	39 (13)
17	15	Tittabawassee, below Dow dam	83 (44)
35	15	Tittabawassee, below Dow dam	n.d.(54)
18	16	Tittabawassee, at Consumer's Power Plant	301 (13)
19	16	Tittabawassee, at Consumer's Power Plant	129 (21)
20	17	Tittabawassee, at Freeland	66 (13)
21	18	Tittabawassee, below Saginaw Twp. Sewage Treatment Plant	64 (56)
22	18	Tittabawassee, below Saginaw Twp. Sewage Treatment Plant	125 (52)
23	18	Tittabawassee, below Saginaw Twp. Sewage Treatment Plant	135 (10)
34	19	Tittabawassee, at Wicke's Park	n.d.(35)
24	19a	Saginaw River, below Saginaw Sewage Treatment Plant	319 (190)
25	20	Saginaw Bay, near mouth of Saginaw R.	288 (105)
26	21	Saginaw Bay, near mouth of Saginaw R.	43 (26)
27	21	Saginaw Bay, near mouth of Saginaw R.	172 (92)
28	21	Saginaw Bay, near mouth of Saginaw R.	28 (8)
33	21	Saginaw Bay, near mouth of Saginaw R.	n.d. (50)
37	22	Cass River, at Frankenmuth	n.d. (40)
38	22	Cass River, at Frankenmuth	n.d. (9)
39	23	Flint River, below Flint	n.d. (100)
40	24	Flint River, Holloway Reservoir	n.d. (24)
8	25	St. Clair River, at Decker's Landing	586 (81)

Table 17 cont'd

Sample Number	Map I.D.	Location	2,3,7,8-TCDD ng/kg
41	26	Clinton River at Avon Rd.	n.d. (45)
42	27	Belle River at Gratiot Rd.	n.d. (76)
62	28	Detroit River at Belle Isle	n.d. (44)
43	29	Huron River at Superior Rd.	n.d. (90)
44	30	Huron River at Flat Rock	246 (100)
7	30	Huron River at Flat Rock	n.d. (50)
45	31	Raisin River at Monroe	n.d. (52)
46	31	Raisin River at Monroe	n.d. (24)
9	-	Lake Erie at Port Clinton OH	75 (42)
59	-	Lake Erie at Port Clinton OH	n.d. (30)
60	-	Lake Erie at Port Clinton OH	n.d. (18)
61	-	Lake Erie at Port Clinton OH	n.d. (11)

Number in parenthesis ( ) indicates the limit of detection of 2,3,7,8-TCDD for that specific sample based on the mass of internal standard present in the injection aliquot. The limit of detection is reported as ng/kg 2,3,7,8-TCDD.

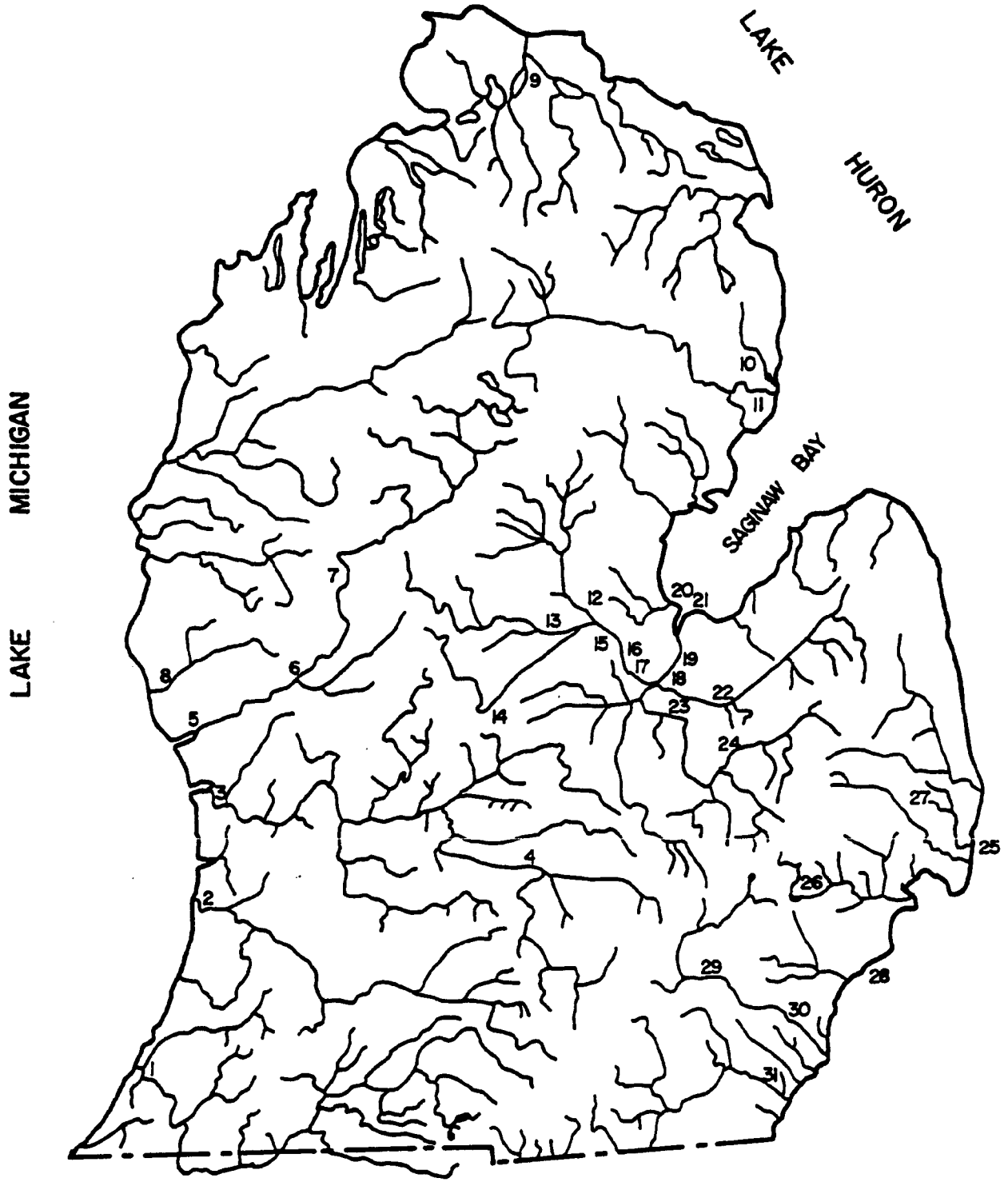


Figure 8. Map of Michigan Identifying Sample Sites

V. DISCUSSION

A. Evaluation of the Analytical Technique and Results

At the time of initiation of the research, the analytical technique employed was judged by a panel of independent consultants as reliable for the determination of dioxin residues in biological media at the part per trillion level and representing the state of the art in trace analysis (185). The procedure evaluated was the one used in this work (183), with the exception that a packed column was used for gas chromatography. The packed column did not have the capability to resolve 2,3,7,8-TCDD from a number of the other tetrachlorinated isomers of TCDD. The final recommendations of the panel stressed that specificity towards 2,3,7,8-TCDD needed to be achieved. Attainment of the chromatographic specificity for 2,3,7,8-TCDD was a major goal of this work. As evidenced by the results from the previous section, that goal was realized, providing satisfactory confirmation of the residues as 2,3,7,8-TCDD.

All of the samples with adequate recoveries were quantified on the basis of the total area of a completely resolved chromatographic peak with a response at or above 2.5 times the noise level. No samples were quantified as part of a shoulder or multiple peak. A sample judged suitable for quantification required the presence of all four characteristic ions, eluting at the proper gas chromatographic retention time and with masses 319.9 and 321.9 at the correct isotope ratio ($\pm$25%).

Only one of the procedural blanks was found to be contami-

nated by 2,3,7,8-TCDD. The contamination was traced to incomplete rinsing of the HPLC sample loop following calibration by 2,3,7,8-TCDD. A record of the order in which samples were purified by HPLC was maintained. Additional instances of this type of contamination were ruled out by closely inspecting the final GC/MS chromatograms of other samples which were processed following HPLC calibration. It turned out that most of these samples were not used due to low recoveries of internal standard.

The absence of background contamination in the rest of the procedural blanks indicates with some certainty that the residues detected in the fish samples do not represent "false positive" values.

A major problem encountered during analysis which seriously effected the interpretation of data was caused by poor sample recoveries. The validity of samples with quantifiable residues of native 2,3,7,8-TCDD was not conpromised by the poor recoveries. If the recovery of tracer is assumed to accurately reflect losses of native TCDD, any quantifiable recovery of the tracer can be used to calculate a corrected TCDD concentration. However, problems are encountered when the internal standard is quantifiable, but sample recovery is too low to allow detection of native 2,3,7,8-TCDD at a reasonable level of sensitivity. In such a case, the sample is determined to be negative for residues of 2,3,7,8-TCDD at a given limit of detection. The conclusion that the sample is negative for 2,3,7,8-TCDD at given limit of detection can be misinterpreted as indicating that the sample is entirely free of 2,3,7,8-TCDD. A sample with 10 ng/kg TCDD cannot be adequately compared to one which is negative at a

minimum detectable level of 100 ng/kg.

The theoretical limit of detection of 1 ng/kg 2,3,7,8-TCDD was not attained in any of the samples analyzed. Although the GC/MS sensitivity (20 pg) would have facilitated detection at this level, it requires that the sample be processed at 100% recovery. The average sample recovery achieved in this work was 4.5%, based on the amount of tracer present in the injection aliquot.

A recovery of 100% requires extremely efficient cleanup and transfer steps. It is virtually impossible to extract and concentrate the entire residue into 2 ul of solvent for final GC/MS quantification. There is some confusion in the literature with respect to the reporting of sample recoveries. Many investigators report recoveries based on the final volume of extract, a portion of which is injected for GC/MS analysis. This can be done when you have a substantial amount of residue and utilize a larger mass of internal standard (ca. 20 ng). This scheme could not be utilized in this study because of low total recoveries and, due to limited amounts of tracer available, the incorporation of only 5 ng of ^{13}C-2,3,7,8-TCDD.

Many of the problems encountered during this study were caused by attempting to process the samples before GC/MS confirmation of the procedure could be performed. Analysis was carried out in stages, with the entire set of samples being processed together. Ideally, a batch of no more than 20 samples should be processed through the entire procedure, including GC/MS quantification, in as short a period of time as possible. In this way, problems caused by contamination, interferences or recoveries

can be immediately detected and corrected. The samples could not be processed in this manner for a number of reasons. Probably the major factor preventing the analysis in an integrated fashion was the limited availability of the necessary equipment and standards. The HPLC was utilized for a number of different projects and had to be modified for HPLC cleanup. Additionally, before quantification could be performed, the specificity of the procedure towards 2,3,7,8-TCDD had to be verified. This required the synthesis, identification and maximization of chromatographic resolution of the TCDD products by GC/MS.

Problems encountered during the computer interfacing of our DuPont 321 GC/MS system and an unusually large number of major breakdowns rendered the GC/MS unavailable for use during most of the pericd of research. A decision was made to enter into a cooperative agreement with the M.S.U. Mass Spectrometry Facility for the limited use of a GC/MS. Use of a Hewlett-Packard 5985 GC/MS for a period of 4 weeks was arranged. During this time, the pyrolysis products were identified and resolved, mass spectrometric sensitivity was maximized and all of the sample extracts were quantified.

Over 300 samples of fish were processed. 166 samples were determined to have suitable cleanup, as evidenced by their HPLC chromatograms. These 166 sample extracts were analyzed by GC/MS. Only 90 samples displayed recoveries suitable for quantification. Losses were most likely introduced during sample reconstitution and transfer and the result of adsorption, volatization and photodegradation during the long period of sample storage. In some cases the extracts had been stored for over two years.

Major adsorptive losses have been reported to occur if the samples are not analyzed immediately (185).

Although the samples were processed according to established protocols and the utmost effort was expended to prevent the appearance of false positive values, there is still a slight possibility that the sample extracts may have contained materials with characteristic ions which could not be resolved from those of TCDD by low resolution mass spectrometry. The major interfering ions arise from the presence and coelution of a component of Arochlor 1254 which has a characteristic ion of m/e 321.8679, by DDE ion at m/e 321.929 or by tetrachlorinated benzy phenyl ethers which display nominal mass fragments at 320, 322 and 324.

However, the erroneous quantification of these interfering ions should have been eliminated by the column and HPLC cleanup steps which are reported to efficiently remove them at a 2×10^6 fold excess (186). On this basis, a fish sample with a 20 mg/kg concentration of DDE would be sufficiently cleaned up to allow proper quantification of a sample with 10 ng/kg native 2,3,7,8-TCDD without interference. Purification of the extracts by HPLC functioned to clean up the samples even further and separated the TCDDs from the other chlorinated dioxins.

Additionally, the characteristic ions of TCDD are 500 times more intense than the interfering ions of DDE or PCB (187) and about 150 times as intense as those of the chlorinated benzyl phenyl ethers (186). Although the capillary chromatographic resolution of 2,3,7,8-TCDD from these interfering compounds has not been demonstrated in this work, it should provide an additional safeguard against the introduction of false positive values

by some of these materials. Ultimate verification of the residues could be performed by use of a high resolution instrument. Confirmation of a portion of samples by high resolution GC/MS would be a valuable addition to a quality assurance program, but in most cases should not be a requirement of all routine analyses.

Based on the author's experiences from this work, the following recommendations should be considered by persons attempting to perform dioxin analyses: 1) Analyses should be performed by a team of conscientious, patient technicians who are knowledgeable in all aspects of the procedure. 2) The team should be divided into four groups responsible for optimization of one facet of the procedure:

a) extraction and cleanup

b) HPLC purification

c) GC/MS analysis

d) reagent preparation and quality control.

3) Sufficient quantities of all 22 isomers of TCDD as well as the ^{13}C and ^{14}C analogs of 2,3,7,8-TCDD must be available. 4) Samples should be processed with at least 10% procedural blanks and 5% duplication. 5) Samples should be processed and analyzed within the shortest time possible. 6) If possible, provisions should be made to verify at least 5% of the samples by high resolution GC/MS.

B. Geographic Distribution and Possible Sources of Residues

The 2,3,7,8-TCDD residue levels listed in Table 17 agree with previous reports of TCDD in fish from the Tittabawassee and Saginaw rivers and Saginaw Bay (Table 10). Quantifiable levels of 2,3,7,8-TCDD were detected in almost all of the fish sampled downstream to the Dow-Midland facility. Samples of carp and sucker collected from the Tittabawassee and Chippewa rivers, eight km upstream to the Dow facility and samples collected from the Pine river, 40 km upstream to Midland also contained residues as high as those detected in samples originating below Midland. Presence of 2,3,7,8-TCDD in the upstream samples rules out aqueous effluent from Dow as the sole source of TCDD detected in the Saginaw watershed.

Fish samples originating in entirely different watersheds, over 160 km from Midland were also determined to contain quantifiable residues of 2,3,7,8-TCDD. The most significant aspect of the results of this survey is that 2,3,7,8-TCDD was detected in samples of fish collected from areas which did not contain trichlorophenol manufacturing sites. The detection of TCDD at these sites suggests that other processes may be contributing to the presence of the 2,3,7,8-TCDD residues.

This study was initially designed to provide a data set of 2,3,7,8-TCDD determinations in fish from every major river in Michigan. However, a large number of samples were lost due to low recoveries. Samples with non-detectable TCDD residues at high limits of detection are also not suitable for direct comparison.

The relatively small sample size remaining limits statistical

treatment of the data to a Z-test for proportions (188). This test is carried out on the basis of whether a given member of a population contains a quantifiable residue and does not address the magnitude of that residue. The data set can be divided into the following two populations:

A) Samples collected within an 8 km radius of the Dow-Midland facility and extending downstream into the Saginaw River and Saginaw Bay.

B) Samples collected from other portions of the state.

The proportions of samples in each population with quantifiable residues of 2,3,7,8-TCDD are compared aqainst the null hypothesis:

H_o: There is no difference between the two populations.

We can designate a sample with a residue level of greater than 50 ng/kg as "positive", while a sample with no detectable residues at or below a limit of detection of 50 ng/kg is classified as "negative". According to this criterion, any sample which contains a quantifiable residue below 50 ng/kg is treated as a "negative" observation, while samples with limits of detection above 50 ng/kg which do not have quantifiable residues of TCDD are not included in the test.

There were 12 "positive" samples in a total of 19 qualifying samples comprising the "A" population (sample numbers 10, 13 through 28, 33, 34 from Table 17 and 18). The remaining 30 qualifying samples (sample numbers 1 through 9, 11,12,30,31,32, 37,38,40,41,44,47,48,50,52,53,57 through 62) comprise the "B" or control population. This population contained 11 "positive" residues.

The value of Z calculated by comparison of these two popula-

tions is 1.80. This value allows rejection of the null hypothesis at a level of confidience of 0.05. We can conclude that there is a significant difference between the two populations, with a greater proportion of positive samples appearing in the population of samples collected from the Midland area extending into Saginaw Bay.

The following calculations and formulas were used:

$$Z = \frac{(p_1' - p_2') - (p_1 - p_2)}{p^* \times q^* \times (1/n_1 + 1/n_2)}$$

$$p^* = \frac{X_1 + X_2}{n_1 + n_2} \qquad q^* = 1 - p^*$$

p_1' = proportion of positive samples in "A" population = 12/19

p_2' = proportion of positive samples in "B" population = 11/30

n_1 = total number of samples in "A" population = 19

n_2 = total number of samples in "B" population = 30

H_o: $p_1 - p_2 = 0$

Calculated value of Z = 1.80

$Z_{0.05} = 1.64$

Conclusion: $p_1 > p_2$; Reject null hypothesis.

Aside from this somewhat weak Z-test, the data does not hold any other statistical significance. Sample sizes at each of the sites are too small to predict the level or presence of 2,3,7,8-TCDD in additional samples of fish. Samples originating outside of the Midland area can only be evaluated on an individual basis. The detection of residue of 2,3,7,8-TCDD does not indicate that a particular river is "polluted" by dioxins. It does

indicate that there may be a source near the site or somewhere in the watershed which can result in the establishment of residues of 2,3,7,8-TCDD in fish.

Although one can only speculate as to the sources of the dioxins in the Tittabawassee and Saginaw Rivers, circumstances would render the chlorophenol production facility the most obvious contributor. There is no question as to the potential for formation of dioxins during synthesis of chlorophenols by the alkaline hydrolysis method; even as associated with a modern trichlorophenol reactor (107). Production of 2,4,5-trichlorophenol has been carried out in Midland for a number of decades. Process design, standards of quality control and accepted practices of waste disposal have changed dramatically during this period.

The TCDD could have entered the environment around Midland with aqueous wastes or through aerial deposition as a result of inefficient incineration of reactor wastes as well as through the formation of new TCDD from chlorophenols present in the wastes being combusted.

The disposal of chlorophenol wastes by landfilling is another potential source of the environmental residues of TCDD. A single container of still bottom from an early chlorophenol reactor which produced high levels of 2,3,7,8-TCDD has the potential to cause widespread contamination at the part per trillion level if disposed of improperly. No records of chlorophenol waste disposal are available. Independent waste haulers have been known to dispose of chemical wastes by various means, including application to rural roadways for dust control. It is possible that some of the dioxin-containing wastes were landfilled at various sites

throughout the state and are contributing to the residues detected at sites far removed from Midland.

The formation of dioxins during combustion is also a valid potential source of environmental residues of 2,3,7,8-TCDD. Studies cited in the previous sections clearly indicate that significant amounts of dioxins are produced during combustion of municipal waste. An estimated 77 ug of 2,3,7,8-TCDD can be produced during the incineration of a ton of municipal waste (see page 50 for reasoning and citations). This value would be expected to vary, depending on the type of waste and conditions in the incinerator, but it is presently the best estimate available. On the basis of information derived from air emission permits issued by the Air Quality Division of the Michigan Department of Natural Resources, a total of 400,000 tons of municipal waste, 800,000 tons of dry sewage sludge and 40,000 tons of liquid chemical waste is disposed of by incineration annually in Michigan. An additional 500,000 tons of waste is burned in smaller, privately-owned incinerators. The total of 1,740,000 tons of waste could result in the formation of 134 grams of 2,3,7,8-TCDD yearly.

The amount of additional 2,3,7,8-TCDD entering the environment through the combustion of fossil fuels is difficult to estimate. There are no data available on the yields of dioxins produced during the burning of coal for heating and power generation. If levels similar to those encountered during waste incineration are produced, these processes could be providing a major input of TCDD into the environment. Results of laboratory-scale combustion experiments suggest that dioxins are formed

during the burning of coal, but no studies have demonstrated the presence of 2,3,7,8-TCDD in fly ash or airborne emissions from coal-fired power plants. One could conclude that dioxins are being produced during the burning of coal on the basis of reaction schemes presented in Figure 4.

The 134 grams of 2,3,7,8-TCDD estimated as being produced in Michigan during waste incineration is equivalent to the amount of 2,3,7,8-TCDD present in 4.4 metric tons of 2,4,5-T with an average 2,3,7,8-TCDD level of 30 mg/kg, or 1340 metric tons of 2,4,5-T with an average concentration of 2,3,7,8-TCDD of 0.1 mg/kg. About 3400 metric tons of 2,4,5-T were applied in the U.S. in 1974. If a similar amount were applied prior to 1971, it could represent an annual nationwide 2,3,7,8-TCDD input ranging from 0.340 to 102 kg, assuming respective TCDD levels in 2,4,5-T ranging from 0.1 to 30 mg/kg. Use of 2,4,5-T has since been suspended, but synthesis of ortho chlorophenols continues, primarily due to the extreme usefulness of these important chemical intermediates. If one assumes that dioxin generation during chlorophenol production is currently being minimized and carefully monitored, inputs from combustion can now be considered major contributors to residues of 2,3,7,8-TCDD.

The entry of 2,3,7,8-TCDD to the environment from combustion processes can be minimized by efficient particulate collection and the proper landfilling of fly ash. The fly ash may contain levels of other toxic chlorinated dioxins and dibenzofurans which render its toxic potential over 10 times greater than based on the level of 2,3,7,8-TCDD alone (142). Failure to confine fly ash may result in its distribution in the environment,

resulting in the biological attenuation of the chlorinated dioxins and furans adsorbed onto it.

The results of this work demonstrate the need for continued research into the formation of dioxins during combustion and its impact on the appearance of environmental residues of 2,3,7,8-TCDD. A critical question to be addressed is whether coal-fired power plants produce dioxins at significant levels. Studies into the presence of TCDD in aquatic sediments and soils should also be conducted to establish background levels of TCDD around the state.

The residue values are especially difficult to interpret because of the variety of potential dioxin sources in Michigan. A similar study should be conducted in an area which contains an industry that relies heavily on fossil fuel energy but does not support a chlorophenol facility. The significance of the residues detected is also tenuous because they are among the first observations conducted in the environment for a specific compound at the part per trillion (ng/kg) level. At this level of sensitivity, many compounds previously undetected and thought to be absent from certain portions of the biosphere appear with apparent ubiquity.

C. Ecological and Public Health Significance of the Residues

This study was primarily concerned with detecting the presence of 2,3,7,8-TCDD in aquatic environments. Fish were chosen as a test animal because they are very good indicators of environmental contamination by recalcitrant, lipophilic organic compounds. They can accumulate many of these materials to levels orders of magnitude higher than generally present in the environment. Fish attenuate TCDD biologically and represent an organism which is a human food source. They can be easily sampled and contain enough tissue for multiple analyses.

Carp and sucker were chosen as test species because they feed on organisms and materials primarily associated with mud and sediment. Sediment has a high adsorption affinity towards 2,3,7,8-TCDD and can represent a major reservoir for dioxins in the aquatic environment. Because carp and sucker cycle so much sediment and bottom-dwelling organisms, they are expected to contain higher levels of TCDD than other species of fish.

Available studies of the cycling of 2,3,7,8-TCDD in model ecosystems report a fish concentration factor of about 10^3, calculated relative to the concentration of TCDD present in the water column (168). Because we are assuming that carp and sucker derive most of the TCDD from sediment during feeding, calculation of a water concentration for TCDD based on the residue levels detected in fish should result in a high approximation. The range of TCDD residue values measured in this work was 17 to 586 ng/kg. Corresponding estimates of water levels based on the model ecosystem data range from 0.017 to 0.588 ng/l.

Based on the results of static fish bioassays, concentrations of 2,3,7,8-TCDD in the water column within the estimated range can be toxic to fish. A water level of 1.0 ng/l TCDD caused complete mortality of mosquito fish (39). Exposure of young coho salmon for 96 hours at a water concentration of 5.6 ng/l resulted in 55% mortality within 80 days postexposure, while water levels as low as 0.056 ng/kg (0.054 ng/g body weight) TCDD for 96 hours resulted in a 10% increase in mortality (189). Exposure of developing yolk sac fry and juvenile rainbow trout and pike to a static water concentration of 0.100 ng/kg 2,3,7,8-TCDD resulted in decreased embryonic development, decreased growth and increases in skeletal malformations as well as death (36).

These studies demonstrate that very low levels of 2,3,7,8-TCDD in water are capable of inducing deleterious effects in exposed populations of aquatic organisms. Although estimates of the water concentration may be high, the residues detected in this study indicate that TCDD may be present in certain rivers in Michigan at concentrations near these action levels.

Fish can also function as a source of TCDD to terrestrial populations which use them as a source of food. An experiment to assess the magnitude of TCDD taken up by mink fed a diet of carp originating in Saginaw Bay was conducted as part of this research. The mink were fed ground Saginaw Bay carp for at least 30 days as part of an experiment to assess the impact of PCBs present in the carp on mortality and reproductive success (190). Samples of the feed and livers from the exposed mink were provided by the investigators conducting the bioassay. Table 19 lists the levels of 2,3,7,8-TCDD detected in he mink livers. All livers contained

detectable residues of 2,3,7,8-TCDD ranging from 39 to 87 ng/kg. A sample of pooled livers from the control population and samples of the feed were not suitable for comparison due to low recoveries. An average residue of 57 ng/kg in a mean liver weight of 12 g represents a total mass of 684 pg of 2,3,7,8-TCDD per liver. The extent of accumulation cannot be evaluated without knowledge of the average TCDD content of the feed. The 684 pg of 2,3,7,8-TCDD could have been provided to the mink by ingestion of a total of 34 g of carp with a mean 2,3,7,8-TCDD content of 20 ng/kg.

Table 18

2,3,7,8-TCDD in Livers of Mink Fed Carp from Saginaw Bay

Sample	2,3,7,8-TCDD (ng/kg)	Limit of detection (ng/kg)
A	39	12
B	87	8
C	46	23

An epidemiological assessment of the hazards associated with the ingestion of TCDD-contaminated fish has been conducted by researchers at the U.S. Food and Drug Administration (191). They used data from a 2-year rat feeding study (20) as the basis for determining human hazards associated with the ingestion of small quantities of 2,3,7,8-TCDD present in fish. The chronic rat feeding study reported no observable effects to rats at a lifetime exposure of 1 ng/kg body weight per day; hyperplasia of the liver and lung cells at a dose level of 10 ng/kg-day and an increase in liver carcinomas at a dose level of 100 ng/kg-day.

The corresponding 2,3,7,8-TCDD doses to a 70 kg human subject would be 70, 700 and 7000 ng/day.

A comprehensive fish consumption study gathered information from about 25,000 individuals in the Great Lakes area. A ranked distribution of those persons who consumed fish from the Great Lakes, including inland lakes and rivers, revealed that a person in the 99th percentile (those consuming the most fish) consumed 36.8 g of fish daily, or 257.6 g per week. An individual in the 90th percentile consumed an average of 15.7 g of fish per day (109.7 g/week).

An individual in the 99th percentile consuming 36.8 g of fish daily with 2,3,7,8-TCDD consistently at the highest level detected in this study (586 ng/kg) would be ingesting TCDD at a dosage of 0.31 ng/kg body weight per day, or a dose of 21.6 ng/day. This dose is less than one-third of the 70 ng/day no-effect level. If the TCDD level was equal to the average positive residue level detected in this survey (178 ng/kg) the individual would be ingesting 6.5 ng TCDD/day, which is less than 10% of the no-effect level. The no-effect level would not be exceeded unless the concentration of 2,3,7,8-TCDD in the fish consistently exceeded 1902 ng/kg, or 1.9 ppb. A fish consumer belonging to the 90th percentile would have to eat 15.7 g of fish daily with an average TCDD level of greater than 4.4 ug/kg (4.4 ppb) 2,3,7,8-TCDD in order to exceed the no-effect level.

At the 99th percentile, the concentration required to induce hepatic injury would be 19 ug/kg. The TCDD present in the fish would have to exceed 190 ug/kg to reach the carcinogenic effect level.

These estimates are based on the individual consuming fish with the respective TCDD residues daily over his entire lifetime. The probability of every meal of fish exceeding the threshold concentration is very low. Few fish would be able to survive a body burden as high as 1.9 ug/kg 2,3,7,8-TCDD.

An additional margin of safety would be realized following cooking of the fish. Table 20 lists the results of cooking experiments conducted on samples of fish with known concentrations of 2,3,7,8-TCDD. Reductions in TCDD content ranged from 26 to 67% following broiling. The reductions were calculated relative to the final weight of the cooked fish. If the loss of moisture (ca. 20%) is considered, the reductions in TCDD content relative to the raw fish are even greater.

Table 19

TCDD Residues in Fish Before and After Broiling

2,3,7,8-TCDD in raw fish, ng/kg	2,3,7,8-TCDD in fish following broiling, ng/kg	% reduction
136 (36)	45 (24)	67
83 (44)	61 (38)	26
64 (56)	36 (18)	44
287 (84)	141 (11)	51

( ) = limit of detection of TCDD

A more conservative estimate of the hazard associated with ingestion of 2,3,7,8-TCDD contaminated fish can be extrapolated from the minimum cumulative toxic dose of 0.1 ug/kg calculated

by Stevens (192). His calculations are based on observations made on human subjects suffering from Yusho disease, induced by 2,3,7,8-tetrachlorodibenzofuran. He reasons that since TCDF is 20 times less toxic than 2,3,7,8-TCDD in most species, the minimum toxic dose of 2 ug 2,3,7,8-TCDF/kg should be equivalent to 0.1 ug/kg 2,3,7,8-TCDD, or 7 ug of 2,3,7,8-TCDD in a 70 kg human.

An individual in the 99th percentile, consuming 36.8 g of fish daily with the highest detected residue of 586 ng/kg would exceed the toxic threshold in about 324 days. If the level of TCDD in the fish was 178 ng/kg (average positive level observed), it would take 1077 days to reach the threshold, while consumption of fish with a TCDD residue of 20 ng/kg would require 26 years to exceed the threshold. These calculations neglect mechanisms of excretion and metabolism which would further prevent the effective dose from being reached. They also neglect the safety factor introduced during cooking.

On the basis of these calculations, humans should not be significantly effected by eating reasonable amounts of fish containing 2,3,7,8-TCDD. However, the margin of safety realized by humans as a result of omnivorous eating habits, cooking practices and a relatively high body weight would not apply to wildlife species that consume fish as a major component of their diet. Assuming equal sensitivity to TCDD, a 5 kg raccoon consuming 100 g of fish per day with an average 2,3,7,8-TCDD level of 500 ng/kg can exceed the 0.1 ug/kg threshold in 10 days. A 750 g mink ingesting 50 g of fish daily with a residue level of 500 ug/kg would take in 25 ng TCDD per day and reach the toxic

dose in 3 days. Ingestion of 50 g of fish daily with an average TCDD level of 150 ng/kg would result in a dosage of 10 ng/kg-day and exceed the toxic dose in 8 days. Fish-eating birds such as diving ducks and gulls would be exposed to similar hazards from the consumption of dioxin-contaminated fish.

The potential stresses imposed upon populations of wildlife as a result of consumption of dioxin-contaminated fish is considerably greater than those imposed upon the human population. This hazard would be magnified by the presence of other toxic compounds in the fish, such as PCBs, chlorinated dibenzofurans and other chlorinated dioxin congeners. The result of the stresses can be a reduction in the reproductive capacity of the exposed individuals, increased mortality, impaired predator avoidance behavior and a number of other factors which could ultimately cause a decline in the size of the population.

The calculated assessments of the risk posed to wildlife as a result of environmental contamination are based on toxicity data from different species and on a few observations of TCDD residues present in fish. Further investigations into the concentrations of chlorinated dioxins and dibenzofurans present in fish and the inclusion of fish-eating wildlife species in residue studies will result in a better understanding of the potential hazards of environmental residues of these materials. Investigation of the acute and chronic toxicity of dioxins to wildlife should be conducted to assess the sensitivity of those species to the toxic effects of 2,3,7,8-TCDD.

VI. CONCLUSIONS

The analytical method utilized in this research can be successfully used to quantify residues of 2,3,7,8-TCDD in fish at the part per trillion (ng/kg) level with absolute chromatographic specificity towards 2,3,7,8-TCDD. The technique is difficult to carry out and requires the availability of extensive dedicated instrumentation. A strict quality control program should be maintained to detect and prevent contamination which may result in high background values or the appearance of interfering compounds. Standards of ^{12}C-, ^{13}C- and ^{14}C-2,3,7,8-TCDD as well as the 21 other isomers of TCDD are crucial to method validation and final sample quantification. Poor sample recoveries result in decreasing the sensitivity of the procedure. Recovery can be maximized through careful sample evaporations and transfers. Processing and quantifying the samples in as short a period of time as possible minimizes volatization and adsorption of TCDD onto glassware during storage.

The results indicate that residues of 2,3,7,8-TCDD ranging from 17 to 586 ng/kg can be detected in fish from many rivers in Michigan which flow through industrialized urban areas. A greater proportion of samples containing quantifiable levels of 2,3,7,8-TCDD originated in the Tittabawassee and Saginaw Rivers, downstream to Midland. These residues are presumably the result of the large amount of chlorophenol manufacturing which has taken place in Midland over many years. However, the formation of dioxins during combustion of municipal wastes and fossil fuels cannot be ruled out as contributing to the existence of the

residues. Additional toxic dioxin and dibenzofuran congeners can be produced during combustion, which may significantly increase the potential hazard of combustion as a dioxin source. Studies into the formation of chlorinated dioxins and dibenzofurans in coal-fired power plants must be intensified to properly evaluate the magnitude of combustion as a source of environmental residues of TCDD.

The concentration at which 2,3,7,8-TCDD was detected in fish suggest that 2,3,7,8-TCDD could be present in the aquatic environment at levels potentially toxic to aquatic organisms. The residues detected in fish should not pose a human health risk to individuals consuming fish in moderate amounts. An added margin of safety is gained during cooking, which reduces TCDD content. However, wildlife which normally consumes large amounts of fish may be exposed to potentially harmful doses of 2,3,7,8-TCDD and other toxic materials.

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APPENDIX

The following section outlines the methods used for quantifying the concentration of 2,3,7,8-TCDD in the fish samples and presents examples of how selected GC-MS chromatograms were interpreted and quantified. Quantification was based on the mass spectrometric response for ions at m/e 322 and 332 of ^{12}C-2,3,7,8-TCDD and ^{13}C-2,3,7,8-TCDD, respectively. The following formula was utilized:

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

where:

A = peak area of native 2,3,7,8-TCDD in the sample
B = peak area of added ^{13}C-2,3,7,8-TCDD in the sample
C = peak area of ^{12}C-2,3,7,8-TCDD in the standard
D = peak area of ^{13}C-2,3,7,8-TCDD in the standard
E = mass of ^{12}C-2,3,7,8-TCDD in the standard (ng)
F = mass of ^{13}C-2,3,7,8-TCDD in the standard (ng)
G = mass of ^{13}C-2,3,7,8-TCDD added to the sample
H = mass of the fish sample

This formula is expressed as two terms. The first term calculates the concentration of 2,3,7,8-TCDD in the fish sample, based on the response of the injection aliquot at m/e 322 relative to the response of the most recently injected standard. The second term is used to correct for sample recovery based on the recovery of the ^{13}C-2,3,7,8-TCDD internal standard spiked into the ground fish sample prior to digestion and cleanup. Quantification is carried out relative to the response

of a standard, usually 50 pg of 2,3,7,8-TCDD. Response from this standard is reestablished every fifth injection to correct for variations in mass spectrometric response caused by instrumental drift.

The equation used for calculating concentrations assumes that mass specrometric response is linear over the range in which the samples to be quantitated respond. This assumption was verified by performing a linearity check at the outset of the quantification period and at the beginning of each day. Figure A-1 is a standard curve of the response measured for five consecutive injections of standards of increasing concentration. It demonstrates that linearity was achieved and validates the assumption. The calculated corelation coefficient for linear response was 0.9 indicating acceptable linearity over the range investigated.

Figures A-2 through A-11 are examples of output from the GC-MS which were used to calculate the TCDD concentrations. The first three figures are examples of output from a method blank, the ^{13}C-2,3,7,8-TCDD surrogate and a mixed standard while the remaining figures are GC-MS specific ion chromatograms of fish sample extracts. They are presented as examples of different types of output and are followed by copies of the data sheets used for calculations. Each sample represents a special case in data interpretation. They have been chosen to present the range of outputs encountered in this work.

Figure A-2 is the specific ion chromatogram of a procedural blank carried through the entire procedure. The signal at

m/e 332 is from the ^{13}C-2,3,7,8-TCDD internal standard. Absence of a signal at ions of m/e 320, 322 and 324 indicates that the sample was free of artifacts or cross contamination. The signal area of 535 units corresponds to about 0.71 ng of the spike, or a 14.1% recovery of the surrogate. This recovery refers to the amount of surrogate present in the injection aliquot, and represents a theoretical limit of detection of 7.1 ng/kg, assuming a minimum detectable quantity of 20 picograms of 2,3,7,8-TCDD and fish sample weight of 20 grams.

Figure A-3 is a GC-MS specific ion chromatogram of 1 ng of ^{13}C-2,3,7,8-TCDD. This is a 1 ul aliquot of the solution used for spiking the fish samples as well as a primary standard for quantification. The chromatogram demonstrates that at a level of 1 ng of the ^{13}C surrogate, there is no ^{12}C-2,3,7,8-TCDD background contributed by ^{12}C-2,3,7,8-TCDD impurities in the surrogate.

Figure A-4 is a mixed standard containing 50 picograms of each of the ^{13}C- and ^{12}C-2,3,7,8-TCDD standard. Some background signal is observed, especially in the region of 18 minutes at m/e 322 and 324. This background did not appear consistently in the standard, each time it was injected. Because this chromatogram was generated between runs during quantitation of samples, the peaks probably are the result of "ghosting". However, they do not show signals at all 4 ions monitored nor do they interfere with the 2,3,7,8-TCDD peaks. The ratio of the area for the m/e 320 ion to the area at m/e 322 for this standard is 0.96. This ratio is higher than expected. The

mean 320/322 ratio calculated for standards injected during the quantitation period was 0.79, and ranged from 0.55 to 0.96.

The next set of chromatograms represents samples determined not to contain any native 2,3,7,8-TCDD. The first chromatogram, figure A-5, is a Carp sample originating in Lake Erie. It shows an injection aliquot recovery of 73% and a theoretical limit of detection of 13 ng/kg, but does not reveal any evidence of native ^{12}C-2,3,7,8-TCDD. There is some contamination at ions 322 and 324, but none corresponding to the retention time of 2,3,7,8-TCDD. Also notable is the absence of any siganal at m/e 320 eluting with the impurities. This sample can be interpreted as containing no 2,3,7,8-TCDD at the stated limit of detection of 13 ng/kg.

The next sample, presented as figure A-6, is somewhat more difficult to interpret. It contains very weak signals at m/e 320 and 322 and a minimal signal at m/e 332. This signal is difficult to quantify because it is near the limit of detection. The area contribution below the top of the background signals must be estimated. The area value of 50 units corresponds to 65 picograms of the surrogate standard, or a limit of detection of 76 ng/kg. The ion signals at m/e 320 and 322 suggest a trace of 2,3,7,8-TCDD. However, because a signal was not observed at m/e 324, the sample cannot be reported as containing any 2,3,7,8-TCDD.

The next figure (A-7) is an example of a sample which had no recovery of the internal standard and was therefore non-quantifiable. This sample was most likely the result of an

elution through an alumina column with improper activity or the product of a series of inefficient transfer steps. It is useless for any type of quantitative determination.

The remaining examples represent samples which contained detectable residues of 2,3,7,8-TCDD. Figure A-8 is an example of a sample with a signal at the limit of detection. The measured area of 6 units for the ion at m/e 332 indicates a very low recovery while the response at ions 320, 322 and 324, although quantifiable, is difficult to differentiate from the background. For this reason, the sample was judged to be too poor to qualify for reporting as a positive sample. The quantified level of 2,3,7,8-TCDD would have been 738 ng/kg at a limit of detection of 638 ng/kg. Because of the high correction factor incorporated due to the low recovery (0.16%), the calculated value is of very low precision and cannot be accepted with any certainty.

Figure A-9 is output fron the sample reported as containing the highest observed residue of 2,3,7,8-TCDD. It originated in the St. Clair River. Review of the output indicates a strong signal at ions of masses 320, 322 and 324 accompanied by a signal at 332 corresponding to 60 pg of surrogate, or a theoretical limit of detction of 81 ng/kg. The ions show acceptable response by all criteria established for quantification. The 320/322 isotope ratio is a little high. However this is due to the way the 322 ion peak was integrated. The baseline for the 320 ion was determined to lie directly along the bottom of the leading and tailing edges of the peak, while the 322 ion baseline

was interpreted to exist above the tangent point of the baseline. It is likely that most of the area above the baseline is due to TCDD, and could be requantified as such. This would result in a lower 320/322 isotope ratio and a higher calculated TCDD concentration.

Figure A-10 is output from a sample of fish originating in the Tittabawassee River. It represents a sample quantified near the mass spectrometric limit of detection. The area at m/e 322 corresponds to 33 pg of 2,3,7,8-TCDD. Corrected for surrogate recovery of 2.6%, the concentration of 2,3,7,8-TCDD in the fish sample sample was calculated as 83 ng/kg.

The last figure (A-11) is output from a sample showing a good recovery and strong signals at all ions representing native 2,3,7,8-TCDD. This sample originated in the Muskegon River. This type of output was the easiest to interpret and holds the greatest degree of precision.

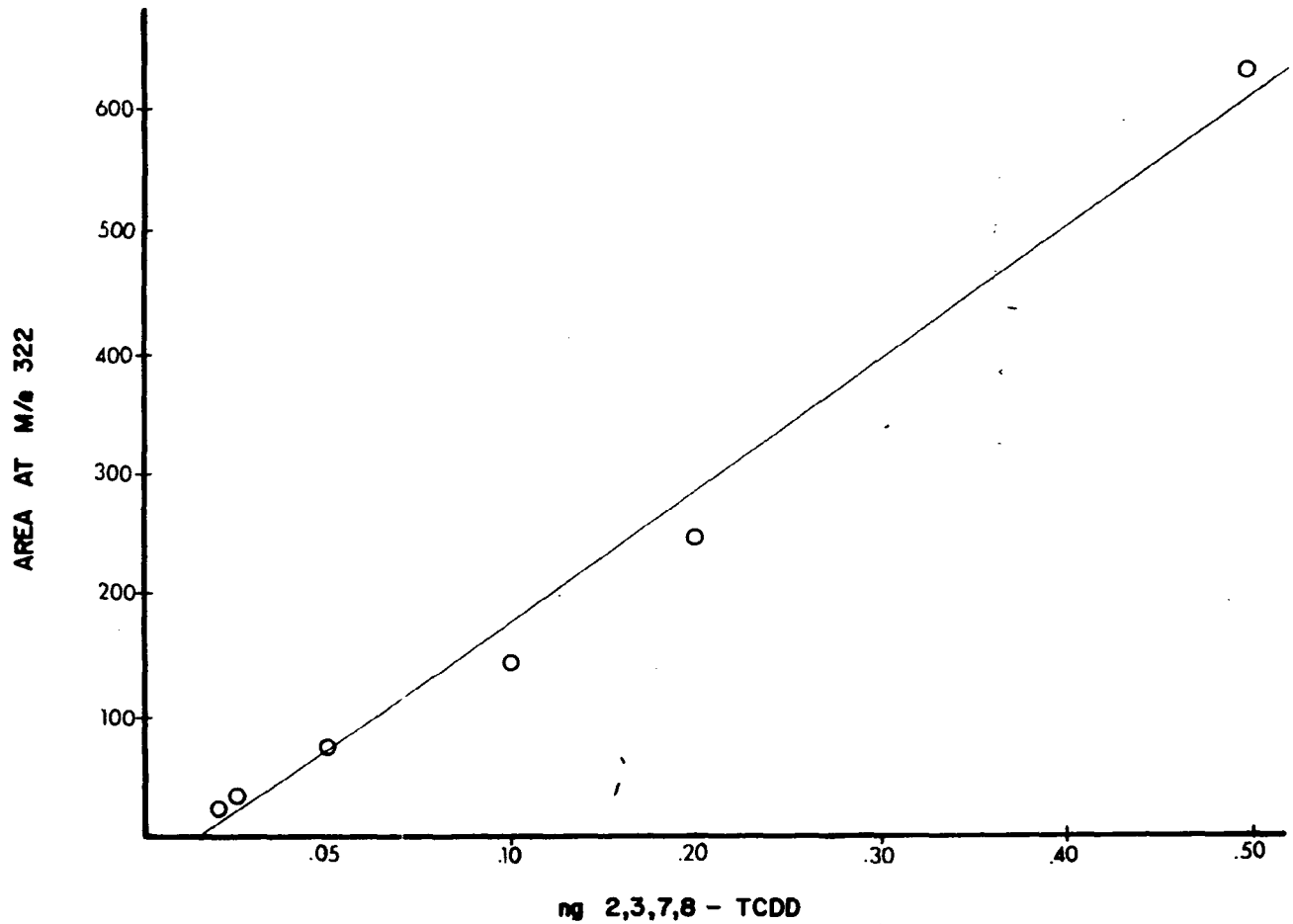

Figure A-1 GC-MS Standard Curve of 2,3,7,8-TCDD.

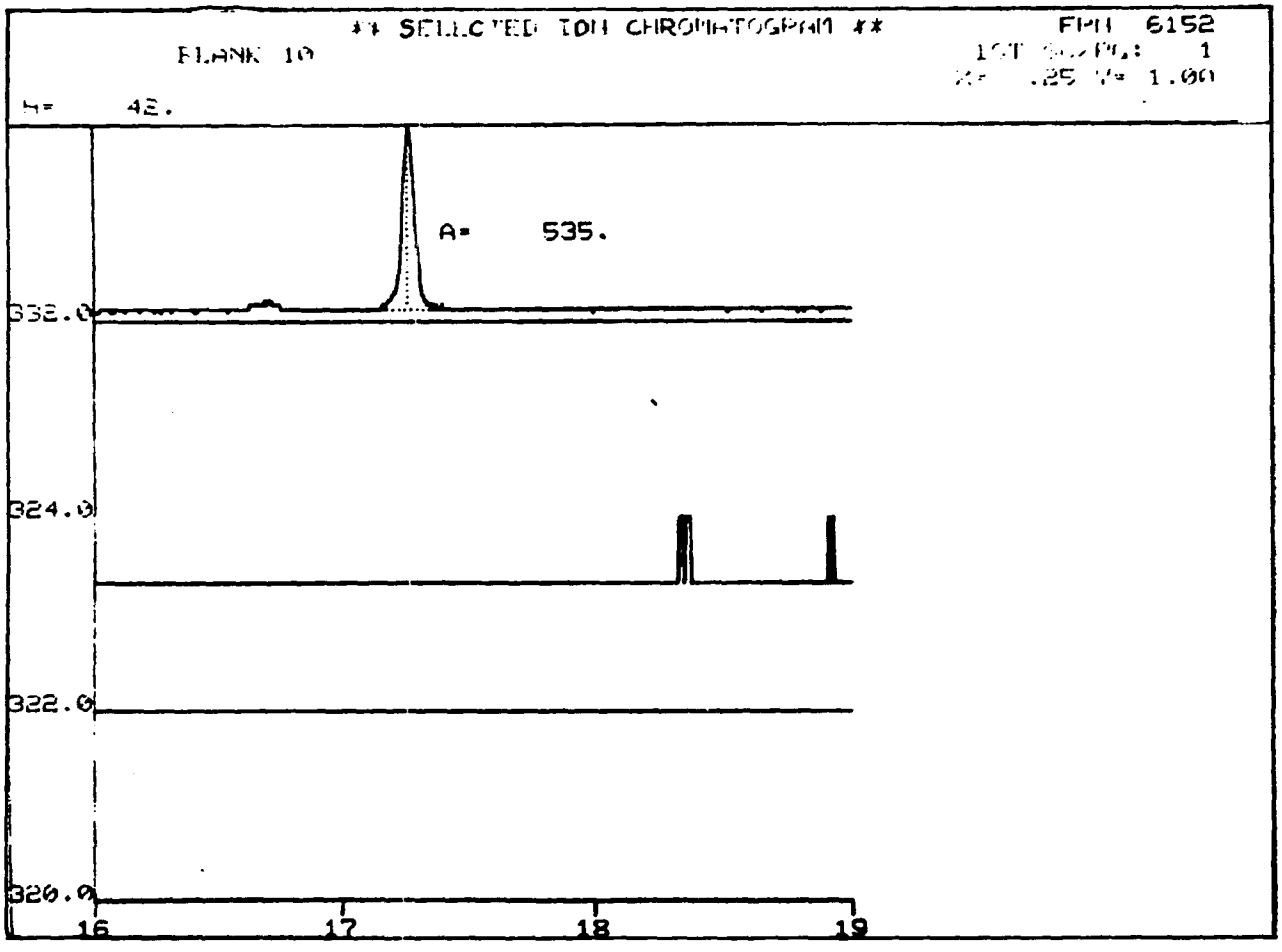

Figure A-2 SIM GC-MS Chromatogram of Procedural Blank.

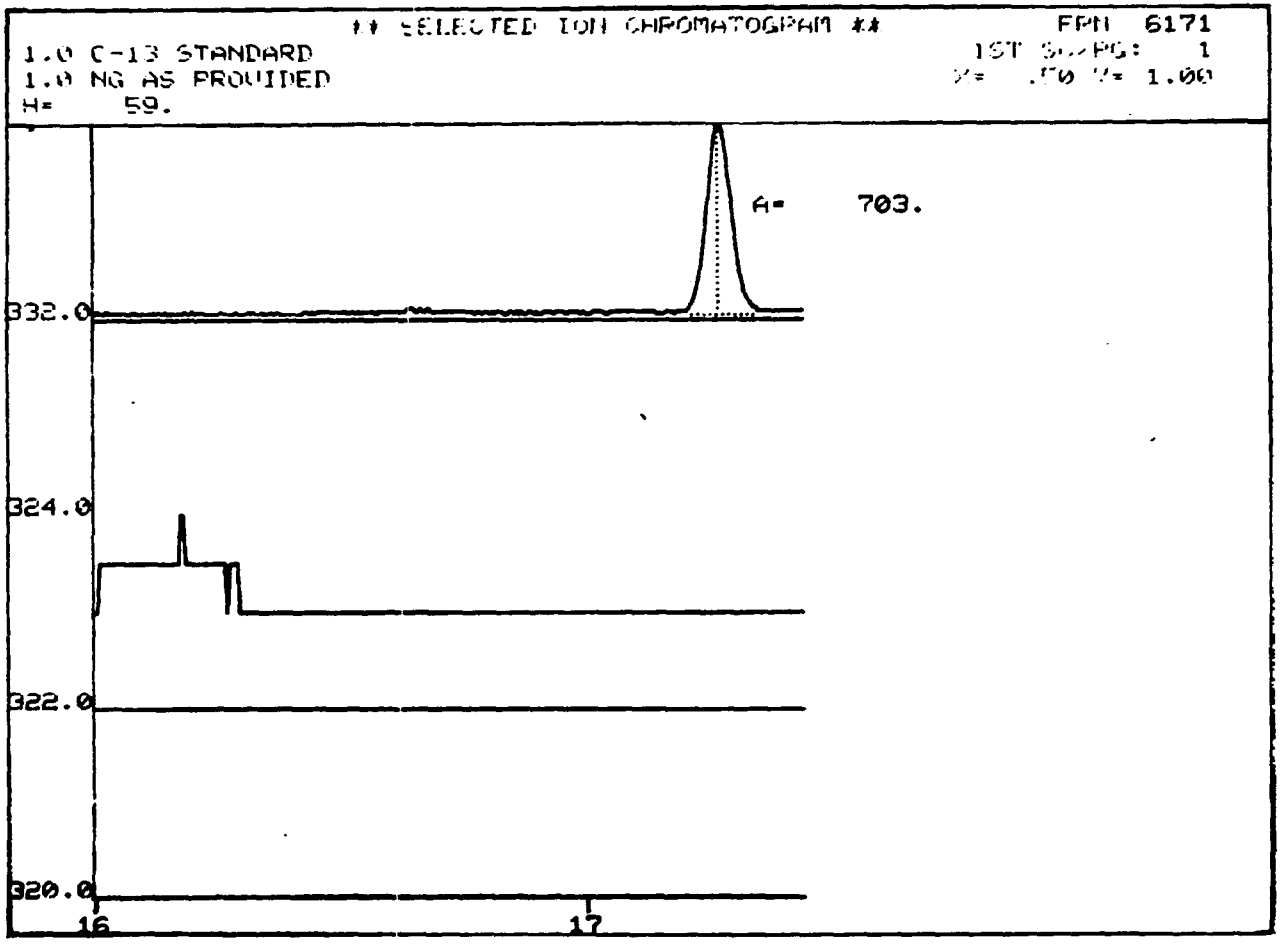

Figure A-3 SIM GC-MS Chromatogram of 1 ng of ^{13}C-2,3,7,8-TCDD

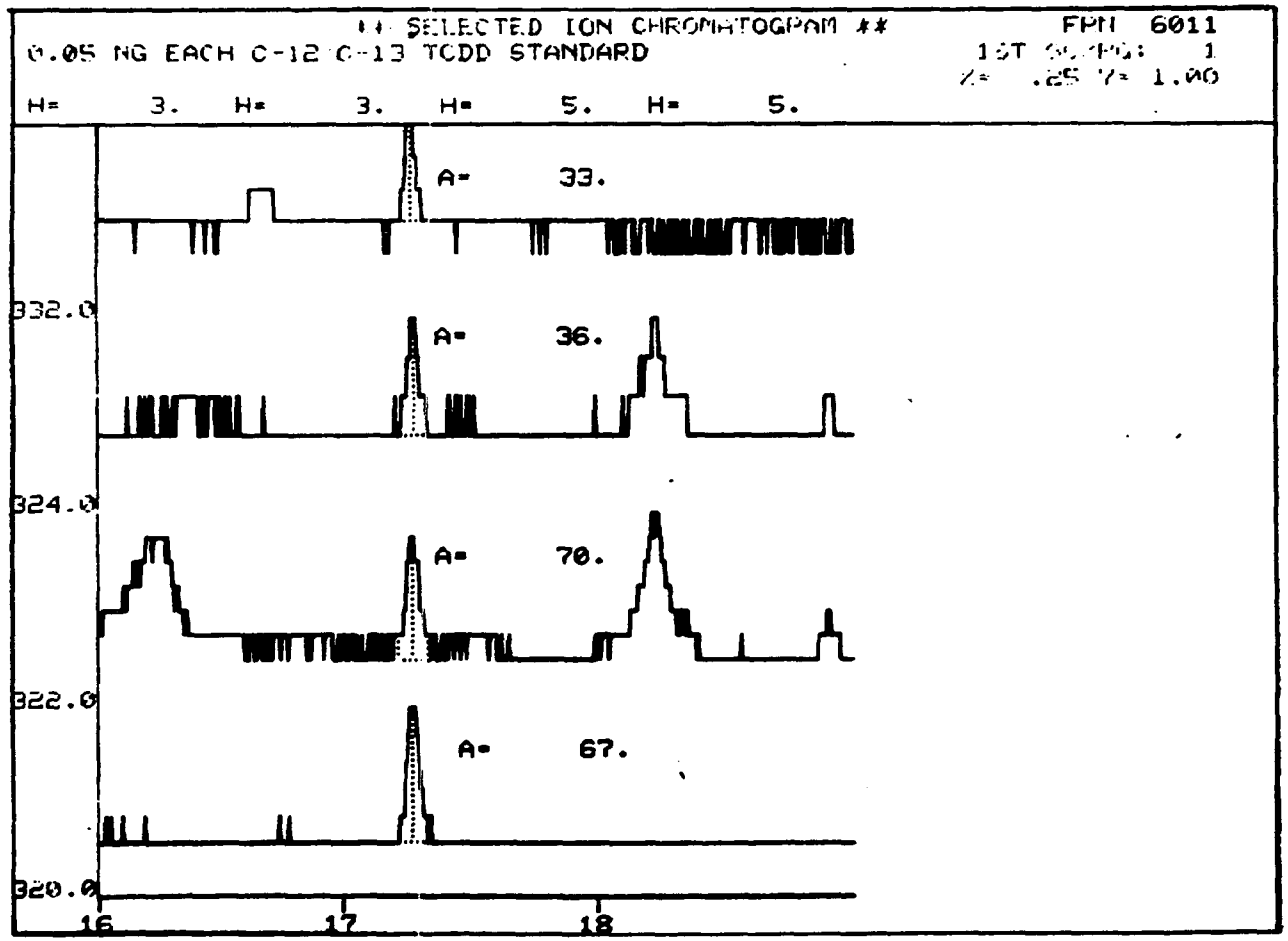

Figure A-4 SIM GC-MS Chromatogram of 50 pg Mixed Standard.

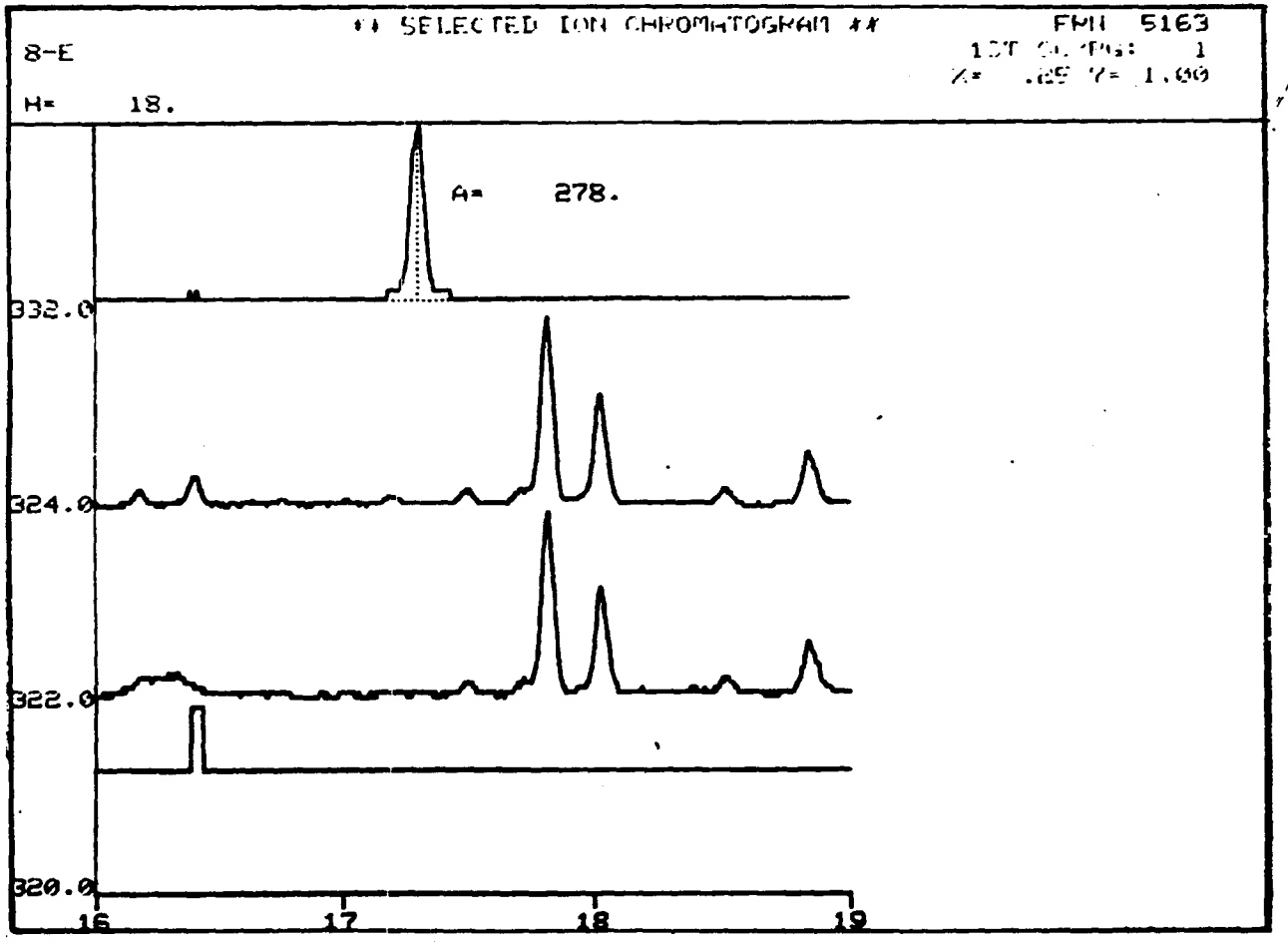

Figure A-5 SIM GC-MS Chromatogram of Sample with No Detectable 2,3,7,8-TCDD

SAMPLE I.D. 8·E

LOCATION Lake Erie

SPECIES Carp

WEIGHT (kg) 1.42

LENGTH (cm) 44.6

SOURCE MSU - Food Science

SAMPLE WEIGHT 25 g

% FAT —

% MOISTURE —

AGE 4

FRN 5163

CARTRIDGE 9

RESPONSE

332 278

320 —

322 —

324 —

ISOTOPE RATIO —

STANDARD RESPONSE

mass 50 pg

332 38

322 64

% RECOVERY $\frac{278}{38}\left(\frac{.05}{5}\right)(100) = 7.31$

LMIT OF DETECTION $\left(\frac{100}{7.31}\right)\left(\frac{20}{25}\right) = 10.9$

CONCENTRATION

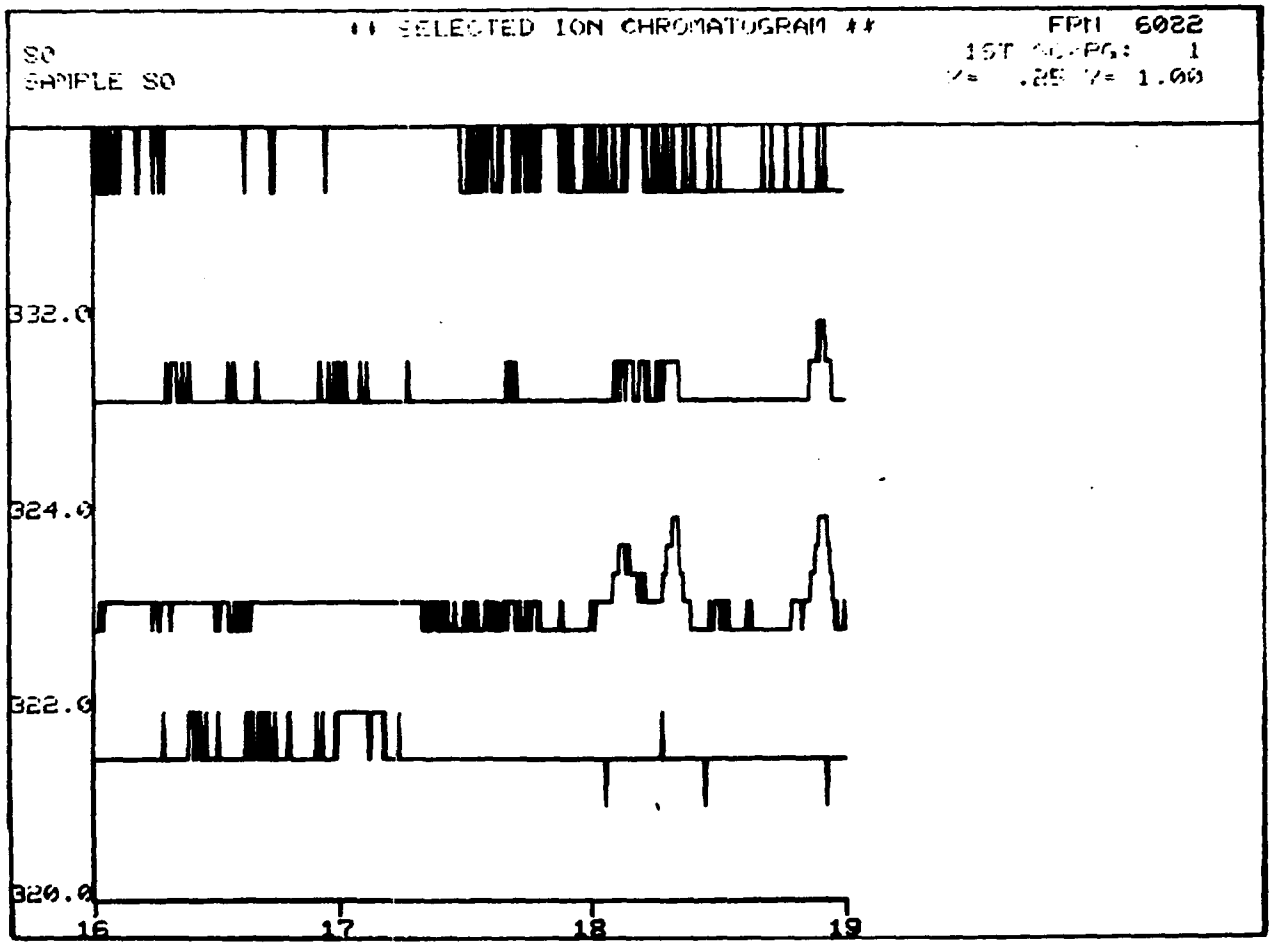

Figure A-6 SIM GC-MS Chromatogram of Sample with No Detectable 2,3,7,8-TCDD

SAMPLE I.D. 149

LOCATION Kalamazo at Saugatuck

SPECIES W. Sucker

WEIGHT (kg) 1.020

LENGTH (cm) 43.6

SOURCE DNR

SAMPLE WEIGHT 20

% FAT 4.0

% MOISTURE 80.1

AGE 4

FRN 5121

CARTRIDGE 9

RESPONSE

332 50

320 –

322 –

324 –

ISOTOPE RATIO _____

STANDARD RESPONSE

mass 50

332 38

322 72

% RECOVERY $\frac{50}{38}\left(\frac{.05}{5}\right)100 = 1.3$

LMIT OF DETECTION $\frac{100}{1.3} = 76.9$

CONCENTRATION

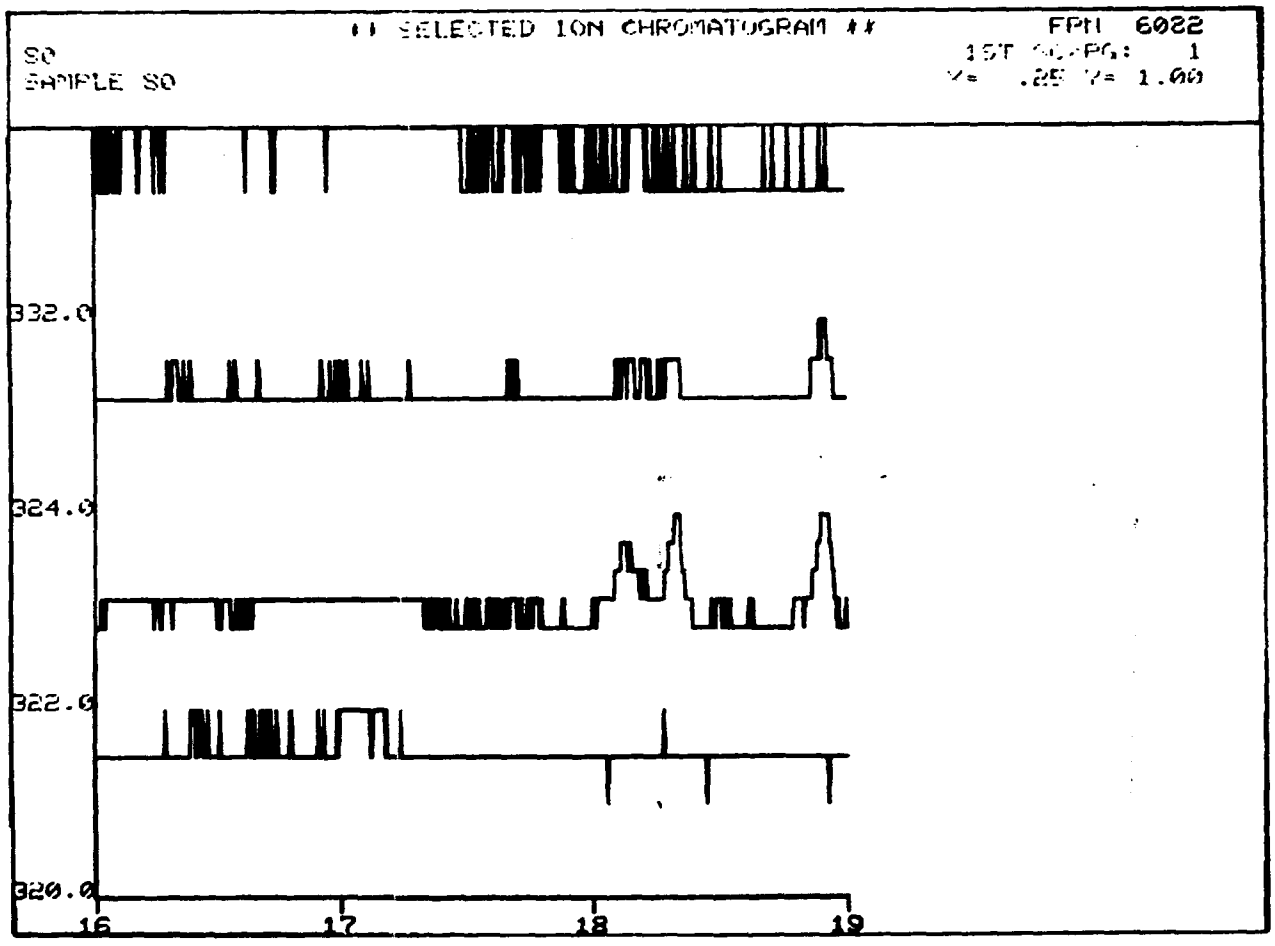

Figure A-7. SIM GC-MS Chromatogram of Sample with No Quantifiable Recovery

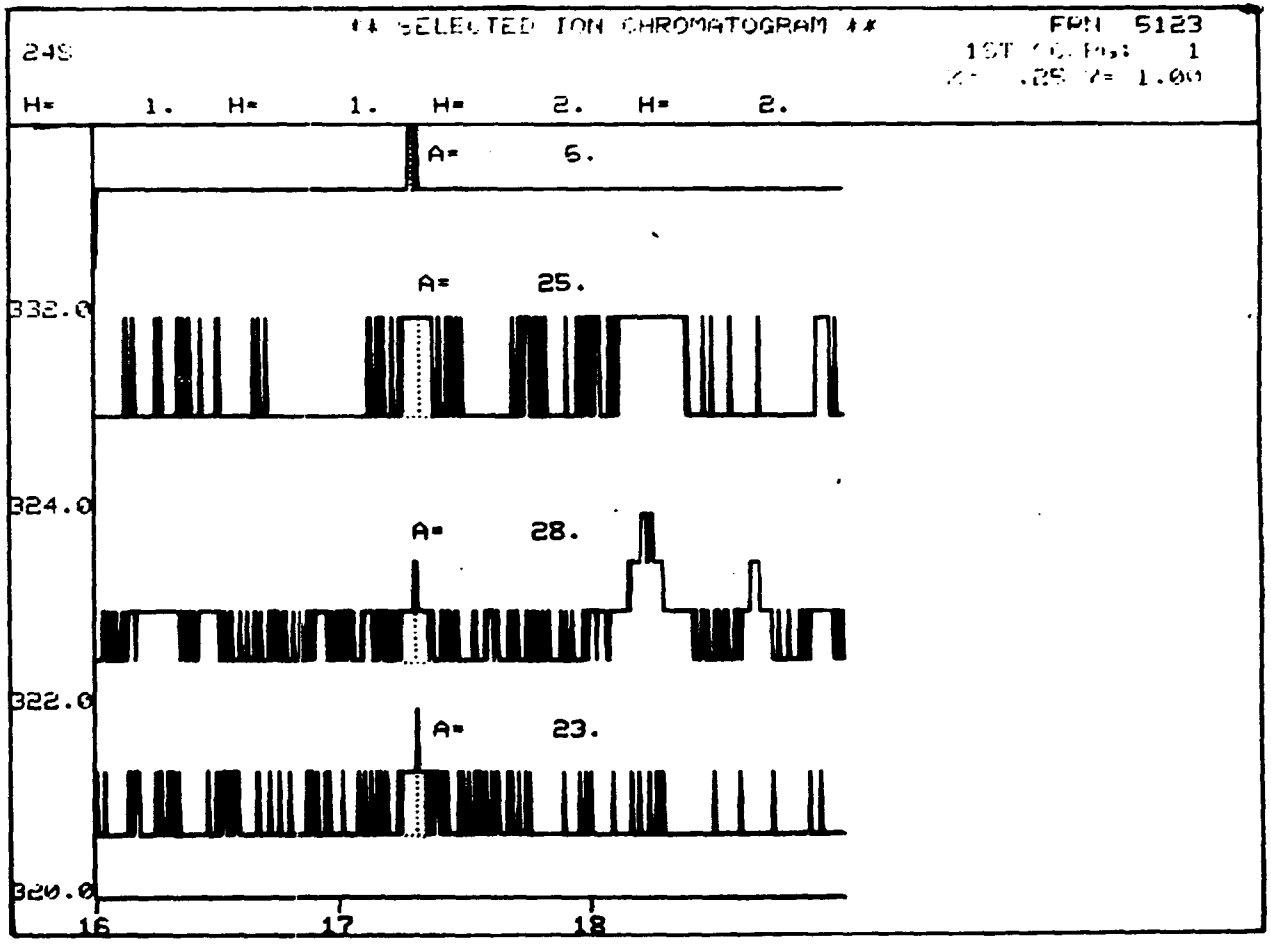

Figure A-8. Example of Sample at the Limit of Detection

SAMPLE I.D. 248

LOCATION Tittabawasse - Saginaw Twp Pk.

SPECIES Carp

WEIGHT (kg) 1.35

LENGTH (cm) 44.4

SOURCE DNR - SK

SAMPLE WEIGHT 20

% FAT 8

% MOISTURE 73.3

AGE 6

FRN 5123

CARTRIDGE 9

RESPONSE

332 6

320 23

322 28

324 25

ISOTOPE RATIO 0.89

STANDARD RESPONSE

mass 50 pg

332 38

322 70

% RECOVERY $\frac{6}{38}\left(\frac{.05}{5}\right)100 = 0.16$ Unacceptable

LMIT OF DETECTION $\frac{100}{.16} = 625$

CONCENTRATION

$$\frac{\frac{28}{60}(.05)(625)}{0.020} = 738.5$$

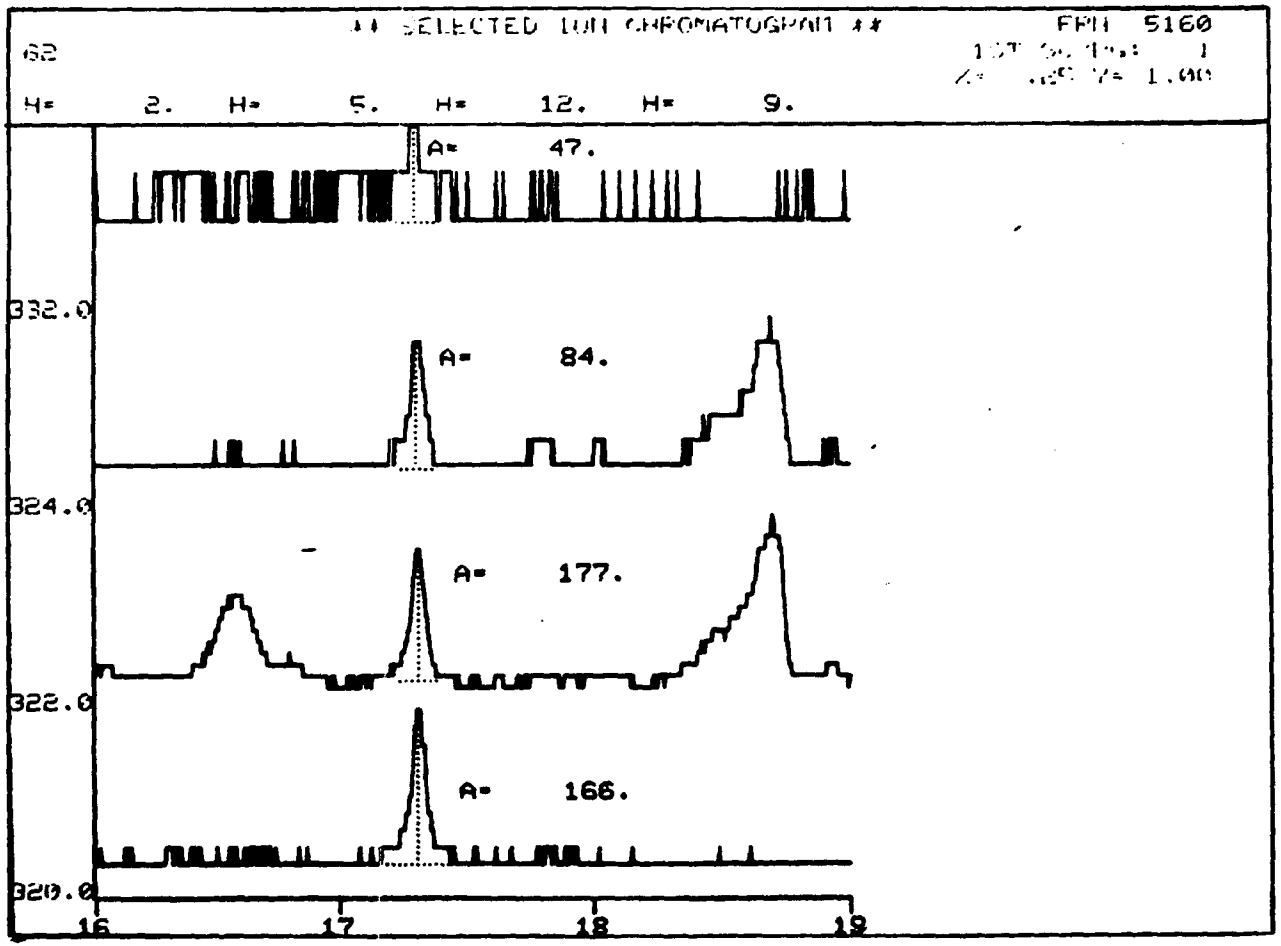

Figure A-9. SIM GC-MS Chromatogram of Sample with the Highest Quantified Level of 2,3,7,8-TCDD

SAMPLE I.D. 62

LOCATION St. Clair at Dechers Landing

SPECIES Carp

WEIGHT (kg) 1.84

LENGTH (cm) 51.8

SOURCE DNR - Pontiac

SAMPLE WEIGHT 20

% FAT 3.5

% MOISTURE 79.4

AGE 7

FRN 5160

CARTRIDGE 9

RESPONSE

332 47

320 166

322 177

324 84

ISOTOPE RATIO 0.94

STANDARD RESPONSE

mass 50 pg

332 38

322 61

% RECOVERY $\left(\frac{47}{38}\right)\left(\frac{.05}{5}\right)100 = 1.24$

LMIT OF DETECTION $\frac{100}{1.24} = 80.8$

CONCENTRATION $\frac{\frac{177}{61}(.05)(80.8)}{0.02} = 586.1$

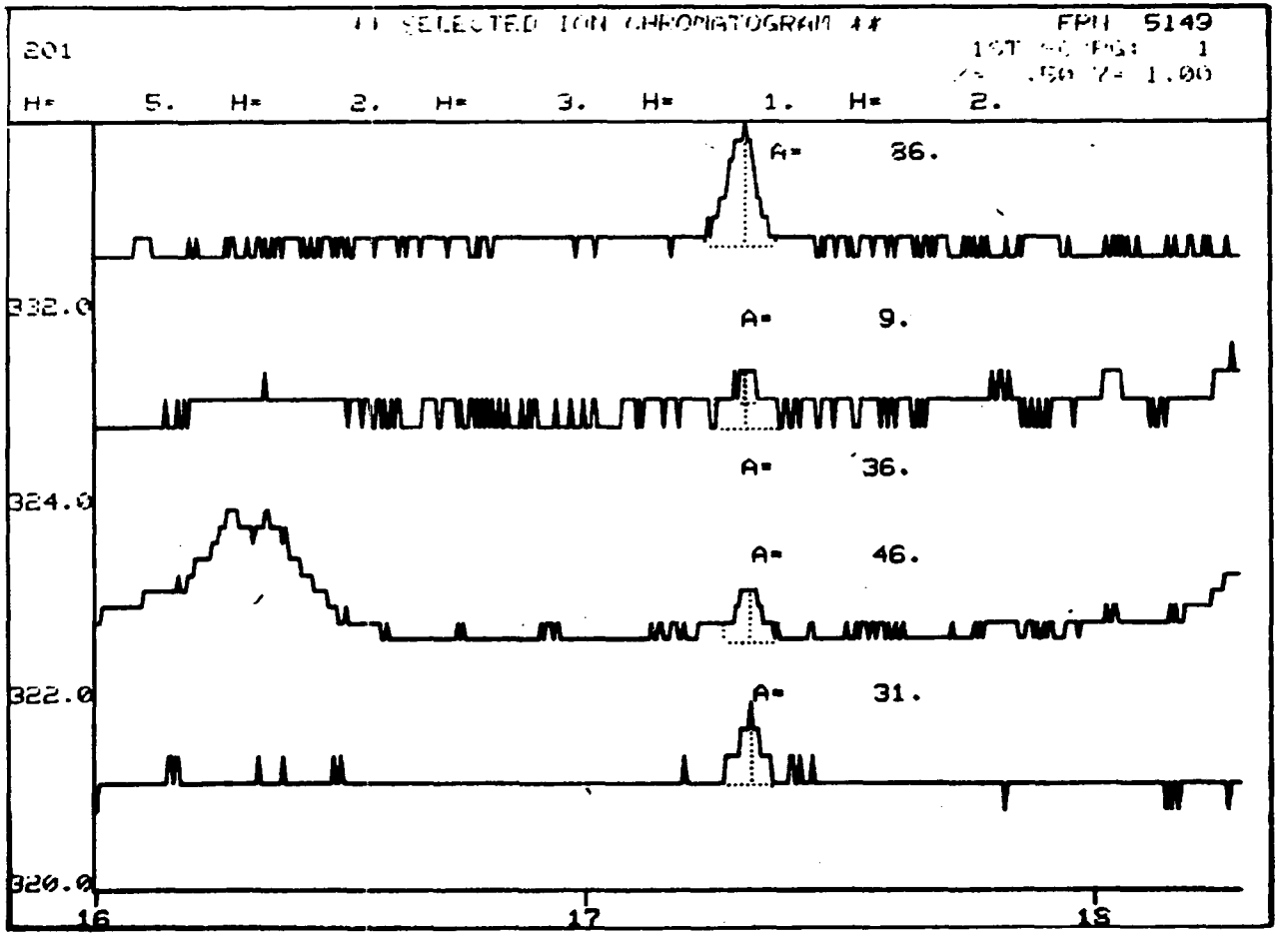

Figure A-10. SIM GC-MS Chromatogram of Sample Quantified at the Limit of Detection

SAMPLE I.D. 201

LOCATION Tallabawarra at Dow Dam

SPECIES Carp

WEIGHT (kg) 0.52

LENGTH (cm) 31.7

SOURCE S.K.

SAMPLE WEIGHT 20

% FAT 3.2

% MOISTURE 80

AGE 9

FRN 5149

CARTRIDGE 9

RESPONSE

332 86

320 36

322 46

324 31

ISOTOPE RATIO 0.78

STANDARD RESPONSE

mass 50 pg

332 38

322 61

% RECOVERY $\frac{86}{38}\left(\frac{.05}{5}\right)100 = 2.3$

LMIT OF DETECTION $\frac{100}{2.3} = 44.2$

CONCENTRATION

$$\frac{\frac{46}{61}(.05)(44.2)}{.02} = 83.3$$

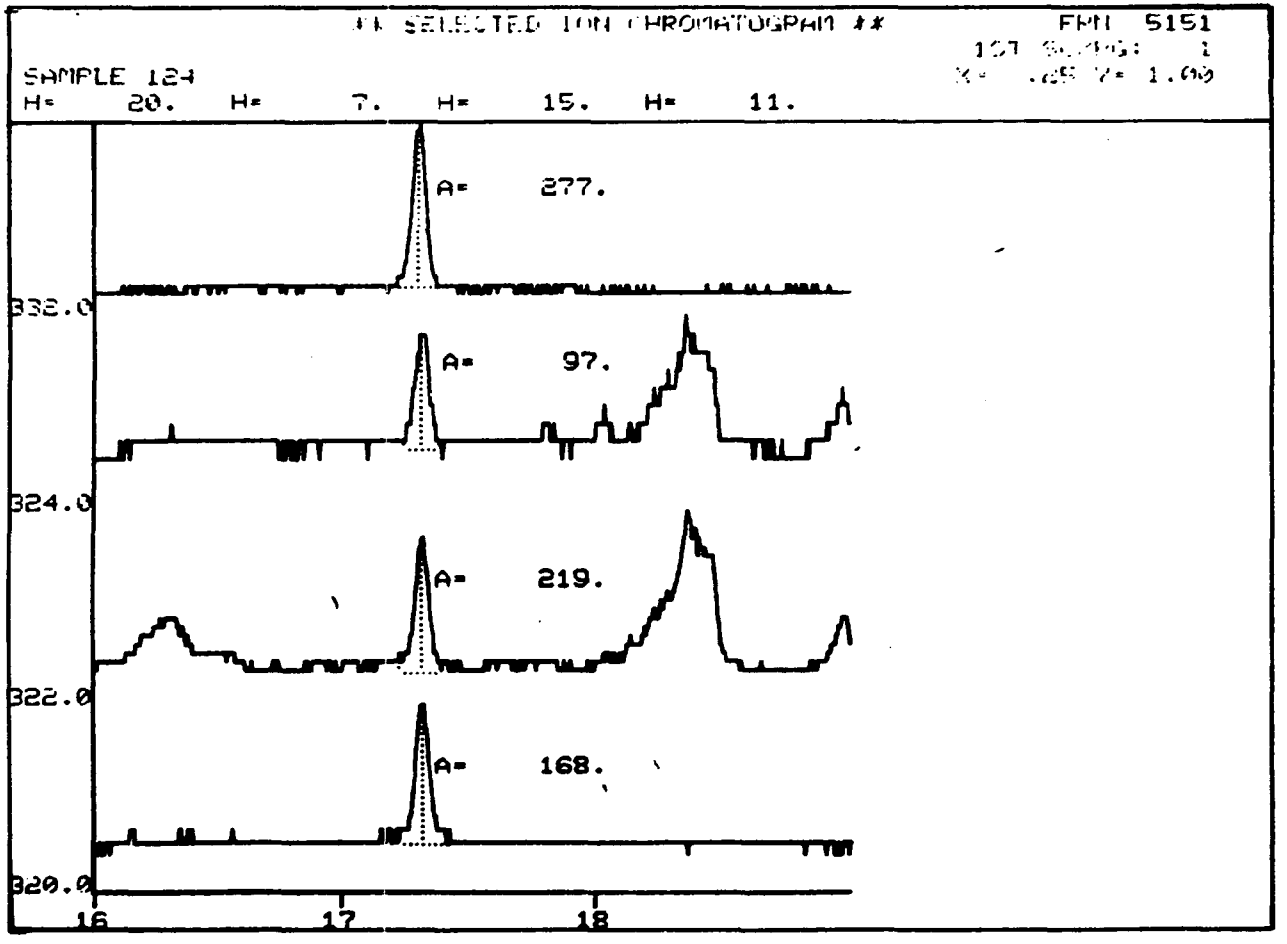

Figure A-11. SIM GC-MS Chromatogram of Sample Exhibiting Optimal Response and Recovery

SAMPLE I.D. 124

LOCATION Muskegon Lake at Cobb Power Plant.

SPECIES Redhorse Sucker

WEIGHT (kg) 0.80

LENGTH (cm) 46.3

SOURCE DNR - Grand Rapids

SAMPLE WEIGHT 20

% FAT 0.9

% MOISTURE 85.1

AGE 5

FRN 5151

CARTRIDGE 9

RESPONSE

332 277

320 168

322 219

324 97

ISOTOPE RATIO 0.77

STANDARD RESPONSE

mass 50 pg

332 38

322 61

% RECOVERY $\frac{277}{38}\left(\frac{.05}{5}\right)(100) = 7.3$

LMIT OF DETECTION $\frac{100}{7.3} = 13.7$

CONCENTRATION

$$\frac{\frac{219}{61}(.05)(13.7)}{0.02} = 122.9$$