unpublished report but released info

FEB 1 1981

not to be cited in literature

TCDD (2,3,7,8 - Tetrachlorodibenzo-p-dioxin) in Great Lakes Herring Gulls

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ABSTRACT

Pooled samples of 10 Herring Gull (Larus argentatus) eggs collected in 1980 from each of 12 colonies scattered throughout the Great Lakes and one colony on the Atlantic Coast in the Bay of Fundy were analyzed by high resolution gas chromatography/low resolution mass spectrometry for tetrachlorodibenzo-p-dioxin isomers. Only 2,3,7,8-TCDD was found. It ranged from 43 to 86 ng/kg in Saginaw Bay colonies, from 44 to 68 ng/kg in Lake Ontario colonies, and from 9 to 14 ng/kg in colonies on the other Great Lakes. The Bay of Fundy colony had a level of approximately 3 ng/kg. Levels of TCDD in archived Lake Ontario eggs collected in 1971-72 were in the order of 1 μg/kg (ppb). TCDD levels declined exponentially in Lake Ontario eggs with a half-life of 2.2 yr from 1974-1980.

Ultra-trace quantities of chlorinated dibenzo-p-dioxins have recently attracted the attention of analytical chemists and toxicologists due to the extreme toxicity of the 2,3,7,8-tetrachloro-isomer, hereinafter referred to as TCDD. TCDD has been recognized in Europe and North America as an ultra-trace (ng/kg) contaminant in soils, sediments, plants and animals. It is particularly found in areas subjected to production, industrial accidents or industrial disposal of 2,4,5-trichlorophenol used in the formulation of herbicides 2,4,5-T and fenoprop and the disinfectant hexachlorophene. Examples include Seveso, Italy (1, 2), the Love Canal at Niagara Falls, N.Y. (3) and Saginaw Bay near Midland, Michigan (4). TCDD persists and bioaccumulates in ecosystems which have been subjected to repeated dosages of herbicide formulations such as Silvex or Agent Orange, which contain 2,4,5-T (4-6). Examples include rangeland studies (4), experimental plots, including U.S. airforce bases (5) and South Vietnam (6). TCDD has also been found in activated sludge and flyash (7) although in low relative amounts ($< 10\%$) to other TCDD isomers. Other TCDD isomers have not been found to be present in environmental samples.

There has been, however, no systematic study of TCDD contamination in aquatic ecosystems, in which organochlorine contaminants often accumulate to high levels. Herring Gulls have been used successfully as indicators of organochlorine

contaminants in Great Lakes aquatic ecosystems (8, 9, 10). An early attempt in 1973 to find chlorinated dibenzodioxins in Herring Gull eggs was unsuccessful (11). However, unconfirmed reports on the finding of TCDD in Lake Ontario fish prompted us to analyse Herring Gull eggs from the Great Lakes for TCDD and other tetrachloro dioxin isomers recently developed, using more sensitive techniques.

We report here the levels of 2,3,7,8-TCDD determined in pools of 10 eggs collected in May 1980 from Herring Gull colonies in each of the Great Lakes (Fig. 1): two in L. Superior, one in L. Michigan, three in L. Huron, two in L. Erie, and four in L. Ontario, including one colony in north central L. Ontario for which archived samples were available retrospectively to 1971, and one colony on the Atlantic coast in the Bay of Fundy. A pooled sample of breast muscle from 5 adult Herring Gulls from Saginaw Bay was also analysed. Samples were analysed for TCDD isomers by high-resolution capillary chromatography/low resolution mass spectrometry (12).

All Herring Gull egg and muscle samples analysed contained detectable levels (> 2-3 ng/kg) of TCDD (Table 1). No other compounds present demonstrated the correct ion abundance ratios for m/z 320, 322 and 324, characteristic of the molecular ion of TCDD isomers. The identity of TCDD was determined by retention time of an authentic standard on three

different capillary columns (methylsilicone, SE-54 and OV-17). On all three columns, TCDD was well-resolved from the 1,2,3,4-; 1,3,6,8-; and 1,3,7,9-TCDD isomers. TCDD isomer interferences with 2,3,7,8-TCDD on methylsilicone and OV-17 columns are completely different (13). Since only one peak was present in the TCDD region of the chromatogram for at least two (usually all three) of the ions measured, these results constitute isomer specific analysis of great certainty. High resolution mass spectral analysis (resolution 20,000, 10% valley) confirmed the structure of TCDD. This degree of resolution is twice that required to distinguish between TCDD and tetrachlorobiphenyl-methyl ethers, or tetrachlorobenzyl- phenyl ethers, which have the same molecular weight and ion abundance ratios, and are potential interferences in analysis of gull samples (14). Confirmatory analyses on selected samples were also performed through U.S. Environmental Protection Agency, and U.S. Fish and Wildlife Service.

Recovery studies were carried out using 1,2,3,4- and 2,3,7,8-TCDD spiked to chicken eggs at the 0, 10 and 50 ng/kg level just prior to the extraction stage. The recoveries were: 50 ng/kg, 80-100% (1,2,3,4-TCDD), 94% (2,3,7,8-TCDD); 10 ng/kg, 84-86% (1,2,3,4-TCDD), 66-67% (2,3,7,8-TCDD). Although the recoveries were reproducible, no correction factors were applied to the analytical results. Repeatability

of analysis of naturally contaminated samples was 13% at the 50 ng/kg level (Muggs Isl., L. Ontario) and 17% at the 10 ng/kg level (Middle Isl., L. Erie).

Eggs from colonies on Lakes Erie, Huron (except [illegible] Bay), Superior and Michigan contained 9-14 ng/kg of TCDD. These colonies are highly contaminated with other organochlorines, particularly PCBs. The ratio of TCDD to PCB (Table 1) was relatively constant, ranging from 3 to 7 x 10^{-7}, in this Great Lakes "baseline" concentration group. It is noteworthy that the ratio in the Bay of Fundy was consistent with the Great Lakes baseline, although the former area is relatively uncontaminated and remote from major potential industrial, agricultural or silvicultural sources of TCDD. The major exogenous source of PCBs in the Great Lakes is atmospheric deposition (14). Our data therefore suggest that TCDD, like PCBs, may be a ubiquitous contaminant, cycling at ultratrace levels between various components of the air, water, sediment and biota of the biosphere.

The TCDD/PCB ratio was approximately 7 times higher in Saginaw Bay and approximately 3 times higher in Lake Ontario than the "baseline" ratio in the other colonies. Both are clearly localized areas of elevated TCDD contamination. The elevated levels in Saginaw Bay gulls corroborate the finding of 2,3,7,8-TCDD in fish collected in 1978 from the

Saginaw and Tittabawassee river system (4), since the Channel Shelter Island colony is situated at the mouth of the Saginaw River. Dow Chemical Co. in the Midland and Bay City, Michigan area is situated on the Saginaw-Tittabawassee river system and has been a major producer of 2,4,5-trichlorophenol and 2,4,5-trichlorophenoxy acid herbicides. The possible influence of this point source of TCDD is reflected in relatively high TCDD levels in eggs from the Little Charity Island colony 50 km from the mouth of this river system. Gulls at Chantry Island, on the opposite shore of Lake Huron were not highly contaminated, indicating that lake-wide distribution of TCDD has not occurred.

The low variability (Relative SD 16%) of TCDD levels in the four Lake Ontario colonies in 1980 suggests that lake-wide contamination of the fish species (mainly alewives and smelt) which comprise the main aquatic portion of the Herring Gull's diet in the Great Lakes (16) has occurred. Combustion of organic compounds at temperatures below 1000°C in the presence of chlorine-containing compounds, such as during the incineration of municipal garbage, has been shown to yield chlorinated dioxins (5, 17). However, the 2,3,7,8-TCDD isomer is a relatively minor constituent ($< 10\%$) of the tetrachloro isomers produced, and no isomer other than 2,3,7,8-TCCD was found in gull eggs. It would also be unusual for widely distributed atmospheric sources such as refuse

incinerators to have contaminated only Lake Ontario. Another possible source of TCDD to Lake Ontario is application of herbicides to agricultural and forest lands, but neither 2,4,5-T nor fenoprop herbicides have been used extensively in Ontario in recent years (18). Spraying of 2,4,5-T in Vietnam and on experimental plots has resulted in detectable levels of TCDD in biota, but only when the application rate was far in excess of usual agricultural practice (4,6,7). Fish levels in Vietnam were only in the 10 ng/kg range, similar to the baseline concentrations which we detected in Great Lakes Herring Gulls outside of Lake Ontario and Saginaw Bay. Some formulations of the herbicide 2,4-D have recently been shown to contain µg/kg levels of 1,3,6,8- and 1,3,7,9-TCDD (19). Since 2,4-D has been used in much larger quantities than 2,4,5-T in the Great Lakes basin and since these isomers of TCDD were not found in the gulls, it appears that waste disposal or major accidents during the manufacturing of 2,4,5-trichlorophenol are the likely sources of the higher levels of TCDD found in Lake Ontario. Hooker Chemical Co. at Niagara Falls, N.Y. manufactured 2,4,5-trichlorophenol and it is known from studies on mirex in gull eggs and fish that widespread contamination of Lake Ontario can occur from such a point source in the Niagara River (10, 20).

The retrospective study of TCDD levels in eggs from 1971 to 1980 at Scotch Bonnet Island in Lake Ontario is shown

in the insert of Fig. 1. Levels decreased throughout this period from 800 - 1,300 ng/kg in the early 1970's to 68 ng/kg in 1980. Most organochlorine residues in Lake Ontario also decreased during this period, presumably due to restrictions on use (9). Prior to 1975 the TCDD/PCB ratio was 45 to 52 x 10^{-7} (except for a low result of 18 x 10^{-7} in 1973). From 1976 onwards the ratio has stayed roughly the same (15 to 24 x 10^{-7}), indicating that both contaminants were being cleared from the ecosystem at similar rates. The data from this later period have a good fit to an exponential decay model (r^2 = 0.9886, log-linear regression) with a half-life of 2.2 years. This half-life is identical to that found for mirex in Lake Ontario as determined from similar Herring Gull egg data (9). It is encouraging that TCDD levels in Lake Ontario gull eggs will likely decline to the "baseline" level of ca. 10 ng/kg within 5-7 years. TCDD levels in fish relative to those in gull eggs have yet to be established, but we expect the rate of decline in fish to be similar.

We shall continue to monitor Herring Gull populations for TCDD in the Great Lakes. On a more widespread basis, we intend to test the hypothesis that 2,3,7,8-TCDD is a ubiquitous contaminant by comparing its relative abundance to such global contaminants as PCBs in a range of Canadian biota.

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12. Ten fresh eggs collected from each colony were homogenized individually and stored as 10 g subsamples at -40°C until further processing. One subsample of each egg was pooled and homogenized just prior to extraction. These 100 g pools were triturated with Na_2SO_4 until a free-flowing powder was formed, and poured into 3 cm ID glass columns. Lipids were eluted from each column with 800 ml of hexane. Hexane was removed by rotary evaporation. Most of the lipid from each half sample was then removed by acetonitrite:H_2O:hexane: dichloromethane partitioning, the residue from each half combined, and the organochlorines fractionated on a large Florisil column (21). TCDD isomers appeared in fractions #4 and #5 from this procedure. After two alumina-column cleanups, the fractions were analysed separately. When necessary, the following procedure was used to remove remaining interferences in fraction 5: Two glass columns (3 cm ID) were packed with 35 g 44% H_2SO_4/silicagel (w/w), and each column prewashed with 50 ml dichloromethane/hexane (1:1). Fraction #5 was dissolved in 200 ml dichloromethane/hexane (1:1), divided into 2 equal portions and transferred to the acid/silicagel column. Each column was washed with 50 ml dichloromethane.

Eluates from the 2 columns were combined, and the solvent was removed by rotary evaporation. The residue was redissolved in 0.5-1 ml hexane, cleaned up on an alumina microcolumn, combined with fraction #4, transferred quantitatively into a small (0.3 ml) reacti-vial equipped with a mininert valve, taken just to dryness, redissolved in 100 µl of hexane and stored in the dark until analyzed. The samples were analyzed by high-resolution GC/low-resolution MS (a Hewlett-Packard 5992B) employing a 25 m flexible fused-silica WCOT column (methyl silicone phase, 0.2 mm i.d., Hewlett Packard), splitless injector and open coupling. Helium carrier gas was used at a linear velocity of 90 cm/sec. Injection port temperature was 250°. Samples (up to 5 µl, in hexane) were injected in the splitless mode and the oven programmed from 100° to 150° at 10°/min., and 150° to 250° at 2.5°/min. After screening of samples for the absence of 1,2,3,4-TCDD, all samples were analyzed by SIM at m/z 320, 322 and 324 by coinjection of an amount of 1,2,3,4-TCDD which gave responses within 30% of the native 2,3,7,8-TCDD responses at these ions. The amount of 2,3,7,8-TCDD was calculated using 1,2,3,4-TCDD as an internal standard. The retention indices of sample peaks, authentic 2,3,7,8-TCDD, and 1,2,3,4-TCDD were 2306.8, 2304.8, and 2294.4, respectively, in a typical run. The corresponding indices were 2345.9 $\pm$ 1.8, 2345.7 $\pm$ 0.7, and 2337.7 $\pm$ 0.6, on a J. & W. Scientific 25 m (0.25 mm

i.d.) SE-54 column, and 2534.4 ± 1.6, 2535.3 ± 1.5 and 2543.6 ± 1.0 on a Canadian Capillary 25 m (0.30 mm i.d.) OV-17 column.

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TABLE 1

LEVELS OF 2,3,7,8-TCDD IN POOLED[1] 1980 GREAT LAKES HERRING GULL EGGS

LAKE - COLONY (Colony No. in Fig. 1)	N[2]	2,3,7,8-TCDD[3] (ng/kg)	RELATIVE ABUNDANCE (%) TO BASE PEAK[4] m/z 324	m/z 320	PCB[5] (mg/kg)	TCDD/PCB x10[7]
L. SUPERIOR						
GRANITE ISL (1)	6	11 ± 2.3	46	79	21	5
AGAWA ROCK (2)	4	10 ± 1.3	45	73	ND	ND
L. MICHIGAN						
BIG SISTER ISL. (3)	3	9 ± 1.0	47	71	25	3
SAGINAW BAY						
CHANNEL SHELTER (4)	4	86 ± 2.5	49	77	32	27
(muscle)	4	90 ± 7.2	48	80	41	22
L. CHARITY ISL. (5)	4	43 ± 3.6	49	76	ND	ND
L. HURON						
CHANTRY ISL. (6)	3	14 ± 2.4	50	75	19	7
L. ERIE						
MIDDLE ISL. (7)	2	13 ± 0.7	46	77	ND	ND
(repeat analysis)	4	11 ± 0.6	49	71	35	3
PORT COLBOURNE (8)	2	9 ± 0.5	44	73	34	3

TABLE 1 (cont'd)

LAKE ONTARIO						
MUGGS ISL. (9)	5	50 ± 2.6	47	76	ND	ND
(repeat analysis)	3	44 ± 1.0	46	74	39	11
PRESQU'ILE (10)	3	64 ± 3.8	49	77	39	17
SCOTCH BONNET ISL. (11)	5	68 ± 4.1	47	76	42	16
SNAKE ISL. (12)	7	55 ± 4.7	44	75	42	13
BAY OF FUNDY						
KENT ISL.	3	3 ± 0.3[6]	54	73	5	6
CHICKEN EGG	3	< 2	ND	ND		ND

1 No. of eggs in pool was 10

2 N is number of replicate injections of each sample

3 Results on a fresh weight basis calculated from m/z 322 using a 1,2,3,4,-TCDD internal standard; mean ± SD of replicate injections, uncorrected for recovery;
ND = not determined. Repeat analysis of colony (7) and (9) eggs began with a second 100 g subsample

4 Base peak is m/z 322; experimental relative abundances for 2,3,7,8-TCDD were 49% (m/z 320) and 77% (m/z 324)

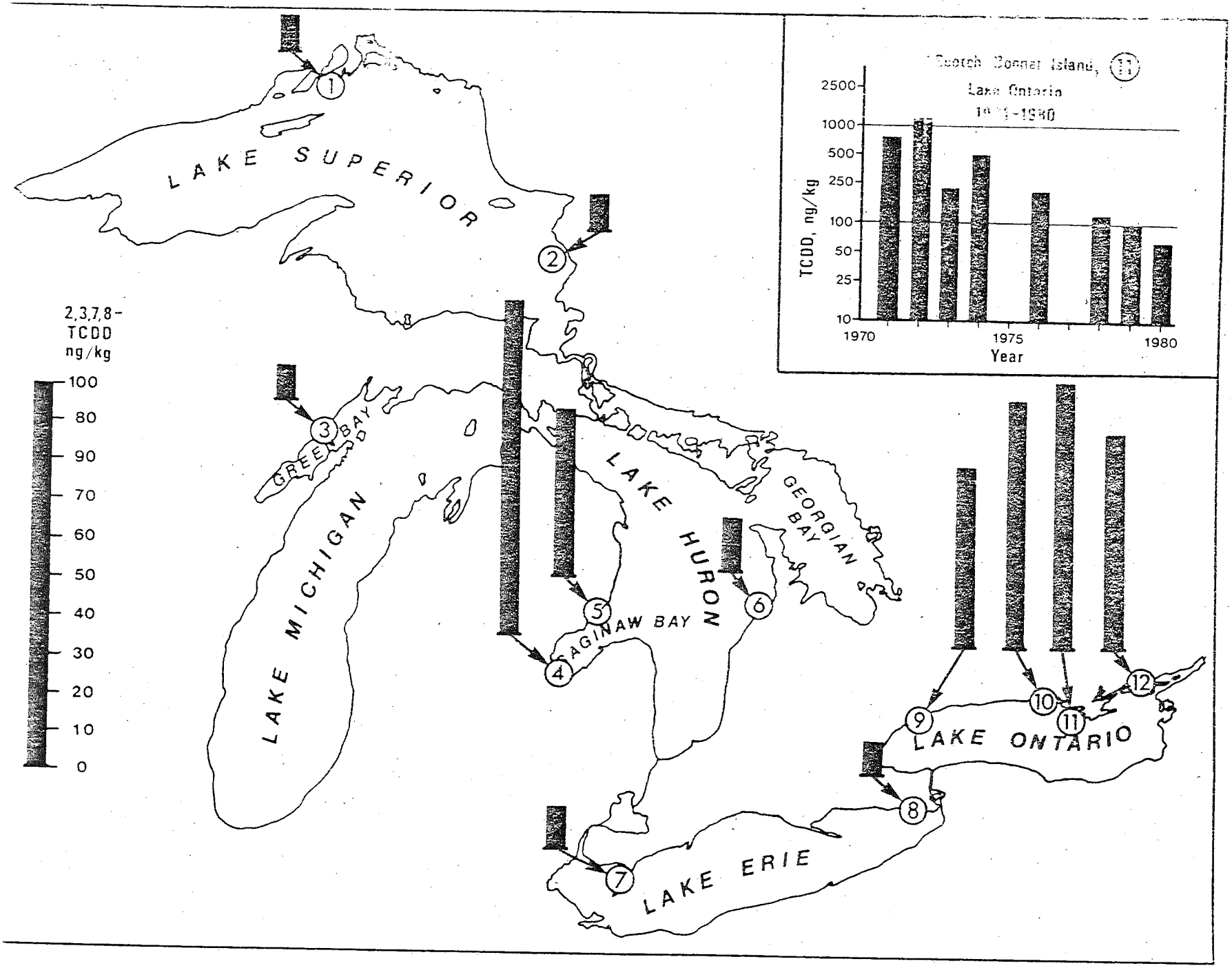
5 Results calculated as Aroclor 1254

6 Due to closeness to detection limit the accuracy of this result is likely poor. TCDD was confirmed by retention time, but not by high resolution GC/MS

FIGURE LEGEND

Fig. 1 Sites of Herring Gull colonies from which eggs were collected in 1980. Colony names can be identified from the numbers in parentheses in column 1, Table 1, which correspond to those shown on the map. Levels (ng/kg fresh weight) of 2,3,7,8-TCDD in pools of 10 eggs are indicated by the height of the bars next to each colony.

Inset: Retrospective analysis of gull egg pools from Scotch Bonnett Island, Lake Ontario, for 2,3,7,8-TCDD. Bar height is proportional to the logarithm of the TCDD level (ng/kg fresh weight).


LAKE SUPERIOR
LAKE MICHIGAN
LAKE HURON
LAKE ERIE
LAKE ONTARIO
GREEN BAY
SAGINAW BAY
GEORGIAN BAY
2,3,7,8-
TCDD
ng/kg
100
80
90
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
Scotch Bonnet Island, 11
Lake Ontario
1970-1980
TCDD, ng/kg
2500
1000
500
250
100
50
25
10
1970
1975
1980
Year