

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND RELATED DIOXINS AND FURANS IN
SNAPPING TURTLE (CHELYDRA SERPENTINA) TISSUES

FROM THE UPPER ST. LAWRENCE RIVER

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Abstract

Fat and liver samples from three snapping turtles (Chelydra serpentina) from the upper St. Lawrence River were analysed for tetra- up to octa-chlorinated dibenzo-p-dioxins (PCDDs) and chlorinated dibenzofurans (PCDFs) by two gas chromatography - mass spectrometry techniques and by a bioassay. The tissues contained only 2,3,7,8-chlorine substituted PCDDs and PCDFs with high levels occurring for 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). On a wet weight basis, fat levels of 2,3,7,8-TCDD were 4 to 7 times higher than liver levels with up to 500 pg/g present in turtle fat. One fat sample also contained over 3 ng/g of 2,3,4,7,8-pentachlorodibenzofuran (PnCDF) suggestive of a PCB source of contamination. The TCDD levels in the turtles are consistent with the consumption of a fish diet containing about 20 pg/g 2,3,7,8-TCDD, a level currently found in eel samples from the same region. Comparison of this limited snapping turtle data with that of other aquatic species from the same and different bodies of water suggest that the Niagara River via Lake Ontario could be a possible source of contamination of 2,3,7,8-TCDD for the St. Lawrence River. The results also show that turtles are excellent monitors for PCDD and PCDF contamination. Human consumption of turtle tissue samples containing the highest levels of 2,3,7,8-TCDD and PCDFs so far detected in wildlife samples may constitute a hazard.

INTRODUCTION

2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) occurs as a contaminant

in some fish species from the Great Lakes, particularly Lake Ontario and the Saginaw Bay area of Lake Huron (1-3). Typically positive values range between 5 and 20 parts per trillion (ppt; pg/g) with the occasional higher value. Other chlorinated dibenzo-p-dioxins (PCDDs) and chlorinated dibenzofurans (PCDFs) have been reported less frequently and in smaller concentrations (4,5). There has been no documentation of the occurrence of 2,3,7,8-TCDD or various PCDDs in other aquatic species from the Great Lakes. However, tissues of the snapping turtle (Chelydra serpentina) from New York State waters are known to accumulate and tolerate remarkably high fat and liver levels of PCBs (6,7). In some cases the concentration of PCB in adipose tissue was almost 1% making the turtle an excellent biological monitor for the presence of this type of chemical contamination. In addition, high levels of PCDFs have been identified in the fatty tissue of a snapping turtle taken in the Hudson river, New York State, in the vicinity of a former capacitor manufacturing plant (8) (site 10 in Fig. 1).

Since we were surveying fish populations from the Great Lakes for PCDDs and PCDFs and because turtles appeared to be such good accumulators of chlorinated aromatic compounds, we analysed some turtle tissues for the PCDDs and PCDFs. This report gives the result of the analyses of liver and fat samples from three snapping turtles taken in the upper St. Lawrence River near Lake Ontario. Extracts of two of the turtle fat samples were also analysed by a second gas-chromatography (GC)-mass spectrometry (MS) method and by a newly developed in vitro bioassay for dioxinlike activity (9). The results show some of the highest 2,3,7,8-TCDD and PCDF levels for any biological sample from the Great Lakes area.

EXPERIMENTAL

Tissue Collection

Abdominal fat and liver tissues were taken from three snapping turtles, two collected in June 1984 in the upper St. Lawrence River near Lake Ontario, sites 1 and 2 in Fig. 1, and a third taken in June 1985 in the same river near East Massena, site 3. Liver tissue from a fourth turtle but a different species was collected in the Ottawa River near Ottawa in July 1980 (site 4). More detailed information on the tissue samples is given in Table 1. Samples of eel (Anguilla rostrata) were obtained in 1982 and 1983 by Fisheries and Oceans Canada from two areas in the middle (site 5) and lower (site 6) St. Lawrence River in Quebec, one of its tributaries the Richelieu River (site 7), and the eastern part of Lake Ontario (site 8).

Analysis

Analysis of tissues was carried out to effect measurement of all tetra-through octa-PCDDs and PCDFs (up to 136 analytes). Crude tissue was first homogenized to a uniform consistency and an aliquot (2 to 3 g for fat and 5 g for liver and fish) taken for analysis. The sample was then homogenized or

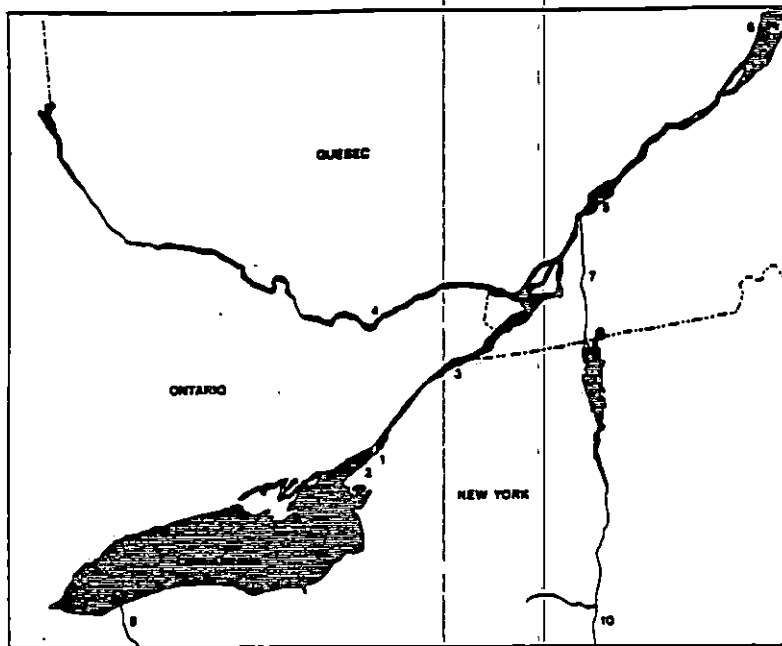


Fig. 1 - Map of area from which the turtle and fish samples were collected. Numbers refer to specific sites mentioned in text.

Table 1. Information on turtle tissues from the St. Lawrence and Ottawa Rivers.

Turtle Number	Date Collected	Location	Species	Tissues	Live Weight Kg	Age Years	Sex
1	June 1984	Steele's Bay Clayton, St. Lawrence River, site 1 ^a	snapping, <u>Chelydra</u> <u>serpentina</u>	Fat Liver	6.4	16+	M
2	June 1984	Goose Bay East, Alexandria, St. Lawrence River, site 2	snapping, <u>Chelydra</u> <u>serpentina</u>	Fat Liver	3.6	8+	F
3	June 1985	East Massena, St. Lawrence River, site 3	snapping, <u>Chelydra</u> <u>serpentina</u>	Fat Liver	7.1	14+	F
4	July 1980	Ottawa River, Ottawa, site 4	Blandings, <u>Emydoidea</u> <u>blandingi</u>	Liver	2.1	-	M

a - refers to Figure 1.

shaken with 120 mL of acetone-hexane (2:1) and spiked with about 100 pg each

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of a mixture of stable isotopically enriched PCDDs and PCDFs in order to calculate losses in sample preparation. The sample extract was then partitioned against water and the aqueous and solid phases extracted a second time with hexane (10). After washing the combined hexane phases with water, a 10% portion was taken, evaporated to dryness in a rotary evaporator under vacuum and weighed. This weight represented the lipid content of the tissue. The remaining hexane extracts were then defatted with sulfuric acid and chromatographed on disposable glass columns containing Florisil as adsorbant. This latter step removed the less polar contaminants such as PCBs and chlorinated diphenyl ethers as outlined previously (11). Further cleanup of some sample extracts was carried out by reversed phase high pressure liquid chromatography (HPLC) (12) with methanol as eluent and by collection of two fractions. Some sample extracts were purified after Florisil by the recently developed chromatographic procedure (13) of activated carbon dispersed on silica gel. In the latter case, a mixture of cyclohexane-dichloromethane eluted the non-planar aromatics followed by the elution of the planar aromatics such as PCDDs and PCDFs with toluene.

Measurement of the PCDDs and PCDFs in sample extracts was effected by GC-MS. The GC was a Varian 3700 fitted with a 25 m DB-5 (J & W, 0.25 mm I.D.) bonded phase fused silica capillary column. The column was heated in steps rapidly from 100 to 180°C (ca 1 min) and then more slowly to 240°C at 5°C per min such that 2,3,7,8-TCDD and octachlorodibenzo-p-dioxin (OCDD) eluted at about 11 and 22 min, respectively. The GC was coupled directly to a VG-Analytical ZAB-2F MS operating at low resolution (2,000) for liver and fish extracts and at high resolution (10,000) for fat extracts. Compounds eluting from the GC were ionized by electron impact and detected by selected ion monitoring of ions from each congener group as described (14,15). Quantitation of the analytes in extracts was achieved by comparing the response of external standards injected either before or after sample extracts. Values reported were corrected for manipulative and purification losses using the isotopically labelled internal standard recoveries. Detection limits were about 2 ppt for most analytes except for OCDD which, due to higher reagent blanks and lower recoveries, was about 10 ppt. Method performance was monitored by the simultaneous processing of laboratory reagent blanks in each set of five samples, by the use of seven carbon-13 labelled PCDD and five chlorine-37 labelled PCDF internal standards and by the participation in interlaboratory studies.

Confirmation of residues in the turtle fat

Separate 4 g aliquot samples of crude turtle fat from animals 1 and 2 taken at the same time as above tissues were spiked with internal isotopic standards, extracted with dichloromethane and partitioned against sulfuric acid. After neutralization and passing through a column of KOH treated silica, the extract was processed through a semi-automated system containing

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acidic alumina, eluted with dichloromethane-hexane (1:1) onto charcoal in Celite, and reverse flow eluted with xylene onto a neutral alumina column. Final elution of the neutral alumina column was carried out with 1:1 dichloromethane hexane. The GC-MS was equipped with a 60 m SP-2330 polar stationary phase column and MS resolution was 10,000. This technique (16) is specific for 2,3,7,8-TCDD due to the complete separation of this isomer from the other 21 TCDD isomers on the polar GC column.

In vitro bioassay procedure

The sample was extracted with dichloromethane, treated with sulfuric acid and neutralized as described above, and the extract was solvent exchanged to benzene to a final volume of 1 mL. Dimethylsulfoxide (DMSO; 50 uL) was added and the benzene evaporated under a flow of dry nitrogen. The DMSO extract was diluted 1:1000 in culture medium and a series of ten-fold dilutions were added to XBF epithelial cells which were initiated one day previously with irradiated 3T3 mouse fibroblast cells. Treated as well as positive and negative control cultures were incubated for 14 days with refeeding every 3 to 4 days with freshly made sample dilutions. The cultures were then evaluated for altered growth and morphology as discussed elsewhere (9,17).

RESULTS

The PCDDs and PCDFs found in the seven tissues from the four turtles are listed by increasing degree of chlorination in Table 2. Only 2,3,7,8-chlorine

Table 2. PCDD and PCDF levels (pg/g) in four turtle fat and liver samples.

Turtle No. ^a	1		2		3		4
Tissue	Fat	Liver	Fat	Liver	Fat	Liver	Liver
2378-Fb	12	ND(2) ^d	6.0	ND(2)	330	74	ND(2)
2378-DC	474	107	232	32	370	74	ND(2)
12378-F	ND(2)	ND(2)	ND(2)	ND(2)	600	100	ND(2)
23478-F	152	29	95	13	3020	480	ND(2)
12378-D	63	12	33	4.6	104	22	ND(2)
Total HxF ^e	ND(2)	ND(2)	ND(2)	ND(2)	890	160	ND(2)
Total HxD ^f	23	3.0	16	6.4	102	18	ND(2)
1234678-F	ND(2)	ND(2)	ND(2)	ND(2)	69	13	ND(2)
1234678-D	2.3	4.5	17	4.0	3.7	3.7	ND(2)
12346789-D	34	27	36	ND(10)	17	ND(10)	16
Lipid %	93	11	71	9.2	85	16	-

a - cf. Table 1; b - chlorinated dibenzofuran; c - chlorinated dibenzo-p-dioxin; d - not detected followed by detection limit in pg/g in brackets; e - hexachlorodibenzofuran including the 123478-, 123678-, and 234678-isomers; f - hexachlorodibenzo-p-dioxins including the 123678-, 123478-, and 123789-isomers.

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substituted PCDDs and PCDFs were detected in these samples (Figure 2) with the tetra- and penta-chlorinated congeners predominating in concentration over

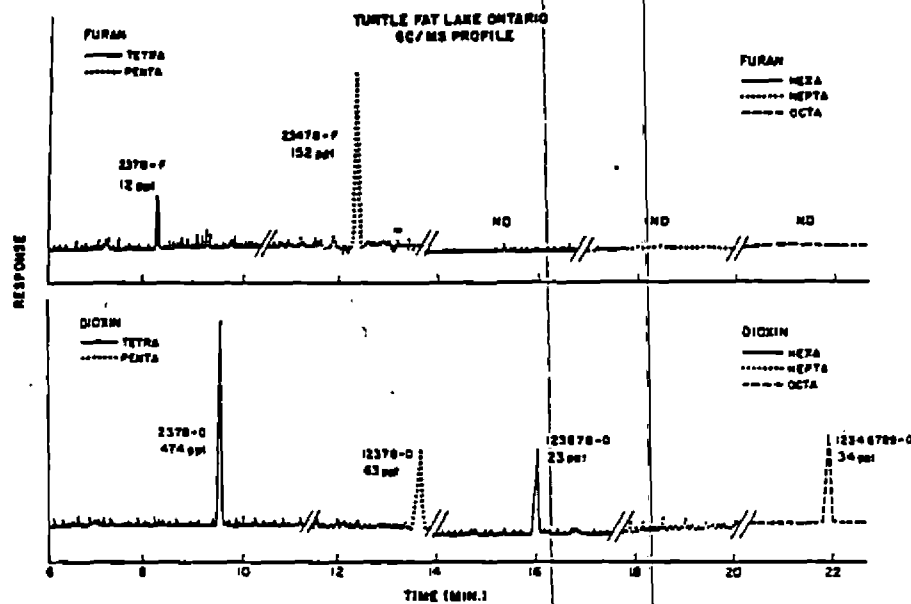


Fig. 2 - GC/MS profile of fat sample from turtle 1 showing presence of 2,3,7,8-chlorine substituted PCDDs and PCDFs; peak responses are reported at different attenuations for each congener group.

the higher chlorinated groups (hexa-, hepta- and octa-). The presence of only 2,3,7,8-chlorine substituted PCDDs and PCDFs is similar to that found in human tissues and in fish from the Great Lakes (1-4,15). However, the distribution of PCDDs and PCDFs among the various congener groups in the turtle tissues is comparable to that found in fish (tetra- and penta- highest and higher chlorinated PCDDs and PCDFs lower) and different to that found in human tissues (for PCDDs, tetra- lowest and octa- highest; for PCDFs, penta-, hexa-, hepta- similar concentration) (15). Although the number of samples is small, a distinction in tissue level exists between the snapping turtle tissues from the St. Lawrence River and the Blandings turtle liver sample from the Ottawa River. The latter sample showed only a small amount of OCDD near the detection limit of 10 ppt, a difference which no doubt reflects the localities from which the animals were obtained. The tissues from turtle 3 contain the same types and about the same concentration of PCDDs as those from turtles 1 and 2. However, turtle 3 shows a different PCDF profile than do turtles 1 and 2. Firstly, additional PCDF (1,2,3,7,8-PnCDF, hexachlorodibenzofurans (HxCDF), and heptachlorodibenzofuran (HpCDF)) are present in turtle 3 and, secondly, the levels of some of the PCDF (particularly 2,3,4,7,8-PnCDF and 2,3,7,8-

tetrachlorodibenzofuran (TCDF)) are much higher in this animal. On a wet weight basis, the 2,3,7,8-TCDD content of the three fat samples was 4 to 7 times higher than the liver samples. However, on a lipid basis the liver samples contained 970, 350, and 462 pg/g 2,3,7,8-TCDD in turtles 1, 2 and 3, respectively, levels which are 1 to 2 times higher than the fat sample levels.

To confirm the 2,3,7,8-TCDD concentrations in some of the positive turtle fat samples, analysis of turtle fats 1 and 2 was also carried out by two separate laboratories using, in one case, a different chromatographic cleanup and GC-MS instrumentation and, in the other case, a completely unrelated in vitro bioassay which measures 2,3,7,8-TCDD like activity. The results from the three different procedures are given in Table 3. The agreement between the two chemical-physical techniques is quite acceptable given the level being measured and the use of different fat from the same animals. The results of the in vitro bioassay indicate that both samples of turtle fat have 2,3,7,8-TCDD-like activity. Presentation of the bioassay data as a range rather than a discrete number is a consequence of using a ten-fold dilution. However, the semi-quantitative values are consistent with the results generated by chemical analysis.

In order to compare the 2,3,7,8-TCDD values found in the turtle samples with other biota, results of the analysis of eel samples from Lake Ontario and the St. Lawrence River area are given in Table 4. Eel is a fatty fish (average lipid content of the eleven samples in Table 4 was 24%) and contains relatively high levels of 2,3,7,8-TCDD (1). The average values found here on a wet tissue basis of about 20 ppt 2,3,7,8-TCDD with smaller amounts of other PCDDs and PCDFs are at least an order of magnitude lower than those found in the turtle fat samples. Although the sampling is limited, the two eels from the Richelieu River (site 7) which does not receive an input from Lake Ontario contained no measurable 2,3,7,8-TCDD.

Table 3. Comparison of 2,3,7,8-TCDD levels in pg/g in turtle fats 1 and 2 using two GC-MS methods and a bioassay (2,3,7,8-TCDD equivalents).

Turtle Fat No.	GC-MS		Bioassay
	HFB ^a	NYS ^b	NYS ^c
1	474	586	200-2000
2	232	203	100-1000

a - Health Protection Branch, results from Table 2; b - New York State Dept. Health, fat from the same turtle but using different sample preparation and GC-MS conditions; c - the same sample as in (b) but after extraction and lipid removal with sulfuric acid

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Table 4. 2,3,7,8-TCDD levels in American eel samples^a collected in 1982 and 1983 from the Great Lakes region.

Origin	Number Samples	Number Positive	Concentration ppt	Average positives in ppt
Lake St. Pierre, St. Lawrence River, site 5b	2	2	16,29	23
Kamouraska, St. Lawrence River, site 6	2	2	15,22	19
Richelieu River, site 7 (tributary of St. Lawrence)	2	0	ND ^c , ND	-
Eastern part Lake Ontario, site 8	5	4	19,15,10,3.7,ND	12

a - average length and weight of river eel samples was 960 mm and 2.1 kg;

b - refer to Fig. 1; c - not detected

DISCUSSION

This is the first report to document the presence of 2,3,7,8-TCDD in turtle tissues. The concentrations found in turtles from the St. Lawrence River are some of the highest known for wildlife biots. Rappe et al. (8) reported high levels of PCDFs (up to 3,000 ppt total) in the fat of a turtle taken from the Hudson river. 2,3,7,8-TCDF and 2,3,4,7,8-PnCDF were measured at 45 and 620 ppt, respectively, but the presence of PCDDs was not assayed than the Hudson river turtle. It is of interest to note that turtle 3, in addition to containing a high PCDD load similar to that of turtles 1 and 2, also had PCDF levels (especially 2,3,4,7,8-PnCDF) even higher than those of the Hudson River turtle. Recently, Korfmacher et al. (18) reported qualitative results of over 500 ppt 2,3,7,8-TCDD in the fat and eggs of a snake, which was taken from a contaminated lake in Arkansas. All these reports on tissue levels in reptiles for 2,3,7,8-TCDD are somewhat lower than the 2.7 and 16 parts per billion (ug/g) found in the liver and adipose, respectively, of a snake from the Seveso area of Italy (19) after the accidental release of 2,3,7,8-TCDD from a trichlorophenol explosion.

Comparison of these results with that reported in other wildlife shows that fish contain lower amounts of 2,3,7,8-TCDD with one of the more contaminated species, eels, from Lake Ontario showing levels of about 20 ppt. Eggs of the herring gull (*Larus argentatus*), a fish eating bird, contain higher levels of 2,3,7,8-TCDD (presently about 80 to 100 ppt in Lake Ontario) than fish do, but neither species appears to accumulate and store 2,3,7,8-TCDD as much as the snapping turtle does. As far as we are aware, PCDF levels of the magnitude found in the tissues of turtle 3 have not been seen in wildlife samples.

Indeed this animal species appears to be unique in its ability to tolerate these contaminants. Because of their non-mobile habits, turtles would appear to be an excellent biological screen for PCDDs and PCDFs. This ability of turtles to accumulate toxic chemicals has been noted by Stone et al. (6) in working with the related contaminant PCB. They found very high fat levels (over 1000 µg/g) of PCBs in certain cases from apparently healthy snapping turtle specimens. Yawith et al. (20) also noted that another aquatic turtle, the Caspian terrapin (Clemmys caspica), contained PCB levels over 20 times higher than that found in two bird species taken from the same area.

The detection of dioxin-like activity determined by the bioassay and the semiquantitative correspondence with the chemical procedure suggests that the bioassay could be used as an initial indicator for the presence of PCDDs and PCDFs upon which a priority ranking for subsequent chemical analysis could be based. This bioassay is based on the 2,3,7,8-TCDD induced reversible inhibition of cell growth and alterations in morphological characteristics of a line of mouse epithelial cells co-cultured with irradiated mouse fibroblast cells (17). This assay is potentially useful as a rapid and inexpensive semiquantitative broad screen for aggregate biological activity even in the presence of a large excess of PCBs and can be used as a supplement to more specific chemical analysis.

The liver/adipose ratios for 2,3,7,8-TCDD in these three turtles varies between 0.14 and 0.22 on a wet weight basis. This ratio is somewhat higher than that found by Stone et al. (6) for PCB (0.04 to 0.07) and DDE (0.03 to 0.09) in the same species from the same state and by Pearson et al. (21) for dieldrin (0.03 to 0.09) in the box turtle (Terrapene carolina) 70 days after a single injected dose. The higher relative amount of 2,3,7,8-TCDD in the turtle liver than in the fat when compared to the other chlorinated compounds is in agreement with experimental data in rats (22) and may reflect a recent exposure to the former contaminant or an exposure close to one causing adverse effects.

Chelydra serpentina are omnivorous and feed on significant amounts of both animal and plant food. The TCDD levels in the turtles are consistent with the consumption of a fish diet containing about 20 ppt 2,3,7,8-TCDD, a level currently found in eel samples from the same region and suggest that turtles bioaccumulate 2,3,7,8-TCDD to the same extent as do laboratory rats (23) and beef cattle (24). In the former case Kociba et al. (23) fed laboratory rats continuously for 2 years a diet containing 22 ppt 2,3,7,8-TCDD (about 1 ng/kg body weight per day) which resulted in a tissue residue level of 540 ppt in both fat and liver. In the other case, Jensen et al. (24) fed beef cattle for 28 days a diet containing 24 ppt 2,3,7,8-TCDD (about 0.8 ng/kg body weight per day) and this resulted in a fat level of about 80 ppt which was increasing and estimated to peak at about 600 ppt. It is also noteworthy that the older turtles in this report (turtles 1 and 3) had the higher fat 2,3,7,8-TCDD residues suggesting that the levels from specimens from the same location may

increase with age and/or size - a relationship which is also suggestive for PCB (6).

It appears that much of the aquatic biota from Lake Ontario and the St. Lawrence River contains appreciable amounts of 2,3,7,8-TCDD (1-3,25). More limited data from two tributaries of the St. Lawrence River, the Ottawa and Richelieu Rivers, appear to show that they do not contribute TCDD to this watershed. It is possible that 2,3,7,8-TCDD is entering the St. Lawrence River from Lake Ontario which in turn could have originated in the Niagara River (site 9) currently the only confirmed source of 2,3,7,8-TCDD in the area (26). Sources of the other PCDDs (excluding 2,3,7,8-TCDD) and the PCDFs have not been identified. Turtle 3 contained much higher levels of PCDFs than did turtles 1 and 2 (in addition to the PCDD residue levels). This result suggests a second component as a source for the levels in turtle 3 possibly from PCB. In support of this, the PCBs in the fat of turtle 3 were over 800 µg/g, more than 20x the levels found in the fat of other two animals. Certainly the low levels of the higher chlorinated PCDDs and PCDFs in turtle and fish samples would not indicate pentachlorophenol as the source.

The turtles from which these tissue levels of 2,3,7,8-TCDD and other PCDDs/PCDFs were found appeared to be normal in both appearance and behavior. Whether they have been subjected to any long term adverse effects such as reproductive abnormalities or chronic disease is presently not known. Current regulations for 2,3,7,8-TCDD in fish are based on a residue level between 10 and 20 ppt. Thus the habitual consumption of these turtle tissues by humans may constitute a health risk.

CONCLUSION

Analysis of turtle fat samples by both chemical and bioassay techniques has shown the presence of up to 500 ppt levels of 2,3,7,8-TCDD in samples from the St. Lawrence river along with lesser amounts of other PCDDs and PCDFs. One sample also showed a very high PCDF residue level suggestive of PCBs as an additional source. The 2,3,7,8-TCDD concentrations in turtles are higher than those found in eel samples from the same area and suggest turtle tissues are an excellent monitor for this type of contamination. The source of the 2,3,7,8-TCDD levels could be the Niagara river via Lake Ontario.

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