Bull. Environ. Contam. Toxicol. (1986) 36:452–459

Environmental Contamination and Toxicology

Nonaccumulation of Chlorinated Dioxins and Furans by Goldfish Exposed to Contaminated Sediment and Flyash

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One or more isomers from the two related classes of chemical compounds, polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), are often present as contaminants in industrial chemicals such as polychlorinated biphenyls (PCBs) (Bowes et al. 1975) and chlorinated phenols (Buser and Bosshardt 1976) and in emissions from incinerators (Lustenhouwer et al. 1980). Several of the PCDD/PCDF isomers exhibit potent toxic properties, in particular 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) (Moore et al. 1979). In the aquatic environment 2,3,7,8-TCDD and/or 2,3,7,8-TCDF have been found in fish from the Great Lakes (O'Keefe et al. 1983; Ryan et al. 1983) and in biota collected in the vicinity of chemical plants (Harless et al. 1982; Mitchum et al. 1980).

Investigations in the laboratory have shown that fish in aquaria or ponds can bioaccumulate 2,3,7,8-TCDD with bioaccumulation factors from water exceeding 10^3. However, in these experiments 2,3,7,8-TCDD was added in a water-miscible solvent either directly to the water column (Tushimoto et al. 1982) or to soils before addition of water (Yockim et al. 1978). When 2,3,7,8-TCDD is present in a matrix as a result of environmental contamination it is conceivable that bioaccumulation factors might differ from those found with artificially contaminated matrices. The work reported here was undertaken to study the bioavailability to goldfish of TCDD/TCDF isomers present in municipal incinerator fly ash and in sediment collected in the vicinity of a chemical waste disposal site.

MATERIALS AND METHODS

Exposure of large (70–300 g) goldfish (Carassius auratus, obtained from Grasse Fork Fish Co., PO Box 1554, Martinsville, IN 46151) took place over a 10 week time period in static 76 L aquaria. Each aquarium held 9–12 fish and had an individual air pump.

The tops of the tanks were covered by glass plates which were sealed to the tanks to minimize volatilization of organics. Goldfish were conditioned to feed at the water surface (Tetra Min solid fish food flakes) to minimize ingestion of experimental substrates. The

control aquarium had a substrate of natural sand. This sand, after rinsing with acetone and hexane, was air dried and then heated to 260°C to complete the drying process. The control aquarium water was filtered using a standard power filter system composed of glass wool and activated charcoal.

Substrates in the two exposure aquaria were respectively fly ash from a municipal incinerator and sediments from Cayuga Creek which receives drainage from the Love Canal waste disposal site in Niagara Falls, NY. Each of these aquaria had a porous bottom nylon net applied directly on the substrate to prevent the fish from ingesting sediment. The netting had a mesh size of 0.1 mm^2 and covered the entire bottom surface of each tank. Some resuspension of sediments occurred but this was minimized by the presence of the netting. All substrates were present to heights of 6 cm above the base of the aquaria.

At each sampling point one or two fish were sacrificed to provide 15-25 g of whole fillet minus skin and scales for analyses. The fillets were stored individually frozen in aluminum foil packages. Samples of sediment, flyash, sand and water were stored under refrigeration in glass jars with Teflon-lined caps. The fish and particulate samples were extracted and then cleaned up by a flow-thru system of basic alumina, carbon and acid alumina columns (O'Keefe et al. 1985). At the time the present study was carried out the system had not been automated and all chromatography steps were carried out manually. In the case of the fly ash samples an acid alumina column was used in place of the basic alumina column. Water samples (1 L) were centrifuged at 2,000 rpm for 10 min to remove particulate matter. The samples were then spiked with 6 parts per trillion (ppt) of the labelled internal standards [U-^{13}C] 2,3,7,8-TCDD and [2,3,7,8-^{37}Cl] TCDF in 0.5 mL acetone and extracted three times with 50-mL volumes of hexane in a 2-L separatory funnel. The combined hexane extracts were cleaned up by the three-column sequence described above.

Cleaned-up extracts were analyzed by ion monitoring mass spectrometry using a cyanosiloxane-coated capillary gas chromatography column coupled directly to a Kratos MS-50 mass spectrometer. Criteria for acceptable TCDD and TCDF signals have been described in an earlier publication (O'Keefe et al. 1983).

RESULTS AND DISCUSSION

Concentrations of TCDD and TCDF isomers in the fly ash and sediment used in the present study are shown in Table 1. The fly ash was obtained as a subsample from the 24 hour collection of an electrostatic precipitator in a municipal incinerator. In agreement with other investigations of PCDD/PCDF concentrations in municipal incinerator fly ash (Lustenhouwer et al. 1980) we found a number of TCDD and TCDF isomers with, in each case, the 2,3,7,8-substituted isomers accounting for approximately 10% of the total tetra isomers. In contrast the sediment contained 2,3,7,8-TCDD as the only TCDD

Table 1. Concentrations (ppt) of TCDD and TCDF isomers in fly ash, sediment and sand before and after exposure in aquaria.

Exposure time (weeks)	Analytes	Matrix[1,2]		
		Fly ash	Sediment	Sand
0	2,3,7,8-TCDD	230;167;140	190;178;167	No samples
	Total TCDD	1,624		
	2,3,7,8-TCDF	1,400;922;435	ND(68);3;7;ND(10)	
	Total TCDF	4,194		
6	2,3,7,8-TCDD	96	No samples	ND(20)
	2,3,7,8-TCDF	1,500		ND(20)
10	2,3,7,8-TCDD	No samples	107	
	2,3,7,8-TCDF		ND(13)	

[1]ND = Not detected with the detection limits shown in parentheses based on 2.5 times noise.

[2]Values separated by semi-colons are from the cleanup and analysis of replicate amounts of a sample.

isomer with a trace of 2,3,7,8-TCDF. This sediment was collected from Cayuga Creek, a small stream which receives discharges from a storm sewer system surrounding the Love Canal chemical dump site. The dump site, situated in Niagara Falls, NY, was the subject of considerable public concern in 1978 when high groundwater levels resulted in migration of chemicals into the basements of homes surrounding the canal. We found part-per-billion concentrations of 2,3,7,8-TCDD in soil collected from the Love Canal and in sediments taken from the storm sewer system (Smith et al. 1983), results which are consistent with the reported dumping of over 200 tons of trichlorophenol wastes into the site from the early 1940's to 1953.

Concentrations of 2,3,7,8-TCDD and 2,3,7,8-TCDF were determined in the fly ash and the sediment after respectively 6 and 10 weeks exposure in the aquaria. It would appear from Table 1 that there was a 40 to 50% reduction in the concentrations of 2,3,7,8-TCDD in both the fly ash and the sediment after exposure in the aquaria. However, at this time, in view of the limited number of samples, we cannot attach any significance to concentration changes of only 50%. Sand, which was used in the control aquarium, showed no evidence of contamination with 2,3,7,8-TCDD or 2,3,7,8-TCDF after 6 weeks exposure. Other tetra isomers were not determined in these samples since it was discovered that while high recovery values could be obtained for 2,3,7,8-TCDD, some other tetra CDD isomers were not adequately recovered. Subsequent modifications of the cleanup method enabled us to obtain recoveries exceeding 70% for all tetra CDD isomers (O'Keefe et al. 1985). Unfortunately, samples for re-analysis by the modified cleanup method were only available from the original fly ash and sediment. In the overall context of the study the low recoveries for certain tetra CDD isomers were of minimal importance since it has been found that only 2,3,7,8 substituted CDDs and CDFs are accumulated in significant concentrations by aquatic biota (Stalling et al. 1982).

As shown in Table 2, the difference between estimated and analytical values did not exceed 30% when goldfish flesh was fortified with 13 ppt of both 2,3,7,8-TCDD and 2,3,7,8-TCDF. Unfortified samples showed no evidence of contamination with 2,3,7,8-TCDD or 2,3,7,8-TCDF at limits of detection of 2.4 and 1.1 ppt respectively. Yockim et al. (1978) found concentrations of 11.7 and 5.9 parts per billion (ppb) 2,3,7,8-TCDD in mosquito fish and channel catfish respectively when they were placed for 15 days in aquaria containing a silt loam soil treated with 100 ppb 2,3,7,8-TCDD. In the same experiments, concentrations of 2-4 ppt 2,3,7,8-TCDD were found in water samples taken over a period of 32 days. On this basis we would expect to find approximately 14 ppt 2,3,7,8-TCDD in the goldfish exposed to the sediment and fly ash. The extrapolated value should be considered an upper limit since we analyzed skinned fillets whereas Yockim et al. (1978) analyzed whole fish. Our detection limits for 2,3,7,8-TCDD in water (~0.02 ppt) would prevent us from finding the expected value of 0.007 ppt. As shown in Table 3, neither 2,3,7,8-TCDD nor 2,3,7,8-TCDF were detected in the fish and water samples taken after 10 weeks exposure to the

contaminated matrices. In contrast, fish collected in Cayuga Creek near the sediment sampling point contained more than 30 ppt 2,3,7,8-TCDD.

Although this study involved the analysis of samples containing ppt to ppb concentrations of PCDD/PCDF isomers, our laboratory frequently analyzes environmental samples containing part-per-million (ppm) concentrations of these compounds. Therefore the presence of 3.8 ppt 2,3,7,8-TCDD in the control goldfish could be explained by contamination from previous analysis of an environmental sample containing 2,3,7,8-TCDD at the ppm level. On the few occasions we have encountered this type of contamination we have attributed it to the GC on-column injection system where the sample is injected directly onto the capillary column at ambient temperatures.

Table 2. Quality control data for goldfish muscle tissue used in TCDD/TCDF bioavailability studies.

Analyte	Concentration(ppt)[1] Added	Found
2,3,7,8-TCDD	0	ND(2)
	13	14.1
	13	8.2
2,3,7,8-TCDF	0	ND(1.1)
	13	17
	13	17

[1]ND = Not detected with the detection limits shown in parentheses based on 2.5 times noise.

Several hypotheses can be advanced to explain the failure of the goldfish to bioaccumulate 2,3,7,8-TCDD or 2,3,7,8-TCDF from the sediment and fly ash. The first hypothesis relates to the rates of desorption of PCDD/PCDF compounds into the water column. It is conceivable that the desorption rates could be so slow that the exposure time of the fish in the laboratory is inadequate for bioaccumulation. Kooke et al. (1981) found that PCDD/PCDF compounds were tightly bound to fly ash and their extraction could only be achieved by refluxing in a Soxhlet apparatus with an aromatic solvent such as benzene or toluene. In a study, similar in many aspects to our own study, Kuehl et al. (1984) exposed carp in aquaria to two different fly ash samples. One sample, a composite from five different incinerators, contained 2000 ppt 2,3,7,8-TCDD in

Table 3. Bioavailability of TCDD/TCDF isomers to fish exposed to fly ash and TCDD contaminated sediment in laboratory aquaria for 10 weeks with a comparison to environmental bioaccumulation.

Matrix	Sample Type	Concentration (ppt)[1] 2,3,7,8-TCDD	2,3,7,8-TCDF
Fly ash	Goldfish	1.9	0.7
	Water	ND(0.03)	ND(0.03)
Sediment	Goldfish	ND(0.8)	0.7
	Water	ND(0.024)	ND(0.009)
Control (sand)	Goldfish		ND(1.7)
	Water	ND(0.02)	ND(0.02)
Cayuga Creek, Love Canal[2]	Carp/Goldfish	39	--
	Pumpkinseed	31	--

[1]ND = Not detected with the detection limits in parentheses based on 2.5 times noise.

[2]Fish taken from the site for collection of the bioavailability sediment.

addition to other TCDD isomers. No 2,3,7,8-TCDD was found in the carp after they were exposed to this fly ash for times varying from 15 to 90 days. When the carp were exposed to the second fly ash sample which contained 160 ppt 2,3,7,8-TCDD they accumulated approximately 30 ppt 2,3,7,8-TCDD after 30 days. Desorption of PCDD/PCDF compounds from sediments with a high organic carbon content could also be a slow process. The organic content of the sediment used in the present study was 29% which was almost 20 times greater than the organic content of the soil used by Yockim et al. (1978).

The second hypothesis involves consideration of the potential role of food chain organisms in the bioaccumulation of PCDD/PCDF compounds by fish. Bruggeman et al. (1981) have suggested that ''food chain accumulation in fish is likely to be an important process only for persistant chemicals with extremely low water solubility''. With a water solubility of 200 ppt and a half life in sediments greater than 1 year 2,3,7,8-TCDD would appear to meet these criteria for food chain bioaccumulation. In our study bacteria were undoubtedly present in the sediment but there were no organisms of dietary significance to the goldfish in either the sediment or fly ash exposures.

To understand the discrepancies between field and laboratory data for PCDD/PCDF bioaccumulation we are proposing to carry out

controlled long term experiments on the effects of diet and environmental factors on PCDD/PCDF bioaccumulation in fish species in both natural habitats and in the laboratory. We would hope that information obtained from this work might provide a basis for predicting the movements of PCDD/PCDF compounds in aquatic environments.

Acknowledgements. This work was supported by in-house grant no. 65038-01 from Health Research, Inc., Albany, NY.

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Received May 3, 1985; accepted June 13, 1985.