A Search for 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in an Environment Exposed Annually to 2, 4, 5-Trichlorophenoxyacetic Acid Ester (2, 4, 5-T) Herbicides

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Herbicides containing esters of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) have contained trace amounts of an impurity, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is toxic to laboratory animals (SCHWETZ et al. 1973) and has been a cause of dermatitis (chloracne) in chemical plant workers (POLAND et al. 1971). TCDD in concentrations up to 800 parts per trillion (ppt) was reported to be in Vietnamese fish collected in 1970 after extensive use of a 2,4,5-T containing herbicide in South Vietnam (BAUGHMAN and MESELSON 1973a and b). However, examination of later samples (1973) did not show the presence of TCDD (limits of detection 20 to 150 ppt) (BAUGHMAN 1974). As part of a study to determine whether TCDD is accumulating in environments exposed to the approved domestic uses of 2,4,5-T herbicides on rangeland and rice fields, samples of catfish, bass, walleyed pike, mud, water and human milk were obtained from two locations where TCDD would most likely accumulate. These were an impoundment from the drainage of a watershed in Texas where 2,4,5-T herbicides had been used for several years for brush control and from a pond in Arkansas used as a reservoir for irrigating rice fields treated with 2,4,5-T herbicides and which collected the drainage from these rice fields. Human milk was obtained from mothers residing in the area of the Texas impoundment. The samples were analyzed by GC-MS after extensive cleanup involving alkaline digestion, H_2SO_4 treatment and chromatography on silica gel and alumina. Other studies on the accumulation of TCDD in the environment are underway and one has been reported (MAHLE et al. 1976).

EXPERIMENTAL

Reagents

Distilled-in-glass and pesticide-grade solvents were used. The silica gel was Curtin high purity 100-120 mesh. The alumina was Fisher A540 activated at 140°C. Inorganic reagents were ACS grade. The TCDD standard was 98% tetrachloro by gas chromatography and

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mass spectrometry (MUELDER and SHADOFF 1973). The ^{37}Cl labeled TCDD was prepared by the chlorination of dibenzo-p-dioxin in chloroform using $^{37}Cl_2$ generated from $Na^{37}Cl$.

Instrumentation

LKB-9000 GC-MS, 6-ft 3% OV-3 silicone column, operated at a resolution of 400.

AEI-MS 30 interfaced to a Pye 104 GC using a silicone membrane separator. A 6-ft 3% OV-101 column was used in the GC. The MS 30 was operated at a resolution of 3000 (medium) or a resolution of 9000 or 12,000 (high). For operation at high resolution a narrow mass range was repeatedly scanned under control of a Varian 1024 channel analyzer during the time when TCDD would elute from the column. The output from the multi-channel analyzer was examined for a peak at the exact mass of TCDD. The low, medium and high resolution measurement of TCDD has been described by SHADOFF and HUMMEL (1975).

Sampling

Samples of water, mud, catfish, and bass were collected in the heart of the Arkansas rice growing area near Grady, Arkansas from a 125-A pond. The pond water is used to flood rice fields which have been sprayed with the equivalent of 1.25 lb/A of 2,4,5-T acid 4 to 8 weeks previously. Later, when the water is drawn off the fields, it is pumped back into the pond for reuse. Water in this pond also collects run-off from surrounding rice fields during the rainy season and is supplemented by wells. This cycle had been in use (including the proper use of 2,4,5-T herbicide) for 18 years up to the time of this study. The fish from this pond were caught by line, eviscerated, washed with well water, wrapped in aluminum foil shiny side out, and frozen in Dry Ice immediately. The removed viscera were wrapped in foil and packaged with the fish. The mud and water samples were sealed in glass containers (previously washed with benzene and air dried) and frozen in Dry Ice.

Water, mud, catfish, and walleyed pike were also collected from the San Angelo, Texas, Reservoir which is an impoundment of the North Concho river and has as its water shed large acreages that have been sprayed with 2,4,5-T herbicides for the control of mesquite (0.5 lbs/A 2,4,5-T acid equivalent) and brush (3-4 lbs/A 2,4,5-T acid equivalent). Human milk was obtained from mothers residing in the general area. The fish were netted, placed in tubs filled with water, and transported to the Texas Parks and Wildlife Labora-

tory where they were eviscerated and packed as described for the Arkansas fish. Water and mud samples were placed in metal cans (previously washed with acetone and air dried) and frozen in Dry Ice.

All samples were shipped frozen to Midland, Michigan and were stored frozen until they were analyzed. At the time of analysis, the head, tail and fins of a fish were removed, and a symmetrical half of the body was cut into 1/4 to 1/2 inch cubes. A representative sample was weighed and the remaining partially thawed pieces of fish were refrozen. Mud samples were thawed, mixed, and a portion weighed for analysis. The entire water sample was analyzed.

Sample Preparation - Fish Tissue

Ten g of fish prepared as described in the Sample Handling and Preparation Section (including bones, skin, and scales, if present), 10 ml of ethanol (20 ml for catfish tissue) and 20 ml of 40% KOH were heated in a flask on a steam bath for one hour. When cool, the KOH solution was extracted with four 10-ml portions of hexane. Ethanol was added to break emulsions if any formed. The combined hexane extracts were washed with 10 ml of water and then with 10 ml portions of concentrated H_2SO_4 until the acid layer was nearly colorless (usually 4 washes). After a 10 ml water wash, the hexane was evaporated using a stream of air. A 4 x 50 mm silica gel column was prepared using a disposable pipet. The residue from the hexane extraction was dissolved in 0.5 ml of hexane and added to the column along with two 0.5 ml hexane washes of the container. The effluent was discarded, and the column then eluted with 2 ml of 20% benzene in hexane (v/v). The effluent containing the TCDD fraction was evaporated just to dryness using a stream of air and the residue dissolved in hexane and added to an alumina column of the same size as the silica gel column. The column was washed with 12 ml of 20% carbon tetrachloride in hexane (v/v) followed by one ml of hexane. The washings were discarded. The column was then eluted with 4 ml of 20% methylene chloride in hexane (v/v). The effluent was evaporated to 0.2 ml using a stream of air and the residual solution was transferred to a 0.3 ml cone-shaped vial using hexane as solvent. The solvent in the vial was evaporated to dryness under a stream of air and the vial capped with a septum cap.

Milk

Milk samples were digested and cleaned up by the procedure for fish tissue, using a 20 g sample.

Mud

A 10-gram portion was weighed into a screw-cap bottle, 7 ml of 1% ammonium chloride and 100 ml of 1 + 1 acetone-hexane were added and the bottle placed on a shaker for 2 hours (NASH et al. 1973). The acetone was removed from the hexane by washing with water, the hexane was evaporated and the residue cleaned up by chromatography on silica gel and alumina as described for fish tissue.

Water

The entire sample (500 ml) was extracted with 200 ml of 1+1 acetone-hexane which had been used to wash the sample container. The hexane separated from the water-acetone solution was evaporated and the residue cleaned up by chromatography on silica gel and alumina as described for fish tissue.

GC-MS Measurement of TCDD

The instrument was tuned to monitor the m/e 320 and 322 molecular ions of TCDD and the response calibrated with standard solutions of TCDD (CRUMMETT and STEHL 1973). The sample residue was dissolved in 10 or 20 µl of xylene and a 2-to 5-µl aliquot was injected into the LKB 9000 GC-MS. Results were calculated using the response for both m/e 320 and 322. If the results did not agree the lower value for TCDD was used. When a result on the LKB 9000 indicated an apparent presence of TCDD, or when interferences were present, yielding an unacceptably high limit of detection, the sample extract was also analyzed using the MS 30 at medium or high resolution.

Calculation of Results

Results were calculated and interpreted using criteria developed at a meeting of academic, governmental, and industrial scientists called to discuss the determination of low levels of TCDD (COLLIER 1973). Criteria which apply to these samples are (1) the signal-to-noise ratio should be at least 2.5 to 1, otherwise the result will be "not detected", and (2) samples with a signal to noise ratio between 2.5 to 1 and 10 to 1 should be rerun a second time.

Recovery Determinations

Known amounts of TCDD or ^{37}Cl TCDD were added to samples in the digestion flask. The TCDD was determined as described in the Experimental section. (The ^{37}Cl TCDD was monitored at m/e 328). Recovery values are given in Table I (fish) and Table II (milk).

TABLE I

RECOVERY OF TCDD ADDED TO FISH

	TCDD, ppt		
	Added	Found	Recovery, %
Catfish	100	75*	75
	50	39,42,34*35*,47*	78,84,68,70,94
	25	17,14,13*,8*,10*	68,56,52,32,40
	10	10	100
	5	6,7,n.d.	120,140,0
Bass	100	87,40*	87,40
	50	35,28*,30*,29*,24*	70,56,60,58,48
	25	19	76
	5	7,6,5	140,120,100
Walleye	100*	81,75	81,75
	25	13	52
	10	5	50
	5	7,4,6	140,80,120

*^{37}Cl TCDD ppt = parts per trillion
n.d. = not detected, peak less than 2.5 times noise

TABLE II

RECOVERY OF TCDD ADDED TO MILK

Added	Bovine Milk		Human Milk	
ppt	Found, ppt	%	Found, ppt	%
2.0	1.7,1.6,n.d.	85,80,0		
5.0	4.2	84		
10.0	6.5	65		
50*	28,40	56,80	16,39,32	32,78,64
100*			95,110**	95,110

*^{37}Cl isomer of TCDD ** Interference present
n.d. = not detected, peak less than 2.5 times noise.

RESULTS

No evidence was found that TCDD is accumulating in the environment under the conditions of annual 2,4,5-T use described in this study. All determinations on Texas samples by both low and high resolution GC-MS were "not detected" by criteria 1 (calculation of results section). Average limits of detection obtained with the LKB 9000 were 7 ppt for 10 catfish, 5 ppt for 10 walleyed pike, 3 ppt for 2 samples of mud, 0.1 ppt for 2 samples of water and 3 ppt for 6 samples of human milk (Table III).

TABLE III

DETECTION LIMITS FOR TEXAS SAMPLES

Sample	No. of Samples	No. of Det'ns	Detection Limit (ppt)* Avg.	σ	High	Low
Catfish	10	24	6.6	2.2	12	2
Walleyed Pike	10	26	5.4	2.1	10	2
Human Milk	6	8	3	1.7	6	1
Mud	2	2	3	---	3	3
Water	2	2	0.1	---	0.1	0.1

*No TCDD was detected in any sample. Detection limits defined as 2.5:1 signal to noise.
ppt = parts per trillion.

All determinations on the Arkansas bass, water, and viscera were "not detected" by criteria 1. The mud and eggs showed an apparent presence of TCDD on on the LKB but all results on these samples with the MS-30 were "not detected" at a lower limit of detection. Average limits of detection were 7,0.2,10,5 and 6 ppt respectively (Table IV).

TABLE IV

DETECTION LIMITS FOR ARKANSAS SAMPLES

Sample	No. Spls.	No. Det'ns	Detection Limit (ppt)* Avg.	σ	High	Low
Bass	10	26	7.2	4.0	14	1
Catfish Viscera	1	1	10	---	---	---
Catfish Eggs	1	1	6	---	---	---
Mud	2	2	5	---	6	4
Water	2	2	0.2	---	0.2	0.2

*No TCDD was detected in any sample. Detection limit defined as 2.5:1 signal to noise.
ppt = parts per trillion

Of the 10 catfish analyzed (Table V) none met the criterion of duplicate analyses for positive results between 2.5 and 10 times noise. Determination number 2 for the second 16 oz fish in Table V was an apparent positive on the LKB but the same extract was "not detected" on the MS-30 with a lower limit of detection. Similarly determination number 1 for the third 16 oz fish was an apparent positive on the MS-30 but was "not detected" on the LKB with a lower limit of detection. Determination number 2 for the first 16 oz fish was not analyzed by high resolution GC-MS because the extract was used up in an unsuccessful photolysis experiment.

TABLE V

ANALYSIS OF ARKANSAS CATFISH FOR TCDD

Weight of Fish	Detn. No.	LKB, ppt Apparent TCDD	LKB, ppt Limit of Detection	MS 30, ppt Apparent TCDD	MS 30, ppt Limit of Detection
40 oz.	1	n.d.	11	n.d.	22
	2	n.d.	18		
	3	n.d.	18		
	4	n.d.	4	n.d.,10*	8,10*
	5	n.d.	5		
	6	n.d.	8		
	7	n.d.	9		
	8	n.d.	8		
	9	n.d.	6		
	10	n.d.	12		
13	1	12	4	n.d.	5
	2	n.d.	18	n.d.,n.d.*	13,5*
	3	n.d.	22	n.d.	10
	4	n.d.	5		
	5	n.d.	8		
18	1	n.d.	18		
	2	n.d.	18		
22	1	n.d.	6	n.d.	9
	2	n.d.	13		
	3	n.d.	10		
16	1	n.d.	13	n.d.	6
	2	9	4		
48	1	8	4	n.d.,n.d.*	6,10*
	2	n.d.	7	n.d.**	2**
	3	7	2	n.d.**	10**
24	1	n.d.	7	n.d.	7
	2	n.d.	4	n.d.	6
16	1	9	4	7	6
	2	7	3	n.d.	5
19	1	n.d.	5		
	2	n.d.	12		
16	1	n.d.	3	6	5
	2	3	2	5	4

ppt - parts per trillion n.d. - not detected. Limit of detection 2.5 times noise *MS-30 result at 12000 resolution **9000 resolution All other MS-30 results at 3000 resolution

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SUMMARY

As part of a broad study to determine whether 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is accumulating in the environment due to approved uses of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) based herbicides, samples of fish, water, mud and human milk were collected from areas in Arkansas and Texas where 2,4,5-T herbicides are used and were analyzed for TCDD. No TCDD was detected by a GC-MS procedure with a detection limit which averaged less than 10 ppt.

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