A Search for the Presence of 2,3,7,8 Tetrachlorodibenzo-*p*-Dioxin in Beef Fat

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INTRODUCTION

Formulations of chlorophenoxyacetic acid (e.g., 2,4,5-trichlorophenoxyacetic acid: 2,4,5-T) esters are used extensively to control broadleaf weeds and woody plants. These esters can contain an impurity, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Studies have shown that TCDD is toxic to laboratory animals (SCHWETZ et.al. 1973) and has been reported to be a cause of chloracne in chemical plant workers (POLAND et.al. 1971).

TCDD was reported to be present in South Vietnam fish collected in 1970 (BAUGHMAN and MESELSON 1973), but was not found in fish collected in 1973 (BAUGHMAN 1974). Studies on domestic fish (SHADOFF and HUMMEL 1975) and bovine milk (MAHLE et.al. 1977) from areas where 2,4,5-T was used, showed no TCDD at detection limits of 10 ppt and 1 ppt respectively.

This surveillance study was undertaken to determine if low level concentrations of TCDD could be detected in beef fat taken from cattle grazing on land where 2,4,5-T had been applied.

EXPERIMENTAL

Safety

TCDD standards were handled according to established procedures (DOW CHEMICAL CO. 1970) to avoid chemical exposure.

Sample Collection and History

Samples 129971 through 129981 (Table I) were obtained at the Texas Department of Corrections farm at Sugarland, Texas, under the direction of P. D. Ludwig of The Dow Chemical Company. This farm was spot sprayed in May, 1974 with a mixture of one gallon of "2,4,5-T" added to 100 gallons of water.

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Table I
INFORMATION ABOUT FAT SAMPLES FROM STEERS GRAZING ON LAND TREATED WITH 2,4,5-T

Sample Number	Location County	State	Type of Steer	2,4,5-T Application Method	Application of 2,4,5-T lb ae/A (B = Burned)[a] >71	71	72	73	74	ASI[b] (months)
129971	Fort Bend	TX	Unknown	Spot Sprayed					[c]	2
129972	Fort Bend	TX	Unknown	Spot Sprayed					[c]	2
129973	Fort Bend	TX	Unknown	Spot Sprayed					[c]	2
129974	Fort Bend	TX	Unknown	Spot Sprayed					[c]	2
129975	Fort Bend	TX	Unknown	Spot Sprayed					[c]	2
129976	Fort Bend	TX	Unknown	Spot Sprayed					[c]	2
129977	Fort Bend	TX	Unknown	Spot Sprayed					[c]	2
129978	Fort Bend	TX	Unknown	Spot Sprayed					[c]	2
129979	Fort Bend	TX	Unknown	Spot Sprayed					[c]	2
129980	Fort Bend	TX	Unknown	Spot Sprayed					[c]	2
129981	Fort Bend	TX	Unknown	Spot Sprayed					[c]	2
129929	Taney	MO	Angus	Unknown			2B			24
135020	Hughes	OK	Charolais-Brahma	Aerial		2	2			24
135021	Hughes	OK	Charolais-Brahma	Aerial		2	2			24
135022	Douglas	MO	Hereford	Aerial				2		7
135023	Douglas	MO	Hereford	Aerial	6B	2B	2B	2B		12
135024	Irion	TX	Angus	Aerial						
135025	Irion	TX	Angus	Aerial					½	2[d]
135026	Irion	TX	Angus	Aerial					½	2[d]
135027	Irion	TX	Angus	Aerial					½	2[d]
135028	Irion	TX	Angus	Aerial					½	2[d]
135029	Irion	TX	Angus	Aerial					½	2[d]
135030	Irion	TX	Angus	Aerial					½	2[d]
135031	Irion	TX	Angus	Aerial					½	2[d]

[a] ae – acid equivalent. Burning occurred generally in the fall of the same year of application. Repeat applications were to different areas of the same pasture.
[b] Approximate interval from last application of 2,4,5-T to slaughter of animal. In most cases the dates of treatment are not accurately known.
[c] Farm was heavily sprayed with a mixture of one gallon of 2,4,5-T product in 100 gallons of water.
[d] Animals put on pasture 10 days after treatment, removed 30 days later and held on untreated area 2 weeks before slaughter.

Samples 129929 and 135020 through 135023 were collected from various farms in Missouri and Oklahoma by Mr. H. M. Elwell, retired rangeland management professor from Oklahoma State University, of Oak Grove, Missouri. Samples 135020 and 135021 were taken from the same animal.

Samples 135024 through 135031 were obtained in Mertzon, Texas by Dr. R. Steger and G. Hoffman of Texas A&M University, Range Science Department. At this location, a 1300 acre fenced lot was aerially sprayed with 2,4,5-T. Before the treatment, the animals had been fed on dry grass of the same species as the treated grass. Ten days after the herbicide treatment, the animals were placed on the treated lot and allowed to graze for 30 days. After this time, they were withdrawn and placed on an untreated area and fed for an additional two weeks before slaughter. The animals were slaughtered and the samples were obtained, wrapped, and frozen at the Wendi-Pak packing plant in San Angelo, Texas, under the direction of the Federal inspector. The carcasses passed inspection with closer scrutiny than given non-test animals. Sample 135024 was a control which was slaughtered at the beginning of the study.

No samples of the herbicides used in these programs were available for analysis. Although the specification level of TCDD in herbicides containing 2,4,5-T was changed in 1972 from a maximum concentration of 1 ppm to 0.1 ppm on an acid equivalent basis, it is not possible to tell with certainty if the herbicide used was produced before or after this change.

In some of the treated areas, the brush had been burned following treatment. This is generally done after the brush dies following spraying. Where done, this is indicated by "B" (Table I) following the rate of application of 2,4,5-T. After burning, the land is often seeded in the fall to establish desirable grass species for grazing.

A common practice in treating with 2,4,5-T herbicide is to treat part of a range or pasture each of several years, although an entire pasture could be treated at one time. In practice, it is usually not necessary to retreat the same area the next year. Hence, in Table I where the history of the use of 2,4,5-T indicates treatment of successive years, different parts of the range or pasture were treated each year. This makes the experimental conditions used in the Mertzon, Texas trial somewhat different than general usage, as the cattle were confined entirely on treated pasture.

The animals were slaughtered between December, 1973 and August, 1974. Frozen samples from these animals were shipped to The Dow Chemical Company, Midland, Michigan. Some samples were then obtained by trimming the fat from muscle tissue. Each sample was then homogenized in a Robot-Coupé macerator and refrozen until analyzed.

ANALYSIS

The procedure used was essentially that of SHADOFF and HUMMEL (1975). A ten-gram sample from each specimen was weighed and digested in an ethanol-KOH solution. The digested material was extracted four times with hexane and the extracts were combined, washed once with water, then washed with concentrated sulfuric acid until the acid was only lightly colored (usually four 10 ml portions). The hexane solution was washed once more with water, then evaporated under flowing air. The residue was taken up in hexane and applied to a 4 mm x 50 mm microcolumn containing silica gel. The sample was then eluted with 20% benzene/hexane (v/v), dried under flowing air, taken up in hexane and applied to a 4 mm x 50 mm microcolumn containing alumina (activated at 130°C in air). This column was washed sequentially with hexane, 20% carbon tetrachloride/hexane, and hexane, then eluted with 20% methylene chloride/ hexane. This final eluent was evaporated and the residue was dissolved in hexane and placed in a 0.3 ml vial, where the solvent was again evaporated. Later 20 µl of o-xylene was added to dissolve the sample and 5 µl portions were injected on a 6 ft. 3% OV-3 gas chromatographic column in a low-resolution LKB-9000 gas chromatograph-mass spectrometer (GC-MS) where the mass chromatogram was observed at m/e 320 and 322. Calibrations were effected using known solutions of TCDD.

Interferences in these GC-MS determinations are always positive; therefore, when there was no measurable response using the low-resolution GC-MS technique, then there was no need for further confirmatory measurements. However, whenever there was an apparent positive response for TCDD using low-resolution GC-MS (in duplicate samples prepared independently by two of the authors) then a fresh sample was prepared and analyzed using a high-resolution GC-MS system. Thus the readily available low-resolution system was used for screening while the more sophisticated high-resolution spectrometer was used for confirmation of the presence of TCDD. While no analytical system can be entirely free of interferences, it is felt that these two techniques are sufficiently different that

compounds which could give false positive results on the low-resolution system would not interfere on the high-resolution system.

The high-resolution system utilized a 6 foot 5% OV-101 gas chromatograph column interfaced with an AEI MS-30 high-resolution mass spectrometer. This spectrometer was modified to scan a range of 0.4 mass units in the vicinity of the m/e 322 molecular ion peak of TCDD containing one ^{37}Cl atom and three ^{35}Cl atoms, using a resolution of 9000. The computer averaging system was set to collect data during the retention time span of TCDD as determined by the response to a standard material. The peak height at the exact m/e value of 321.8936 for TCDD was used to determine the amount present, using calibrations from standard solutions which were injected alternately with the samples.

RESULTS

A series of seven recovery measurements were made using fat samples which had been shown to be free of TCDD (Table II). Each sample was prepared by adding 0.10 nanograms of TCDD to ten grams of fat before the KOH digestion step. The average recovery value of 76% is consistent with previous environmental samples (SHADOFF and HUMMEL 1975, MAHLE et.al. 1977).

TABLE II

2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) Recovery from Beef Fat

	TCDD		
Sample	Added, ng	Found, ng	Recovery, %
129971	0.10[a]	0.08	80
129974	0.10	0.07	70
129976	0.10	0.08	80
129979	0.10	0.08	80
135025	0.10	0.09	90
92075	0.10	0.06	60
85595	0.10	0.07	70
		Average	76 ±10

[a] Equivalent to 10 ppt in the fat sample.

The results of surveillance samples from Texas, Missouri, and Oklahoma are shown in Table III. The limit of detection is defined as 2.5 times the peak to peak noise as shown on the chromatogram. To be a positive response, the following criteria must be met: (1) a response with signal/noise greater than or equal to 2.5; (2) the retention time matches that of 2,3,7,8-TCDD; (3) the response is observed in replicate samples using the low-resolution GC-MS system; and (4) a measurement of an independent preparation of the same sample using the high-resolution GC-MS system gives the same response.

Measurement of tissue samples from cattle in Oklahoma, Missouri, and the Texas Department of Corrections in Sugarland, Texas showed no TCDD response at detection limits of 3 to 6 ppt. Low resolution GC-MS measurements of eight samples from a ranch in Mertzon, Texas showed no apparent TCDD response in three samples (one of which was a control animal slaughtered before the study began) and apparent positive TCDD responses in the remaining five samples. Fresh preparations of these five samples were analyzed using the high resolution GC-MS technique and three of the five apparent positive TCDD responses were verified at the detection limits of 3 or 4 ppt.

SUMMARY

Specimens of fat taken from steers which had grazed on rangeland previously treated with 2,4,5-T herbicides were analyzed for the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). A cleanup procedure resulting in a 500-fold concentration was followed by a gas chromatography-mass spectrometry detection technique. The limit of detection of TCDD (2.5 times peak to peak noise) was found to be in the 30-60 picogram range (3-6 ppt in beef fat using 10 gram samples).

None of the sixteen samples comprising two of the three studies showed any response for TCDD. In the third study, in which the animals were confined to a fenced pasture sprayed in its entirety with a 2,4,5-T herbicide, samples from three of the seven animals gave a positive response at the extremely low level of 3 to 4 ppt TCDD, which is at the detection limit.

Table III
RESULTS OF ANALYSIS OF THE SURVEILLANCE BEEF FAT SAMPLES

Sample Number	State	Determination Number	Low Resolution GC-MS[a] Apparent TCDD (ppt)[b]	Low Resolution GC-MS[a] Detection Limit (ppt)	High Resolution GC-MS[a] Apparent TCDD (ppt)	High Resolution GC-MS[a] Detection Limit (ppt)
129971	TX	1	N.D.[c]	4		
129972	TX	1	N.D.	4		
129973	TX	1	N.D.	4		
129974	TX	1	N.D.	4		
		2	N.D.	4		
129975	TX	1	N.D.	4		
129976	TX	1	N.D.	4		
129977	TX	1	N.D.	4		
129978	TX	1	N.D.	4		
129979	TX	1	N.D.	6		
		2	N.D.	3		
129980	TX	1	N.D.	6		
129981	TX	1	N.D.	5		
129929	MO	1	N.D.	4		
135020	OK	1	N.D.	5		
135021	OK	1	N.D.	5		
135022	MO	1	N.D.	5		
135023	MO	1	N.D.	5		
		2	N.D.	4		
135024	TX	1	N.D.	6		
		2	N.D.	6		
135025	TX	1	N.D.	7		
		2	N.D.	6		
135026	TX	1	7	4		
		2			4	4
135027	TX	1	4	3		
		2	11[d]	3		
		3			3	3
135028	TX	1	N.D.	4		
		2	N.D.	4		
135029	TX	1	5	4		
		2	4	4		
		3	5	3		
		4	11[e]	4		
		5	13	6		
		6			N.D.	5
135030	TX	1	4	4		
		2	N.D.	4		
		3	14[d]	3		
		4	8[e]	3		
		5			N.D.	4
135031	TX	1	N.D.	5		
		2	15[d]	3		
		3	10	3		
		4			4	4

[a] Data corrected for recovery
[b] ppt = parts per trillion (w/w)
[c] N.D. = Not detected
[d] Interferences present at both m/e 320 and 322. Numbers are upper limits to the amount of TCDD which could be present.
[e] Data indicates there may be an interference in at least one mass. Numbers are upper limits to the amount of TCDD which could be present.

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REFERENCES

BAUGHMAN, R. W., Ph.D. Thesis, Harvard University, Cambridge, Mass., 1974.

BAUGHMAN, R. W. and M. MESELSON, Environ. Health Persp. #5, 27 (1973).

DOW CHEMICAL CO., "Summary of Safe Handling of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Laboratory," Biochemical Research Laboratory, Midland, Michigan, 1970.

MAHLE, N. H., H. S. HIGGINS, M. E. GETZENDANER, Bull. Environ. Contam. Toxicol., in press.

POLAND, A., D. SMITH, G. METTER, P. POSSICK, Arch. Environ. Health, 22, 316 (1971).

SCHWETZ, B. A., J. M. NORRIS, G. L. SPARSCHU, V. K. ROWE, P. J. GEHRING, J. L. EMERSON, C. G. GERBIG, Advances Chem. Ser., 120, 55 (1973).

SHADOFF, L. A. and R. A. HUMMEL, 170th National Meeting of the American Chemical Society, Chicago, 1975.