

Search for the Presence of 2, 3, 7, 8-Tetrachlorodibenzo-p-Dioxin in Bovine Milk

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Grazing animals could possibly ingest residues of phenoxy compounds, such as 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) or their impurities from pasture or rangelands, when these compounds are applied to control broadleaf weeds and brush (LENG 1972). The determination of 2,4,5-T in bovine milk has been reported (BJERKE et. al. 1972), but nothing has been reported on the possible presence of the toxic impurity 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). This surveillance study was undertaken to determine if low level concentrations of TCDD could be detected in milk from cows grazing on grass where 2,4,5-T had been applied following normal agricultural practice.

EXPERIMENTAL

Sample Collection and History

Mr. H. M. Elwell, a retired rangeland management professor from Oklahoma State University, of Oak Grove, Missouri, collected the samples at the farms where the animals had been grazing on treated pasture or range. Milk was obtained from both dairy and beef-type cows in Missouri, Arkansas, and Oklahoma, each sample from a different farm. These areas were selected as representative of those where 2,4,5-T is used for broadleaf weed and brush control in pasture and rangeland. Applications of 2,4,5-T are generally made in the spring when the plants to be controlled are growing vigorously.

All applications were made by aircraft, with the possible exception of one location in Boone County, Arkansas, where the method of application was not recorded (Table I, Sample 129912).

TABLE I
INFORMATION ABOUT THE SURVEILLANCE MILK SAMPLES FROM COWS GRAZING ON GRASS TREATED WITH 2,4,5-T

Sample Number	County	Location	State	Type of Cow	Water Source	History of 2,4,5-T Use lb ae/A ("B" = Burned) ^{1/}				Grass	ASI ^{2/}
						>71	71	72	73		
124948	Hughes	OK	Beef	Treated ponds	4 ^{3/}	2	2		1.5	Bluestem	23 months
124949	Washington	OK	Beef	Treated ponds	5 ^{3/}					Bluestem	5 days
124950	Osage	OK	Beef	Treated ponds						Bluestem	48 months
124951	Osage	OK	Beef	Treated ponds		2	2	2		Bluestem	12 months
124952	Osage	OK	Beef	Treated ponds					2	Bluestem	3 days
124953	Johnson	OK	Beef	Treated ponds		2	2	2		Bluestem	12 months
124954	Pittsburg	OK	Beef	Treated ponds		2	2	2		Bluestem	12 months
124955	Osage	OK	Beef	Treated ponds		2	2	2		Bluestem	12 months
124956	Osage	OK	Beef	Treated ponds		2			2	Bluestem	10 days
129912	Boone	AR	Dairy					2B	2B	Fescue & Bluestem	6 months
129913	Carroll	AR	Dairy					2	2	Fescue & Orchard	18 months
129914	Boone	AR	Beef	Treated ponds & Springs			2B			Fescue	30 months
129915	Boone	AR	Beef	Springs					2B	Fescue & Native	6 months
129916	Boone	AR	Beef	Treated ponds & Springs		2B			3	Fescue & Native	6 months
129917	Izard	AR	Beef	Treated area						Fescue	18 months
129918	Fulton	AR	Beef/Beef/Dairy	Treated area		2B	2B	3B	2B	Fescue	18 months
129919	Osage	OK	Beef				2	2		Bluestem, Indian & Switch	14 months
129920	Randolph	MO	Beef	Treated pond					2	Fescue & Bluestem	15 months
129921	Polk	MO	Dairy			2B	2B			Fescue & Orchard	27 months
129922	Cedar	MO	Beef			2B	2B		2	Bluestem & Fescue	4 months
129923	Taney	MO	Dairy			2B	2B	2B	2	Bluestem & Fescue	4 months
129924	Taney	MO	Dairy/Dairy/Beef			2B	2B	2B		Fescue	27 months
129925	Wright	MO	Dairy					2.5		Mixed	18 months
129926	Wright	MO	Dairy	Treated area drainage					2.5		6 months
129927	Taney	MO	Beef					2	3	Mixed	5-6 months

^{1/} Burning occurred generally in the fall of the same year as spring application. Repeat applications were to different areas of the same pasture. ae = acid equivalent.
^{2/} Approximate interval from last application 2,4,5-T to sampling of milk. In most cases the dates of treatment are not accurately known.
^{3/} Pounds applied prior to 1971.

Commercial 2,4,5-T formulations were applied to rangeland on which mixed hardwoods were growing. The specific formulations and dates of manufacture of the 2,4,5-T are not known. However, it is reasonable to assume that the TCDD content of the herbicide was less than 1 ppm. Applications made in 1973 and 1974 could have been material manufactured with a TCDD content of less than 0.1 ppm, although they might have been made with herbicides manufactured earlier.

Samples were collected between May 26 and September 20, 1974. The interval between the last application of 2,4,5-T and collection of samples ranged from five days to 48 months, with 14 collected within a year (Table I). Most of the cows had been exposed to 2,4,5-T treated pasture for several years. In many 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 the same time. In practice, it is usually not necessary to re-treat the same area the next year. Hence, in Table I where the history of use of 2,4,5-T indicates treatment on successive years, different parts of the range or pasture were treated each year.

Many of the samples were a composite of milk from more than one cow. Dairy animals received the normal minerals, supplements, and dairy ration in addition to the grass they consumed. In addition, most of the cows had been treated with approved, but unspecified insecticides for fly and tick control.

Milk samples were collected in polyethylene bottles, frozen soon after collection and maintained frozen until they were analyzed after they were shipped to The Dow Chemical Company, Midland, Michigan.

Collection of milk from the non-dairy type cows was generally accomplished with much difficulty as these animals were not accustomed to being milked by humans.

Water consumed by the animals was often from supplies within the treated areas which were treated along with the brush. Where this was documented, it is recorded as "treated pond," etc. in Table I.

Analysis

Milk was analyzed for TCDD using a gas chromatography-mass spectrometry (GC/MS) technique. The clean-up procedure was essentially that of SHADOFF AND HUMMEL (1975). To attain a lower limit of detection, forty gram samples instead of the usual ten gram samples were used. To insure a representative sample, the entire milk sample was shaken vigorously after thawing, and a portion was removed. The sample was then digested with an alcoholic potassium hydroxide solution. The digestion time was increased from one hour to four and one-half hours to insure complete hydrolysis of the larger sample. This solution was then extracted four times with 15 ml portions of hexane.

The hexane extracts were shaken with 10 ml portions of concentrated sulfuric acid until the acid layer was only lightly colored (usually four 10 ml portions) and the hexane was then evaporated at room temperature using a stream of air. The residue was dissolved in hexane and transferred to a 50 mm column of silica gel held in a 4-mm diameter disposable transfer pipet. The silica gel was eluted with 20% benzene in hexane and the eluate evaporated. The residue was dissolved in hexane and transferred to a 50 x 4 mm column of alumina that had been activated at 130°C. The alumina was washed with 12 ml of 20% carbon tetrachloride in hexane and then eluted with 20% methylene chloride in hexane. The 20% methylene chloride eluate was evaporated to 0.1 ml and transferred to a 0.3 ml cone-shaped vial, using hexane as solvent. The solvent in the vial was evaporated at room temperature using a stream of air.

Gas chromatography-mass spectrometry was used to determine if TCDD was present. A 1.8-m 3% OV-3 silicone column was used in an LKB-9000 instrument which was tuned to monitor the m/e 320 and 322 molecular ions of TCDD. To get maximum sensitivity one ion was monitored per injection. Ten or 20 microliters of o-xylene were added to the cone-shaped vial containing the sample extract and a two or five microliter aliquot was injected into the GC column. The ³⁷Cl TCDD, when added to determine recovery, was monitored at m/e 328 during a separate injection. Results were calculated from the ratio of sample peak heights to standard peak heights.

RESULTS AND DISCUSSION

Recovery data for TCDD added to the control and various samples can be found in Table II. ^{37}Cl TCDD was added in some cases, to confirm recovery from some of the surveillance samples. The limit of detection given in Table III with the results is 2.5 times the noise level shown on a strip chart recorder from that sample.

TABLE II. RECOVERY OF TCDD ADDED TO BOVINE MILK

Sample	TCDD Added (ppt) ^{a/}	TCDD Found (ppt) ^{b/}	Percent Recovery
Reagent Blank	--	ND ^{c/}	--
Control	--	1	--
Control	--	ND	--
Control	2	2	100
Control	2	2	100
Control	2	1	50
Control	5	3	60
Control	5	3	60
Control	5	4	80
Control	25 ^{d/}	17	68
124949	25 ^{d/}	18	72
124951	25 ^{d/}	17	68
124954	25 ^{d/}	12	48
			Average = 71

^{a/} ppt - Parts per trillion

^{b/} Not corrected for blank or control

^{c/} ND - Not detected

^{d/} ^{37}Cl TCDD used

The control milk used in this study was homogenized whole milk purchased at a Midland, Michigan supermarket, in a plastic-coated cardboard container. The average recovery of 71% over the range of 2 to 25 ppt of added TCDD is consistent with previous studies on other environmental samples (SHADOFF AND HUMMEL 1975).

The results of these analyses are given in Table III. Variation in the limit of detection can be attributed to variations in the gas chromatographic column and/or mass spectrometer as well as to differences in the samples.

The results of the analyses of milk from the cows grazing on grass treated with 2,4,5-T are indistinguishable from the control milk. This control milk obtained from a Midland, Michigan supermarket would have had very little likelihood of contact with 2,4,5-T. The variation of ND (none detected) to 1 ppt apparent TCDD occurred in both cases. Because of the variation in the control milk, a positive finding of TCDD in the surveillance samples would require a response well above the 1 ppt level.

Typical mass chromatograms can be seen in Figure 1. Because of the low sensitivity limit requirement, only one m/e value (usually m/e 320) was recorded for each injection. If an apparent positive response is encountered, then a second m/e value (usually m/e 322) was recorded.

CONCLUSION

Surveillance samples of milk from the states of Oklahoma, Arkansas, and Missouri were collected from cows grazing on pasture or rangeland treated with normal applications of 2,4,5-T. These samples and control samples were analyzed for TCDD by GC/MS. A detection limit of 1 ppt was achieved. With this sensitivity the control samples were indistinguishable from those from treated areas. Hence, TCDD was not found.

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TABLE III

RESULTS OF ANALYSIS OF THE SURVEILLANCE MILK SAMPLES FROM COWS GRAZING ON GRASS TREATED WITH 2,4,5-T.

Sample Number	State	Last Application of 2,4,5-T lb ae/A Date	ASI ¹ / Date	Limit of Detection ppt	Apparent Gross ² / Net ³ / TCDD, ppt
124948	OK	2 6/72	23 months	1	ND
124949	OK	1.5 6/74	5 days	1	1
124950	OK	1 -/70	48 months	0.5	ND
124951	OK	2 -/73	12 months	1	ND
124952	OK	2 6/74	3 days	1	1
124953	OK	2 -/73	12 months	1	ND
124954	OK	2 -/73	12 months	0.5	ND
124955	OK	2 -/73	12 months	0.5	ND
124956	OK	2 6/74	10 days	1	ND
129912	AR	2 -/74	6 months	1	ND
129913	AR	2 -/73	18 months	1	1
129914	AR	2 -/72	30 months	1	ND
129915	AR	2 -/74	6 months	1	ND
129916	AR	3 -/74	6 months	1	1
129917	AR	2 -/73	18 months	1	ND
129918	AR	2 -/73	18 months	1	ND
129919	OK	2 -/73	14 months	1	ND
129920	MO	2 -/73	15 months	1	ND
129921	MO	2 -/72	27 months	1	ND
129922	MO	2 -/74	4 months	1	1
129923	MO	2 -/74	4 months	1	ND
129924	MO	2 -/72	27 months	1	ND
129925	MO	2.5 -/73	18 months	1	ND
129926	MO	2.5 -/74	6 months	1	1
129927	MO	3 -/74	5-6 months	1	ND

¹/ ASI = Last application - sampling interval (approximate)²/ ND = None detected³/ Gross minus largest control of 1 ppt. Control milk from animals presumed not to be grazing on grass sprayed with 2,4,5-T showed an apparent 1 ppt TCDD response in one of two analyses. (See Table II)

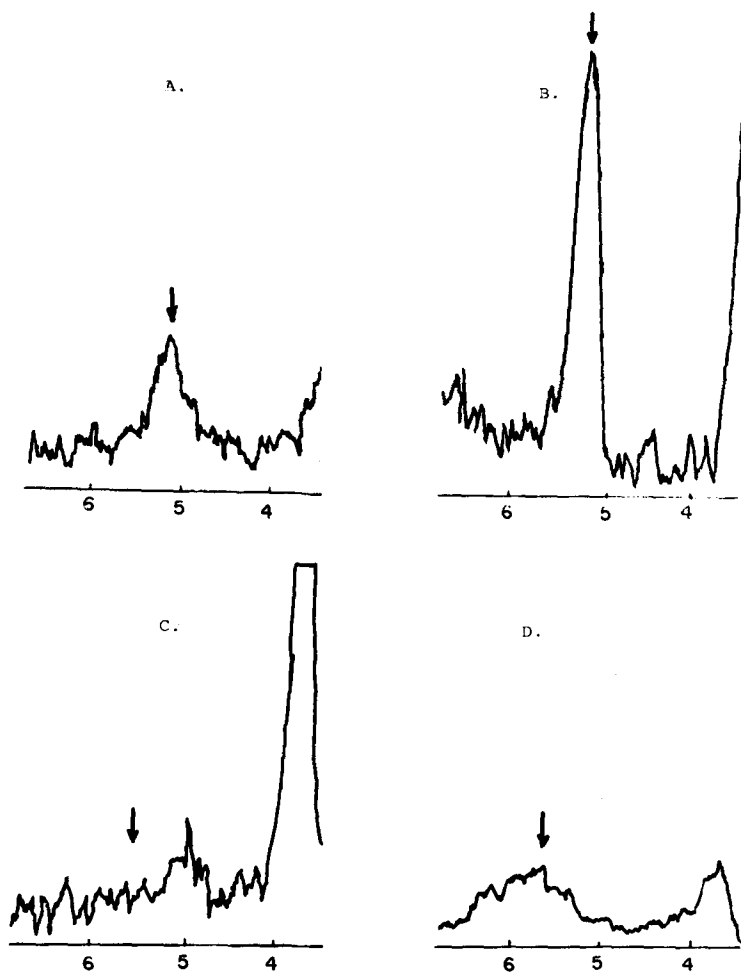


Figure 1. Mass Chromatograms ($m/e = 320$) (\downarrow = TCDD Retention Time)
(Time (min.))

A. TCDD standard 5 μ l of 2 ppb = 10 pg
 B. Control milk + 5 ppt TCDD
 C. Sample 129921. Limit of detection = 1 ppt of TCDD. No TCDD response.
 D. Sample 129913. Limit of detection = 1 ppt of TCDD. 1 ppt TCDD response.

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