Bull. Environm. Contam. Toxicol. 29, 440–446 (1982)

Secretion of TCDD in Milk and Cream Following the Feeding of TCDD to Lactating Dairy Cows

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The secretion of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) into milk of dairy cows fed 2,4,5-T has been studied (BJERKE et al. 1972), but there have been no studies on the levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) secretion into milk of dairy cows fed TCDD. The level of secretion of hexachloro-, heptachloro- and octachlorodioxin have been determined in milk from cows fed pentachlorophenol (FIRESTONE et al. 1979). This study was designed to determine the TCDD levels which would occur in milk and cream when cows were continuously fed various levels of 2,4,5-T containing TCDD for two or three weeks. This study was also designed to determine the rate of decline of TCDD secretion in milk following removal of TCDD from the diet.

EXPERIMENTAL

Animals, Dosing and Sampling

Six Holstein dairy cows weighing from 998 to 1,240 pounds, were confined in pens at the Agricultural Research Center, Midland, Michigan in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care. The cows were conditioned for two weeks on a complete dairy ration. A total of 36 pounds of feed was given to each cow daily, half at each milking. The fortified feeds were prepared by blending 10 to 25% (w/w) concentrates of 2,4,5-T acid on silica gel with sufficient feed to make rations containing 10, 30, 100, 300 or 1000 ppm 2,4,5-T in the feed. The corresponding TCDD levels were 5, 15, 50, 150 and 500 ppt. The concentrates were prepared by mixing an acetone solution of 2,4,5-T with silica gel. The 2,4,5-T used was a special research sample containing 0.5 ppm TCDD. These are artificially exaggerated TCDD feeding levels when compared to potential field exposures.

Following a two-week conditioning period, three cows were continued on basal rations while three were given feed containing TCDD at 5 ppt, 15 ppt, 50 ppt, or

0007-4861/82/0029-0440 $01.40

150 ppt for 14 days consecutively at each level, and finally at 500 ppt for 21 days for a total treatment period of 11 weeks. Two of these cows were continued on basal rations for an additional 83 days making possible a study of the rate of decline of TCDD residues in milk.

All milking was done by machine according to the same daily schedule. The control cows were all milked with one machine which was washed after milking each cow. Individual milking machines were used for the animals on treated feeds. Separate milk samples from each cow were obtained at predetermined intervals by combining one-half pint from the evening milking with an equal amount from the milk collected the following morning. Samples were stored frozen in one pint screw-cap tin cans until analyzed. Composite cream samples were collected from morning milk by pooling 1-1/2 gallons of milk from each cow of its respective group. The composited milk was separated on a DeLaval, Model 100 electric farm separator which was adjusted to give medium heavy cream. Samples were collected in one pint tin cans and immediately frozen. All excess milk and cream were destroyed by incineration.

Analysis - TCDD

The extraction and cleanup procedure used was described by HUMMEL (1977) and the gas chromatography-mass spectroscopy used was given by SHADOFF AND HUMMEL (1978). The analytical procedure was verified by adding known amounts of unlabeled TCDD or ^{37}Cl TCDD to samples before analysis. A sample was analyzed monitoring m/e 328 for ^{37}Cl TCDD giving a recovery value, and was reinjected monitoring m/e 320 and 322 to determine the unlabeled TCDD in the sample. Separate injections were necessary because m/e 320, 322 and 328 could not be monitored simultaneously on this instrument due to high signal background at m/e 328. Control milk and cream was fortified with 2 to 50 ppt TCDD for milk and 2 to 500 ppt for cream followed by analysis. The average recovery of TCDD was 75% from milk and 88% from cream (Table I). Due to the parts-per-trillion sensitivity of the method, the accuracy of any given results varies ±20% at 10 ppt or above for milk and ±30% at 10 ppt or above for cream. Thus, a residue given as 20 ppt would actually be 20±4 ppt.

RESULTS AND DISCUSSION

No adverse effects were observed in the cows due to feeding of 2,4,5-T containing 0.5 ppm of TCDD as evidenced by milk production, body weights, feed consumption, and observations by a veterinarian. The

TABLE I

RECOVERY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ADDED TO MILK AND CREAM

MILK			CREAM		
TCDD, ppt		Recovery	TCDD, ppt		Recovery
Added	Found	%	Added	Found	%
0	ND[a](1)[b], 1(1), ND(2)	--	0	ND(5), 4(2)	--
	ND(3), ND(1), ND(2)	--		3(3), ND(3)	--
	ND(2)	--			
2	ND(2), 2, 2	0, 100, 100	2	3	150
	2, 1, 2	100, 50, 100	4	5	125
			5	8	160
5	4, 3, 3, 4	80, 60, 60, 80	10	6, 6	60, 60
10	8, 7	80, 70	25	17, 14	68, 56
20	14, 15	70, 75	50	18, 54, 39[c]	56, 108, 78
25[c]	21, 17, 18	84, 68, 72	100	75[c], 83[c]	75, 83
	17, 12	68, 48			
			200	146	73
50[c]	41, 40, 38, 42	82, 80, 76, 84			
	43, 43, 37, 40	86, 86, 74, 80	500	380	76
	45, 38, 47, 41	90, 76, 94, 82			
	38, 33, 36, 40	76, 66, 72, 80			
	39, 38, 28, 40	78, 76, 56, 80			

[a] ND - not detected, peak less than 2.5 times recorder noise level.

[b] ( ) limit of detection.

[c] ^{37}Cl isomer of TCDD ppt - parts per trillion

cows consumed all the feed presented to them throughout the test.

The TCDD residues found in milk and cream from cows on feed containing TCDD increased in proportion to the TCDD in the diet, Table II. For milk, residues ranged from non-detectable at the 5 ppt diet level to 89 ppt at the 500 ppt diet level. Levels of TCDD in cream were about 10 times larger than those found in milk and ranged from 11 ppt to 780 ppt at the highest feeding level. The higher concentration in cream is to be expected because TCDD is more soluble in fat than in water. To check this concept, the fat content of cream composites from the 150 ppt and 500 ppt diet levels and milk samples collected on the same days from cow 36 were estimated by extracting 100 ml of the sample with hexane in a manner similar to the AOAC method 16.052. The ratio of fat in cream/milk obtained were 9.4 and 10.2, respectively. These ratios are similar to the 9.4 and 8.8, respectively, found for the cream/milk ratios for TCDD residues at these levels, suggesting that TCDD was associated only with the fat portion of milk and cream.

Data for the decline in TCDD residues in milk following removal of TCDD from the diet are given in Table III. It has already been observed that a single compartment model (linear regression) was adequate to describe the elimination of TCDD from rats (ROSE et al. 1976) and dioxins from cows (FIRESTONE 1979). Therefore, dissipation data in this experiment were evaluated in this manner yielding a linear regression equation, $\ln c = 3.98-0.015t$. A correlation coefficient of 0.89 was obtained from these data indicating that a linear model is a reasonable fit for these data. This equation yields a half-life of 41 days for TCDD in milk, which is very similar to the half-life values of 41 to 51 days found for hexachloro, heptachloro-, and octachlorodioxins secreted in milk (FIRESTONE et al. 1979).

This was strictly a laboratory study to determine the relationship between TCDD diet level and resultant milk level. In actual field situations the initial TCDD level on grass would be small, around 5 ppt (MORTON et al. 1967), since current 2,4,5-T has less than one-tenth the TCDD level present in the 2,4,5-T used in this study. In addition, the half-life of TCDD on grass is only one week (NASH and BEALL 1980) so TCDD residues would be continuously declining. Thus continuous feeding of TCDD at levels from 5 to 500 ppt constitutes an exaggeraged feeding rate when compared to low level TCDD residues which rapidly decline. It is interesting to note that no TCDD was found in milk even after two weeks of continuous feeding at 5 ppt.

TABLE II

RESIDUES OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN MILK AND CREAM FROM COWS CONTINUOUSLY FED A DIET CONTAINING EXAGGERATED LEVELS OF TCDD

TCDD in Feed, ppt	Days on Feed[a]	Cow #	Apparent TCDD[b], ppt[c] Milk	Cream
0	0	9078	ND[d](2)[e]	ND(5), 3
		0	96	ND(2) --
5	14	36	ND(2)	10, 13
	14	7417	ND(2)	--
15	14	36	3	18, 26
	14	7417	3	--
50	14	30	11	99
	14	7417	10	--
150	3	30	11	--
	12	30	16	--
	14	30	16	180
	14	36	16, 22	--
	14	7417	14	--
500	3	36	42	--
	3	7417	42	--
	16	30	50, 44	--
	16	36	89	--
	16	7417	69	--
	21	30	79	780
	21	7417	68	--

[a] The amount of TCDD in the diet was increased every 14 days until 500 ppt level was reached.
[b] Corrected for approximately 80% recovery of TCDD.
[c] ppt - parts per trillion.
[d] ND - not detected.
[e] detection limit calculated as 2.5 times detector noise level.

TABLE III

DECLINE OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) RESIDUES IN MILK FOLLOWING COMPLETION OF 21 DAYS FEEDING AT THE EXAGGERATED LEVEL OF 500 PPT OF TCDD

	Apparent TCDD, ppt[a] Days no TCDD in the diet											
Cow #	3	7	17	31	40	48	60	62	68	75	80	90
36	86	59	43	35	32	29	16,12	14	15	18	14	14
7417	--	38	31	25	26	22	18,21	22	21	21	20	19

[a] Parts per trillion.

CONCLUSIONS

This study has shown that the level of TCDD secreted into milk and cream are such as to suggest that the residue found is proportional to the TCDD diet level and to the fat content of the milk or cream. After TCDD is removed from the diet the amount of TCDD secreted into milk decreases with time having a half-life of 41 days.

ACKNOWLEDGEMENT

The diet preparation, care, feeding, milking of the cows, and sample collection by the personnel under the direction of Dr. Charles Johnston, sample collection and handling coordination by D. Ervick, and H. S. Higgins, L. Lamparski, E. Madrid and J. Turley assistance with sample analysis is gratefully acknowledged.

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Accepted August 24, 1982