A Residue Study on Beef Cattle Consuming 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

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Seven beef cattle were fed for 28 days on a complete ration which was fortified with 24 parts per trillion (ppt) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). This continuous feeding of 24 ppt of TCDD is an artificially exaggerated feeding level. Distribution of TCDD in the major edible tissues was studied by sacrificing three treated and three control animals at the end of the 28-day feeding period and analyzing for residues of TCDD in fat, liver, kidney, and muscle. Dissipation of TCDD from fat was studied in four of the treated animals by analyzing biopsy fat samples at intervals up to 36 weeks after withdrawal of TCDD from the feed and at the 50-week sacrifice time. These studies showed that at this exaggerated TCDD feeding level, residues occurred primarily in fat and these residues were actively dissipated after removal of TCDD from diet.

Concern about the potential health hazards of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has led to numerous studies in animals fed exaggerated levels in the total diet. In several cases, tissues were analyzed for residues of TCDD. Fries and Marrow (1975) reported that after 6 weeks of feeding rats, 90% of the steady-state TCDD retention was reached at ~10.5 times the average daily intake. The TCDD level in the liver was somewhat greater than in the fat, and both levels were ~10 times higher than in other tissues. Rose et al. (1976) found a total TCDD body retention of ~15 times the average daily intake after 7 weeks of feeding. Allen et al. (1975) also found the major TCDD residue occurring in rat liver. The distribution of TCDD in both rat and monkey tissues was compared by Van Miller et al. (1976). They found that the TCDD level in rat liver was higher than in other rat tissues, while in monkeys, TCDD concentration was higher in fat than in liver. In rainbow trout, TCDD residues were approximately the same as the level of TCDD in the food which they consumed (Hawkes and Norris, 1977). These studies have shown that animals which ingest TCDD will have TCDD in certain body tissues for as long as exposure continues.

It is also clear that the body burden decreases after TCDD exposure stops. Piper et al. (1973), Allen et al. (1975), Fries and Marrow (1975), and Rose et al. (1976) all found a half-life ranging from approximately 12 to 30 days for TCDD retention in rats.

The fate of (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) in cattle has been reported by Clark et al. (1975), but studies on TCDD in beef cattle have not been published to date. The objectives of the work reported herein were to determine the distribution of TCDD in edible tissues and to determine the rate of dissipation of TCDD from fat.

EXPERIMENTAL SECTION

Feeding and Sampling. Uniform distribution is difficult to accomplish when chemicals are mixed in cattle feeds at the part per trillion (ppt) level. This problem can be minimized by diluting the test chemical in a larger amount of "carrier" compound prior to addition to the feed. Since 2,4,5-T can contain TCDD as a trace contaminant, 2,4,5-T was chosen to serve as a carrier for the TCDD in this study. Feed was prepared by adding 2,4,5-T containing 0.06 ± 0.02 ppm of TCDD as follows: 1633.5 g of 25% 2,4,5-T on silica gel was added to 1.4 kg of chicken feed and blended with 90.7 kg of "Purina Cattle 12" ration in a 180 kg capacity Marion mixer. This mixture was blended in a Kelly-Duplex upright mixer with sufficient feed to produce a total of 1361 kg of finished feed. Analysis of the feed showed it contained 24 ± 5 ppt of TCDD using a modification of the analytical procedure of Hummel (1977), in which the sample was extracted with hexane for 4 h in a Soxhlet extractor, the hexane evaporated, and the residue analyzed in the normal manner.

Twelve young beef animals were selected from a herd at the Dow Chemical U.S.A. Research Farm at Lake Jackson, TX. These facilities are fully accredited by the American Association for Accreditation for Laboratory Animal Care. The calves were weaned, acclimated to the pens, and fed basal ration for 3 weeks before treatment. The treated animals consisted of seven calves which were individually penned in concrete-floored partially covered pens. Control animals were divided into groups of three and two each, penned, and fed basal ration only throughout the test. All animals were fed ad libitum. Throughout the tests observations were made at frequent intervals by a veterinarian to note any changes in the animals due to the treatment.

The experiment consisted of two parts: a tissue distribution study and a dissipation study. All treated animals were fed rations containing 24 ppt of TCDD for 28 days. The distribution of TCDD was studied by analyzing tissues of three of the treated calves and three control calves sacrificed within 24 h after feeding ceased. Samples of muscle, fat, liver, and kidney were taken from each animal and shipped frozen to Midland, MI. Samples were then ground in a Hobart food grinder, subsampled into polyethylene bags, and frozen until analyzed.

Dissipation of TCDD from fat was studied by analyzing fat samples taken by biopsy from the cattle at various intervals following discontinuance of TCDD in the diet. These animals were turned to pasture following the treatment period and given a daily grain supplement to maintain fat levels. The extra grain supplement was ended after the 36-week sampling. The biopsy of 20–40 g of omental or tail head fat was performed by a veterinarian, with each sample placed in aluminum foil (shiny side out) and stored frozen until analysis. The animals were sacrificed 50 weeks after TCDD was discontinued in the diet, and samples of fat, liver, kidney, and muscle were taken and prepared as above. The dissipation of TCDD residue was followed only in fat samples because TCDD levels were too low in other tissues taken at the end of the feeding period. The fat tissue was found to contain ~9 times more TCDD than other tissues.

Analysis. The sample cleanup procedure for muscle, liver, and fat was essentially that which was developed by

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Table I. Weights and Feed Intake of Beef Animals Fed 24 ppt of TCDD for 28 Days before Slaughter

calf no.	animal wt, kg initial	final	av	feed eaten, kg	av feed per head per day, kg	TCDD feeding rate, μg kg^{-1} day^{-1}	TCDD total fed, μg
190	349	386	368				
191	298	313	306	658[a]	7.8	–	–
192	300	317	309				
193	190	205	198	140	5.0	0.00061	3.4
194	190	217	204	174	6.2	0.00073	4.2
195	200	231	216	176	6.3	0.00070	4.2

[a] Fed as a group of three animals.

Table II. Weights and Feed Intake of Beef Animals Fed 24 ppt of TCDD for 28 Days Followed by 50-Weeks Feeding with No TCDD in the Diet before Slaughter

calf no.	animal wt, kg initial	end of treatment	50 weeks	treatment av[a]	av feed per head per day, kg	TCDD feeding rate, μg kg^{-1} day^{-1}	TCDD total fed, μg
196	198	228	349	213	5.26	–	
197	190	216	–[b]	203			
198	175	193	295	184	6.41	0.00083	4.3
199	183	205	314	194	6.30	0.00078	4.2
200	203	219	335	211	7.19	0.00082	4.8
203	173	201	307	187	6.50	0.00083	4.4

[a] Average weight during treatment period. [b] Animal was lost due to complications from the first biopsy.

Table III. Recovery of TCDD Added to Beef Tissues

TCDD added, ppt[a]	TCDD recovered, % fat	liver	kidney	muscle
5	0, 0	100, 120	80, 80	90, 80
10	70, 80, 70, 80, 80, 90, 60	40, 100, 90	100, 80	60, 70
20	80	–	70, 70	65, 75
25	–	68		84
40	65	–		
50	70	64[b]	79[b]	68, 64[b]
100	78, 60, 54, 63, 74,[b] 75,[b], 69[b]	68,[b] 90,[b] 59,[b] 80,[b] 68,[b] 53,[b] 58,[b] 30[b]	82[b]	79[b]
200	78		77,[b] 74[b]	
250	–	82[b]		
300	74, 61[b]	73		
930	72	–		
av:	71 ± 4[c]	av: 73 ± 12	av: 79 ± 6	av: 74 ± 7

[a] Parts per trillion. [b] Recovery using [^{37}Cl]TCDD. [c] 95% confidence level for the mean.

Hummel (1977). A 10-g sample was digested by saponification with an aqueous ethanolic potassium hydroxide solution and extracted with hexane. The combined hexane extracts were washed with concentrated sulfuric acid. Further cleanup was accomplished by silica gel and alumina column chromatography.

When this cleanup procedure was applied to control beef kidney fortified with TCDD, recovery was ~25%. The presence of unsaponifiable matter caused early elution of part of the TCDD from the alumina column. This severe loss of TCDD was overcome by inserting a cleanup step utilizing Florisil adsorbent between the silica gel and alumina steps. The entire effluent from the silica gel column was collected and the solvent evaporated. The residue was transferred to a 5 × 50 mm column of Florisil (activated at 150 °C) with three 1-mL portions of hexane, the column was washed with 4 mL of 20% benzene in hexane (v/v), and the eluant was discarded. The column was eluted with 4 mL of methylene chloride, the solvent was evaporated, and the residue was carried through the alumina cleanup step.

Gas chromatography–mass spectrometry (GC–MS) (Shadoff and Hummel, 1978; Kocher et al., 1978) was used for TCDD quantitation. An LKB 9000 or 9000S GC–MS operating at a resolution of 400 was used to analyze the fat and liver extracts. Muscle and kidney extracts were analyzed by using a high-resolution mass spectrometer to increase sensitivity (AEI MS-30 at a resolution of 1000).

The fat content of the muscle sample was determined by the official AOAC Method 24.005 (Association of Official Analytical Chemists, 1975).

RESULTS AND DISCUSSION

No adverse effects were observed in the animals during the 50 weeks of the feeding study as evidenced by body weights, feed consumption (Tables I and II), and observations by a licensed veterinarian. The consumption of TCDD ranged from 0.0006 to 0.0008 μg of TCDD (kg of body weight)$^{-1}$ day^{-1} for the seven treated animals, for a total of ~0.02 μg/kg in 4 weeks.

The efficiency of the analytical procedure was determined by analyzing control samples fortified with known amounts of TCDD with natural chlorine isotope abundance, or [^{37}Cl]TCDD varying from 5 to 930 ppt. The average recovery of TCDD was 71% from fat (10–930 ppt), 73% from liver, 79% from kidney, and 74% from muscle (Table III).

The TCDD residues found in fat, liver, kidney, and muscle following continuous feeding of 24 ppt of TCDD for 28 days are shown in Table IV. The level of TCDD found in liver, kidney, and muscle was considerably lower than the TCDD diet level. However, the level found in

Table IV. Residues of TCDD in Various Tissues of Cattle Fed 24 ppt of TCDD for 28 Days (No Withdrawal)

animal no.	TCDD diet level, ppt[a]	TCDD found in tissues, ppt[a]			
		fat	liver	kidney	muscle
190	0	ND (10)[b]	ND (3)[b]	ND (2)[b]	ND (2)[b]
191	0	ND (10)	ND (2)	ND (2)	ND (2)
192	0	ND (5)	ND (2)	ND (2)	ND (2)
193	24	66	10, 8	6	2 (2)
194	24	91	8	8	2 (2)
195	24	95	7, 8	7	2 (2)

[a] Parts per trillion, corrected for recovery. [b] ND = not detected; numbers in parentheses are detection limits (2.5 times recorder noise level).

fat was ~4 times higher than the diet level. These results suggest that beef fat is the most sensitive beef tissue to use in monitoring for TCDD exposure as was done in the Dioxin Implementation Plan ("Pesticide Chemical News", 1976).

The TCDD level in muscle should be proportional to its fat content. The average fat content of muscle samples from three treated and three control steers was determined to be 2%. Taking an average TCDD level in fat of 90 ppt multiplied by 2% fat in muscle would give a residue of 1.8 ppt in muscle. This agrees with the 2 ppt (detection limit of 2 ppt) actually found for these samples.

These data represent residues obtained following an artificially exaggerated TCDD ingestion rate when compared to the intake that might be obtained grazing grass sprayed with 2,4,5-T. Typically, the initial deposit of 2,4,5-T on grass is 100 ppm for each pound of herbicide applied per acre (Morton et al., 1967). Since 2,4,5-T presently contains <0.05 ppm of TCDD, the treated grass could have a maximum initial TCDD level of 5 ppt. This residue decreases with time, having a half-life on grass somewhere between the 4-h value found on excised leaf surfaces (Crosby and Wong, 1977) and a value of 1 week found for grass in a microagroecosystem (Nash and Beall, 1978) and in a field experiment where grass was sprayed with a 2,4,5-T formulation (Getzendaner and Hummel, 1975). Assuming these half-lives, the TCDD level would decline to between <0.001 and 0.3 ppt in 1 month. Thus, TCDD residue levels obtained in this study in which a continuous 24-ppt diet level was fed should not be confused with the greatly reduced potential for TCDD residues in normal herbicide use.

The variability of these data in Table V reflects two problems inherent in a study of this type. The first is the variability in response of the test animals to the TCDD in the diet, and the second is the variability when analyzing less than 100 ppt of TCDD in unrendered beef fat samples. This variability made it essential that the data be examined as a whole and not in parts. Therefore, all of these data were examined by using a kinetic model in which the

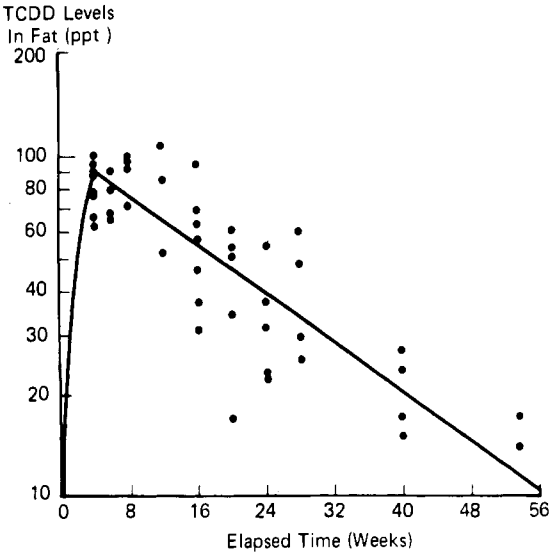

Figure 1. TCDD uptake and dissipation in beef fat. This curve was generated by using a kinetic model and a nonlinear estimation computer program. (The withdrawal portion of this experiment started at the fourth week of elapsed time.)

parameters of the model were estimated by using a statistical weighting scheme employing a nonlinear estimation computer program (Gehring et al., 1976; Reilly et al., 1977). This computer program generated the dissipation curve shown in Figure 1 from which the dissipation half-life ($t_{1/2}$) was estimated to be 16.5 ± 1.4 weeks and the elimination rate constant was determined to be 0.042 ± 0.003 week^{-1}.

This kinetic model also allows estimation of the uptake of TCDD in beef fat. The number of daily doses, assuming each dose was essentially consumed at one time, necessary to reach 95% of the steady-state residue level would be 499 ± 42, and the maximum residue at steady state consuming 24 ppt of TCDD would be 594 ± 62 ppt. This is close to the 540 ppt found in the fat of rats fed 22 ppt of TCDD for 2 years (Kociba et al., 1978).

The results of this feeding study provide a foundation of ruminant animal data supporting the conclusion that residues of TCDD would be detected in beef fat if range animals had been exposed to measurable levels of TCDD. However, no TCDD has been found at levels above the detection limit in beef samples taken from areas which would maximize exposure to TCDD through treatment with 2,4,5-T, except for 3 samples out of 89 from treated areas ("Pesticide Chemical News", 1976; Kocher et al., 1978; Mahle et al., 1977; Shadoff et al., 1977). These three all came from Missouri, in the general area of a trichlorophenol plant from which TCDD-contaminated waste oil was used for dust control on roads and horse arenas (Carter et al., 1975). No TCDD was found in animal and fish samples taken from forest areas treated with 2,4,5-T (Newton and Snyder, 1978; Newton et al., 1978; USDA–

Table V. Residues of TCDD in Beef Fat at Various Time Intervals following Discontinuance of TCDD in the Diet after 28-Days Continuous Feeding of 24 ppt of TCDD

animal no.	TCDD found, ppt,[a] at week										
	0	2	4	8	12[b]	16	20[b]	24	28	36	50
196	ND (10)[c]	–	ND (3)	–	ND (9)	–	ND (10)	–	ND (6)	–	ND (5)
197[d]	ND (8)	–	ND (8)	–	–	–	–	–	–	–	–
198	63, 100	91	100	85	46	61	37	60	–	15, 17	14
199	80	66	92	52	69	54	31, 54	48	–	26	17
200	86	68	71	108	63, 57, 95	51	37	25	–	23	ND (10)
203	77	80	97	85	31, 37	17, 34	22, 23	29	–	15	ND (10)

[a] Parts per trillion, corrected for recovery. [b] Tail head sample; all others are omental fat. [c] ND = not detected; numbers in parentheses are detection limits (2.5 times recorder noise level). [d] Animal died due to complications from first biopsy.

EPA, 1979). These negative monitoring results are expected because animals in the field are not generally exposed to measurable amounts of TCDD. TCDD is not ingested by most animals because of the combined effects of very low application rate, rapid photodecomposition rate (Crosby and Wong, 1977; Getzendaner and Hummel, 1975; Nash and Beall, 1978), and very strong binding to soil, k_{oc} = 486 000 (Kenaga, 1980).

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Pesticide Mutagenicity in *Bacillus subtilis* and *Salmonella typhimurium* Detectors

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Four pesticides, captan [*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide], folpet [*N*-[(trichloromethyl)thio]phthalimide], naled (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), and triallate [*S*-(2,3,3-trichloroallyl) diisopropylthiocarbamate], were evaluated for their ability to induce mutations in *Salmonella typhimurium* mutants (*TA1535*, *TA1536*, *TA1537*, *TA1538*, *TA98*, and *TA100*) and *Bacillus subtilis* mutants (*TKJ5211* and *TKJ6321*) with and without a rat liver microsomal activation system. These pesticides were more mutagenic in *TKJ6321* or *TKJ5211* than in *TA100* or *TA1535* (base pair substitution mutants). None of the pesticides required metabolic activation, but they were significantly detoxified by this metabolism.

Captan [*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide) and its isomeric relative, folpet [*N*-[(trichloromethyl)thio]phthalimide), possess valuable antifungal properties and have been used commercially on a large scale in agriculture and horticulture. They are widely applied as a spray, both to control various fungal diseases and to prevent spoilage of fruit and vegetables during storage and transit. Naled (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) is used on numerous crops, also against flies in barns, poultry houses, and kennels, and in and around food processing plants. Triallate [*S*-(2,3,3-trichloroallyl) diisopropylthiocarbamate] is used for preemergence application control of wild oats in barley, drum wheat, spring wheat, winter wheat, green peas, field-dried peas, and lentils. Because of the widespread application of these pesticides, humans may be exposed to these chemicals through their occupation or by consuming food containing residues of these chemicals.

Although procedures for establishing risk criteria for such things as acute or chronic toxicity can be readily determined, the risk for long-term effects such as cancer and deleterious human or animal heritable defects are much more difficult to assess. In recent years, simple, rapid, and economical short-term microbial mutagenicity

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