Polychlorodibenzo-*p*-dioxin and Pentachlorophenol Residues in Milk and Blood of Cows Fed Technical Pentachlorophenol

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Three cows were fed 20 mg kg^{-1} day^{-1} of commercial technical-grade pentachlorophenol (PCP) for 10 days and then 10 mg kg^{-1} day^{-1} of the PCP for an additional 60 days. No clinical effects were observed during the treatment period or during the 165-day posttreatment period. Although the commercial PCP contained seven dioxins, only three dioxins, 1,2,3,6,7,8-hexachlorodioxin, 1,2,3,4,6,7,8-heptachlorodioxin, and octachlorodioxin, were found in milk, body fat, and blood samples. Hexachlorobenzene (HCB), present in the commercial PCP, was also found in the samples. PCP in composite whole milk and HCB and total dioxins in composite milk fat rose to 4 mg/kg, 200 μg/kg, and 85 μg/kg, respectively, during the treatment period. When PCP feeding was stopped, PCP in the milk and blood declined within a few days to basal levels of less than 0.1 mg/kg. HCB and dioxin half-life was approximately 50 days.

Pentachlorophenol (PCP) is used extensively in the United States as a wood preservative and for various industrial applications as a bactericide and fungicide to protect products such as adhesives, paper and paperboard, leather, latex paints, textiles, rope, and ink. However, most of the approximately 50 million pounds of PCP produced in this country is used for wood preservation. The use of PCP as a preservative for wood used in construction of barns, corrals, feed bunks, and similar structures has led to reported instances of dairy herd contamination (*Chem. Eng. News*, 1977; *Chem. Mark. Rep.* 1977; Moore, 1977).

Commercial technical-grade PCP is a mixture of about 85–90% PCP and several percent each of tetrachlorophenol and chlorinated phenoxyphenols; it also contains a number of impurities including chlorinated dibenzo-*p*-dioxins (dioxins), chlorinated dibenzofurans (furans), and chlorinated diphenyl ethers (Firestone et al., 1972). Recently, milligrams/kilogram levels of PCP and micrograms/kilogram levels of hexachlorodioxin (HCDD), heptachlorodioxin (HpCDD), and octachlorodioxin (OCDD) residues were found in food-grade gelatin presumably prepared by alkaline hydrolysis of cattle hides preserved with PCP (Firestone, 1977). The presence of dioxins in food materials is of particular concern because they are fat-soluble, are resistant to biological degradation, and tend to accumulate in the food chain. In addition, many dioxins produce severe toxicological responses in monkeys, rats, mice, and guinea pigs (Allen et al., 1977; Harris et al., 1973; McConnell et al., 1978; Schwetz et al., 1973). On the other hand, Walters (1952) found that cattle were unaffected by exposure to PCP-treated fencing, housing, and feeding equipment. Also, Harrison (1959) reported that the acute fatal and minimum toxic daily dose rates of PCP to calves were 140 and 35 mg/kg body weight, respectively. Van Gelder (1977) determined that the biological half-life of PCP in dairy cows was 1.5 days.

The objectives of this study were to determine levels of residues of (a) PCP, pentachloroanisole (PCA), a metabolite of PCP, and dioxin in milk of lactating dairy cows during and following oral administration of commercial grade PCP, (b) the relationship between PCP and dioxin residues in blood and milk, and (c) the depletion rate of dioxins in blood and milk. Residue levels of hexachlorobenzene (HCB) were also determined in blood and milk since the PCP used in the study contained 80 mg/kg of HCB (Roman, 1977).

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Table I. Days in Lactation, Weight, and Milk Production of Cows at Start of Study

cow	days lactation	wt, kg	milk, kg/day
1	198	704.5	10.4
2	139	643.2	15.7
3	135	448.2	15.2
4[a]	73	576.3	26.7

[a] Control cow.

EXPERIMENTAL SECTION

Cows. Four lactating Holstein cows (three treated, one untreated) were used for the study, which was initiated in July, 1977. The cows were held in a pasture lot adjacent to the milking parlor. The pasture was provided with shelter consisting of a three-sided shed. Water and no. 2 alfalfa hay were available ad libitum in the pasture lot. Milking, except for sample collection, was carried out at approximately 7:00 a.m. and 4:00 p.m. daily with an automatic milker. During milking each cow received approximately 2.7–5.4 kg of a grain ration (USDA 20c, Agricultural Research Center, Beltsville, MD) formulated for lactating dairy cattle at each milking. Days in lactation, weight, and milk production data for the cows at the start of the study are shown in Table I. Average daily milk production of each of the test cows during the course of the study (235 days) was 10 kg. Daily milk production of the control cow during the study was about 23 kg.

Dosing and Sampling. Acclimation of the cows to the study regimen began 3 weeks before the treatment was started. During the acclimation phase, pretreatment milk and blood samples were collected for chemical analysis. Beginning on day 0 of the 70-day treatment period, three cows received PCP administered orally by gelatin capsule at a dosage rate of 10 mg/kg twice daily (20 mg kg^{-1} day^{-1}). A fourth cow, given a gelatin capsule containing only ground corn, served as a control. After day 10 and for the remainder of the 70-day treatment period, the test cows received only 10 mg of PCP kg^{-1} day^{-1} administered as a single dose after the morning milking.

Milk samples were collected twice weekly (Monday p.m. milking and Thursday a.m. milking) throughout the treatment period and for 13 weeks after treatment was stopped. Milk samples were obtained from each cow by hand-milking 50 mL (discarded) and then 175–200 mL from each quarter (total of about 750 mL) into 1-qt glass sample jars. Milking was then completed with the automatic milker. Milk from the three treated cows was composited for analysis of PCP, PCA, HCB, and dioxins. Blood samples (200 mL) were collected in 50-mL glass

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Table II. Dioxin and Furan Content of PCP Composite MB 419 (Buser, 1977)[a]

dioxin (D)		ppm	furan (F)	ppm
Cl_4-D		0.1	Cl_4-F	0.9
Cl_5-D		0.5	Cl_5-F	4
Cl_6-D	(1,2,4,6,8,9-)	2.0	Cl_6-F (1 major, 3 minor isomers)	32
Cl_6-D	(1,2,3,6,8,9-)	7.8		
Cl_6-D	(1,2,3,6,7,8-)	10	Cl_7-F (2 isomers)	120
Cl_6-D	(1,2,3,4,6,8,- and 1,2,3,7,8,9-)	0.7	Cl_8-F	130
Cl_7-D	(1,2,3,4,6,7,9-)	75		
Cl_7-D	(1,2,3,4,6,7,8-)	205		
Cl_8-D		690		

[a] GLC conditions: column temperature 200 °C, 3 min isotherm., then 2 °C/min to 240 °C; splitless injection (0.3 min), in tetradecane, 2-μL aliquot injected corresponding to 0.20- and 0.04-mg samples; same response for furans assumed as for dioxins.

Vacutainers containing 1 mL of 7.5% EDTA and were analyzed individually. Biopsy samples of fat were obtained just before treatment was stopped and at the end of the posttreatment period, at which time the cows were sacrificed. (Cows 1 and 3 were sacrificed 165 days after the study was begun. Cow 2 calved 221 days after the study began and was sacrificed 14 days later.) All samples were refrigerated after collection. Analytical samples were weighed into suitable containers within several hours for storage or analysis. Precautions were taken during collection, preparation, and storage of samples to avoid contamination.

Composition of PCP Used in Feeding Experiment. The technical PCP was an equal weight composite (lot MB419) prepared by Monsanto Industrial Chemicals Co. from the typical product of each of the domestic producers of technical grade PCP (Monsanto Industrial Chemicals Co., Sauget, IL; Reichhold Chemicals Inc., Tacoma, WA; and Vulcan Materials Corp., Wichita, KN). It was analyzed for dioxin and furan content at the Swiss Federal Research Station for Fruit-Growing, Viticulture, and Horticulture (Buser, 1977). The procedure of Buser and Bosshardt (1976) was followed except that a 25 m × 0.37 mm i.d. glass capillary column coated with OV-17 was used for gas–liquid chromatography–mass spectrometry (GLC–MS) analysis. The analytical results are shown in Table II. The composite PCP contained no detectable level of PCBs (polychlorobiphenyls; limit of detection, GLC–MS, 2 mg/kg); however, 80 mg/kg of HCB was present in the material (Roman, 1977).

PCP Analysis. Milk samples were analyzed as follows (results reported on whole-milk basis): Five milliliters of concentrated sulfuric acid was added slowly to 10 g of milk in a 25 × 150 mm screw-cap test tube (Teflon liner). The tube was sealed with the screw cap, and the contents were mixed by careful swirling. The mixture was allowed to stand 10–15 min, and 10 mL of hexane–2-propanol (8:2) was added to the reaction mixture. The test tube was resealed and shaken at 10-min intervals for 0.5 h and then centrifuged (900*g* for 3 min). The upper hexane layer was removed with a Pasteur pipet, and the aqueous layer was extracted twice more with 10-mL portions of hexane–2-propanol. The combined hexane extracts (24 mL) were then shaken with 5 mL of 1.0 N KOH. The upper hexane layer was removed and discarded. The aqueous layer was washed with an additional 10-mL portion of hexane. Two milliliters of 6 M sulfuric acid was added to the aqueous layer and the acidified solution was extracted first with 6 mL of hexane and then with two additional 2-mL portions of hexane. The hexane extracts were combined for GLC analysis. A Packard 7000 series gas chromatographed equipped with a tritium source concentric type electron capture (EC) detector was used with a 0.6 m × 4 mm i.d. glass column packed with 2% SP1000 plus 1% phosphoric acid on 80–100 mesh Chromosorb WAW at column, injector, and detector temperatures of 175, 205, and 200 °C, respectively. Carrier gas (nitrogen) flow rate was 120 mL/min. The absolute retention time of PCP was about 3 min. The identity of PCP was confirmed by preparation of the methyl ether and EC–GLC analysis on a 2 m × 4 mm i.d. glass column packed with 10% OV-101 on 80–100 mesh Chromosorb W HP, according to U.S. FDA (1968) Pesticide Analytical Manual, Vol. I, Section 301 (revised 7-1-75).

Feces were analyzed according to the procedure for milk. Urine samples were acidified and extracted with hexane–2-propanol as described for milk, and the combined hexane extracts (24 mL) were concentrated for GLC determination.

Blood samples were analyzed as follows: Five milliliters of 6 M sulfuric acid was added to 2 mL (2.05–2.10 g) of whole blood and 6 mL of hexane in a 50 × 120 mm test tube with Teflon-lined screw cap. The capped tube was shaken to mix the contents and placed in a water bath at 100 °C for 45 min, with shaking at 15-min intervals. The tube was then removed, allowed to cool to room temperature, and centrifuged (900*g* for 3 min). The upper hexane layer was transferred with a Pasteur pipet to a 10-mL volumetric flask and the aqueous layer was extracted by shaking with two additional 2-mL portions of hexane for 2 min each time and centrifuging before removing the hexane layer. The aqueous layer was then centrifuged one more time and the remaining hexane was removed. PCP in the blood extracts was determined by EC–GLC as described for the milk extracts. Recoveries of PCP from fortified milk samples were 95 ± 5% (0.01 mg/kg level) and 101 ± 2% (5 mg/kg level). Recoveries from fortified blood samples were 93 ± 5% (0.05 mg/kg level) and 103 ± 4% (15 mg/kg level).

PCA and HCB Analysis. Milk samples were cleaned up according to U.S. FDA (1968) Pesticide Analytical Manual, Vol. I, Section 211.13h. Blood samples were analyzed using U.S. EPA (1977) pesticide residue methodology for blood [Section 5, A, (3), (a)]. PCA and HCB were determined by EC–GLC on a Hewlett-Packard Model 5700 instrument equipped with ^{63}Ni EC detector. A 1.8 m × 4 mm i.d. glass column packed with 2.8% Silar 10C on 80–100 mesh Chromosorb W HP was used at 145 °C to determine PCA and HCB in milk. A 1.8 m × 4 mm i.d. glass column packed with 5% OV-101 on 80–100 mesh Chromosorb W HP was used at 210 °C to determine PCA and HCB in blood. The carrier gas was a 95% argon–5% methane mixture with a 60 mL/min flow rate. Injector and detector temperatures were 250 and 350 °C, respectively. PCA and HCB levels in the milk fat were calculated from whole-milk values and fat content of the milk. Recoveries of HCB from fortified milk samples (100 μg/kg) were 76 ± 3%. Recoveries of PCA from fortified blood samples (1 μg/kg level) were 98 ± 5%.

Dioxin Analysis. Samples of milk, blood, and feces (20 g) and fat (1–5 g) were cleaned up and analyzed by EC–GLC as previously described (Firestone, 1977), except that hexane extracts were chromatographed on a column of activated alumina (neutral, Brockman activity 1, 80–100

Table III. Dioxin Content of Cow Feces[a]

cow	dioxins, ppb						
	1,2,3,6,8,9-HCDD	1,2,3,6,7,8-HCDD	1,2,3,7,8,9-HCDD	1,2,3,4,6,7,9-HpCDD	1,2,3,4,6,7,8-HpCDD	OCDD	total dioxins
1	0.45	0.61	0.10	29.9	33.1	412	476
2	0.37	0.63	0.05	21.3	23.1	290	335
3	0.37	0.47	0.10	27.9	29.6	429	487

[a] Collected on day 28 of the 70-day treatment period; analysis by EC-GLC.

mesh, Fisher Scientific Co. No. A950) before chromatography on the Florisil column. The alumina column, prepared as described for the Florisil column, was eluted with 12 mL of 20% carbon tetrachloride in hexane (fraction 1, discarded), followed by 5 mL of methylene chloride (fraction 2; solvent evaporated and replaced with 0.5–1.0 mL of hexane for the Florisil chromatography). Each set of four–five samples included a control sample fortified with HCDD, HpCDD, and OCDD. Dioxin levels in the milk fat were calculated from whole-milk values and fat content of the milk. Recoveries of dioxins from milk fortified with 100 ng/kg of HCDD, HpCDD, and OCDD were 85 ± 6, 85 ± 7, and 72 ± 6%, respectively. Recoveries of dioxins from blood fortified with 100 ng/kg of HCDD, HpCDD, and OCDD were 80 ± 5, 79 ± 4, and 68 ± 5%, respectively.

Confirmation of PCP, HCB, and Dioxins by GLC–MS. The presence of PCP, HCB, PCA, and dioxins in selected treatment and posttreatment samples was confirmed by GLC–MS using a Finnigan 3300F quadrupole mass spectrometer interfaced to a Finnigan 9500 gas chromatograph through a steel capillary bore isolation valve. Negative ion chemical ionization was employed with methane reagent gas. GLC–MS conditions were as follows: glass column, 1 m long × 2 mm i.d., packed with 3% SP-2100 on 80–100 mesh Supelcoport; column, injector, separator, and transfer line temperatures, 220, 190, and 225 °C (PCA or HCB analysis) or 270, 260, and 250 °C (dioxin analysis), respectively. The mass spectrometer was scanned repetitively under computer control at 2-s intervals (from 200 to 300 amu, PCA or HCB analysis) or 1–3-s intervals (230–480 amu, dioxin analysis), starting 2 min after sample injection. With methane carrier gas pressure of 1 torr, filament emission of 0.5 ma at 140 eV, transfer line temperature of 250 °C, and source temperature of 140 °C, the negative ion spectra of HCB, dioxins, and furans were characterized by abundant molecular ions and some fragmentation due to loss of chlorine. In addition, an M – 19 fragment ion of low abundance was observed; the fragment resulted from addition of oxygen coupled with loss of chlorine from the molecular ion. The negative ion spectrum of PCA contained an abundant molecular ion as well as an abundant M – 19 fragment ion.

RESULTS AND DISCUSSION

Nature of the Residues. Low concentrations (1–2 ng/kg) of PCA were present in blood and composite milk samples from test cows during the 70-day treatment period, as were higher (nanograms/kilogram) levels of PCP. HCB, present in the technical PCP, was also found in the blood and composite milk from test cows during and after the treatment period.

The technical PCP used in the study contained varying levels of four HCDDs, two HpCDDs, and OCDD (Table II). However, only three dioxins, 1,2,3,6,7,8-HCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD, were found in the milk, blood, and tissue fat of treated cows. The presence of these dioxins in samples from treated cows was confirmed by GLC–MS, as was the presence of hexa-, hepta-, and octachlorofuran. No dioxins were detected in the milk, blood, or tissue fat from the control cow. The limits of detection in milk and blood samples (EC–GLC; 2.5% of full-scale deflection at 4× attenuation) were about 0.002, 0.004, and 0.008 ng/kg for HCDD, HpCDD, and OCDD, respectively. The limits of detection in fat samples were about 0.010, 0.020, and 0.040 ng/kg for HCDD, HpCDD, and OCDD, respectively. It is noteworthy that only biologically active dioxins with halogens at the four lateral ring positions (Poland and Glover, 1973) were detected in the samples. This suggests that dioxin congeners with low or zero biological activity (lateral ring positions incompletely filled with halogen atoms) are rapidly metabolized and/or excreted, whereas the biologically active congeners with halogen in all lateral ring positions are relatively stable after absorption by the animal.

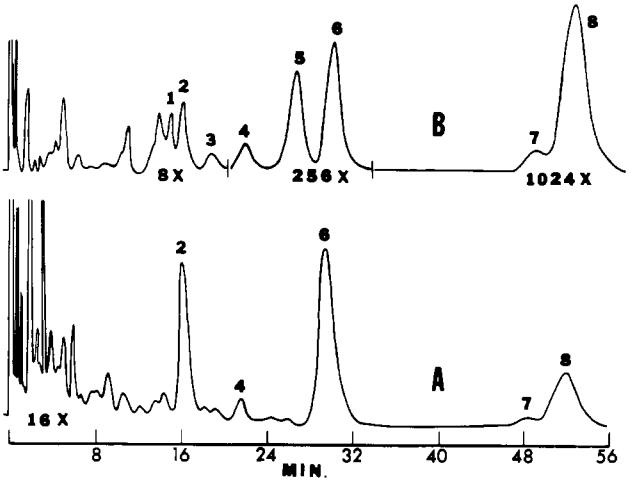

Figure 1. Gas chromatograms of extract (Florisil eluate II) from 1 g of (A) composite milk and (B) cow 3 feces, collected on day 28 of the 70-day treatment period: (1) 1,2,3,6,8,9-HCDD, (2) 1,2,3,6,7,8-HCDD, (3) 1,2,3,7,8,9-HCDD, (4) heptachlorofuran, (5) 1,2,3,4,6,7,9-HpCDD, (6) 1,2,3,4,6,7,8-HpCDD, (7) OCDF, and (8) OCDD (see text for GLC conditions).

Typical gas chromatograms of the dioxin extract from the feces and composite milk samples (treated cows) are shown in Figure 1. EC–GLC analysis, confirmed by GLC–MS, demonstrated that hexachlorofuran, heptachlorofuran, and octachlorofuran were present in the milk samples in addition to 1,2,3,6,7,8-HCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD, whereas 1,2,3,6,8,9-HCDD, 1,2,3,6,7,8-HCDD, 1,2,3,7,8,9-HCDD, 1,2,3,4,6,7,9-HpCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD were found in feces as well as hexa-, hepta-, and octachlorofuran. The dioxin content of feces collected from the three test cows on day 28 of the 70-day treatment period is shown in Table III. OCDD comprised 87% of the various dioxins present in the feces.

Residues in Treatment Period. Blood and composite milk samples obtained prior to dosing did not contain more than 0.05 mg/kg of PCP. This low, fairly constant level of PCP was also found in blood and milk samples from the control cow as well as in the feed (alfalfa hay, grain, grass).

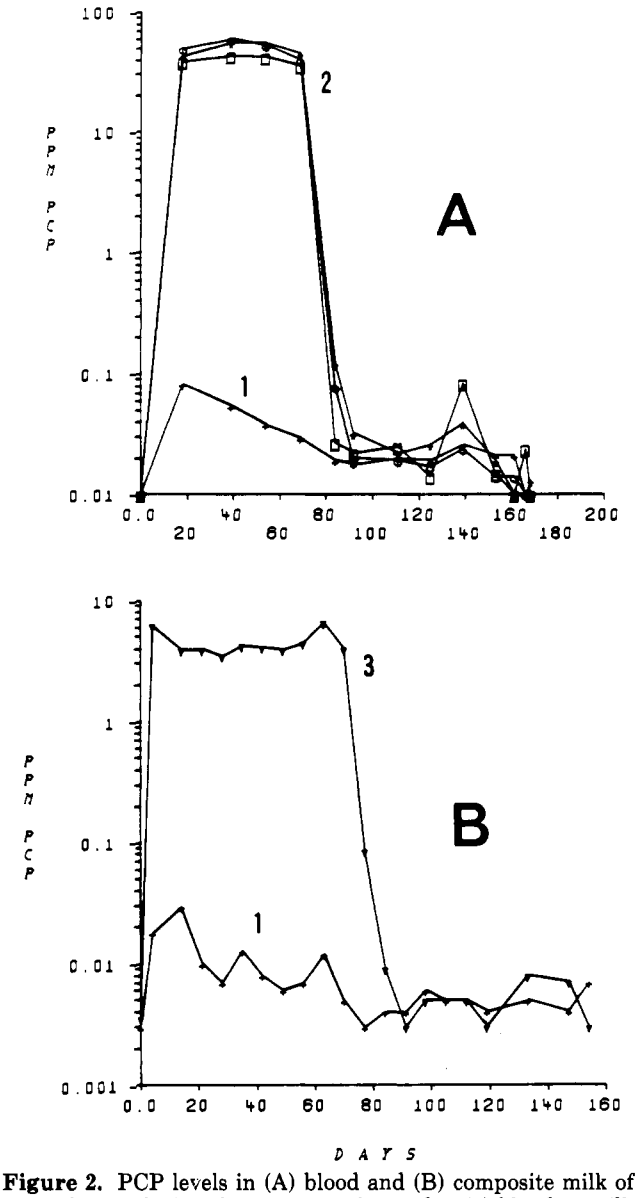

Figure 2. PCP levels in (A) blood and (B) composite milk of treated cows during the course of the study: (1) blood or milk from control cow, (2) blood from test cows, and (3) composite milk from test cows. Treatment ended on day 69. ppm = mg/kg = 1 part in 10^6 parts.

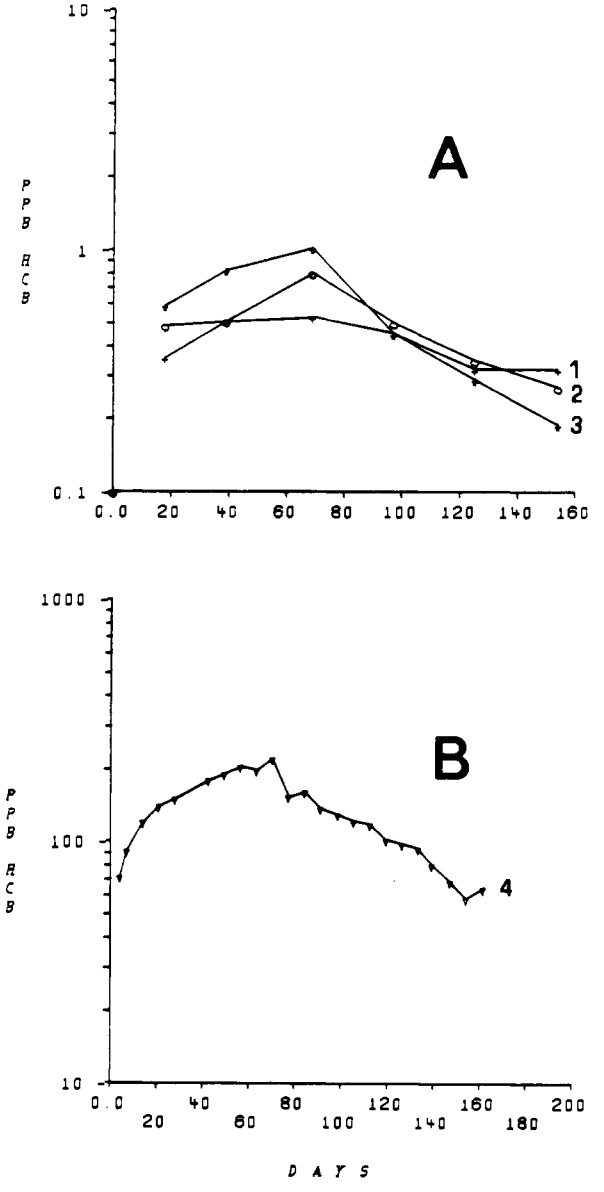

Figure 3. HCB levels in (A) blood and (B) composite milk fat of treated cows during the course of the study: (1) blood from cow 1, (2) blood from cow 2, (3) blood from cow 3, and (4) composite milk from test cows. Treatment ended on day 69. ppb = μg/kg = 1 part in 10^9 parts.

The levels of PCP detected in blood and milk during the course of the study are presented in Figure 2. PCP in the blood of treated cows rose rapidly to a steady-state level of about 40 mg/kg after dosing began, whereas PCP in the composite milk from treated cows rose to a steady-state level of about 4 mg/kg. Levels of PCA in the milk fat of treated cows were about 1–2 μg/kg. HCB levels in blood and composite milk fat of treated cows (Figure 3) rose to about 0.5–1.0 and 200 μg/kg, respectively.

Samples of urine, feces, and milk collected on day 28 of the 70-day treatment period contained 225, 5, and 4 mg/kg PCP, respectively, indicating that urine is the primary route for PCP excretion. Jakobson and Yellner (1971) found that 73–83% of PCP-^{14}C administered to mice was excreted in the urine in 4 days. Ahlborg et al. (1974) found about 80% of administered ^{14}C activity in the urine 40 h after intraperitoneal injection of PCP-^{14}C in rats and mice. When given orally, the radioactivity recovered from urine was about 40% in rats and 25% in mice. Braun and Sauerhoff (1976) observed that excretion by the kidneys was the main route of elimination of PCP in rhesus monkeys. The half-life value for excretion in the urine after oral administration was 92 h for females and 41 h for the males.

Average concentrations of 1,2,3,6,7,8-HCDD, 1,2,3,4,-6,7,8-HpCDD, and OCDD in the composite milk fat of treated cows rose to 20, 40, and 25 μg/kg, respectively (Figure 4). Concentrations of octachlorofuran in the composite milk fat rose to about 2 μg/kg. Although the fat content of successive fractions of the milk varied from about 1 to 5.5% during the course of the milking (average fat content, about 4%), the dioxin content in the milk fat from various fractions did not vary by more than 10%. Levels of dioxins in the milk or body fat of treated cows were about 1000 times those in the blood (Figure 5). Concentrations of dioxins in individual samples of body (shoulder) fat from the treated cows at day 69 averaged 13, 24, and 32 μg/kg, respectively, of 1,2,3,6,7,8-HCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD, not greatly different from levels found in the composite milk fat at day 69 (19, 39, and 24 μg/kg, respectively). Average daily excretion of the hexa-, hepta-, and octachlorodioxins in the milk

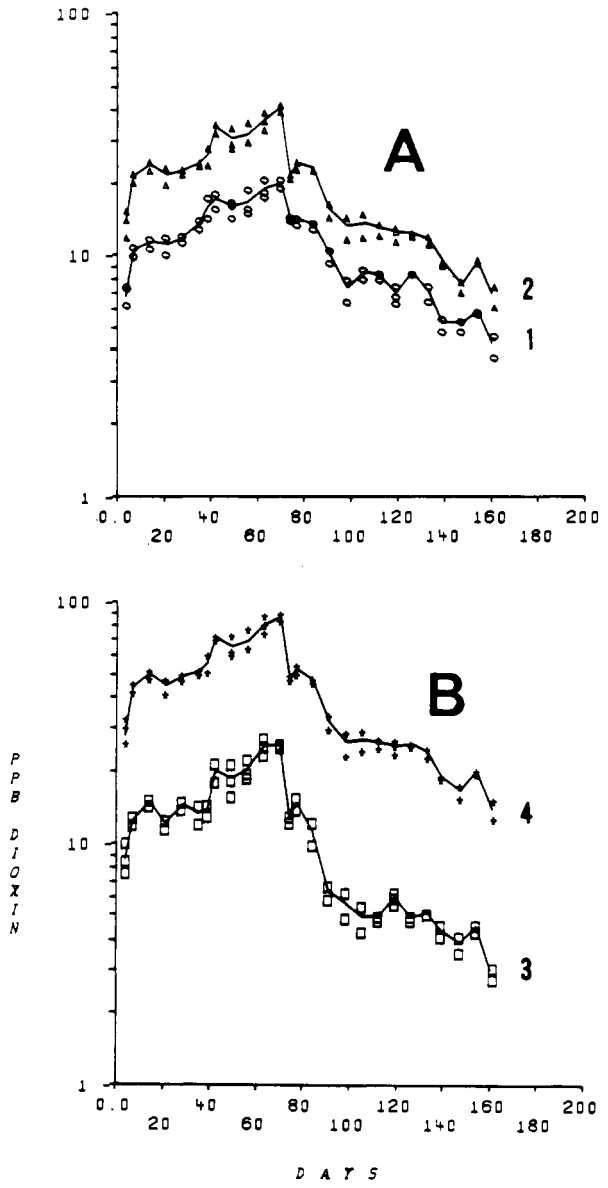

Figure 4. Hexa- and heptachlorodioxin levels (A) and octachloro and total dioxin levels (B) in composite milk fat of treated cows during the course of the study: (1) 1,2,3,6,7,8-hexachlorodioxin, (2) 1,2,3,4,6,7,8-heptachlorodioxin, (3) octachlorodioxin, and (4) total dioxins. Treatment ended on day 69. ppb = μg/kg = 1 part in 10^9 parts.

during days 40–70 was about 20, 40, and 23 μg, approximately 33, 3, and 0.6% of daily intake of the dioxins. The high levels of OCDD in the feces of treated cows (Table III) suggested that a large portion of ingested OCDD was not absorbed by the cow. This is in agreement with observations with Sprague-Dawley rats (Norback et al., 1975) that over 90% of the total dose of a radioactive analogue of OCDD administered to the rats was recovered in the feces as unabsorbed material.

Residue Elimination in Posttreatment Period. The levels of PCP in the milk and blood of treated cows declined within a few days to basal levels (0.02–0.08 μg/kg) when PCP feeding was stopped (Figure 2). After dosing was stopped, the low levels (1–2 μg/kg) of PCA in the composite milk fat of treated cows declined to below the limit of quantitation (<1 μg/kg), and levels of both HCB and dioxin in the composite milk fat and blood declined slowly. The decline in concentration of HCB (Figure 3) and dioxins (Figures 4 and 5) is described by first-order elimination rate constants and the following equation:

$$C = C_1 e - kt \quad (1)$$

$$\ln C = \ln C_1 - kt \quad (2)$$

where C is the concentration at any time, C_1 is the initial concentration, k is the rate constant, and t is the time in days. Statistical parameters describing the elimination of HCB and dioxins in composite milk fat and blood, determined by the method of least squares, are given in Table IV. The parameters presented in the table are for composite milk fat and for blood from cow 2. Slopes determined for OCDD in the blood of the other two test cows were not statistically significant because of the lack of data points for a sufficiently long period of time. Statistical parameters for HCB in blood were not determined because too few data points were available.

Data obtained from analysis of the composite milk fat were suggestive of an initial rapid decline in the levels of dioxins when PCP dosing was stopped, which could be described as the first component of a two-component first-order system as observed by Fries et al. for elimination of DDE (1969) and PCB (1973) from lactating cows. However, an insufficient number of data points were obtained in this study to verify the operation of a two-component system in the elimination of dioxins (and HCB). On the other hand, Piper et al. (1973), Fries and Marrow (1975), and Rose et al. (1976) observed that elimination of TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) in rats was adequately described by a single compartment model with first-order rates and half-lives varying between 12 and 31 days. The rates of decline of HCB and the three dioxins in the composite milk fat and blood from cow 2 (Table IV) did not vary too greatly (half-lives of 41–88 days), with the exception of OCDD in blood (cow 2, =274; correlation coefficient, 0.78). It was estimated from the rate equation that a period of 264 days would be required to reduce the concentration of HCB in the composite milk fat from 166 μg/kg at the end of the treatment period to 10 μg/kg. In addition, it was determined that 300, 316, and 238 days would be required to reduce the HCDD, HpCDD, and OCDD, respectively, from 15, 26, and 14 μg/kg at the end of the treatment period to 0.25 μg/kg (0.01 μg/kg in whole milk with 4% fat).

At 100 days after PCP feeding was stopped, average levels of dioxins in the shoulder fat of cows 1 and 3 were 2.5, 6.6, and 5.6 μg/kg, respectively, of 1,2,3,6,7,8-HCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD, compared to 4.3, 6.9, and 3.0 μg/kg of the dioxins in the composite milk fat. These results indicate that the dioxin content in the composite milk fat is similar to that in body fat. Cow 2 calved 151 days after PCP feeding was stopped, and the cow and calf were sacrificed 14 days later. No dioxins were found in the calf's blood at calving, whereas blood from the cow contained 0.01, 0.02, and 0.02 μg/kg, respectively, of hexa-, hepta-, and octachlorodioxin. Dioxin content in the blood and body fat of the cow and calf at sacrifice are shown in Table V. Dioxin levels in the milk fat are also recorded in the table and were approximately half of those in the cow's body fat. The relative concentrations of OCDD in the calf's blood and body fat (Table V) were considerably lower than in the cow's blood and body fat, apparently because the calf excreted more ingested OCDD than the other dioxins.

The resistance of HCB and dioxins to metabolic degradation and elimination is in marked contrast to the rapid excretion of PCP. The results reported here demonstrate that the absence of PCP in milk or biological tissue affords no guarantee of the absence of biologically active dioxins.

Van Gelder (1978) reported that there were no clinical effects with cattle fed 5 mg of PCP kg^{-1} day^{-1} for 14 days.

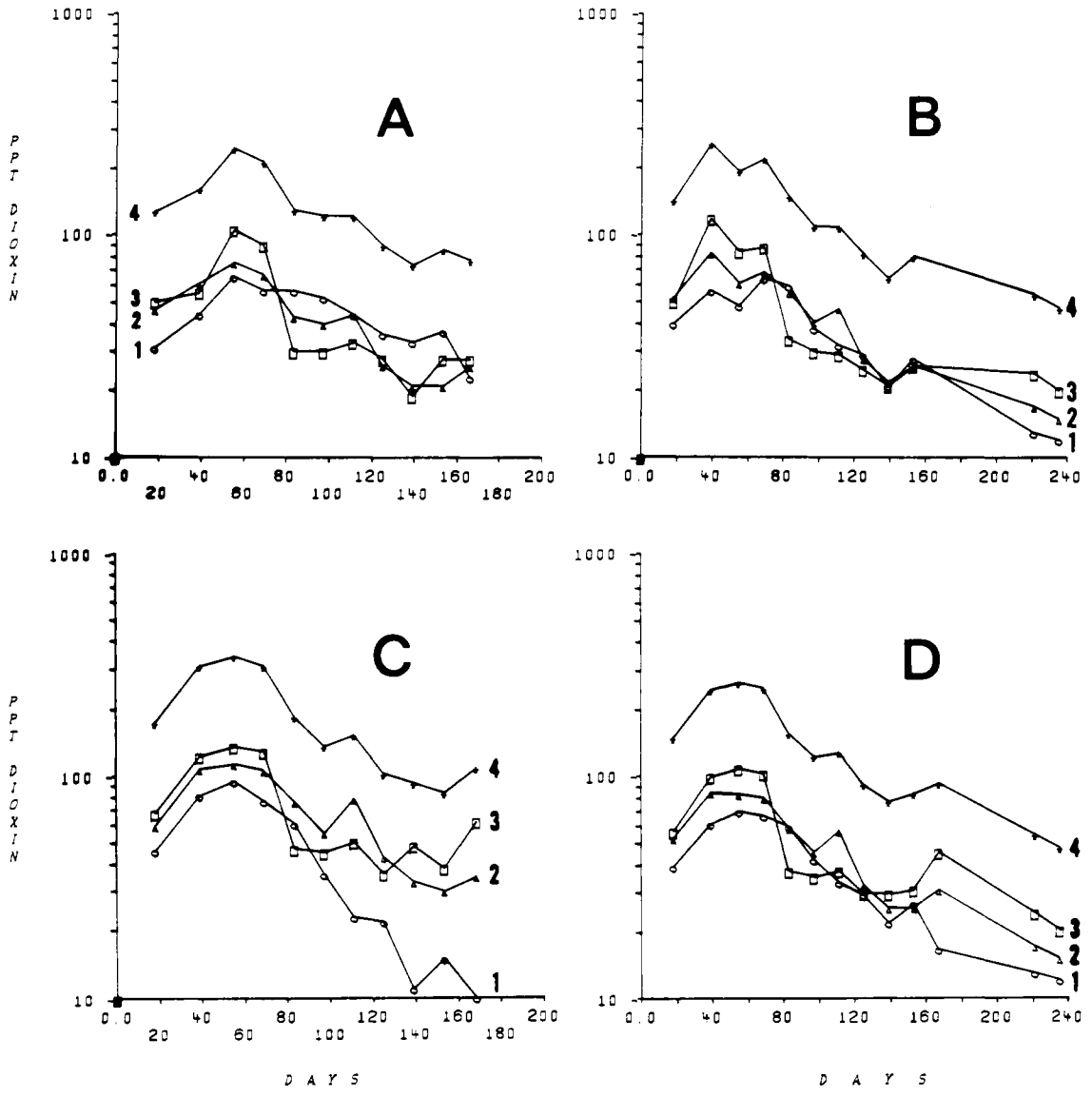

Figure 5. Dioxin levels in blood of treated cows during the course of the study. (A) Cow 1, (B) cow 2, (C) cow 3, and (D) average values from the three treated cows: (1) 1,2,3,6,7,8-hexachlorodioxin, (2) 1,2,3,4,6,7,8-heptachlorodioxin, (3) octachlorodioxin, and (4) total dioxins. Treatment ended on day 69. ppt = ng/kg = 1 part in 10^{12} parts.

Table IV. Least-Squares Statistical Parameters[a] for Elimination of HCB and Dioxins from Composite Milk Fat and Blood of Cow No. 2

	HCB	1,2,3,6,7,8-HCDD	1,2,3,4,6,7,8-HpCDD	OCDD
composite milk fat				
C_i (intercept, μg/kg	193.4	15.2	26.4	13.7
k (slope, day^{-1})	−0.0128	0.0137	0.0147	−0.0168
s_e (SE estimate)	0.0792	0.169	0.190	0.290
s_a (SD intercept)	0.0408	0.0554	0.0625	0.0953
s_b (SD slope)	0.00075	0.00106	0.00119	0.00182
r (correlation coefficient)	0.98	0.92	0.92	0.87
n (no. of data points)	14	31	31	31
τ (half-life, days)	54.1	50.6	47.1	41.3
blood (cow 2)				
C_i (intercept, ng/kg)		51.8	52.1	31.3
k (slope, day^{-1})		−0.00916	−0.00739	−0.00253
s_e (SE estimate)		0.173	0.191	0.121
s_a (SD intercept)		0.109	0.120	0.076
s_b (SD slope)		0.00117	0.00130	0.00082
r (correlation coefficient)		0.95	0.93	0.78
n (no. of data points)		8	8	8
τ (half-life, days)		75.7	87.8	274

[a] See Davis et al. (1978) for discussion of statistical parameters used to interpret least-squares results.

However, McConnell et al. (1979) found that technical grade PCP given in the diet to female yearling heifers for 160 days (20 mg kg^{-1} day^{-1} for 42 days followed by 15 mg kg^{-1} day^{-1} for 118 days) caused a significant clinicopa-

Table V. Dioxin Content in Blood, Body Fat, and Milk Fat of Cow 2 and Calf 165 Days after End of PCP Dosing[a]

tissue	dioxins, ppb 1,2,3,-6,7,8-HCDD	1,2,3,4,-6,7,8-HpCDD	OCDD	dioxins
blood				
cow	0.012	0.015	0.020	0.047
calf	0.027	0.014	0.006	0.047
body fat				
cow (shoulder)	4.8	11.1	6.1	22.0
calf (hind quarter)	2.3	1.9	0.5	4.7
milk fat (cow)	2.2	4.4	3.3	9.9

[a] Samples collected 14 days after calving (165 days after end of PCP dosing) at which time the animals were sacrificed.

thologic syndrome suggestive of dioxin and/or furan intoxication.

No clinical evidence of toxicosis associated with PCP administration was observed during the course of the study. Clinical findings will be discussed in a separate report.

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Identification of the Main Metabolite of Ethylenethiourea in Mice

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The degradation of 2-imidazolidinethione (ethylenethiourea) in mice involves oxidation at the sulfur atom, giving 2-imidazolin-2-yl sulfenate as the major product. This compound was synthesized by irradiation of an aqueous solution of ethylenethiourea with Co-60 γ rays and purified from the irradiation mixture by high-pressure liquid chromatography. The synthesis was verified by TLC, IR, NMR, and mass spectrometry methods. Other possible mechanisms for degradation are discussed.

The ethylenebis(dithiocarbamates), including Maneb, Zineb, Mangozeb, Nabam, and Amobam, constitute an important class of fungicides widely used for controlling crop diseases. Ethylenethiourea (ETU), which is a contaminant in these fungicides (Bontoyan and Looker, 1973) and a primary metabolite in environmental degradation and in test organisms (Engst and Schnaak, 1974; Watts et al., 1974; Marshall, 1977) has been reported to be goitrogenic (Seifter and Ehrich, 1948; Graham and Hansen, 1972; Graham et al., 1973, 1975), carcinogenic (Innes et al.,

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