Metabolic Fate of [U-^{14}C]Pentachlorophenol in a Lactating Dairy Cow

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A lactating Holstein-Friesen dairy cow was administered a single oral dose of [U-^{14}C]pentachlorophenol (PCP). To better simulate chronic exposure conditions on a typical dairy farm, the cow was fed 0.2 mg/kg body wt/day technical PCP for 95 days prior to [U-^{14}C]PCP administration and for the 4 days postadministration. Both absorption and elimination of PCP obeyed first-order kinetics and had half-lives of 4.3 and 43 h, respectively. Over the 76-h postadministration, 75% of the radiolabel was eliminated in urine, 5% in milk, and 5% in feces. The radiolabel was distributed widely into various tissues, but the highest levels were in liver, kidneys, and lungs. Skeletal muscle and adipose tissue contained among the lowest levels. In milk, the fat fraction contained the greatest amount of the radiolabel but the whey fraction represented the largest pool (62.2%). In whey and casein, both conjugated and unconjugated forms were quantified. There was no evidence of phase 1 metabolism of PCP, whereas phase 2 metabolism (conjugation) was highly evident. In urine, two-thirds of the PCP was present in a conjugated form, whereas in serum about 80% was present as unconjugated PCP. These data suggest that efficient conjugation and elimination of PCP prevent its accumulation in cattle tissues.

Pentachlorophenol (PCP) and its sodium salt (Na PCP) have in the past been extensively used as wood preservatives. Total U.S. production in 1980 was approximately 46.8 million pounds (U.S. International Trade Commission, 1981). Most of the manufactured PCP is used in the preservation of wood (76%) or wood products (6%) such as plywood and fiber board (Cirelli, 1978). Livestock facilities are often constructed out of wood treated with technical PCP (penta) which is a mixture of 85–90% PCP and several other chemicals including various chlorodibenzodioxins (CDD) (Firestone et al., 1979; McConnell et al., 1980; Kinzell et al., 1981). Approximately 50% of Michigan Grade A dairy farms have used penta-treated wood in construction of various components of their livestock facilities (Shull et al., 1981). Exposure of dairy cattle to treated wood on one farm resulted in residues of both PCP and CDD in liver and adipose tissue (Hass et al., 1978). Because of concern that extensive usage of penta-treated wood on cattle farms may result in hazardous residues in products consumed by humans several studies have been conducted in cattle (Firestone et al., 1979; Parker et al., 1980) but none have focused on the metabolic fate of PCP.

The pharmacokinetics of PCP has been investigated quite extensively in various laboratory species such as the mouse (Jakobson and Yllner, 1971), rat (Ahlborg et al., 1974; Braun et al., 1977), and monkey (Braun and Sauerhoff, 1976). Species differences have been observed, notably in metabolism of PCP. In the rat tetrachlorohydroquinone was a major metabolite in urine (Ahlborg et al., 1974) but was not detected in monkey urine (Braun et al., 1976).

The purpose of the present study was to determine the metabolic fate of a single dose of [U-^{14}C]PCP in a lactating dairy cow. The data include kinetics of absorption and elimination, distribution into tissues and milk, and partial characterization of metabolism.

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MATERIALS AND METHODS

Labeled and Unlabeled Pentachlorophenol. Uniformly ring-labeled [^{14}C]PCP with a specific activity of 5.0 mCi/mmol was purchased from New England Nuclear, Labelled Chemical Division (Boston, Mass.). Purity as determined by thin-layer chromatography (TLC) and autoradiography was approximately 96%. A 10 mg/mL stock solution of [^{14}C]PCP in methanol was diluted to 10%, 1%, and 0.1% in methanol and 1-μL aliquots of each solution were spotted on the plates. The TLC system was comprised of 20 × 20 cm silica gel GF plates (Fisher Scientific, Pittsburgh, PA) and a solvent system composed of toluene–acetone–ammonium hydroxide (50:40:1, v/v/v) developed to a distance of 18 cm. Autoradiographic analysis was performed with 20 × 25 cm sheets of X-ray film (X-Omat, R-Film, XR-5, Eastman Kodak, Rochester, NY) which was exposed to the developed TLC plates for 108 h followed by development and fixation of the film for visualization.

The [^{14}C]PCP was purified with an HPLC system (Water's Associates, Inc.) equipped with a C_{18} μ-Bondapack column (3.9 mm × 30 cm) and a UV absorbance detector. The operating conditions were as follows: solvent, methanol–water (72:28, v/v, containing 0.5 M acetic acid); flow rate, 1 mL/min. The fraction corresponding to an analytical reference standard of unlabeled PCP (>99% purity) was collected and the purity of the combined fractions was found to be greater than 99% with the TLC–autoradiographic system described above.

The unlabeled technical PCP was a composite (MB-528 American Wood Preserves Institute) of commercial preparation from three chemical companies. The chlorophenol and CDD content of this composite which was reported previously (Kinzell et al., 1981) is pentachlorophenol (85–90%), tetrachlorophenols (4–8%), trichlorophenols (0.1%), other chlorophenols (2–6%), octa-CDD (1000 ppm), hepta-CDDs (378 ppm), hexa-CDDs (173 ppm), and tetra-CDDs (0.035 ppm).

Experimental Cow. A 400-kg Holstein-Friesen cow in early lactation was confined in a tie stall with water available ad libitum and fed a diet of alfalfa haylage–high moisture corn (60:40, w/w) supplemented with a 38% protein concentrate. Technical PCP at a dosage of 0.2 mg/kg body wt/day was mixed with the concentrate and fed for 99 days. This dosing regime was selected to simulate environmental exposure of dairy cattle housed in

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facilities constructed of PCP containing lumber. Daily doses were split and fed in conjunction with morning and evening feedings. The cow was fed and milked by automatic milker at 0700 and 1900 h. Three days (day 92 of experiment) prior to the administration of a single dose of [^{14}C]PCP, the cow was placed in a metabolism stall and fitted with an indwelling urethral catheter for total urine collection and a jugular cannula for serial collection of blood. On day 95, 5.4 mL of methanol containing 815 μCi (2 μCi/kg body wt.) of [^{14}C]PCP was mixed with α cellulose and placed in a gelatin capsule. At 1900 h, the capsule was administered per os using a balling gun. The same feeding and dosing (with 0.2 mg/kg of body weight/day of technical PCP) regime was continued after administration of the [^{14}C]PCP dose for 76 h at which time the cow was necropsied. No attempt was made to account for ^{14}C in expired air because published reports indicated <0.05% was eliminated by this route in rodents (Jakobson and Yllner, 1971; Larsen et al., 1972).

Samples. Blood and representative samples of urine, milk, and feces collected a few hours prior to administration of [^{14}C]PCP served as zero time samples. After administration of the radiolabeled PCP, accumulated urine and feces were removed and quantitated at 4 h intervals. The cow was completely milked-out every 4 h with an automatic milking machine. Blood was collected every 30 min during the first 10 h, every 1 h for 10–16 h, every 2 h from 16 to 24 h, and every 4 h from 24 to 76 h. Serum was obtained by centrifugation (4250g for 15 min) and frozen. Representative samples of all the collected samples were stored frozen (−20 °C) in tightly sealed containers. The experiment was terminated 76 h after administration of [^{14}C]PCP. The cow was stunned by a captive bolt pistol followed by exanguination. Organs were trimmed of excess fat, weighed, and samples frozen.

Gas Chromatographic (GC) Analysis of PCP in Biological Fluids and Tissues. Pentachlorophenol concentrations in selected fluids and tissues were quantified as total PCP (i.e., the combined fraction of conjugated and unconjugated PCP). All extractions were carried out in 16 × 125 mm culture tubes sealed with Teflon-lined screw-caps on a Fisher Rotorack (Fisher Scientific Co., Pittsburg, PA) operating at 70 rpm. All centrifugations were conducted in an IEC centrifuge (International Equipment Co., Needham Hghts, MA) equipped with a swinging bucket rotor, at 4250g for 15 min.

One milliliter of serum was combined with 0.22 mL of concentrated H_2SO_4 and mixed (Vortex-Genie, Scientific Industries, Bohemia, NY) for 5 s. For urine, 0.1 mL of 4 N H_2SO_4 was added to 1 mL of urine. The mixtures were heated in a water bath (85 °C) for 3 h. After cooling to room temperature, 1 mL of 30% saline and 3 mL of benzene (containing 0.5 μg of lindane/mL of benzene) were added and the mixtures extracted for 2 h. The lindane served as an internal standard. After setting at room temperature for at least 8 h the mixtures were centrifuged and a subsample of the benzene phase was analyzed by GC for PCP.

Frozen milk was thawed slowly at room temperature and tissues were thawed in a water bath (25 °C). Tissues were minced and 2-g subsamples were homogenized (Willems Polytron homogenizer, Lucerne, Switzerland) in double-distilled deionized (dd) H_2O (3:1, tissue to dd H_2O, w/v). Replicate aliquots of either milk or tissue homogenates were then extracted as follows: 1 mL of samples were combined with 1 mL of 30% saline, 0.3 mL of 1 N NaOH, and 10 mL of hexane. This mixture was extracted for 30 min and the hexane layer discarded. This process was repeated two additional times. After the final hexane extraction, 1-mL aliquots of the aqueous phase were acid hydrolyzed, extracted, and GC analyzed for PCP by using the same procedure described above for serum.

Extraction efficiencies of PCP from tissue homogenates and fluids were determined in samples from untreated animals and spiked to various levels with PCP in benzene. Spiking levels of PCP in benzene were 150, 100, 50, 10, 5, 1, 0.5, 0.3, 0.1, and 0 ppm for the fluids and the same levels below 50 ppm, plus 20 ppm, for the tissues. A 10-g sample of tissue or 10-mL sample of fluid was spiked to the given level and was then subsampled (1-g or 1-mL subsamples) six times. These subsamples were then acid hydrolyzed, extracted, and analyzed by the same procedure as the experimental samples. The recovery percentages over the range of spiking levels were smoothed by fitting to a low degree (1–3) polynomial, which was subsequently used to correct the experimental data for recovery.

Benzene extracts (3 mL) from various tissues and fluids were analyzed for PCP by a Varian 3700 GC equipped with a ^{63}Ni electron capture detector. The column was coiled glass (6 ft × 2 mm i.d.) packed with 1% SP-1240DA on 100/120 Supelcoport (Supelco Inc., Bellefonte, PA). Operating temperatures were 200 (injector), 160 (column), and 350 °C (detector). Carrier gas was N_2 (99% pure) at a flow rate of 40 mL/min. GC electrometer range and recorder (Houston Omniscribe strip chart recorder) attenuation settings were 10 and 16, respectively. injection volume was 0.5 μL. Quantitation of peak areas was accomplished with a Varian CDS 111 microprocessor. Lindane (0.5 μg/mL of benzene) served as an internal standard.

Fractionation, Extraction, and Analysis of Whole Milk. Frozen milk was thawed in a water bath (37 °C) and homogenized with the Polytron homogenizer operated at a speed setting of 3. Aliquots (40 mL) were then centrifuged at 4000g for 2 h at 4 °C. The fat pad was carefully removed, blotted dry, and weighed. Replicate 0.1-g samples of the fat were dissolved in 5 mL of aqueous counting scintillant (ACS; Amersham, Chicago, IL) and quantitated for ^{14}C activity. The volume of the whey fraction was recorded; 0.4 mL was added to 5 mL of ACS and quantitated for ^{14}C activity. The surface of the casein pellet was carefully rinsed (twice) with 10 mL of dd H_2O. Replicate 0.4-mL aliquots of these rinses were counted in 5 mL of ACS. The casein pellet was resuspended in 30 mL of dd H_2O with the Polytron and the homogenate centrifuged (4000g for 1 h). Replicate 0.4-mL aliquots of the supernatant were counted and the remainder discarded. The pellet was placed in a preweighed container, the weight recorded, a 1:4 homogenate (casein–dd H_2O, w/v) prepared, and 0.2 mL added with 5 mL of ACS and quantitated for ^{14}C activity.

Whey and casein were also analyzed for both total and unconjugated PCP. One milliliter of a 1:4 homogenate (casein–dd H_2O, w/v) of casein and 1 mL of whey were analyzed by GC for total PCP by using the same procedure as described for serum. For extraction of unconjugated PCP, 0.22 mL of 4 N H_2SO_4, 1 mL of 30% saline, and 3 mL of benzene were combined with 1 mL of replicate samples of whey of casein homogenate. These mixtures were blended (Vortex-Genie) for 5 s and then let stand for 15 min; this process was repeated two additional times. After centrifugation, subsamples of the benzene phase were analyzed by GC for PCP. Concentrations of conjugated PCP were determined by difference, i.e., by subtracting the amount of unconjugated PCP from the amount of total PCP. ^{14}C activity was also measured in the same benzene extracts that were prepared for GC analysis. Replicate

Table I. Recovery of ^{14}C in Urine and Serum after Sample Cleanup Prior to HPLC Separation

	urine		serum	
	52-h sample	76-h sample	24-h sample	48-h sample
amount of ^{14}C introduced onto the column,[a] dpm	23964 ± 871[b]	17275 ± 476	112950 ± 261	65269 ± 502
amount of ^{14}C eluted,[c] dpm	23631 ± 843	16610 ± 311	100808 ± 2870	62760 ± 1803
overall recovery, %	98.6	96.2	89.3	96.2

[a] Before Sephadex G-10 cleanup. [b] Mean (±SE). [c] Methanol (eluting solvent) was evaporated and the residue solubilized in 1% acetic acid.

0.2-mL aliquots of benzene in 5 mL of ACS were counted by liquid scintillation counting. Extraction efficiencies were determined in samples of casein homogenate and whey spiked with 7500 dpm [^{14}C]PCP. Recoveries were 97.6 ± 1.4% (SE) for the 1:4 casein homogenate and 100.4 ± 1.1% (SE) for the whey; these values did not differ significantly for the two extraction procedures (total PCP and unconjugated PCP).

Cleanup and HPLC Separation of ^{14}C Fractions in Serum and Urine. Urine (1 mL) was acidified with 100 μL of HPLC grade glacial acetic acid. Serum (1 mL) was diluted with 2 mL of dd H_2O and 1 mL of glacial acetic acid. Of these mixtures, 100 μL in 5.0 mL of ACS was quantitated for ^{14}C activity. Another aliquot (1.0 mL) of each mixture was placed onto a 5 mm × 8 cm long glass column packed with Sephadex G-10 (Pharmacia Fine Chemicals, Uppsala, Sweden). This packing was preconditioned in dd H_2O containing 1% glacial acetic acid. The first elution was with 15 mL of dd H_2O containing 1% glacial acetic acid. Radiolabeled fractions were eluted with 10 mL of methanol containing 1% glacial acetic acid. An aliquot (100 μL) of the methanol eluate was quantitated for ^{14}C activity which provided an evaluation of recovery (Table I). The remainder of the methanol eluate was placed under a stream of N_2 gas to remove most of the methanol. The aqueous residue was made up to 4 mL of dd H_2O containing 1% glacial acetic acid. One-milliliter aliquots of these mixtures were then injected onto an HPLC (Waters Associates, Inc.) equipped with a Model U6K injector, Model 6000A pumps, a Waters Data Module, a Waters System controller, a Model 440 absorbance detector (254 λ), and a Bio-Sil ODS-18 reversed-phase column (4 mm × 250 mm, Bio-Rad Laboratories). The solvents used were dd H_2O containing 1% HPLC grade glacial acetic acid (J. T. Baker, Phillipsburgh, NJ) (solvent A) and spectro grade glass-distilled acetonitrile (Burdick and Jackson, Muskegon, MI) containing 1% HPLC grade glacial acetic acid (solvent B). The following gradient was run: 0–5 min isocratic with solvent A, 5–30 min with a linear gradient to 100% solvent B, and 30–55 min isocratic with 100% solvent B. Column effluent was collected at 1.0-min intervals with a fraction collector (ISCO, Model 328) equipped with a flow interrupter valve (ISCO, Model 590). ACS (5 mL) was added with 100 μL of each fraction and ^{14}C activity was quantitated.

To determine whether PCP was metabolized, the retention time (R_t) of the ^{14}C fractions in urine and serum were compared to a number of possible metabolites including di-, tri-, and tetrachlorophenols, tetrachlorohydroquinone, and pentachloroanisole. Analytical standards of these chemicals (RFR Corp., Hope, RI) were dissolved in acetonitrile containing 1% glacial acetic acid. One milliliter was injected onto the HPLC which was operated according to the same conditions described above. Figure 1 shows a typical chromatogram. Congeners within each of three groups of chlorophenols (di-, tri-, and tetrachlorophenols) were found to coelute, (e.g., 2,3,4,5-tetrachlorophenol has the same R_t as 2,3,4,6-tetrachlorophenol). To determine whether PCP or possibly its metabolites were conjugated, samples of urine and serum were subjected to acid hydrolysis according to the method described above. The same procedure for cleanup and HPLC separation was used following acid hydrolysis.

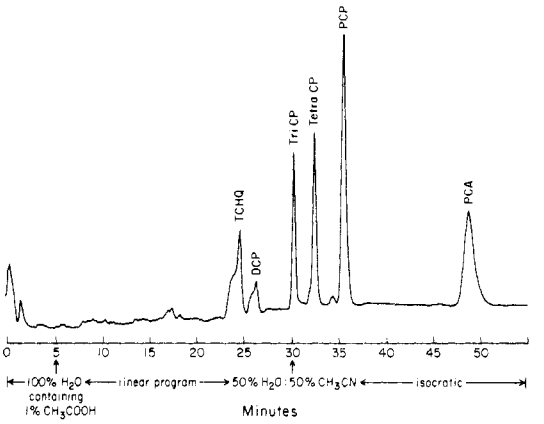

Figure 1. HPLC profile of various chlorophenols, tetrachlorohydroquinone (TCHQ), and pentachloroanisole (PCA). Individual isomers of dichlorophenols (DCP), trichlorophenols (TriCP), or tetrachlorophenols (TetraCP) were not separable under the HPLC conditions utilized.

Quantitation of ^{14}C Activity. Radioactivity was measured with a Isocap 300 liquid scintillation counter (LSC) (Tracor Analytic, Chicago, IL) by using sample channel ratios (SCR) to correct for sample quench. For quantitation of total ^{14}C activity in fluids including serum, urine, milk, lymph, and bile, 0.1 mL was combined with 0.3 mL of dd H_2O and 5 mL of ACS and counted. Aqueous homogenates of feces (1:3, feces–dd H_2O, w/v) were prepared with the polytron and subjected to the same extraction procedure used for total PCP analysis in milk. Aliquots (0.2 mL) of benzene extract were added with 5 mL of ACS and counted. Recovery percentages were experimentally determined by using [^{14}C]PCP spiked into nonradioactive feces. Dry weights of fecal samples were determined by drying in a convection oven (60 °C) for 2 days. For analysis of tissues, pipettable aqueous homogenates were prepared with the Polytron. Measured volumes were combusted for 4 min in a Biological Materials Oxidizer (Harvey Co.). The CO_2 was trapped in 2 mL of a solution of 5 M ethanolamine in 2-methoxyethanol to which was added 5 mL of liquid scintillant (permafluor V–Carbsorb II, 2:1 v/v Packard Chemicals) and the mixture was quantitated for radioactivity by LSC.

RESULTS

The maximum concentration of ^{14}C activity in serum occurred at about 10 h after the cow was administered a single oral dose of [^{14}C]PCP (Figure 2). First-order kinetics for both elimination and absorption is indicated by the semilogarithmic plots of the data presented in Figures 2 and 3, respectively. The extrapolated line shown in Figure 3 which was derived by using the curve-stripping method of Gehring et al. (1976) allows better visualization of absorption kinetics. The rate constants and half-life values ($t_{1/2}$) of absorption (k_a) and elimination (k_e) calculated from the straight lines are $k_a = 0.162\ h^{-1}$ and $t_{1/2}$ =

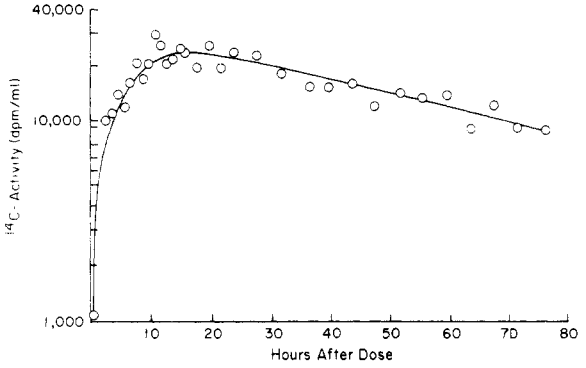

Figure 2. ^{14}C activity in serum after administration of a single dose of [U-^{14}C]PCP.

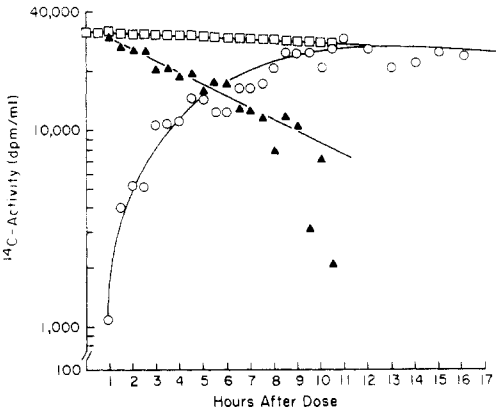

Figure 3. Application of "curve stripping" to serum data for the purpose of estimating the absorption rate constant (k_a) and absorption half-life ($t_{1/2}$).

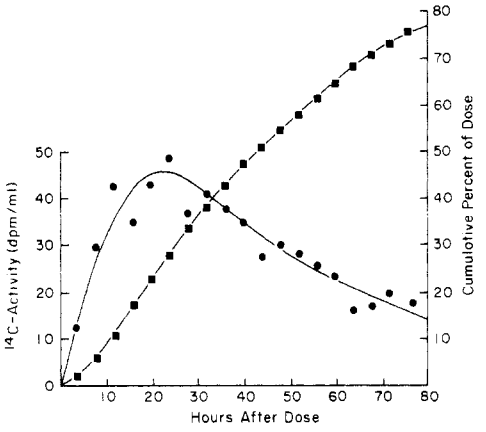

Figure 4. ^{14}C activity in urine and cumulative percentage excreted into urine measured at 4-h intervals after administration of a single dose of [U-^{14}C]PCP.

4.3 h and k_e = 0.016 h^{-1} and $t_{1/2}$ = 42.8 h.

As shown in Figure 3, the primary route of elimination of the ^{14}C was the urine. Approximately 75% of the dose was excreted into the urine during the 76-h period. Maximum ^{14}C activity in urine was observed at about 24 h. The k_e and $t_{1/2}$ calculated from the descending portion of the semilogarithmic curve was 0.017 h^{-1} and 40.33 h, respectively.

Compared to urine, feces was a minor route of elimination, accounting for only 5% of the ^{14}C dose (Figure 4). The greatest activity in feces was observed at about 36 h.

Milk also represented a minor excretory route for PCP in the cow with about 5% of the administered ^{14}C activity accounted for in milk over 76 h (Figure 5). Maximum ^{14}C activity was achieved at about 16–20 h and remained

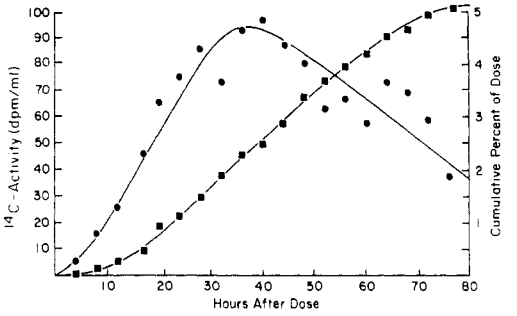

Figure 5. ^{14}C activity in feces and cumulative percentage excreted into feces measured at 4-h intervals after administration of a single dose of [U-^{14}C]PCP.

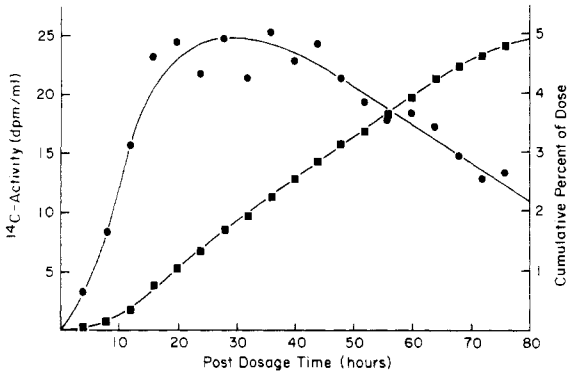

Figure 6. ^{14}C activity in milk and cumulative percentage secreted into milk measured at 4-h intervals after administration of a single dose of [U-^{14}C]PCP.

Table II. ^{14}C Activity and Total PCP Concentration in Various Fluids Collected at Necropsy[a]

fluid	^{14}C activity, dpm/mL	[PCP], ppb
urine	7635	7047 ± 286[b]
serum	9240	6302 ± 132[c]
lymph	8873	*d*
duodenal contents	8755	*d*
bile	5756	*d*
milk	1369	1035 ± 30[b]
spinal fluid	256	*d*
aqueous humor	*e*	*d*

[a] The cow was fed 0.2 mg/kg body wt/day of technical PCP for 99 days. A single dose of 815 μCi [U-^{14}C]PCP was administered on day 95, and the cow was killed on day 99. [b] Mean (±SE) concentrations in the last 7 samples collected at 4 h intervals from 52 to 76 h after administration of [^{14}C]PCP. [c] Mean (±SE) of serum collected at necropsy; triplicate analyses. [d] Not analyzed. [e] None detected.

relatively constant (between 2.0 and 2.5 × 10^3 dpm/mL) for about 24 h. The subsequent elimination phase had a k_e of 0.016 h^{-1} and $t_{1/2}$ of 42.5 h.

The distribution of ^{14}C activity within the three fractions of milk, i.e., casein, whey, and fat, is shown in Figure 6. The whey fraction contained the greatest amount of ^{14}C activity (60.3 ± 1.0%, SE) followed by casein (26.6 ± 0.7) and fat (13.2 ± 0.7). These proportions were relatively constant over the 76-h period. However, the fat and casein fractions were similar in concentration and both were substantially greater than the whey fraction. During the 16–40-h time period when ^{14}C activity in milk was maximum, mean (±SE) radioactivity was fat 6336 ± 715 dpm/g, casein 6528 ± 588 dpm/g, and whey 1402 ± 64 dpm/mL. In 40 mL of whole milk, the amount of each fraction was fat 1.67 ± 0.08g, casein, 4.30 ± 0.13 g, and whey 32.96 ± 0.11 mL.

^{14}C activities measured in various fluids and tissues collected at necropsy are given in Tables II and III, re-

Table III. ^{14}C Activity and Total PCP Concentrations in Various Tissues Collected at Necropsy[a]

tissue	^{14}C activity dpm/g (wet wt)	^{14}C activity % of dose	[PCP], ppb
liver	7964	5.61	1624
kidney	4941	0.35	1792
gall bladder	4047		*b*
lung	3562	0.87	981
adrenals	2878	0.0006	923
mammary gland	2753		*b*
lymph node	2499		942
ovary	1693		*b*
pancreas	1550		*b*
heart muscle	1000	0.13	*b*
spleen	964	0.04	*b*
round muscle	844		409
adipose tissue	343		*b*
brain	343		*b*
spinal cord	210		*b*
thyroid	*b*		1103

[a] The cow was fed 0.2 mg/kg body wt technical PCP for 99 days. A single dose of 815 μCi [U-^{14}C]PCP was administered on day 95, and the cow was killed on day 99. [b] Not analyzed.

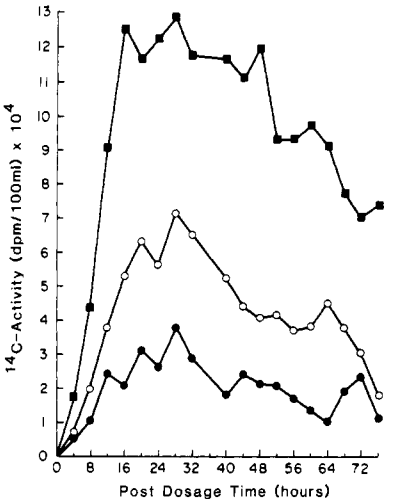

Figure 7. Distribution of ^{14}C activity in whey (■), casein (○), and fat (●) fractions of milk collected at 4-h intervals after administration of a single dose of [U-^{14}C]PCP.

spectively. Of the fluids analyzed, serum and lymph had comparable levels of ^{14}C activity, and both contained greater levels than other fluids. Of the tissues and organs analyzed, liver contained the highest level of ^{14}C activity. Skeletal muscle, adipose tissue, and brain all contained relatively low levels of ^{14}C activity. As shown in Table III, the liver accounted for 5.61% of the administered ^{14}C dose, whereas the other organs were less than 1% in comparison. Concentrations of total PCP (i.e., extractable into benzene following acid hydrolysis under heat) in the various tissues and organs are also tabulated in Table III. These represent steady-state concentrations because the cow was administered technical PCP twice daily for 99 days prior to necropsy. Therefore, while both ^{14}C activity and GC analyzed PCP levels are given for most tissues, the two values cannot be compared directly.

PCP does not appear to be metabolized to lower chlorinated forms (e.g, di-, tri-, or tetrachlorophenols), to tetrachlorohydroquinone, or to pentachloroanisole. Figure 7 part a shows a typical ^{14}C distribution of unhydrolyzed urine into 2 fractions. The first fraction (peak 1) was not present in urine subjected to acid hydrolysis (Figure 7 part b, Table III). The second fraction (peak 2) had the same R_t (35–40 min) as PCP (Figure 1). The exact nature of the PCP conjugate was not determined.

The relative proportions of conjugated (peak 1) and unconjugated PCP (peak 2) at various time intervals are shown in Table IV. As indicated, 83.5% of the ^{14}C activity excreted during the first 4-h period following administration of the [^{14}C]PCP dose was unconjugated PCP. Thereafter, about two-thirds of the ^{14}C activity in urine was associated with the conjugated fraction. In contrast, about 80% of the ^{14}C activity in serum was unconjugated PCP.

The distribution of unconjugated and conjugated fractions (both PCP and ^{14}C activity) in whey and casein is given in Table V. The conjugated fraction was determined indirectly by substracting the unconjugated concentration from the combined conjugated and unconjugated concentrations, both of which were quantitated directly for ^{14}C activity and PCP. The ratios of conjugated and unconjugated forms analyzed for ^{14}C activity and PCP are similar. In whey, a slightly greater percentage was present in the unconjugated form whereas in casein approximately two-thirds of both ^{14}C and PCP were present as the conjugated form.

DISCUSSION

The primary objective of this experiment was to determine the toxicokinetics and metabolism of PCP in a lactating dairy cow under exposure conditions approximating those of typical dairy farms. On modern dairy farms, cattle can ingest PCP directly by licking treated wood such as free-stalls, feed bunks, or splash boards, or indirectly by ingesting contaminated feed that was stored in a treated facility such a bunk silo. To simulate this exposure, the experimental cow was fed 0.2 mg/kg body wt/day of technical PCP for 95 days prior to administration of a single dose of [^{14}C]PCP and also for the 4 days

Table IV. HPLC Profile of ^{14}C in Urine and Serum

sample no., h	unhydrolyzed[a] pre-HPLC ^{14}C, dpm	peak 1,[c] dpm	%	peak 2,[d] dpm	%	acid hydrolyzed[b] pre-HPLC ^{14}C, dpm	peak 1,[c] dpm	%	peak 2,[d] dpm	%
				Urine						
4	20376	3561	17.5[e]	17009	83.5[e]	21692	642	3.0	20438	94.2
28	81038	53250	65.7	27476	33.9	66182	1114	1.7	65858	99.5
52	60646	43707	72.1	18757	30.9	54710	*f*	0	54331	99.3
76	42189	26627	63.1	16041	38.0	36918	*f*	0	36817	99.7
				Serum						
24	23712	1666	7.0	19418	81.9	23945	†	0	23488	99.1
48	16621	1731	10.4	13749	82.7	16150	†	0	14462	89.5
72	9407	802	8.5	7451	79.2	10271	*f*	0	9524	92.7

[a] Samples were acidified and extracted into solvent. [b] Samples were acidified, heated at 85°C for 3 h in a water bath, and then extracted into solvent. [c] Peak 1 corresponds to conjugated products. [d] Peak 2 corresponds to unconjugated PCP. [e] Calculated as total dpm in a peak/total dpm injected onto the HPLC × 100. [f] Not detected.

Table V. Percentages of Unconjugated and Conjugated Fractions in Whey and Casein of Milk Quantitated for Both PCP (GC) and ^{14}C Activity (LSC)[a]

milk fraction	n[b]	unconjugated fraction, %[c]	conjugated fraction, %[d]
whey			
GC[e]	19	54.5 ± 1.6[f]	45.5 ± 1.6
LSC[g]	18	58.4 ± 1.4	41.6 ± 1.4
casein[h]			
GC[e]	19	34.3 ± 1.1	65.7 ± 1.1
LSC[g]	18	27.8 ± 1.3	72.2 ± 1.3

[a] Milk was collected at 4-h intervals over the 76-h period after administration of [^{14}C]PCP. [b] Data from each of the 4-h samples were combined after statistical analysis revealed the absence of a significant time effect. [c] The fraction extracted into benzene under acid conditions. [d] The fraction obtained by difference (see Materials and Methods for description). [e] Gas chromatographic analysis of PCP. [f] Mean (±SE). [g] Liquid scintillation counting of ^{14}C activity. [h] 1:4 casein homogenate (dd H_2O–casein, w/v).

after administration of the radiochemical. Analysis of blood indicated that the experimental cow was in a steady-state condition relative to the body burden of chlorophenols. On the basis of data reported by Firestone et al. (1979), the body burden of the CDDs should also have reached steady-state concentrations. Moreover, an opportunity was provided for adjustment of certain biological processes that could possibly influence metabolic fate of the [^{14}C]PCP such as the rumen microflora (Shull and McCarthy, 1978) or liver drug metabolizing enzymes (McConnell et al., 1980; Shull et al., 1985). However, it is impossible from the present experiment to ascertain the degree to which the kinetic and metabolism data from this cow was influenced by the previous and simultaneous exposure to technical PCP.

PCP was absorbed quite rapidly as indicated by the time-to-maximum plasma concentration (TTMPC) of 10 h and a k_a of 0.162 h ($t_{1/2}$ = 4.3 h). This suggests that the rumen was a major site of absorption which would not be predicted from the pK_a of PCP (4.74), a weak acid. At the usual pH of rumen fluid (6.0–6.4), the calculated percentage of the unionized (i.e., absorbable) form of PCP would be 2.2–5.5%, a condition not favoring rapid absorption of PCP. However, the concentration gradient of the unionized form should favor absorption for two reasons: first, the calculated percentage of unionized PCP at the pH of plasma (7.4) is 0.22%, and second, the influences of metabolism, distribution into tissues, and excretion would serve to decrease plasma concentrations. Although the predicted direction of PCP diffusion is from rumen fluid to plasma, the rate of this diffusion (k_a = 0.162 h^{-1}, $t_{1/2}$ = 4.3 h) is surprising. Also, compared to rate constants and TTMPC data in female rats (k_a = 0.456 h^{-1}, $t_{1/2}$ = 1.52 h, TTMPC = ca. 4 h; Braun et al., 1977) and in female Rhesus monkeys (k_a = 0.383 h^{-1}, $t_{1/2}$ = 1.81 h, TTMPC = ca. 12–24 h; Braun and Sauerhoff, 1976), the cow is similar. However, unlike the cow, rapid absorption would be expected from the stomach of these species due to the higher gastric acidity. An alternative explanation of the cow data is that the [^{14}C]PCP passed quickly from the rumen into the abomasum (pH ≥2) where absorption would be strongly favored. The half-life for emptying the rumen by outflow through the reticular-omasal orifice is about 5–7 h for the liquid component, but is much longer for the roughage component (Dunlop, 1983).

The concentration of PCP in plasma decreased by an apparent first-order rate corresponding to a half-life of 42.8 h. Clearance can be attributed primarily to excretion of PCP and its conjugates into urine; about 75% of the dose was eliminated via this route in 76 h. Another 10% of the dose was eliminated in milk (about 5%) and feces (about 5%). Of the remaining 15%, some was distributed to various tissues such as the liver (5.61%, Table III) and the rest was in the process of biliary excretion as indicated by substantial ^{14}C activity in bile and duodenal contents (Table II).

The shape of the blood elimination curve indicates a single compartment system. There is no suggestion in the data of biphasic elimination (i.e., α and β phases), the β phase being a slowly equilibrating compartment, as was reported in rats given a single dose of [^{14}C]PCP (Larsen et al., 1972; Braun et al., 1977). Interestingly, the elimination half-life in the cow (42.8 h) is similar in magnitude to the β phase half-life reported in the rat, 32.5 h in females, and 40.2 h in males (Braun et al., 1977). Like the cow, Rhesus monkeys exhibited only an α elimination phase corresponding to half-life values of 72.0 h in males and 83.5 h in females (Braun and Sauerhoff, 1976). Braun et al. (1977) suggested that the β phase which represented less than 10% of the administered dose reflected primarily a heterogeneous plasma protein binding and secondarily a small but strongly bound pool of PCP in highly perfused organs such as liver and kidneys. This same rationale could be applied in explaining the elimination kinetics in the cow. One could speculate that had the study continued beyond 76 h, that a β elimination phase would have been eventually observed. Another factor that could have delayed elimination was enteroheptic recirculation. Evidence for this is the substantial quantities of ^{14}C activity measured in bile and duodenal contents. Also, the minor deviations from linearity in serum ^{14}C activity observed during the elimination phase can be partially explained on this basis. A similar phenomenon was observed in Rhesus monkeys (Braun and Sauerhoff, 1976). Theoretically, increases in blood levels would follow a scenario of events intiated by food consumption which would subsequently trigger biliary secretion into the duodenum. No conclusion on the extent of reabsorption from the intestines in the cow can be drawn from the present experiment, but the recovery of 5% of the administered dose in feces confirms that reabsorption was not complete. Braun and Sauerhoff (1976) proposed that the long elimination half-life of PCP in monkeys was the result of enterohepatic recirculation based on evidence of fluctuations in plasma PCP concentrations, a slow but steady elimination of PCP in feces, and large amounts of radioactivity associated with liver and intestinal contents after 360–400 h postadministration.

Low levels of radioactivity were distributed into muscle and adipose tissue (Table III). When viewed together with the rapid rate of elimination, this data indicates that neither PCP nor its metabolites accumulated in edible meat. Similarly, the brain and spinal fluid also contained low levels of radioactivity, indicating poor penetration by PCP of the blood-brain barrier. Two closely associated processes that would explain the data are binding to serum proteins and ionization of PCP in blood. At the pH of plasma (pH 7.4), PCP would be 99.8% ionized, a condition favoring protein binding and restricting transmembrane movement. Braun et al. (1977) observed strong binding of PCP to bovine serum albumin at pH 7.4. Also, 10–20% of the ^{14}C activity in serum was conjugated PCP (Table IV), a form with limited distribution outside of blood. The considerable ^{14}C activity localized in liver can be attributed to the profuse blood supply of this organ and to its involvement in biliary excetion. In rats administered a single oral dose of [^{14}C]PCP, the liver, followed by kidneys, lungs, and adrenals were the organs with the greatest levels of

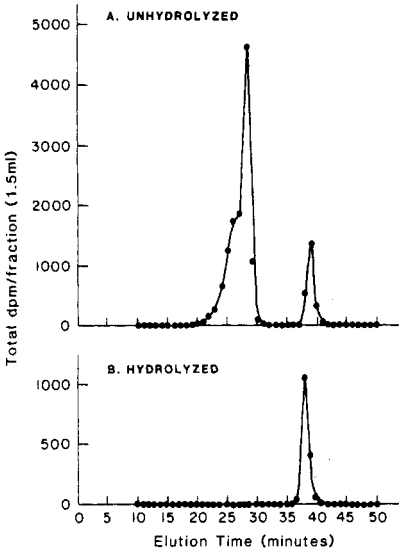

Figure 8. HPLC separation of ^{14}C activity in unhydrolyzed urine (A) and hydrolyzed urine (B). Unhydrolyzed urine was heated (85 °C) in strong acid for 3 h and then extracted into solvent.

^{14}C activity (Larsen et al., 1972; Braun et al., 1977).

There was no evidence that phase 1 metabolism of PCP occurred. Lesser chlorinated phenols and tetrachlorohydroquinone, a known metabolite of PCP in rats (Braun et al., 1977; Ahlborg, et al., 1978), were not found in urine or serum (Table V). In contrast, PCP was conjugated via phase 2 metabolism and was found primarily (about 67%) as such in urine. However, in serum the majority (about 80%) of the PCP was unconjugated and was probably bound to serum proteins. Metabolism of PCP in other species differs markedly from the cow. In rats, as much as 30% of a single dose was recovered in urine as tetrachlorohydroquinone or its glucuronide conjugate (Ahlborg et al., 1978). Moreover, most of the unmetabolized PCP (>60%) was extracted unchanged (Braun et al., 1977; Ahlborg et al., 1978). In Rhesus monkeys, all of the ^{14}C activity in urine was unchanged PCP (Braun and Sauerhoff, 1976). Since none of these experiments utilized animals previously exposed to technical PCP, direct comparisons to metabolism data from this cow are tenuous. There is a suggestion from other studies that significantly lower plasma PCP concentration are found in cattle fed technical PCP vs. their experimental counterparts fed equimolar amounts of purified PCP (Parker et al., 1980). This decrease could result from dioxin/furan induction of liver conjugases such as glucuronyl transferases. Although, technical PCP did not induce *p*-nitrophenol glucuronyl transferase in cattle (McConnell et al., 1980), a significant response was reported in the rat (Goldstein et al., 1977).

The urine was the major route of elimination of unchanged and conjugated PCP. Similar results have been reported in the mouse (Jakobson and Yllner, 1971), rat (Larsen et al., 1972; Ahlborg et al., 1974; Braun et al., 1977), and Rhesus monkey (Braun and Sauerhoff, 1976). Feces and milk represented minor routes of elimination, each accounting for about 5% of the administered [^{14}C]PCP. Interestingly, the elimination half-life in milk was identical with that of blood (42.5 h). Within milk, 62.2% of the ^{14}C activity was associated with the whey fraction, the remainder in the casein (24.4%) and fat (13.3%) fractions. The proportions of conjugated and unconjugated PCP were about equal in whey whereas in casein two-thirds was the conjugated form. Thus, the ratio of conjugated and unconjugated forms of PCP in the whey fraction is similar to that found in serum, whereas the proportion in the casein fraction is more like that found in urine.

Exposure of cattle on farms to PCP by direct or indirect contact with penta-treated wood products presents two forms of hazard: (1) possible adverse health effects in the animals, (2) possible food chain transfer of preservative chemicals via milk and meat. This study addressed primarily the second of these two concerns in the dairy cow. Efficient conjugation and urinary excretion prevented accumulation of PCP. Residue concentrations in milk and edible body tissues, except liver, were quite low. Moreover, the toxicokinetic profile of PCP supports results of toxicological studies in cattle, which show that the toxicity of PCP is not cumulative upon repeated exposure (McConnell et al., 1980; Hughes et al., 1985).

Registry No. PCP, 87-86-5.

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