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DISPOSITION AND METABOLISM OF RADIOLABELLED PENTACHLOROANISOLE IN RATS AND RABBITS

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Abstract—Male Sprague–Dawley rats and New Zealand White rabbits were administered ^{14}C-labelled pentachloroanisole (PCA) in corn oil by gavage as single doses of 25 mg/kg and were then placed in individual metabolism cages for as long as 4 days. Peak blood level of radioactivity occurred 6 hr after administration of the dose to rats and between 3 and 4 hr in rabbits; the blood elimination half-life ranged from 8 to 15 hr in rats and averaged 6 hr in rabbits. Rats excreted an average of 54.2% of the administered radiolabel in the urine and 32.4% in the faeces during the 96 hr following the dose; rabbits excreted an average of 84.2 and 13.1% of the radiolabel in the urine and faeces, respectively, during this time. Examination of the metabolites in the rat showed that 60% of the urinary radioactivity was attributable to tetrachlorohydroquinone (TCH), 3% to free pentachlorophenol (PCP) and 29% to conjugated PCP; faecal metabolites were PCP (85.7%), TCH (4.3%) and polar metabolite(s) (10%). In the rabbit, 58% of the urinary radioactivity was attributable to TCH, 8% to free PCP and 34% to conjugated PCP. Faecal metabolites consisted of PCP and conjugated material.

INTRODUCTION

Pentachloroanisole (PCA) is a metabolite of pentachlorophenol (PCP), which is used as a wood preservative and fungicide. PCA is formed by methylation of PCP by algae and soil micro-organisms; it is found in environmental specimens and occasionally as a contaminant of shellfish. In the Great Lakes, low concentrations of PCA have been found in tissues of lake trout. Glickman and co-workers (1977) reported studies on the uptake, metabolism and disposition of PCA and PCP in rainbow trout, and found that PCA was 24 times more persistent than PCP in selected tissues of rainbow trout.

Vodicnik *et al.* (1980) studied the disposition and metabolism of [^{14}C]PCA in female mice after ip administration. Elimination of carbon-14 from mouse tissues was rapid, with half-lives ranging from 5 to 10 hr in all tissues examined except liver. The radioactivity was excreted primarily by way of the urine (approx. 44–47% of the administered dose); the products found were free and conjugated PCP and an oxidation product, tetrachlorohydroquinone (TCH). The products found in faeces were free and conjugated PCP. No parent PCA was found in the excreta. The data of Vodicnik *et al.* (1980) suggested that PCA was demethylated before excretion.

In studies of the metabolism of ^{14}C-labelled PCP in the mouse, Jakobson and Yllner (1971) found gastric and biliary secretion of PCP and/or its metabolites following sc or ip injection. Most of the activity (72–83% of the dose) was excreted in the urine in 4 days; faecal excretion ranged from 3.8 to 7.7% during this time. The only products found in mouse urine after ip treatment were PCP, both free and conjugated, and TCH. These investigators also observed that PCP was secreted by gastric juice, since the radioactivity after an ip injection was found in the stomach fundus and stomach contents, as shown by autoradiograms. They observed that biliary secretion was also important in the metabolism of PCP.

The pharmacokinetics and metabolism of PCP in Sprague–Dawley rats were reported by Braun *et al.* (1977). In their study, 78.1–80.1% of the orally administered (10 mg/kg) dose was excreted in the urine and 18.8–19.3% in the faeces. At a dose of 100 mg/kg, 72.2 and 24.5% of the dose were excreted by male rats in the urine and faeces, respectively. However, females given PCP at 100 mg/kg excreted 54.4% of the administered radioactivity in the urine and 42.8% in the faeces. The products excreted were 48% unchanged PCP, 6% conjugated PCP, and 10% TCH. Rat blood plasma contained PCP, with a small fraction as the glucuronide. Plasma binding was calculated to be 94.6%. The bulk of the radioactivity was rapidly excreted; 90% or more of the radioactivity was excreted by Day 3 following the dose.

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Abbreviations: PCA = pentachloroanisole; PCP = pentachlorophenol; TCH = tetrachlorohydroquinone; TLC = thin-layer chromatography.

Larsen *et al.* (1972), in studies with rats (strain not specified) given [^{14}C]PCP, reported an average urinary recovery of 68.3% of the orally administered dose (59 mg kg), and faecal recovery ranged from 9.2 to 13.2%. They also observed that serum contained more than 99% of the total activity measured in blood. No sex differences were apparent in tissue concentrations of PCP and/or its metabolites.

Braun and Sauerhoff (1976) administered [^{14}C]PCP in corn oil to Rhesus monkeys as a single oral dose of 10 mg/kg. Some differences were observed between males and females in the elimination of PCP from plasma. Excretion of radiolabel from PCP was mainly by way of the urine, accounting for 68.6–78.0% of the dose; faecal excretion ranged from 11.9 to 23.8%. 15 days after treatment, 11.2–11.7% of the radioactivity administered was still in the bodies of two female monkeys, mainly in the liver and small and large intestines. These investigators reported that monkeys excreted PCP as the unchanged compound in urine.

Because PCP is a ubiquitous environmental contaminant, a comparison of the distribution/excretion data for PCP and its metabolite, PCA, may have toxicological significance. Our study was undertaken (a) to obtain basic distribution/excretion data on PCA when administered to various species of animals, and (b) to compare the distribution/excretion of PCA with that of PCP.

MATERIALS AND METHODS

Test substances

Ring-labelled [^{14}C]PCA (Lot No. 985692) was obtained from ICN Nuclear (Costa Mesa, CA, USA) and had a specific activity of 4.38 mCi/mmol. The radiochemical and chemical purities were both greater than 95%. Just before the radiolabelled compound was used, the purity was checked in our laboratory by thin-layer chromatography (TLC) and was confirmed. Non-radioactive PCA was prepared from purified PCP (Aldrich Chemical Co., Milwaukee, WI, USA) by treatment with diazomethane to form the methyl ether. Purity of the non-radioactive PCA was determined by standard methods and found to be more than 98%. TCH was obtained from Eastman Kodak Co. (Rochester, NY, USA).

Radiolabelled PCA was dissolved in acetone, and aliquots of this solution were pipetted into 125 ml Vitro bottles. An appropriate amount of non-radioactive PCA was weighed and transferred to the bottle, and a minimum amount of acetone was added to dissolve the solids. The acetone was then evaporated by using a gentle stream of nitrogen. The resultant solid was dissolved in Mazola corn oil to yield a solution containing 2.5 mg PCA and 0.84 μCi of ^{14}C activity per ml. This solution was checked for purity by TLC and radiochromatographic scanning as well as for concentration (dpm/ml) prior to use in experiments.

Rats

Male Sprague–Dawley rats were obtained from the Charles River Breeding Laboratories (Wilmington, MA, USA) and weighed 200–250 g when used. After an overnight fast, they were given [^{14}C]PCA in corn oil by gastric intubation as a single dose of 25 mg/kg; administered radioactivity ranged from 1.7 to 2.1 μCi per rat. After treatment, rats were placed in individual stainless-steel metabolism cages designed to separate urine and faeces. Purina Rat Chow No. 5002 (Ralston Purina Co., St Louis, MO, USA) and water were available throughout the study. At the sampling times rats were killed by CO_2 asphyxiation.

To determine the time that radioactivity reached a peak in blood, the rats were bled by heart puncture at various times from 15 min to 12 hr after [^{14}C]PCA administration. The blood was collected in a syringe and then stored under refrigeration in Vacutainer tubes containing ethylenediaminetetraacetic acid. For measurement of radioactivity, the blood was pipetted on to Combusto-Pads, dried and burned in the Packard Biological Sample Oxidizer (Packard Instrument Co., Downers Grove, IL, USA).

In addition, blood was extracted with acetone, and the extracts were concentrated to dryness under nitrogen. The residues were dissolved in methanol and the solutions were filtered through a 0.45 μm filter. The filtrates were analysed by TLC followed by scraping of the TLC plates and liquid scintillation counting to determine the amounts of PCA and PCP in the blood.

For tissue distribution studies, the rats were killed at 6, 12 or 24 hr and the thoracic cavity was opened. Blood was obtained by heart puncture and stored as described above. Tissues were harvested, rinsed with tap water, blotted and stored frozen until analysed.

For recovery studies, excreta were collected every 24 hr for 4 days. At 96 hr after [^{14}C]PCA administration, the rats were killed and exsanguinated. Tissues were harvested, rinsed, blotted and stored frozen until analysed.

Rabbits

New Zealand White male rabbits were obtained from Zartman's Farms (Douglassville, PA, USA) and weighed 2.0–2.6 kg when used. After an overnight fast, they were administered [^{14}C]PCA in corn oil as a single dose of 25 mg/kg (16.8–22 μCi per rabbit). The rabbits were then placed in individual stainless-steel cages. Charles River Rabbit Formula (Agway, Inc., Syracuse, NY, USA) and water were available throughout the study.

For blood level studies, two rabbits were given the radiolabelled PCA and placed in restraints, which were then placed in individual metabolism cages. Blood was obtained from the ear veins at various times between 30 min and 8 hr after dosing to obtain kinetic information following the administration of radiolabelled [^{14}C]PCA. The blood was stored under

refrigeration until analysed as described previously for rat blood.

In the recovery studies, excreta were collected every 24 hr for 4 days. The rabbits were killed 96 hr after [^{14}C]PCA treatment by an overdose of pentobarbital. Tissues were harvested, rinsed, blotted and stored frozen until analysed.

Analysis of tissues, faeces and urine

The tissues obtained from the experimental animals were weighed, and if heterogeneous, homogenized. Portions of the tissues were carefully weighed in duplicate into cellulose Combusto-Cones, dried overnight at room temperature and burned in duplicate to $^{14}CO_2$ in a Packard 306 Biological Sample Oxidizer. Faeces were homogenized with water, and small test portions of the homogenate were weighed into Combusto-Cones, dried and burned in the Biological Sample Oxidizer. The solutions obtained from the Biological Sample Oxidizer were counted by liquid scintillation (Tracor Mark 3, Tracor Instruments, Austin, TX, USA). Aliquots of urine were dissolved in Instagel and counted by liquid scintillation. Quench correction was by external standardization.

Thin-layer chromatographic analyses. TLC was performed by using Fisher Silica Gel GF Rediplates, 5 × 20 cm, 250 μm thickness. Solvent systems used were benzene-methanol, 95:5 (v/v) (Solvent System I); ethyl acetate-isopropanol-NH_4OH, 45:35:20 (by vol) (Solvent System II); and benzene-methanol-glacial acetic acid, 95:4:1 (by vol) (Solvent System III). The approximate mobilities (R_F values) of authentic compounds in Solvent System I were 0.93, 0.48 and 0.42 for PCA, PCP and TCH, respectively. In Solvent System II, the approximate R_F values were 0.96, 0.77 and 0.00 for PCA, PCP and TCH, respectively. In Solvent System III, the approximate R_F values were 0.92, 0.72 and 0.50 for PCA, PCP and TCH, respectively.

After development in solvent, the TLC plates were either scanned on a Packard Radiochromatogram Scanner, or 5-mm sections were sequentially scraped (origin to front) into liquid scintillation vials containing 1 ml *N,N*-dimethylformamide. Scintillation fluid (10 ml) was added to the vials, and the mixture was counted by liquid scintillation under conditions optimized for counting TLC scrapings. The scanner was used when radioactivity was sufficient for measurement by G-M (Geiger-Müller) counting, such as to ascertain the purity of radiolabelled PCA or dosing solutions, and scrapings were typically used to examine biological materials or extracts.

Reverse isotopic dilution studies

Urine excreted during the first 48-hr period was pooled, and radioactivity of the pooled urine (dpm/ml) was determined. A 100-ml aliquot was measured into a 250-ml round-bottomed ground-glass boiling flask, and an accurately weighed portion (generally 1.000 g) of non-radioactive, pure suspected metabolite (e.g. PCP) was added to the urine as an acetone solution. The mixture was acidified with 10 ml concentrated HCl, a three-stage Kuderna-Danish condenser (Kontes Glass Co., Vineland, NJ, USA) was attached to the flask, and the flask was heated on the steam bath for 1 hr to hydrolyse any conjugated metabolites. The hydrolysed urine was then cooled and extracted twice with diethyl ether. The ether solution was dried with anhydrous Na_2SO_4, decolorized with charcoal (Darco G-60, ICI Americas, Inc.), and evaporated to dryness under nitrogen. The resultant solid was recrystallized from various solvents (acetone-water, methanol-water,

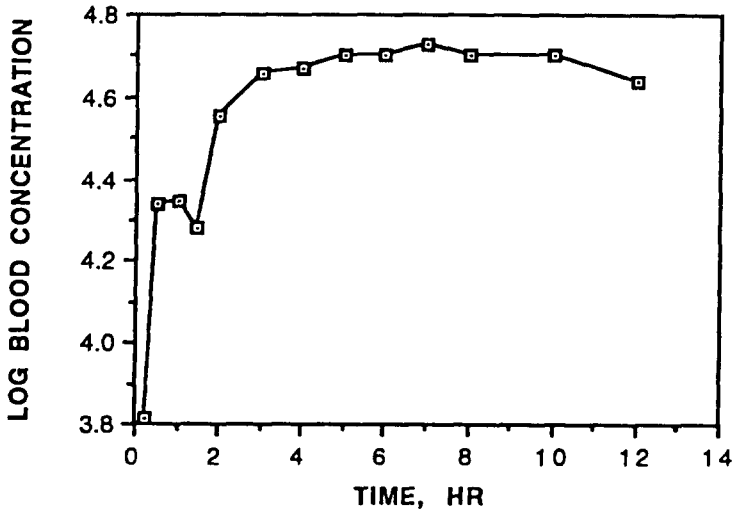

Fig. 1. Semilog plot of radioactivity in rat blood after administration of [^{14}C]pentachloroanisole. Each point is the average for two to four rats. The logarithm of blood concentration (dpm/ml) is plotted against time.

Table 1. Distribution of radiolabelled pentochloroanisole (PCA) in rat tissues*

Tissue	Time (hr) 6 (n = 8)	12 (n = 10)	24 (n = 10)	96 (n = 7)
Blood†	8.21 ± 0.45	6.24 ± 0.2	1.41 ± 0.23	0.01 ± 0.01
Heart	0.31 ± 0.03	0.21 ± 0.01	0.05 ± 0.01	0 ± 0
Lung	0.62 ± 0.06	0.52 ± 0.1	0.13 ± 0.02	0 ± 0
Liver	7.93 ± 0.47	6.05 ± 0.11	3.93 ± 0.14	0.94 ± 0.09
Spleen	0.12 ± 0.01	0.06 ± 0	0.01 ± 0	0 ± 0
GI tract‡	34.16 ± 2.01	29.27 ± 2.53	6.6 ± 1.04	0.15 ± 0.01
Kidney	1.42 ± 0.08	0.88 ± 0.03	0.36 ± 0.05	0.08 ± 0.01
Testes	0.57 ± 0.04	0.35 ± 0.02	0.11 ± 0.02	0 ± 0
Brain	0.14 ± 0.02	0.09 ± 0.01	0.02 ± 0	0 ± 0
Pancreas	0.31 ± 0.09	0.13 ± 0.01	0.04 ± 0.01	0 ± 0
Eyes	0.02 ± 0	0.01 ± 0	0 ± 0	0 ± 0
Carcass	32.22 ± 1.52	20.29 ± 1.07	5.58 ± 0.81	0.35 ± 0.05
Fat§	7509 ± 1077	4134 ± 223	1265 ± 183	49 ± 2.25
Muscle§	5179 ± 289	3546 ± 121	876 ± 138	9.7 ± 4.2
Urine	5.77 ± 0.99	7.62 ± 2.72	34.56 ± 1.77	54.24 ± 2.44
Faeces	0	13.15 ± 0.83	28.41 ± 3.07	32.35 ± 2.45

GI = gastro-intestinal

*Rats were given [^{14}C]PCA in corn oil at 25 mg/kg by gavage. Values are percentages of the administered dose ± SEM.

†Blood was estimated to be 9% of body weight.

‡Plus contents.

§Values for fat and muscle are given as dpm/g and are not included in the percentage of ^{14}C distributed.

acetonitrile–hexane) until constant specific activity was obtained, that is when no further drop in specific activity resulted from repeated recrystallization. From the final specific activity, the total activity attributable to the metabolite (e.g. 1 g PCP) was calculated and related to the radioactivity in the urine.

RESULTS

Rat studies

Blood levels. Analysis of blood indicated that the peak blood level of radioactivity was achieved 6 hr after the gavage of [^{14}C]PCA. The blood elimination half-life of radiolabel ranged from 8 to 15 hr. These results are shown in Fig. 1. In addition, rat blood was analysed for individual radiolabelled compounds by TLC (Solvent System III) followed by scraping of the TLC plates and liquid scintillation counting. The half-life of the administered compound, PCA, was found to be 75 min. The remainder of the radioactivity in blood was attributed to PCP; only traces of TCH were detected in blood collected 2 hr or later after the oral dose. Acid hydrolysis of blood before extraction did not increase the amount of TCH detected. The half-life of the radiolabel, which ranged from 8 to 15 hr, appears to result from the metabolite PCP.

Tissue distribution. Because the peak blood level of radioactivity was achieved at 6 hr and the blood elimination half-life of radioactivity ranged between 8 and 15 hr after [^{14}C]PCA administration, rats were killed and radioactivity in tissues was determined at 6, 12 and 24 hr after dosing. Data summarizing these tissue distribution studies as well as data from the 96-hr recovery study are presented in Table 1.

Recovery studies. To determine whether there was substantial conversion of ring-labelled PCA to CO_2, a rat was given [^{14}C]PCA in corn oil by gavage at a dose of 25 mg/kg and was then immediately placed in a closed glass metabolism cage. All the air from the animal chamber was passed through a trap containing a mixture of monoethanolamine and ethanol (1:1, v/v) to remove any $^{14}CO_2$. Only 0.03% of the administered radiolabel was recovered in the trap in 24 hr. The experiment was repeated with a second rat, and 0.04% of the administered radiolabel was recovered in the trap. A third rat was kept in the enclosed glass metabolism cage for 96 hr, and a total of only 0.09% of the administered radiolabel was recovered as $^{14}CO_2$. This indicated that the PCA phenyl ring was not substantially converted to CO_2 in the rat.

4-day recovery studies were performed in rats given oral [^{14}C]PCA in corn oil at 25 mg/kg. An average of 88.13% of the radiolabel was recovered in the excreta 4 days after the dose. At this time point, an average of less than 1% of the dose remained in the liver; the gastro-intestinal tract (with contents) accounted for 0.15% of the dose, and the carcass accounted for an average of 0.35% of the radiolabel. These results are summarized in Table 1.

Metabolism. Examination of rat urine by TLC indicated that no PCA was excreted at the 25 mg kg dose. In untreated urine, the metabolites of PCA were concentrated at the origin; only 3.1% of the urinary radioactivity was attributable to PCP. Treatment of rat urine with bovine liver β-glucuronidase (Glucurase, Sigma Chemical Co., St Louis, MO, USA) resulted in some increase in PCP, and treatment with a β-glucuronidase/aryl sulfatase mixture from *Helix pomatia* (Product 127698, Boehringer Mannheim Biochemicals, Indianapolis, IN, USA) resulted in further release of PCP. Acid hydrolysis of rat urine resulted in virtual elimination of the peak at the origin and increases in the peaks in the regions

attributable to PCP and TCH. These results are shown in Fig. 2.

Reverse isotopic dilution studies using PCP and TCH were used to confirm the presence of PCP and TCH in rat urine. The procedure indicated that 60% of the urinary radioactivity was attributable to TCH and 32% to PCP (3.1% was unconjugated). Minor unidentified metabolite(s) accounted for the balance of the urinary radioactivity.

Examination of rat faecal extract (50% methanol in water) by TLC revealed radioactive peaks at the origin and in the region attributable to PCP. No PCA was found in the faeces. Acid hydrolysis of the faecal extract and TLC examination of the product in Solvent Systems I and II revealed the presence of PCP (85.7%), TCH (4.3%) and a polar, acid-resistant metabolite at the origin (10%).

Rabbit studies

Blood levels. Two male New Zealand White rabbits were given [^{14}C]PCA in corn oil at a dose of 25 mg/kg and were bled at various times for 8 hr after dosing. The peak blood level occurred between 3 and 4 hr after dosing, and the blood elimination half-life of radiolabel in rabbits averaged 6 hr. These results are shown in Fig. 3. Although a small amount of PCA was detected in the early blood samples, the radioactivity in rabbit blood at the peak blood level (3–4 hr)

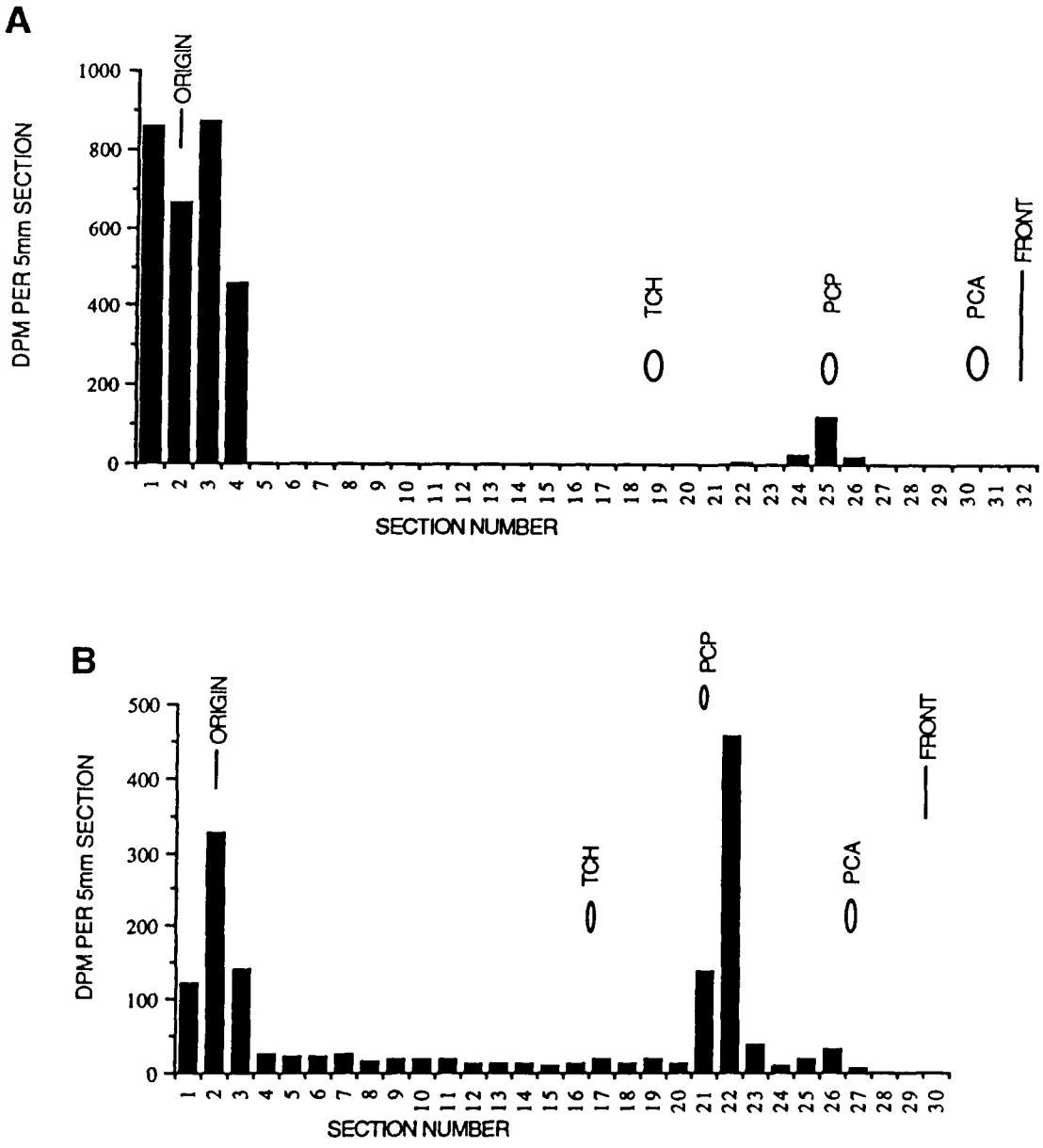

Fig. 2(A) and (B)—*caption overleaf.*

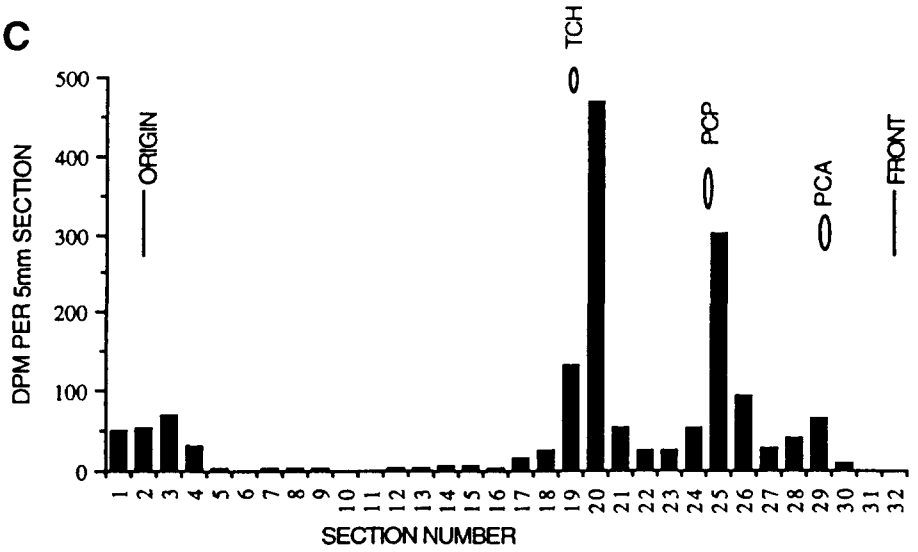

Fig. 2. Thin-layer chromatograms (Solvent System III) of urine from rats given [^{14}C]pentachloroanisole. A, Untreated urine; B, glucuronidase/aryl sulfatase-treated urine; C, acid-hydrolysed urine. TCH = tetrachlorohydroquinone; PCP = pentachlorophenol; PCA = pentachloroanisole.

and later was attributable to PCP; thus the 6-hr half-life of radiolabel in rabbit blood was due to PCP. We were unable to quantify accurately the amount of PCA in the early rabbit blood samples because it was present at concentrations at or below the limit of detection; consequently, we were unable to calculate the half-life of PCA in rabbit blood.

Recovery studies. An average of 84.2% of the radiolabel was recovered in the urine and 13.1% in the faeces in 4 days after [^{14}C]PCA administration to rabbits. None of the tissues sampled at 4 days contained more than 0.1% of the administered radiolabel. The results of these studies are summarized in Table 2.

Metabolism. Examination of rabbit urine by TLC indicated that no PCA was excreted in the urine. Treatment of rabbit urine with β-glucuronidase resulted in release of PCP, and treatment with a β-glucuronidase/aryl sulfatase mixture similarly resulted in release of PCP. The thin-layer chro-

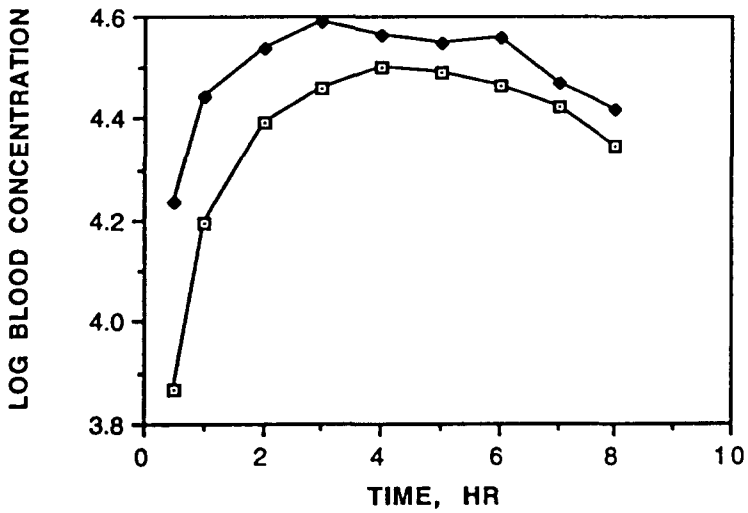

Fig. 3. Semilog plot of radioactivity in rabbit blood after administration of [^{14}C]PCA: rabbit 1, ⊡; rabbit 2, ◆.

Table 2. Recovery of radiolabel from pentochloroanisole (PCA) 96 hr after administration to rabbits*

Tissue	Rabbit no. 1	2	3	4	5	Mean ± SEM
Blood†	0.07	0.06	0.05	0.05	0.13	0.07 ± 0.02
Heart	0	0	0	0	0	0 ± 0
Lung	0	0	0	0	0	0 ± 0
Liver	0.09	0.09	0.01	0.05	0.17	0.08 ± 0.03
Spleen	0	0	0	0	0	0 ± 0
Stomach	0.01	0.02	0.02	0.03	0.03	0.02 ± 0
Large intestine	0.04	0.05	0.05	0.05	0.07	0.05 ± 0.01
Small intestine	0	0	0.01	0	0.03	0.01 ± 0.01
Kidney	0.01	0.01	0	0.01	0.02	0.01 ± 0
Testes	0	0	0	0	0	0 ± 0
Gall bladder	0	0	0	0	0	0 ± 0
Bile	0	0	0	0	0	0 ± 0
Muscle‡	0	0	0.01	0.01	0.3	0.07 ± 0.06
Brain	0	0	0	0	0	0 ± 0
Fat§	0.04	0.04	0.04	0.04	0.06	0.04 ± 0.01
Pancreas	0	0	0	0	0	0 ± 0
Eyes	0	0	0	0	0	0 ± 0
Urinary bladder	0	0	0	0	0.02	0.01 ± 0
Urine	79.25	83.72	79.43	96.88	81.52	84.16 ± 3.28
Faeces	10.92	12.43	15.32	6.85	20.02	13.11 ± 1.20

*Rabbits were given [^{14}C]PCA in corn oil at 25 mg/kg by gavage. Values are percentages of the administered dose.
†Blood was estimated to be 7% of body weight.
‡Muscle was estimated to be 40% of body weight.
§Fat was estimated to be 10% of body weight.

matograms following acid hydrolysis of rabbit urine revealed the release of both PCP and TCH (Fig. 4).

Reverse isotopic dilution studies with rabbit urine were used to confirm the presence of PCP and TCH; these studies indicated that 58% of the urinary radioactivity was attributable to TCH, and 42% to PCP (8% was unconjugated).

Examination of rabbit faecal extract by TLC revealed radioactive peaks at the origin and in the region of PCP. No PCA could be detected in rabbit faeces. Further work-up of rabbit faecal extract was not performed because faecal recovery accounted for only 13.1% of the dose administered.

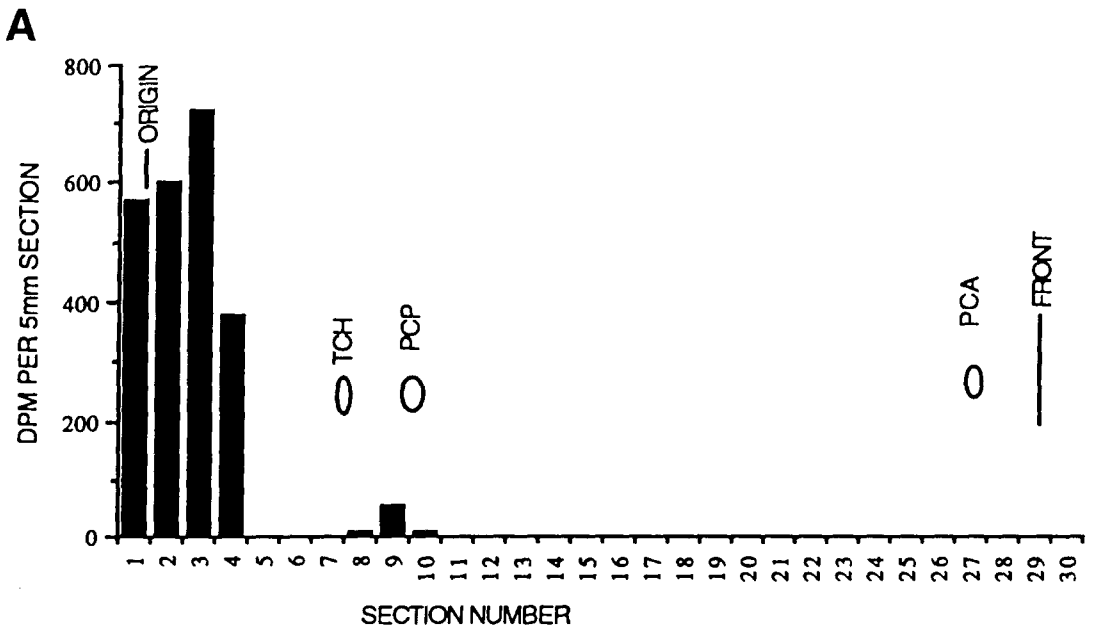

Fig. 4(A)—*caption overleaf.*

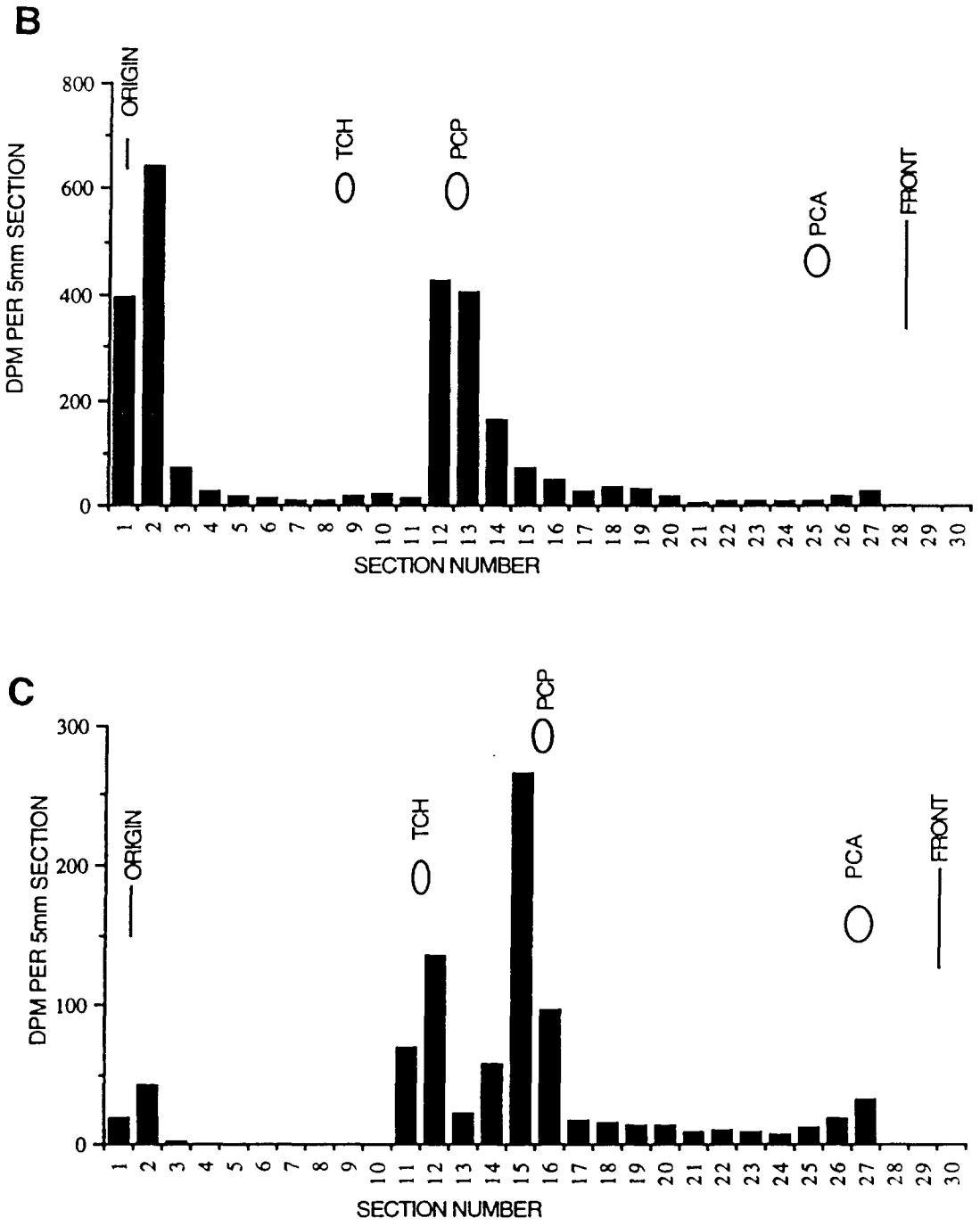

Fig. 4. Thin-layer chromatograms of urine (Solvent System I) from rabbits given [^{14}C]pentachloroanisole. A, Untreated urine; B, glucuronidase aryl sulfatase-treated urine; C, acid-hydrolysed urine. TCH = tetrachlorohydroquinone; PCP = pentachlorophenol; PCA = pentachloroanisole.

DISCUSSION

The rat excreted an average of 54.2% of the radiolabel from the administered [^{14}C]PCA in the urine and 32.4% in the faeces in 96 hr. Most of the radiolabel was excreted during the first 48 hr following the dose. Examination of the blood and excreta after administration of PCA indicated that the compound was metabolized and excreted in the urine. The metabolites found in urine were PCP and TCH, largely in conjugated form. PCP was present as both glucuronide and sulfate conjugates, because although some of the conjugated material was labile to β-glu-

curonidase, additional PCP was liberated by a β-glucuronidase sulfatase mixture. The conjugate of TCH in rat urine, however, was unaffected by treatment with enzymes. TCH was detected in rat urine only after acid hydrolysis of the urine. TLC analysis of untreated rat urine showed that a maximum of 3.1% of PCP was unconjugated. No PCA was found in the urine at the 25 mg/kg dose; however, we have observed small amounts of PCA in the urine of rats given a 50 mg/kg dose (G. J. Ikeda, unpublished observations). Faecal metabolites consisted of free PCP, an unknown polar metabolite and a minor amount of TCH. No PCA was found in faeces. There was probably a small amount of contamination of faeces by urine. Biliary secretion of PCP may also occur in rats, since PCP was found in the faeces.

Ahlborg *et al.* (1974) reported inhibition of bacterial β-glucuronidase by TCH. In our experiments with rat urine, β-glucuronidase from bovine liver or *Helix pomatia* did not appear to be affected by conjugated TCH, and no detectable amount of TCH was released by enzyme treatment. We found TCH in rat urine only after acid hydrolysis of rat urine (1 N in HCl, heated for 1 hr at 100 C). These results suggest three possibilities: (1) that TCH was most probably not present as a glucuronide conjugate; (2) that TCH formed a conjugate that was enzyme-resistant, or (3) that hydrolysis of the glucuronide conjugate and release of a minute amount of TCH aglycone inhibited further hydrolysis of TCH glucuronide. If this last situation prevailed, inhibition of the hydrolysis of PCP glucuronide would also be expected. Since there was no apparent inhibition of the hydrolysis of PCP glucuronide, it appears that TCH either (a) did not form a glucuronide conjugate, or (b) formed a conjugate that was enzyme resistant.

The metabolism of PCA in rats corresponded to that observed by Vodicnik *et al.* (1980) in the mouse: PCA was readily demethylated to PCP, and the products of PCP metabolism were found in the urine. As Vodicnik and co-workers observed in the mouse, we found no free PCA in the urine or faeces of the rat. The metabolites that we found in rat urine also corresponded to the metabolites found by Braun *et al.* (1977) after the administration of PCP to rats; this appears to support the hypothesis of a rapid demethylation of PCA followed by metabolism of the PCP thus formed. We were able to account for 92% of the urinary radioactivity as PCP and TCH.

The radioactivity excreted in rat faeces was largely attributable to free PCP, since acid hydrolysis of faecal extract did not alter the polar material found at the origin of the thin-layer chromatogram. This is in contrast with observations of Vodicnik *et al.* (1980) in the female mouse, in that the polar faecal metabolite could be acid-hydrolysed, yielding PCP.

Rabbits excreted radiolabel from the administration of [^{14}C]PCA rapidly and mainly in the urine; most of the urinary radioactivity was excreted in the first 48 hr of the experiment. Examination of rabbit urine by TLC indicated some unconjugated PCP in untreated urine. Treatment with β-glucuronidase or a mixture of β-glucuronidase and aryl sulfatase resulted in release of the PCP conjugates. As in the rat, TCH was liberated only with acid hydrolysis of the urine.

The radioactivity excreted in rabbit faeces consisted of PCP and conjugated material; some of the faecal radioactivity was probably due to contamination of faeces by urine. However, because some radioactivity could still be found in the large intestine 96 hr after dosing (Table 2), it is probable that some of the radioactivity in the faeces was due to biliary secretion of PCP.

The rabbit readily absorbed and readily excreted radiolabel from ring-labelled [^{14}C]PCA. An average of more than 84% of the administered radiolabel was recovered in the urine. By contrast, rats excreted an average of slightly more than 54% of the administered radiolabel in the urine. The two species appear to readily convert PCA to PCP. The PCP was then metabolized by further oxidation to TCH or conjugated by Phase II reactions and excreted in the urine. At least some of the TCH is conjugated and excreted in the urine. The major metabolites found in the urine of the two species were the same, namely PCP and TCH. The rat excreted a small amount of unknown urinary metabolite that we did not observe in rabbit urine. Because of its structure, TCH may react with other molecules in its environment. Although we did not investigate this possibility, studies of the reaction of TCH with macromolecules could prove to be interesting, and may give clues to the identity of the unknown metabolite(s) in rat urine.

Residual levels of radioactivity in rat and rabbit tissues were different. Whereas the rabbit retained less than 0.1% of the administered radioactivity in liver 4 days after a single 25 mg/kg dose, the rat retained nearly 10 times this level (average of 0.94%) in the liver during this time. Rabbit kidney retained 0.01% of the radiolabel after 4 days, whereas rat kidney retained eight times this level, an average of 0.08%. The residual radioactivity in the rabbit's gastro-intestinal tract (combination of stomach, large and small intestine values) totalled 0.08% of the dose, whereas the rat's gastro-intestinal tract averaged nearly twice this level, at 0.15%. The estimated radiolabel retained in rabbit blood, however, was seven times the estimate in rat blood after 4 days (0.07% *v.* 0.01%).

The residual amounts of administered radiolabel in rat tissues per unit weight were fat > muscle > blood; from Table 1, the amounts observed in these tissues averaged 49:9.7:0.01, or a ratio of 4900:970:1 for fat:muscle:blood. This means that radiolabel (PCP) was almost completely removed from rat blood, whereas 1 g muscle contained 970 times the level of PCP found in 1 ml blood, and 1 g fat contained 4900 times that level. In the rabbit, the residual amounts of radiolabel (PCP) were blood > fat > muscle in the ratio of 46:35:1 for blood:fat:muscle. In the rabbit,

the muscle was essentially depleted of radiolabel in 96 hr, whereas blood and fat still contained an average of 46 and 35 times the level in muscle, respectively.

Braun and co-workers (1977) reported that Sprague–Dawley rats excreted 78.1–80.1% of an orally administered dose of 10 mg PCP/kg in the urine and 18.8–19.3% in the faeces; at a dose of 100 mg/kg, male rats excreted 72.2 and 24.5% of the dose in the urine and faeces, respectively. Female rats dosed with PCP at 100 mg/kg excreted 54.4 and 42.8% in the urine and faeces, respectively. In the present study in which we administered a dose of 25 mg PCA/kg to male rats an average of only 54.2% of the dose was excreted in he urine and 32.4% in the faeces.

Although PCA was found to be readily demethylated to PCP *in vivo*, more of the radiolabel from a PCA dose was excreted in the faeces. Since no unchanged PCA could be found in rat faeces, it is unlikely that the greater excretion of radiolabel in faeces was due to poor absorption of PCA. It appears that PCA was well absorbed, rapidly demethylated to PCP and then either oxidized to TCH or conjugated by UDP glucuronosyl transferase or sulfotransferase. The metabolites were then either secreted in the bile or excreted in the urine. Since more of the radiolabel was excreted through the faeces when PCA was administered orally than when PCP was administered orally, secretion in the bile appears to play a larger role in the disposition of PCA. The enzyme-resistant, acid-labile metabolite of TCH may be formed by another pathway, such as by reaction with glutathione. We did not pursue the characterization of this metabolite.

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