Disposition, Bioavailability, and Serum Protein Binding of Pentachlorophenol in the B6C3F1 Mouse

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The toxicokinetics of pentachlorophenol (PCP) were studied in B6C3F1 mice, a strain in which PCP was previously found to be carcinogenic. In a crossover design, doses of 15 mg/kg were given intravenously (bolus) and orally (gastric intubation) to six animals. Concentrations of PCP in blood, urine, and feces were measured by capillary gas chromatography with electron-capture detection. After intravenous administration, the values of clearance and volume of distribution were 0.057 ± 0.007 L/hr/kg and 0.43 ± 0.06 L/kg, respectively. These two parameters exhibited low intermouse variability (coefficients of variation <14%). The elimination half-life was 5.2 ± 0.6 hr. After oral administration, the PCP peak plasma concentration (28 ± 7 μg/ml) occurred at 1.5 ± 0.5 hr and absorption was complete (bioavailability = 1.06 ± 0.09). The elimination half-life was 5.8 ± 0.6 hr. Only 8% of the PCP dose was excreted unchanged by the kidney. PCP was primarily recovered in urine as conjugates. A portion of the dose was recovered in urine as the mutagen, tetrachlorohydroquinone (5%) (TCHQ), and its conjugates (15%). For both PCP and TCHQ, sulfates accounted for 90% or more of the total conjugates (glucuronides and sulfates).

KEY WORDS: pentachlorophenol; tetrachlorohydroquinone; toxicokinetics; metabolism.

INTRODUCTION

Pentachlorophenol (PCP) is a general biocide (insecticide, fungicide, bactericide, herbicide, algaecide, molluskicide) used mainly as a wood preservative. Because treatment with PCP leads to a fivefold increase in the lifetime of wood (1), the wood industry has used large amounts of PCP (annual world production of 30,000 tons per year) (2). Due to improper disposal, PCP has become an environmental pollutant and is now considered to be ubiquitous (3,4).

Fatal intoxications have been reported in humans after extensive exposure to PCP (2). Clinical signs of acute poisoning (fever, profuse sweating, fast heart rate and breathing) are the result of uncoupled oxidative phosphorylation (5). After chronic exposure, PCP was found to be fetotoxic in rat (6) and carcinogenic in B6C3F1 mice (7).

Pharmacokinetics of the parent compound or its metabolite have been integrated with bioassay data to assess the risk of cancer of several carcinogenic substances (8–10). For PCP, the risk of cancer in humans has been estimated only from bioassay data in mice (11), a species for which pharmacokinetic data are lacking. Furthermore, tetrachlorohydroquinone (TCHQ), a metabolite of PCP in mice (12,13), has been found to be mutagenic (14) and is potentially responsible for the carcinogenicity. Data on the pharmacokinetics of PCP and TCHQ may improve the risk assessment of cancer in humans.

The purpose of the present study was to estimate toxicokinetic parameters (clearance, volume of distribution, bioavailability, half-life, and unbound fraction in serum) of PCP in B6C3F1 mice, the strain of mice used in the carcinogenicity study. The recoveries of PCP, TCHQ and their conjugated metabolites in urine and feces were also investigated to assess the magnitude of the oxidative pathway leading to the formation of TCHQ.

MATERIALS AND METHODS

Animals

Male B6C3F1 mice were obtained from Charles River Breeding Laboratories (Kingston, NY). These animals were maintained at room temperature with a 12-hr light–dark cycle. They were given access to food and water ad libitum during the study. To investigate disposition and bioavailability, blood samples were withdrawn from one group of six animals (group I) that weighed 27 ± 1 g (mean ± SD). To study the excretion of PCP and its metabolites in urine and feces after oral administration, two groups of four animals (groups II and III) were housed in plastic metabolism cages (Nalgene, Nalge Company, Rochester, NY). The mice in groups II and III weighed 27 ± 1 and 26 ± 1 g, respectively.

Dosing Solution

The dosing solution (for both intravenous and oral routes of administration) was made by dissolving 75.76 mg of PCP (99% purity; Aldrich Inc., Milwaukee, WI) in 10 ml of an isotonic pH 9.0 borate solution. This solution (7.5 mg of PCP/ml) was kept at 4°C during this study.

Administration of PCP

Single PCP doses of 15 mg/kg were administered in all groups between 9 and 10 AM. The volume of solution administered was approximately 60 μl. The syringe was weighed before and after administration to determine accurately the dose administered. Mice in group I received PCP intravenously (bolus into the tail vein). After a 1-week washout, the same animals were dosed orally (by gastric intubation). Intravenous doses were administered first because the bolus into the tail vein was found to be difficult to perform (about 50% success rate) with this dark gray strain of mice. Mice in groups II and III were given PCP orally by gastric intubation.

The dose of 15 mg/kg was chosen because it is was high enough to generate measurable blood concentrations for five to six half-lives but was still below the intraperitoneal LD_{50} of 59 ± 4 mg/kg (15).

Collection of Samples

In group I, blood samples were obtained by puncturing a lateral tail vein with a needle and subsequently withdrawing blood with a heparinized precalibrated (44.7-μl) capillary

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tube (VWR Scientific, San Francisco, CA). Blood samples were taken at 0.083, 4, 8, 12, 24, 28, 32, and 36 hr after intravenous administration and 0.5, 1, 2, 3, 6, 12, 24, 30, and 36 hr after oral administration. The blood (accurately measured with a pipette when less than 44.7 μl) was transferred to a culture tube containing 0.3 ml of pH 3 citrate buffer (0.1 *M*). For each of the two administrations, a total volume of about 300 μl of blood was withdrawn from each animal over 36 hr.

In groups II and III, urine and feces were collected daily for 48 hr. A magnetic stirrer was placed under the container designed to collect the urine. The urine was mixed continuously with an initial 2 ml of a solution containing 0.1 *M* ascorbic acid and 0.1 *M* EDTA (pH 7.4) to prevent the degradation of TCHQ (16). After diluting to 50 ml with a solution containing 10 m*M* ascorbic acid and 10 m*M* EDTA and stirring, a 5-ml aliquot was transferred to a culture tube. After weighing the feces, a solution containing 10 m*M* ascorbic acid and 10 m*M* EDTA (pH 7.4) was added so that 1 ml of feces suspension (after homogenization) corresponded to 0.1 g of feces. The biological samples were stored frozen at −20°C until they were analyzed.

Assay of PCP and Its Metabolites

Concentrations of PCP and TCHQ in blood, urine, and feces were measured by capillary gas chromatography with electron-capture detection according to a method described previously (16). Assays in urine were performed before and after hydrolysis (both chemically and enzymatically with β-glucuronidase and sulfatase) to determine unconjugated PCP and TCHQ and to evaluate the proportion of both PCP and TCHQ converted to glucuronide and sulfate conjugates (16). Enzymatic hydrolyses were carried out using β-glucuronidase from *Escherichia coli* (type VII; Sigma) and sulfatase from *Aerobacter aerogenes* (type VI; Sigma). Since commercial β-glucuronidases are considered to contain no sulfatases (17) and the sulfatase used in the present study had no β-glucuronidase activity at pH 7.0, the enzymes hydrolyzed specifically the different conjugates of both PCP and TCHQ. The feces were subjected to chemical hydrolysis to determine the total recovery of PCP and TCHQ.

The intraday and interday precision and accuracy of the assays for PCP and TCHQ have been estimated at three concentrations (between 0.05 and 10 μg/ml): the coefficients of variation were always lower than 11% and the relative error was between −6% and +12% (16). Because of the small blood volume (44.7 μl), the limit of detection in blood was estimated as follows. The mean signal calculated from eight replications of blank blood samples spiked at 0.2 μg/ml (mean of the peak height ratio ± SD = 0.028 ± 0.004) was significantly different ($P < 0.05$) from that corresponding to eight blank blood samples (0.015 ± 0.009). Intraday assay precision was investigated at 0.2 μg/ml; a coefficient of variation of 12% was obtained. Consequently, 0.2 μg/ml was considered as the limit of detection of PCP and TCHQ in blood when a sample of 44.7 μl was used.

Toxicokinetic Analysis

The program Siphar (Simed, Créteil, France) was used to analyze the individual PCP concentration–time data. After intravenous administration, the decline of the concentration in blood, C, with time, t, was monoexponential in each animal and an open one-compartment model was therefore used to fit the data. This model is mathematically equivalent to $C = C_0 \cdot e^{-kt}$, where k is the rate constant of elimination and C_0 is the concentration extrapolated to zero time. These two parameters were estimated by nonlinear regression using a least-squares method and a weighing factor equal to the reciprocal of the concentration calculated by the model. The weighting scheme was selected according to information available on the analytical error. Because neither the standard deviation nor the coefficient of variation for the intraday precision was constant over the range of concentrations, a weighting factor of $1/C$ was used. Clearance (CL) was computed by dividing Dose by area under the curve (AUC). AUC was calculated as the sum $AUC(0 - t_{last}) + (C_{t_{last}}/k)$, where $AUC(0 - t_{last})$ is the area under the curve (estimated by linear trapezoidal rule) from time zero to the last sampling time (t_{last}), and $C_{t_{last}}$ is the concentration at time t_{last}. Volume of distribution (V) was obtained from $Dose/C_0$. Half-life ($t_{1/2}$) was calculated from $t_{1/2} = (\ln 2)/k$.

After oral administration, an open one-compartment model with first-order absorption (k_a = rate constant of absorption) was used to fit the data. This model is mathematically expressed as $C = C_0' (e^{-kt} - e^{-k_a t})$. C_0', k_a, k, and AUC were estimated in the same way as after intravenous administration. The half-lives of absorption ($t_{1/2,abs}$) and elimination ($t_{1/2}$) were obtained from k_a and k, respectively. Maximum plasma concentration (C_{max}) and time to reach this value (t_{max}) were determined by inspection.

Systemic bioavailability (F) was estimated using the following equation:

$$F = \frac{AUC_{PO}}{AUC_{IV}} \cdot \frac{Dose_{IV}}{Dose_{PO}}$$

The subscripts PO and IV refer to oral and intravenous administrations, respectively. Renal clearance (CL_R) was obtained from the ratio of the amount of PCP excreted unchanged in urine for the 48-h collection (assumed equal to $A_{e\infty}$ and obtained in groups II and III) to the mean AUC (calculated in group I). Percentages of the dose excreted in urine as PCP and its metabolites were calculated as the amounts (drug equivalents) excreted in urine (over 48 hr) times 100 divided by the dose administered.

The blood-to-plasma concentration ratio was estimated for each group I animal to estimate the plasma clearance (CL_p) and the plasma volume of distribution (V_p) according to the following equations:

$$CL_p = CL \cdot \frac{C}{C_p} \quad \text{and} \quad V_p = V \cdot \frac{C}{C_p}$$

where C_p is the plasma concentration. The mice were sacrificed a week after oral administration and the blood was withdrawn by cardiac puncture and transferred to a heparinized tube. For each animal, 1 μl of a ^{14}C-PCP solution (100 μCi/ml) was added to 300 μl of blood. After gentle mixing, 50 μl of blood was kept for counting. The rest of the blood was centrifuged at 2000*g* for 10 min and 50 μl of plasma was kept for counting. The samples were prepared for counting by the

addition of 50 μl of hydrogen peroxide (30%). After 12 hr, 2 ml of scintillation cocktail (Scintiverse II, Fischer Scientific, Springfield, NJ) was added and the radioactivity was determined with a liquid scintillation counter (Beckman LS 1801, Fullerton, CA). Uniformly ring-labeled ^{14}C-PCP was obtained from Sigma (St. Louis, MO). The specific activity was 11.9 mCi/mmol and the radiochemical purity was 100% when measured by high-performance liquid chromatography.

Serum Protein Binding

The serum protein binding of PCP was investigated *in vitro* using ultrafiltration. Due to the high lipophilicity of PCP, nonspecific adsorption of PCP on the membrane (and/or the plastic) of the ultrafiltration device was expected (18). A regenerated cellulose membrane (Ultrafree-MC Filter Unit, 30,000 molecular weight cutoff; Millipore Corp., Bedford, MA) exhibited no nonspecific adsorption (0.6 ± 0.9% from six devices). Percentage unbound in serum was measured at a PCP concentration of 10 μg/ml. As it has been shown that PCP binds to albumin (19), saturation of protein binding was not expected at the concentrations observed in this study (20). The protein binding was investigated in serum for each of six B6C3F1 mice that weighed 26 ± 1 g. The animals were sacrificed with carbon dioxide and blood was obtained by cardiac puncture. Serum was used instead of plasma because heparin and other anticoagulants have been shown to interfere with protein binding measurements (21). Phosphate buffer (12 μl, pH 7.4, final concentration of 0.12 *M*) and 3 μl of a ^{14}C-PCP solution (50 μCi/ml) were added to 320 ml of serum. The solution was mixed and 20 μl was kept for counting. The solution was then transferred to the ultrafiltration device and equilibrated at 37°C for 20 min. The samples were spun at 37°C in a fixed-angle rotor centrifuge (Savant Instruments Inc., Farmingdale, NY) for 6, 10, 15, and 30 min. The ultrafiltrate obtained from the first centrifugation was discarded (22). Samples (20 μl) of the other three ultrafiltrates were kept for counting. The numbers of counts measured in the three ultrafiltrates were averaged. The unbound fraction in serum (f_u) was calculated from the ratio of this number to the counts in 20 μl of the initial serum solution. The precision of the ultrafiltration technique (measured from six determinations of the same sample) was 11%.

RESULTS AND DISCUSSION

Mean values of blood PCP concentrations versus time after intravenous and oral administration (15 mg/kg) are presented in Fig. 1. The mean ± SD values of the corresponding toxicokinetic parameters are given in Table I. The volume of distribution of PCP is relatively small. In spite of its high lipophilicity [log of partition coefficient (octanol/water) = 3.32 at pH 7.2 (2)], PCP distribution is restricted, probably as a result of extensive serum protein binding (f_u = 0.014 ± 0.004 at 10 μg/ml). The blood-to-plasma concentration ratio is 0.60 ± 0.03 at a concentration of 2.5 μg/ml.

Absolute bioavailability was estimated assuming that clearance was the same after oral and intravenous administration. As already reported in rats (23,24), PCP can be considered to be completely absorbed when administered in solution by gastric intubation.

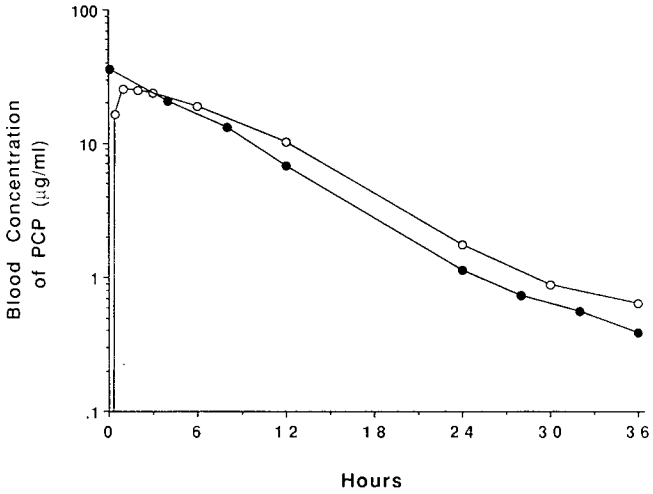

Fig. 1. Time course of blood concentration of pentachlorophenol (PCP) after administration of single doses (15 mg/kg) by intravenous (●——●) and oral (○——○) routes (mean values from six mice).

Plasma renal clearance of PCP is 2.5 ml/hr/kg. Since the average glomerular filtration rate (GFR) in the mouse is 1.2 L/hr/kg (25), PCP renal clearance is lower than the value expected for filtration alone ($f_u \cdot$ GFR = 16.8 ml/hr/kg). This observation supports net tubular reabsorption of PCP. Blood renal clearance is 4.1 ml/hr/kg and represents only 7.6% of the total blood clearance. PCP elimination must occur primarily by nonrenal routes, most likely hepatic metabolism. Assuming that metabolism occurs only in the liver, PCP hepatic extraction (E_H), defined as the ratio of hepatic blood clearance (CL_H) to hepatic blood flow (Q_H), can be estimated. From an average Q_H of 0.16 L/hr in the mouse (26), taking (CL-CL_R) as an estimate of CL_H and assuming no enterohepatic recirculation of PCP, E_H is 0.008. PCP is therefore a low extraction ratio xenobiotic. For such a compound, the hepatic blood clearance is approximated by the product of the unbound clearance (CL_u), the unbound fraction in serum, and the value of C/C_b (27). The high binding to serum proteins greatly contributes to the low clearance value.

Even after the addition of ascorbic acid to prevent TCHQ degradation during blood collection (16), TCHQ was not detectable (detection limit of about 0.2 μg/ml) in blood.

Table I. Toxicokinetic Observations and Parameters (Mean ± SD) of Pentachlorophenol Obtained After Administration of 15 mg/kg by Intravenous and Oral Routes to Six Mice (Group I)

Parameter	Intravenous	Oral
C_{max} (μg/ml)	—	28 ± 7
t_{max} (hr)	—	1.5 ± 0.5
$t_{1/2,abs}$ (hr)	—	0.6 ± 0.5
AUC (μg · hr/ml)	280 ± 33	302 ± 27
F	1	1.06 ± 0.09
CL (L/hr/kg)	0.057 ± 0.007	—
CL_p (L/hr/kg)	0.034 ± 0.004	—
V (L/kg)	0.43 ± 0.06	—
V_p (L/hr)	0.26 ± 0.03	—
$t_{1/2}$ (hr)	5.2 ± 0.6	5.8 ± 0.6

As suggested also by the low urinary concentration of unconjugated TCHQ, this metabolite may undergo rapid conjugation *in vivo*.

Urine and feces were collected for 48 hr after oral administration, which corresponds to approximately eight half-lives of the parent compound. The percentages of the dose excreted in urine and feces as PCP, TCHQ, and their conjugated metabolites are presented in Table II. Because absorption is complete, the percentage of the dose excreted in feces as PCP and TCHQ (after chemical hydrolysis) suggests that biliary excretion contributes to the total elimination (about 8% of the dose, mainly as PCP and/or its conjugates). As the molecular weights of PCP and TCHQ are lower than 300, it is likely that only the conjugates of PCP and TCHQ are extensively excreted in the bile (28).

In urine, approximately 8 and 5% of the dose were recovered as unconjugated PCP and TCHQ (a mutagenic compound), respectively. After chemical hydrolysis, concentrations of PCP and TCHQ in urine were increased about three- and fivefold, respectively. These results show that PCP and TCHQ in urine are primarily conjugated. Treatment of urine with β-glucuronidase and sulfatase indicated that PCP-sulfate and TCHQ-sulfate accounted for 90% or more of the conjugates of PCP. Although the sulfatase enzyme is considered to be specific, the present study gives only indirect evidence for the occurrence of PCP-sulfate and TCHQ-sulfate in urine.

In conclusion, the pharmacokinetics of PCP after oral administration to B6C3F1 mice is characterized by complete absorption, a relatively small volume of distribution (0.45 L/kg), and a low clearance (0.054 L/hr/kg). Elimination of PCP occurs mainly by metabolism, as only 8% of the dose is excreted unchanged in urine. Although not detectable in blood, the metabolite TCHQ is formed, since conjugates of this compound are found in urine. These kinetic results in mice, together with the data obtained in other species, should improve cancer risk assessment in humans after exposure to PCP. Assuming that the same average unbound steady-state concentration measured in plasma gives, for a lifetime exposure, the same risk of cancer in mice and humans, it is possible (using clearance and percent unbound measurements in mice and humans) to estimate the doses in humans equivalent to those given in mice in the carcinogenicity study. Then, using a model appropriate for extrapolation to low doses, it is possible to calculate the risk of cancer in humans due to a given level of exposure to PCP (7).

Table II. Percentage of the Dose Recovered in Urine as Pentachlorophenol (PCP) and Tetrachlorohydroquinone (TCHQ) Before and After Chemical and Enzymatic Hydrolyses[a]

	Group II		Group III	
	PCP	TCHQ[b]	PCP	TCHQ[b]
Urine				
No hydrolysis	6.7	3.6	8.6	5.5
Hydrolysis with[c]				
β-Glucuronidase	7.6	6.6	9.7	5.6
Sulfatase	21.8	21.4	18.9	20.4
Sulfuric acid	23.9	20.2	20.3	20.4
Feces				
Hydrolysis with sulfuric acid	8.6	0.2	6.1	0.3

[a] Urine was collected for 48 hr after oral administration of PCP to two pooled groups of four mice (groups II and III). Percentage of dose recovered in feces after chemical hydrolysis is included.
[b] PCP equivalents.
[c] Value includes unconjugated compound.

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