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TOXICOKINETICS AND METABOLISM

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Fractionation of protein adducts in rats and mice dosed with [^{14}C]pentachlorophenol

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Abstract Pentachlorophenol (PCP) induces liver cancer in mice, possibly due to covalent binding of PCP metabolites to critical macromolecules. In this work, covalent binding was related to PCP biotransformation and specific (cysteinyl) adducts of chlorinated quinones in liver and blood of Sprague-Dawley rats and B6C3F1 mice dosed with [^{14}C]PCP. Using a sequential scheme of scintillation counting along with selective cleavage of cysteinyl adducts by Raney nickel, we quantified total radiobinding, total covalent binding, non-cysteinyl protein binding, and specific protein adducts in liver nuclei (Np), liver cytosol (Cp), hemoglobin (Hb), and serum albumin (Alb). Almost all of the radiobinding to Np (>98%) was attributed to covalent binding in both rats and mice. Regarding Cp, more covalent binding was observed in mice than in rats (100% versus 67%, $P=0.015$). Very little binding was attributed to serum Alb (rats 1.3%, mice 2.6%, $P=0.046$) or Hb (not detected in either species). These results indicate that the liver was the main organ for PCP metabolism and that relatively little of the dose of reactive metabolites became systemically available. Cysteinyl binding accounted for 76–91% of total covalent binding to Np and 68–76% of total covalent binding to Cp. In addition, five times more PCP was bioactivated in the livers of mice than in those of rats (2.14% of the dose bound to Cp in mice and 0.416% in rats). These results reinforce previous studies, suggesting that the liver was a target organ of PCP carcinogenicity and that mice were more susceptible to liver damage than rats. However, the sum of all quantified adducts accounted for only 7–8% of total cysteinyl binding to Np and 2% to Cp, suggesting that other uncharacterized binding species may be important to the toxicity of PCP.

Keywords Pentachlorophenol · Raney nickel · Protein binding · Tetrachlorobenzoquinone

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Introduction

Pentachlorophenol (PCP), a ubiquitous environmental contaminant, is a procarcinogen in rodents and possibly in humans (Seiler 1991; WHO 1987). PCP induced liver cancers in mice and mesotheliomas in rats following 2-year chronic bioassays (Chhabra et al. 1999; McConnell et al. 1991). Human epidemiological studies have linked possible PCP exposures with soft-tissue sarcomas (Hardell and Sandstrom 1979), non-Hodgkin's lymphomas (Greene et al. 1978; Hardell et al. 1994; Pearce et al. 1986), and blood disorders (Roberts 1990).

Although the specific mechanism by which PCP exerts its carcinogenicity remains elusive, metabolism to chlorinated quinones is believed to play a role (Ehrlich 1990; Witte et al. 1985). PCP is metabolized primarily by cytochrome P450 (presumably CYP1A2) to tetrachlorohydroquinone and tetrachlorocatechol, which can be oxidized to their corresponding quinones [tetrachloro-1,4-benzoquinone (Cl_4-1,4-BQ) and tetrachloro-1,2-benzoquinone (Cl_4-1,2-BQ)] and semiquinones (tetrachloro-1,4-benzosemiquinone and tetrachloro-1,2-benzosemiquinone) (Ahlborg et al. 1978; Lin et al. 1999; Renner and Hopfer 1990; van Ommen et al. 1986a, 1988).

The toxicity of quinones and their thioether derivatives has been extensively studied. Two general mechanisms have been proposed for the toxic effects of quinones, namely covalent binding to macromolecules and generation of reactive oxygen species during redox cycling between the quinone and semiquinone forms (Bolton et al. 2000; Bratton et al. 1997; Monks and Lau

1992; Monks et al. 1992; O'Brien 1991). More specifically, quinone metabolites of PCP have been shown to covalently bind to macromolecules (Bodell and Pathak 1998; Ehrlich 1990; Lin et al. 1999, 2001a; van Ommen et al. 1986a, 1986b, 1988; Waidyanatha et al. 1996; Witte et al. 1985) and to produce oxidative damage to genomic DNA (Dahlhaus et al. 1994, 1995, 1996; Jansson and Jansson 1992; Lin et al. 2001b; Naito et al. 1994; Sai-Kato et al. 1995; Umemura et al. 1996, 1999; Witte et al. 2000).

Studies of rats dosed with [^{14}C]PCP showed that liver contained the highest levels of radioactivity, followed by the kidney and blood (Braun et al. 1977; Larsen et al. 1972). Regarding specific covalent products, cysteinyl adducts of chlorinated quinones and two uncharacterized PCP adducts have been investigated in liver and/or blood from rats and mice following gavage administration of PCP (0–40 mg/kg body weight) (Lin et al. 1997, 1999; Waidyanatha et al. 1996). Moreover, covalent binding of Cl_4-1,4-BQ and Cl_4-1,2-BQ has been quantified in microsomal incubations of [^{14}C]PCP (van Ommen et al. 1986a, 1986b) and of unlabeled PCP (Tsai et al. 2001).

Since the proportions of total covalent binding attributable to PCP metabolism and to specific chlorinated quinone metabolites have not yet been elucidated in vivo, we measured total binding and the abundance of particular cysteinyl adducts of PCP metabolites in rats and mice after administration [^{14}C]PCP. The PCP-derived protein adducts were determined in proteins isolated from the livers and blood of these animals after reduction by Raney nickel (Ni), which specifically cleaves carbon-sulfur bonds (Danenberg and Heidelberger 1976; Farnsworth et al. 1990; Perlstein et al. 1971). This allowed us to estimate also the fraction of total covalent products bound to sulfhydryl groups.

Materials and methods

Chemicals

[^{14}C]PCP (>98% radiochemical purity, specific activity 10.4 mCi/mmol), ammonium sulfate, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Company (St. Louis, Mo., USA). Phenylmethylsulphonyl fluoride (PMSF) was obtained from Aldrich Chemical Company (Milwaukee, Wis., USA). All other chemicals were the same as reported previously (Tsai et al. 2001).

Animals and tissue collection

Sprague-Dawley rats and B6C3F1 mice were obtained from Charles River Breeding Laboratories (Raleigh, N.C., USA). To investigate PCP disposition, six male Sprague-Dawley rats (410–430 g) and six male B6C3F1 mice (27–29 g) were assigned to control and dosing groups (three in each). Following gavage dosing (in 10 mM phosphate-buffered saline), rats received [^{14}C]/[$^{12}C_6$]PCP at 20 mg/kg body weight (equivalent to 120 μCi/rat) and mice received [^{14}C]PCP at 20 mg/kg body weight (equivalent to 20 μCi/mouse). (Note that [^{14}C]PCP was administered to mice instead of a [^{14}C]/[$^{12}C_6$]PCP mixture to increase the level of radiobinding). Controls were administered equivalent volumes of 10 mM phosphate-buffered saline by gavage. Animals were killed 24 h after administration. Blood was collected via cardiac puncture into a heparinized syringe and the liver was removed after perfusing with 0.25 M sucrose. Blood and liver samples were processed as described below.

Isolation of hemoglobin (Hb) and albumin (Alb)

Red blood cells were separated from plasma by centrifuging at 800 *g* for 5 min. The red blood cells were washed with saline (0.9% NaCl) and an equal amount of deionized water was added. Samples were frozen at –20°C overnight to lyse the cells prior to isolation of Hb.

Hb and Alb were isolated as described in Rappaport et al. (1993a) with modifications. Briefly, Hb was isolated from lysed red blood cells by centrifuging at 30,000 *g* for 40 min at 4°C followed by dialysis (molecular weight cut-off, MWCO 6,000–8,000) against 4×3.5 l of 1 mM ascorbic acid at 4°C. Globin was precipitated by dropwise addition of the hemolysate to cold acidified acetone (0.1% HCl by volume), washing with ice-cold acetone, and drying to constant weight under vacuum at 37°C. Alb was isolated from plasma by adding an equal volume of saturated ammonium sulfate to precipitate the immunoglobulins. After removing immunoglobulins, the supernatant was purified by dialysis (MWCO 12,000–14,000) against 4×3.5 l of 1 mM ascorbic acid at 4°C. The dialysate was dried, weighed, and stored at –80°C prior to analysis.

Isolation of liver cytosol (Cp) and liver nuclei (Np)

Liver Cp and Np were isolated according to the procedure described in Lin et al. (1999) with modifications. Livers were thawed, sliced, and suspended in 0.25 M ice-cold sucrose containing 1 mM EDTA and 1 mM PMSF. After 10–15 strokes of a tissue grinder, the homogenate was filtered and centrifuged at 1,000 *g* for 10 min. The resulting pellet and supernatant were used to isolate liver Np and Cp, respectively.

Np was isolated from the 1,000 *g* pellet, resuspended in 0.25 M sucrose containing 1 mM EDTA and 0.2 mM PMSF, and underlaid with 2.3 M sucrose containing 1 mM EDTA and 0.2 mM PMSF. The nuclei were isolated by centrifugation at 105,000 *g* for 60 min. The 105,000 *g* pellet was resuspended and centrifuged at 105,000 *g* for another 30 min. The resulting pellet was extracted with 0.25 M HCl, and the extract was dialyzed, dried, weighed, and stored as Np at –80°C prior to analysis. Np from all mice was pooled prior to analyses due to the small amounts of nuclei obtained from each animal. Cp was isolated from the 1,000 *g* supernatant, centrifuged at 15,000 *g* for 20 min, and the resulting supernatant centrifuged at 105,000 *g* for another 60 min. The final supernatant was dialyzed, dried and stored as Cp at –80°C prior to analysis.

Analysis of protein adducts

Cysteinyl adducts of Cl_4-1,4-BQ, Cl_4-1,2-BQ, and two uncharacterized PCP adducts were analyzed following cleavage of Alb, Cp, and Np with Raney Ni as described in Tsai et al. (2001).

Gas chromatography-mass spectrometry (GC-MS) analysis

All samples were analyzed by GC-MS in negative ion chemical ionization mode (GS-NICI-MS) using an HP 5890 gas chromatograph coupled to an HP 5989A mass spectrometer. The GC-MS conditions were the same as described in Tsai et al. (2001).

Radiobinding

Small aliquots of the [^{14}C]PCP dosing solutions or the purified ^{14}C-labeled proteins, dissolved in 1 mM ascorbic acid, were added

to 20 ml scintillant (Econoscint; Fisher Scientific, Pittsburgh, Pa., USA) and counted on a Wallac 1409 liquid scintillation analyzer for 5 min. Three small aliquots, each equivalent to 2 mg protein, were removed for scintillation counting, as shown in Fig1. The first count was performed upon purified protein after exhaustive dialysis against 1 mM ascorbic acid using either 6,000–8,000 or 12,000–14,000 MWCO membrane tubing. This count represents activity from the total covalent and noncovalent binding arising from PCP. The second count was performed after protein digestion and washing with methyl-*t*-butyl ether (MTBE) to remove noncovalently bound and interfering compounds. This count represents covalent binding of PCP-derived reactive metabolites. The third count was performed after reaction with Raney Ni followed by extraction with MTBE to remove the cleaved sulfur-bound species; this count represents non-cysteinyl covalent binding. The difference between counts 2 and 3 represents reactive metabolites bound to free cysteine residues. These cysteinyl adducts were then characterized and quantified by GC-MS following derivatization by *N*-heptafluorobutyrylimidazole (HFBI) as described in Tsai et al. (2001). Levels of particular mono-*S*- an multi-*S*- substituted adducts derived from Cl_4-1,2-BQ or Cl_4-1,4-BQ were combined for reporting. Levels of two uncharacterized adducts, measured as 2,3,4,5- and 2,3,5,6-tetrachlorophenol-HFB were also combined.

Statistical analysis

The means and standard error (SE) were calculated for all triplicate samples. Paired *t*-tests were used to test differences between rats and mice at a two-tailed statistical significance level of $P < 0.05$.

Results

The proportion of total radiobinding that can be attributed to PCP-derived covalent binding was estimated from protein solutions (Cp, Np, and Alb) after digestion and washing with MTBE. Results are summarized in Table 1. More than 97.9% of total radiobinding was covalently bound to Np in rats and mice. Mice showed higher percentages of covalent binding than rats for both Cp (100% versus 67.0%, $P = 0.015$) and Alb (2.63% versus 1.30%, $P = 0.046$). Radiobinding to Hb was indistinguishable from background levels (i.e., 75 dpm) in both rats and mice even when 75 mg Hb was used.

The percentage of total covalent binding (i.e., total protein adduction) attributable to reactions with free cysteine residues was estimated from the fractions of radioactivity released following treatment with Raney Ni. As shown in Table 2, large proportions of PCP-derived adducts were bound to cysteine in both species. The percentage of Cp binding was greater in rats (76.3%) than in mice (68.3%, $P = 0.003$), while that of Alb binding was greater in mice (88.7%) than in rats (26.5%, $P = 0.001$). Subsequently, the concentrations of particular PCP-derived quinone adducts and two uncharacterized cysteinyl adducts were determined. Because [^{14}C]PCP had been administered to mice instead of a [^{14}C]/[$^{12}C_6$]PCP mixture (to increase the level of radiobinding), the particular adduct levels in mice were adopted from a parallel experiment in which 20 mg [$^{12}C_6$]PCP/kg body weight was administered to matching

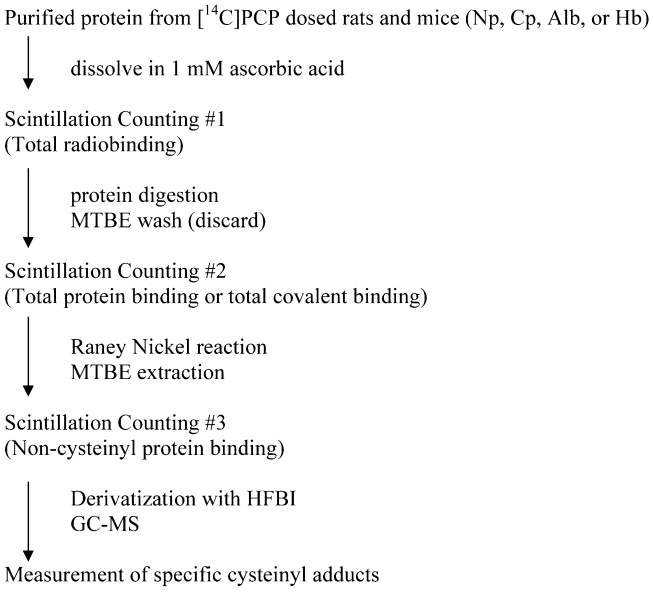

Fig. 1. Scheme for determining radiobinding of [^{14}C]pentachlorophenol (PCP) products by scintillation counting. (*Np* liver nuclei, *Cp* liver cytosol, *Alb* albumin, *MTBE* methyl-*t*-butylether, *HFBI* *N*-heptafluorobutyrylimidazole)

Table 1. Percentages of total radiobinding attributed to [^{14}C]pentachlorophenol (PCP)-derived covalent binding in various tissue fractions in rats and mice following administration of 20 mg PCP/kg body weight. Data represent mean values with SE in parentheses, $n = 3$ (*Np* liver nuclei, *Cp* liver cytosol, *Alb* albumin)

Protein	PCP-derived covalent binding (% of total radiobinding)	
	Rat	Mouse
Np	97.9 (2.00)	100[a]
Cp	67.0 (1.80)	100* (2.65)
Alb	1.30 (0.064)	2.63* (0.287)

[a]No error estimate is available because the liver nuclei proteins in mice were pooled
*$P < 0.05$, significant difference between rats and mice by paired *t*-test

Table 2. Percentages of [^{14}C]pentachlorophenol (PCP)-derived covalent binding associated with cysteine residues, as estimated by treatment with Raney Ni, in various tissue fractions in rats and mice following administration of 20 mg PCP/kg body weight. Data represent mean values with SE in parentheses, $n = 3$ for rats (*Np* liver nuclei, *Cp* liver cytosol, *Alb* albumin)

Protein	PCP-derived cysteinyl binding (% of total covalent binding)	
	Rat	Mouse
Np	91.2 (2.49)	75.5[a]
Cp	76.3 (0.310)	68.3* (0.353)
Alb	26.5 (2.91)	88.7* (2.00)

[a]No error estimates are available because the liver nuclei proteins were pooled
*$P < 0.05$, significant difference between rats and mice by paired *t*-test

animals. The fractions of total covalent binding and of total cysteinyl binding attributable to particular adducts are summarized in Table 3. Adducts of Cl_4-1,4-BQ were the major products in all tissue fractions in both species. Adducts of Cl_4-1,2-BQ were detected in liver and blood proteins of mice, but not of rats. The percentages of total adducts represented by two uncharacterized PCP adducts were small in both species (0.006–0.385% in rats and 0.001% for Cp in mice).

Discussion

The disposition of reactive PCP metabolites in rats and mice was investigated based on the assay described by Tsai et al. (2001), which uses Raney Ni to selectively cleave sulfur bonds, corresponding to cysteinyl protein adducts in this context. Several studies have successfully applied Raney Ni to cleave cysteinyl adducts in various tissues, i.e., those of styrene 7,8-oxide styrene in blood proteins (Ting et al. 1990; Rappaport et al 1993; Yeowell-O'Connell et al. 1996), of benzoquinone in blood and bone-marrow proteins (McDonald et al. 1993; Rappaport et al. 1996), of chlorinated quinones from PCP in blood and liver proteins (Lin et al. 1999; Tsai et al. 2001; Waidyanatha et al. 1996), and of polychlorinated biphenyl quinones in liver and brain proteins (Lin et al. 2000).

By administering [$^{14}C/^{12}C_6$]PCP to animals along with the sequential counting scheme shown in Fig. 1, we estimated the fractions of total radiobinding associated with covalent binding (Table 1), of total covalent binding associated with cysteinyl binding (Table 2), and of total covalent binding/total cysteinyl binding associated with particular metabolites (Table 3). Almost all of the radiobinding was associated with covalent binding to liver proteins in both rats and mice (Table 1). This supports experimental results indicating that the liver was the primary organ for PCP metabolism in both species and was the target organ for PCP toxicity (NTP 1989, 1999). One purpose of this study was to estimate how much PCP had been metabolized in the livers of rats and mice. To answer this, we estimated the covalent binding attributable to PCP binding as the ratio of total Cp radioactivity (count #2 in Fig. 1) to the total radioactivity administered (120 μCi/rat and 20 μCi/mouse). Assuming the liver weight to be 4% of total body weight for rats (420 g) and 5.5% for mice (28 g) (Travis et al. 1990), and that 24.5 mg of purified liver Cp were obtained per gram of liver (Lin et al. 1996), the proportions of PCP bound to liver Cp were 0.416% in rats and 2.14% in mice. For example, in rats:

$$\frac{\frac{5342\ \text{dpm}/2\ \text{mg protein}}{2.2\times10^6\ \text{dpm/mCi}} \times 420\ \text{g body wt} \times \frac{0.04\ \text{g liver}}{\text{g body wt}} \times \frac{24.5\ \text{mg protein}}{\text{g liver}}}{120\ \text{mCi}} \times 100\% = 0.416\%$$

This indicates that mice metabolized about five times more PCP to liver-binding species than rats. Since metabolism is necessary for PCP-induced toxicity, this result supports the finding of liver tumorigenesis observed in mice but not in rats dosed with PCP (Chhabra et al. 1999; McConnell et al. 1991).

Results showed that less than 3% of the radiobinding was covalently bound to Alb in rats and mice (Table 1), and that radiobinding was not detected in Hb. This indicates that very little of the dose of PCP's reactive intermediates was available for extrahepatic transport in the blood. Indeed, the observed covalent binding to Alb could well represent intrahepatic reactions of binding species with prealbumin, which is synthesized in the hepatocyte. Previous studies indicated extensive binding of PCP to serum proteins in rats and mice (>96%) (Braun et al. 1977; Reigner et al. 1993); this probably reflects non-covalent binding that was removed by digestion of proteins followed by extraction with MTBE in our assay (Fig. 1).

In this study, we also determined that most of the covalent binding was associated with cysteinyl adducts, as shown in Table 2. This supports our original idea of using cysteinyl adducts as biological dosimeters of exposure to PCP.

Finally, results indicate that we are currently able to account for specific protein adducts representing 7–8% of total cysteinyl binding to Np and 2% to Cp in rats and mice (Table 3). This suggests that the brunt of covalent products involve other uncharacterized binding species, which may play important roles in the toxicity of PCP.

Table 3. Percentage of total protein adducts and total cysteine-bound adducts accounted for by chlorinated quinones (Cl_4-1,4-BQ and Cl_4-1,4-BQ) and uncharacterized pentachlorophenol (PCP) adducts. Data represent mean values with SE in parentheses, $n=3$ (*Np* liver nuclei, *Cp* liver cytosol, *Alb* albumin, *N.D.* not detected)

Species	Protein	Percentage of total covalent binding			Percentage of total cysteinyl binding		
		Cl_4-1,4-BQ adducts	Cl_4-1,2-BQ adducts	Uncharacterized PCP adducts	Cl_4-1,4-BQ adducts	Cl_4-1,2-BQ adducts	Uncharacterized PCP adducts
Rat	Np	5.28 (0.288)	N.D.	0.305 (0.045)	6.35 (0.736)	N.D.	0.383 (0.066)
	Cp	1.53 (0.080)	N.D.	0.120 (0.010)	2.02 (0.102)	N.D.	0.158 (0.013)
	Alb	7.48 (0.838)	N.D.	0.495 (0.083)	30.8 (4.94)	N.D.	2.06 (0.402)
Mouse	Np	5.88 (1.16)	1.06 (0.037)	N.D.	7.08 (1.40)	1.28 (0.045)	N.D.
	Cp	0.667 (0.071)	0.477 (0.039)	0.001 (0.000)	0.987 (0.108)	0.699 (0.058)	0.002 (0.000)
	Alb	12.3 (0.635)	0.934 (0.006)	N.D.	16.4 (0.463)	1.25 (0.038)	N.D.

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References

Ahlborg UG, Larsson K, Thunberg T (1978) Metabolism of pentachlorophenol in vivo and in vitro. Arch Toxciol 40:45–53

Bodell W J, Pathak DN (1998) Detection of DNA adducts in $B6C3F_1$ mice treated with pentachlorophenol. Proc Am Assoc Cancer Res 39:2266

Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ (2000) Role of quinones in toxicology. Chem Res Toxicol 13:135–160

Bratton SB, Lau SS, Monks TJ (1997) Identification of quinol thioethers in bone marrow of hydroquinone/phenol-treated rats and mice and their potential role in benzene-mediated hematotoxicity. Chem Res Toxicol 10:859–865

Braun WH, Young JD, Blau GE, Gehring PJ (1977) The pharmacokinetics and metabolism of pentachlorophenol in rats. Toxicol Appl Pharmacol 41:395–406

Chhabra RS, Maronpot RM, Bucher JR, Haseman JK, Toft JD, Hejtmancik MR (1999) Toxicology and carcinogenesis studies of pentachlorophenol in rats. Toxicol Sci 48:14–20

Dahlhaus M, Almstadt E, Appel KE (1994) The pentachlorophenol metabolite tetrachloro-*p*-hydroquinone induces the formation of 8-hydroxy-2-deoxyguanosine in liver DNA of male $B6C3F_1$ mice. Toxicol Lett 74:265–724

Dahlhaus M, Almstadt E, Henschke P, Luttgert S, Appel KE (1995) Induction of 8-hydroxy-2-deoxyguanosine and single strand breaks in DNA of V79 cells by tetrachloro-*p*-hydroquinone. Mutat Res 329:29–36

Dahlhaus M, Almstadt E, Henschke P, Luttgert S, Appel KE (1996) Oxidative DNA lesions in V79 cells mediated by pentachlorophenol metabolites. Arch Toxicol 70:457–460

Danenberg PV, Heidelberger C (1976) The effect of Raney nickel on the covalent thymidylate synthetase-5-fluoro-2′-deoxyuridylate-5,10-methylenetetrahydrofolate complex. Biochemistry 15:1331–1337

Ehrlich W (1990) The effect of pentachlorophenol and its metabolite tetrachlorohydroquinone on cell growth and the induction of DNA damage in Chinese hamster ovary cells. Mutat Res 244:299–302

Farnsworth CC, Gelb MH, Glomset JA (1990) Identification of geranylgeranyl-modified proteins in HeLa cells. Science 247:320–322

Greene MH, Brinton L A, Fraumeni J F, D'Amico R (1978) Familial and sporadic Hodgkin's disease associated with occupational wood exposure. Lancet 2:626–627

Hardell L, Sandstrom A (1979) Case-control study: soft tissue sarcomas and exposure to phenoxyacetic acid or chlorophenols. Br J Cancer 39:711–717

Hardell L, Eriksson M, Degerman A (1994) Exposure to phenoxyacetic acids, chlrophenols, or organic solvents in relation to histopathology, stage, and anatomical localization of non-Hodgkin's lymphoma. Cancer Res 54:2386–2389

Jansson K, Jansson V (1992) Induction of micronuclei in V79 Chinese hamster cells by tetrachlorohydroquinone, a metabolite of pentachlorophenol. Mutat Res 279:205–208

Larsen RV, Kirsch LE, Shaw SM, Christian JE, Born GS (1972) Excretion and tissue distribution of uniformly labeled ^{14}C-pentachlorophenol in rats. J Pharm Sci 61:2004–2006

Lin P-H, Waidyanatha S, Rappaport SM (1996) Investigation of liver binding of pentachlorophenol based upon measurement of protein adducts. Biomarkers 1:232–243

Lin P-H, Waidyanatha S, Pollack GM, Rappaport SM (1997) Dosimetry of chlorinated quinone metabolites of pentachlorophenol in the livers of rats and mice based upon measurement of protein adducts. Toxicol Appl Pharmacol 145:399–408

Lin P-H, Waidyanatha S, Pollack GM, Swenberg JA, Rappaport SM. (1999) Dose-specific production of chlorinated quinone and semiquinone adducts in rodents livers following administration of pentachlorophenol. Toxixol Sci 47:126–133

Lin P-H, Sangaiah R, Ranasinghe A, Upton PB, La DK, Gold A, Swenberg JA (2000) Formation of quinonoid-derived protein adducts in the liver and brain of Spague-Dawley rats treated with 2,2′,5,5′-tetrachlorobiphenyl. Chem Res Toxicol 13:710–718

Lin P-H, Nakamura J, Yamaguchi S, Upton PB, La DK, Swenberg JA (2001a) Oxidative damage and direct adducts in calf thymus DNA induced by the pentachlorophenol metablites, tetrachlorohydroquinone and tetrachloro-1,4-benzoquinone. Carcinogenesis 22:627–634

Lin P-H, Nakamura J, Yamaguchi S, La DK, Upton PB, Swenberg JA (2001b) Induction of direct adducts, apurinic/apyrimidinic sites and oxidized based in nuclear DNA of human HeLa S3 tumor cells by tetrachlorohydroquinone. Carcinogenesis 22:635–639

McConnell EE, Huff JE, Hejtmancik M, Peters AC, Persing R (1991) Toxicology and carcinogenesis studies of two grades of pentachlorophenol in B6C3F1 mice. Fundam Appl Toxicol 17:519–532

McDonald TA, Waidyanatha S, Rappaport SM (1993) Production of benzoquinone adducts with hemoglobin and bone marrow proteins following administration of $[^{13}C_6]$benzene to rats. Carcinogenesis 14:1921–1925

Monks TJ, Lau SS (1992) Toxicity of quinone-thiothers. Crit Rev Toxicol 23:243–270

Monks TJ, Hanzlik RP, Cohen GM, Ross D, Graham DG (1992) Contemporary issues in toxicology: quinone chemistry and toxicity. Toxicol Appl Pharmacol 112:2–16

Naito S, Ono Y, Somiya I, Inoue S, Ito K, Yamamoto K, Kawanishi S (1994) Role of active oxygen species in DNA damage by pentachlorophenol metabolites. Mutat Res 310:79–88

NTP (National Toxicology Program) (1989) Toxicology and carcinogenesis studies of two pentachlorophenol technical-grade mixtures (CAS No. 87-86-5) in $B6C3F_1$ mice (feed studies). Technical Report Series No. 349. NIH publication No. 89-2804. US Department of Health and Human Services, Public Health Services, NIH, Research Triangle Park, N.C.

NTP (National Toxicology Program) (1999) Toxicology and carcinogenesis studies of pentachlorophenol (CAS No. 87-86-5) in F344/N rats (feed studies). Technical Report Series No. 483. NIH publication No. 97-3973. US Department of Health and Human Services, Public Health Services, NIH, Research Triangle Park, N.C.

O'Brien PJ (1991) Molecular mechanisms of quinone cytotoxicity. Chem Biol Interact 80:1–41

Pearce NE, Smith AH, Howard JK, Sheppard RA, Giles HJ, Teague CA (1986) Non-Hodgkin's lymphoma and exposure to phenoxyherbicides, chlorophenols, fencing work, and meat work employment: a case-control study. Br J Ind Med 43:75–83

Perlstein MT, Atassi MZ, Cheng SH (1971) Desulfurinzation of sulfur amino acids and proteins with Raney nickel. Biochim Biophys Acta 236:174–182

Rappaport SM, Ting D, Jin Z, Yeowell-O'Connell K, Waidyanatha S, McDonald T (1993) Application of Raney nickel to measure adducts of styrene oxide with hemoglobin and albumin. Chem Res Toxicol 6:238–244

Rappaport SM, McDonald T, Yeowell-O'Connell K (1996) The use of protein adducts to investigate the disposition of reactive metabolites of benzene. Environ Health Perspect 104 [Suppl 6]:1235–1237

Reigner RG, Frederic YB, Tozer TN (1993) Pentachlorophenol carcinogenicity: extrapolation of risk from mice to humans. Human Exp Toxicol 12, 215–225

Renner G, Hopfer C (1990) Metabolic studies on pentachlorophenol (PCP) in rats. Xenobiotica 20:573–582

Roberts HJ (1990) Pentachlorophenol-associated aplastic anaemia, red cell aplasia leukaemia and other blood disorders. J Fla Med Assoc 77:86–90

Sai-Kato K, Umemura T, Takagi A, Hasegawa R, Tanimura A, Kurokawa Y (1995) Pentachlorophenol-induced oxidative

DNA damage in mouse liver and protective effect of antioxidants. Food Chem Toxicol 33:877–882

Seiler JP (1991) Pentachlorophenol. Mutat Res 257:27–47

Ting D, Smith MT, Doane-Setzer P, Rappaport SM (1990) Analysis of styrene oxide-globin adducts based upon reaction with Raney nickel. Carcinogenesis 11:755–760

Travis CC, Quillen JL, Arms AD (1990) Pharmacokinetics of benzene. Toxicol Appl Pharmacol 102:400–420

Tsai C-H, Lin P-H, Waidyanatha S, Rappaport SM (2001) Characterization of metabolic activation of pentachlorophenol to quinones and semiquinones in rodent liver. Chem Biol Interact 134:55–71

Umemura T, Sai-Kato K, Takagi A, Hasegawa R, Kurokawa Y (1996) Oxidative DNA damage and cell proliferation in the liver of B6C3F1 mice exposed to pentachlorophenol in their diet. Fundam Appl Toxicol 30:285–289

Umemura T, Kai S, Hasegawa R, Sai K, Kurokawa Y, Williams GM (1999) Pentachlorophenol (PCP) produces liver oxidative stress and promotes but does not initiate hepatocarcinogenesis in B6C3F$_1$ mice. Carcinogenesis 20:1115–1120

van Ommen B, Adang A, Muller F, van Bladeren PJ (1986a) The microsomal metabolism of pentachlorophenol and its covalent binding to protein and DNA. Chem Biol Interact 60:1–11

van Ommen B, Adang AEP, Posthumus MA, Muller F, van Bladeren PJ (1986b) The microsomal metabolism of hexachlorobenzene. Origin of the covalent binding to protein. Biochem Pharmacol 35:3233–3238

van Ommen B, Voncken JW, Muller F, van Bladeren PJ. (1988) The oxidation of tetrachloro-1,4-hydroquinone by microsomes and purified cytochrome P-450b. Implications for covalent binding to protein and involvement of reactive oxygen species. Chem Biol Interact 65:247–259

Waidyanatha S, Lin P-H, Rappaport SM (1996) Characterization of chlorinated adducts of hemoglobin and albumin following administration of pentachlorophenol of rats. Chem Res Toxicol 9:647–653

WHO (World Health Organization) (1987) Environmental Health Criteria 71. Pentachlorophenol. WHO, Geneva

Witte I, Juhl U, Butte W (1985) DNA-damaging properties and cytotoxicity in human fibroblasts of tetrachlorohydroquinone, a pentachlorophenol metabolite. Mutat Res 145:71–75

Witte I, Zhu B-Z, Lueken A, Magnani D, Stossberg H, Chevion M (2000) Protection by desferrioxamine and other hydroxamic acids against tetrachlorohydroquinone-induced cyto- and genotoxicity in human fibroblasts. Free Radic Biol Med 28:693–700

Yeowell-O'Connell K, Jin Z, Rappaport SM (1996) Determination of albumin and hemoglobin adducts in workers exposed to styrene and styrene oxide. Cancer Epidemiol Biomarkers Prev 5:205–215