Environmental Health Perspectives
Vol. 46, pp. 137-140, 1982

Effects of Transplacental Exposure to Chlorinated Phenols

by Jerry H. Exon* and Loren D. Koller*

Female rats were exposed to 0, 5, 50 or 500 ppm of 2-chlorophenol (2-CP) or pentachlorophenol (PCP). The study was designed to produce progeny which were exposed to the chlorophenolic compounds both prenatally and postnatally. Percent conception, litter size, birth weight, and number of stillbirths was determined at parturition. Hematologic parameters and body weights of the progeny were recorded at weaning age (3 weeks).

Effects on reproduction were observed in both the 2-CP and PCP-exposed groups, as indicated by decreased litter sizes and increased number of stillborn. The data indicate that these chlorinated phenolic compounds may be feto- or embryotoxic at high doses. Effects on hematologic parameters were not observed. Further study involving transplacental and chronic exposures to these chlorophenolic compounds appears warranted.

The chlorophenols are a group of 19 compounds which differ in degree and position of chlorination (*1*). These compounds consist of mono-, di-, tri- and tetrachloro isomers and a pentachlorophenol. The chlorinated phenols are effective disinfectants, antiseptics, fungicides, slimicides, bactericides, wood preservatives, herbicides, insecticides, and molluscicides (*2-9*). The widespread use of these chemicals in industry and agriculture has resulted in contamination of food producing animals and the environment. Inadvertent formation of 2-chlorophenol, 2,4-dichlorophenol and 2,4,6-trichlorophenol as by-products of water chlorination represent significant sources of exposure to these compounds (*1*).

2-Chlorophenol is a commercially produced chemical used as an intermediate in the production of higher chlorinated phenols. The generation of waste sources from the commercial production of 2-chlorophenol, its chemically derived products, and the inadvertent formation of 2-chlorophenol due to chlorination of organics in drinking and waste waters are potential sources of environmental contamination (*1*).

Pentachlorophenol (PCP) and its salts have been widely used in agriculture and industry since 1936 (*10*). Approximately 200 million pounds of PCP were produced worldwide in 1977 (*11*). Principal uses of PCP are as a pesticide and wood preservative (*12*), and contamination of livestock occurs by licking treated wood or drinking from vats used to treat wood (*6*). Industrial exposure and consumption of contaminated food and water are the principal sources of exposure to humans (*13*).

This study was conducted to assess the effects of two chlorophenolic compounds, 2-chlorophenol and pentachlorophenol, on reproduction and hematology. The experimental design included prenatal and postnatal exposure to the chlorophenolic compounds.

Materials and Methods

Female Sprague-Dawley rats were weaned at 21 days of age and divided into groups of 12-20 rats each. The rats were placed on dietary regimens containing 0, 5, 50 or 500 ppm 2-CP (Aldrich Chemicals, 97% pure) in the drinking water or PCP (ICN Pharmaceuticals, K & K Laboratories No. 18497, 95% pure) in the feed. The female rats were housed four per cage in stainless hanging wire racks. Feed and water were available *ad libitum*. The rats were bred at 90 days of age (10 weeks exposure). The dams were transferred to polycarbonate cages with stainless steel lids and hardwood shavings to litter. The study was designed to produce progeny that were exposed to the chlorophenolic contaminants both prenatally and postna-

*Veterinary Medicine, University of Idaho, Moscow, Idaho 83843.

tally. Percent conception, litter size, birth weight, and number of stillbirths was determined at parturition. Body weights and hematologic parameters (white and red cell counts, hemoglobin, packed cell volume and mean corpuscular volume) were recorded at weaning age. Blood samples were analyzed using a Coulter counter, Model ZB_i. The dams were terminated at weaning and liver and kidney tissues were collected for analysis of chlorophenolic content. Gas chromatography was used to analyze 2-CP and PCP in tissues by the methods of Erney (*14*).

Results

Parameters relating to reproductive performance that were observed include percent conception of the dams, litter size, birth and weaning weights, percent stillborn, survival to weaning and body weight gain of the dams (Tables 1–4). Percent conception was greater in all treatment groups as compared to controls (Tables 1 and 2). Litter size was significantly ($p \leq 0.05$) decreased in groups of dams treated with high levels of 2-CP (Table 1). Litter size was also decreased in groups given 500 ppm PCP ($p \leq 0.10$) (Table 2). Percent of stillborn pups born to dams receiving either 2-CP or PCP was generally greater as compared to controls. This increase was significant ($p \leq 0.05$) in the 500 ppm 2-CP group (Table 1). Body weights at weaning were generally decreased in PCP-exposed groups. No consistant effects were observed in regard to survival to weaning (Tables 3 and 4). The body weight gains of dams prior to breeding appeared to be unaffected by the treatments.

Table 1. Effect of 2-chlorophenol (CP) on rats and progeny.

Treatment[a]	Conception, %	Litter size (mean ± SD)[b]	Birth wt., g (mean ± SD)[c]	Stillborn, %	Mean weight gain of dams, g
Control	67 (8/12)	11.4 ± 1.2	2.2 ± 0.4	0 (0/91)	168
CP					
5 ppm	75 (9/12)	11.7 ± 3.5	2.3 ± 0.4	0 (2/105)	170
50 ppm	75 (9/12)	10.1 ± 2.3	2.5 ± 0.4	0 (0/91)	158
500 ppm	86 (12/14)	9.2 ± 4.3	2.4 ± 0.4	0 (6/110)	159

[a]CP was given in the drinking water of the dams from weaning through parturition.
[b]Live and stillborn.
[c]Live pups only.
[d]Significant at $p < 0.05$ to control.

Table 2. Effect of pentachlorophenol (PCP) on rats and progeny.

Treatment[a]	Conception, %	Litter size (mean ± SD)[b]	Birth wt., g (mean ± SD)[c]	Stillborn, %	Mean weight gain of dams, g
Control	58 (7/12)	10.6 ± 2.4	2.6 ± 0.7	1 (1/74)	146
PCP					
5 ppm	77 (10/13)	10.3 ± 3.2	2.5 ± 0.3	7 (7/103)	145
50 ppm	85 (11/13)	10.7 ± 3.5	2.5 ± 0.3	2 (2/110)	130
500 ppm	86 (12/14)	9.2 ± 3.0	2.4 ± 0.5	3 (3/110)	141

[a]PCP was given in the feed of the dams from weaning through parturition.
[b]Live and stillborn.
[c]Live pups only.
[d]Significant at $p < 0.10$ to control.

Table 3. Effect of 2-chlorophenol (CP) on progeny of rats.

Treatment[a]	Weaning wt. (mean ± SD), g	Survival to weaning, %[b]
Control	51.3 (4.4)	100 (91/91)
CP		
5 ppm	54.2 (8.4)	100 (103/103)
50 ppm	54.1 (8.4)	100 (91/91)
500 ppm	53.9 (9.6)	99 (103/103)

[a]2-Chlorophenol was given in the drinking water either prenatally by exposing the dams from weaning through parturition (90 days) and postnatally from parturition.
[b]Exclusive of stillborn pups.

Table 4. Effect of pentachlorophenol (PCP) on progeny of rats.

Treatment[a]	Weaning wt. (mean ± SD), g	Survival to weaning, %[b]
Control	60.0 (9.8)	97 (71/73)
PCP		
5 ppm	56.7 (6.8)	89 (95/96)
50 ppm	55.7 (7.6)	98 (106/108)
500 ppm	54.9 (12.2)	99 (106/107)

[a]Pentachlorophenol was given in the feed prenatally by exposing the dams from weaning through parturition (90 days) and postnatally from parturition.
[b]Exclusive of stillborn pups.

Table 5. Analysis of feed and tissue samples of dams fed pentachlorophenol or 2-chlorophenol for 10 weeks.

	Chlorophenol content, ppm		
	Feed[a]	Liver[b]	Kidney[b]
Control (PCP)	0.21	0.048	0.066
PCP			
5 ppm	4.80	0.102	0.034
50 ppm	71.40	0.081	0.046
500 ppm	411.50	0.099	0.056
Control (2CP)	—	0.16	0.26
2CP			
5 ppm	—	2.20	2.60
50 ppm	—	3.20	2.40
500 ppm	—	0.08	2.00

[a]A pool of three separate bags.
[b]A pool of tissues from five rats/group.

Table 6. Chemical content of pentachlorophenol mixture used to prepare PCP diets.[a]

	Content
Composition by gas chromatography	
2,3,4,6-Tetrachlorophenol	6.8%
2,3,4,5-Tetrachlorophenol	0.6%
Pentachlorophenol	85.5%
Dioxins by liquid chromatography	
Hexachlorodibenzo-*p*-dioxins	8 ppm
Oxtochlorodibenzo-*p*-dioxins	400 ppm

[a]ICN Pharmaceutical, K&K Laboratories, catalog no. 18497; 97% pure.

The accumulation of the chlorophenol compounds in tissues of dams was comparatively minimal (Table 5). Pentachlorophenol levels in liver and kidney tissues were considerably lower than 2-CP residues in similar tissues. Residues in kidney versus liver tissue were approximately equal. Feed samples were also analyzed for PCP content (Table 5). The analysis correlated reasonably well with the target concentrations of 5, 50 and 500 ppm. Purity of the PCP mixture used in preparation of the PCP diet was analyzed by the Dow Chemical Company (Table 6). This analysis incidated that the PCP formulation was only 85.5% pure as compared to the 95% purity advertised by the manufacturer. The dioxin content was also determined (Table 6).

Hematologic parameters examined for all treatment groups included red and white blood cell counts, packed cell volume (hematocrit), hemoglobin and mean corpuscular volume (Tables 7 and 8). No significant effects of chlorinated phenols on hematologic parameters were observed.

Discussion

The chlorophenols tested, 2-CP and PCP, appeared to alter certain reproduction parameters in rats. Stimulated conception rates, reduced litter size at the highest doses (500 ppm), and increased number of stillbirths were observed in chlorophenol-treated groups. These results indicate that high levels of these compounds tend to be feto- or embryotoxic.

Table 7. Hematologic parameters of rats exposed to 2-chlorophenol.[a,b]

Treatment	RBC 10^4/mm^3	Hct, % PCV	MCV, μm^3	WBC, 10^2/mm^3	Hgb, g/dl
Control	747 (46)	37 (3)	49 (2)	163 (46)	16.1 (0.9)
CP					
5 ppm	728 (56)	36 (3)	47 (2)	164 (27)	16.2 (0.9)
50 ppm	778 (38)	40 (2)	48 (2)	160 (34)	16.5 (0.9)
500 ppm	828 (57)	42 (4)	48 (2)	178 (46)	17.5 (1.6)

[a]2-Chlorophenol was given in the drinking water either prenatally by exposing the dams from weaning through parturition (90 days) and postnatally from parturition.
[b]All hematologic parameters are reported as mean (standard deviation).

Table 8. Hematologic parameters of rats exposed to pentachlorophenol.[a,b]

Treatment	RBC 10^4/mm^3	Hct, % PCV	MCV, μm^3	WBC, 10^2/mm^3	Hgb, g/dl
Control	767 (42)	39 (2)	50 (1)	162 (34)	16.2 (0.8)
PCP					
5 ppm	781 (51)	38 (2)	49 (1)	161 (32)	16.2 (0.8)
50 ppm	786 (23)	38 (2)	49 (2)	154 (28)	16.1 (0.5)
500 ppm	789 (66)	37 (2)	47 (2)	192 (57)	15.8 (1.2)

[a]Pentachlorophenol was given in the drinking water either prenatally by exposing the dams from weaning through parturition (90 days) and postnatally from parturition.
[b]All hematologic parameters are reported as mean (standard deviation).

The results are consistent with those published by others (*15*).

The majority of commercially prepared technical-grade PCP is a mixture of about 85–90% PCP and the remainder primarily consists of tetrachlorophenol and chlorinated phenoxyphenols (*16*). Small amounts of other impurities include chlorinated dibenzo-*p*-dioxins, chlorinated dibenzofurans and chlorinated diphenyl ethers. The mixture purchased for this study was listed by the manufacturer to be 95% pure PCP. However, analysis indicated that the mixture contained actually 85.5% PCP and a variety of other phenolics and impurities. Nevertheless, exposure to this substance in the environment would be as prepared containing both the PCP and associated impurities. Once adverse effects are identified from exposure to the manufactured product, then analytical grade PCP must be evaluated to determine if the effects are produced by PCP, its impurities or from a combination of these products.

This work was supported by U.S. Environmental Protection Agency grant R8072670110.

REFERENCES

1. Ahlborg, U. G., and Thunberg, T. M. Chlorinated phenols: occurrence, toxicity, metabolism, and environmental impact. CRC Crit. Rev. Toxicol. 7: 1-35 (1980).
2. Binet, P. Toxicologie comparée des phénols. Rev. Med. Suisse Romande. 16: 449-456 (1896).
3. Baylé, P. Sur la toxicologie du pentachlorophenol. PhD thesis, University of Lyon, 1924.
4. Carswell, T. S., and Nelson, H. K. Properties and usès of pentachlorophenol. Ind. Eng. Chem. 30: 622-629 (1938).
5. Carswell, T. S., and Hatfield, I. Pentachlorophenol for wood preservation. Ind. Eng. Chem. 31: 1431-1440 (1939).
6. Bevenue, A., and Beckman, H. Pentachlorophenol. A discussion of its properties and its occurrence as a residue in human and animal tissue. Residue Rev. 19: 83-134 (1967).
7. Crossland, J., and Shea, K. P. The hazards of impurities. Environment 15: 35-50 (1973).
8. Roushdy, M. Z., E.-Gindy, M. S., Ayad, N., and Mousa, A. H. Effect of sub-lethal doses of molluscicides on the susceptibility of *Bulinus truncatus* snails to infection with *Schistosoma haematobium*. Egypt. J. Bilharziasis 1: 85-89 (1974).
9. Robichaux, T. J. Bactericides used in drilling and completion operations. In: Environmental Aspects of Chemical Use in Well-Drilling Operations. Conference Proceedings, U.S. Environmental Protection Agency, Houston, Texas, 1975, p. 183, EPA-560/1-75-004.
10. Spencer, E. Y. In: Guide to Chemicals Used in Crop Protection. Publication No. 1093, Research Branch, Agriculture Canada, Ottawa, Canada, 1973, p. 542.
11. Cirelli, D. P. Patterns of pentachlorophenol usage in the United States of America—an overview. In: Pentachlorophenol: Chemistry, Pharmacology and Environmental Toxicology, K. R. Rao, Ed., Plenum Press, New York, 1978, p. 13.
12. Arsenault, R. D. Pentachlorophenol and contained chlorinated dibenzodioxins in the environment: A study of fate, stability, and significance when used in wood preservation. Proc. Ann. Meet. Am. Wood Preserv. Assoc. 73: 122-131 (1976).
13. Dougherty, R. C. Human exposure to pentachlorophenol. In: Pentachlorophenol: Chemistry, Pharmacology and Environmental Toxicology, K. R. Rao (Ed.), Plenum Press, New York, 1978, p. 351.
14. Erney, R. Gas-liquid chromatographic determination of pentachlorophenol in milk. J. Assoc. Off. Anal. Chem. 61: 214-216 (1978).
15. Schwetz, B. A., Keeler, P. A., and Gehring, P. J. The effect of purified and commercial grade pentachlorophenol on rat embryonal and fetal development. Toxicol. Appl. Pharmacol. 25: 151-161 (1974).
16. Firestone, D., Ress, J., Brown, N. L., Barron, B. P., and Damico, J. N. Determination of polychlorodibenzo-*p*-dioxins and related compounds in commercial chlorophenols. J. Assoc. Off. Anal. Chem. 55: 85-92 (1972).