THE EFFECT OF PENTACHLOROPHENOL ON THE REPRODUCTIVE PERFORMANCE IN RATS

By

Kuo-Chuan Karen Chou

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Dairy Science

1980

ABSTRACT

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THE REPRODUCTIVE PERFORMANCE IN RATS

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Pentachlorophenol (PCP) is widely used as an anti-fungal agent. There is a paucity of data on the effects of PCP on reproduction. Experiments were carried out to determine the effects of purified PCP and an industrial composite of PCP on reproduction in rats. Dose levels of 0, 0.4, 4 or 40 mg/kg of technical grade pentachlorophenol and the purified pentachlorophenol adjusted to provide the similar levels of PCP were administered intraperitoneally to pregnant Sprague-Dawley rats on days 3, 6, 9, 12 and 15 of gestation. The carrier vehicle was corn oil.

Animals treated with the highest levels of either chemical showed lower maternal body weight gain and higher incidence of fetal resorptions compared to the controls. Survival of the newborn pups from the dams treated with the high dose level of purified PCP decreased.

Sex ratios of the weaning rats showed that fewer female offspring survived from PCP treatments than the male offspring.

The activity of hepatic pyruvate kinase increased more in the dams treated with purified PCP than those treated with technical grade PCP.

Study of the growth rate and reproduction of the offspring from the dams treated with PCP showed no differences between controls and treated animals.

No gross sturctural anomalies were observed among the offspring. Although more toxic effects were observed among the animals treated with purified PCP than with technical grade PCP, the toxic effects were only observed when higher dose levels (4 or 40 mg/kg of body weight) were given. Animals in the environment are not likely to be exposed to PCP at such high levels.

However, the exception is the effect of PCP on the activity of hepatic pyruvate kinase. For dams treated with all levels of PCP, including the lowest level tested (0.4 mg/kg of body weight), pyruvate kinase increased. This suggests possible abnormal glycolysis and gluconeogenesis in the liver. A future study of PCP toxicity in animals should emphasize effects on the pathway of carbohydrate metabolism.

To my husband, Edward,
without whom I would not
have started and could not
have finished this study.

ACKNOWLEDGEMENT

The author wishes to express her deepest appreciation to Dr. Robert Cook, whose guidance and support made the completion of this investigation possible.

Thanks also are extended to the other committee members, Dr. Steven Aust, Dr. Lee Shull, and Dr. William Thomas for their helpful suggestions in completing this study.

Acknowledgement is made to Mr. Mark Jackson for his hard work and help throughout the study.

Finally the author is indebted to her parents for their caring and understanding.

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LIST OF ABBREVIATIONS

ADP	adenosine diphosphate
ATP	adenosine triphosphate
AHH	aryl hydrocarbon hydroxylase
2,6-DCHQ	2,6-dichlorohydroquinone
HCDD	hexachlorodibenzo-p-dioxin
MFO	mixed-function oxygenases
NADH	nicotinamide adenine dinucleotide, reduced form
OCDD	octachlorodibenzo-p-dioxin
PCDDs	polychlorinated dibenzo-p-dioxins
PCDFs	polychlorinated dibenzofurans
PCP	pentachlorophenol
ppm	parts per million
ppb	parts per billion
2,4,5,-T	2,4,5-trichlorophenoxyacetic acid
TCA	tricarboxylic acid
TCBQ	tetrachlorobenzoquinone
TCHQ	tetrachlorohydroquinone
TCH	tetrachlorohydroquinone
TCP	tetrachlorophenol
Tri-CHBQ	trichlorohydrobenzoquinone
Tri-CP	trichlorophenol

INTRODUCTION

Pentachlorophenol (PCP) is a synthetic compound first synthesized in the 1930's. Registered as a pesticide agent, it has been used extensively as a fungicide, insecticide, herbicide, and antiseptic for more than forty years. In the last twenty years, it has become the most widely used wood preservative in the United States.

Sixty-nine million pounds of pentachlorophenol were produced in 1975, eighty percent of which was used by wood preservative processors. Over twenty-five million cubic feet of lumber and fenceposts were treated with pentachlorophenol at 155 wood preservation plants in thirty-one states. This compound has recently received attention in the animal industry because of the increasing usage of PCP treated wood in animal facilities. Morton Buildings, Inc., the largest farm building company in the United States, reported that approximately 15,000 buildings are built with PCP treated wood every year, and most of these are farm buildings. Livestock of all kinds housed in these buildings are either directly or indirectly exposed to the PCP treated wood.

The potential toxicity of PCP to livestock was recently brought to the scientific community when a

Michigan farm had high mortality in dairy cows (70/232) and even higher mortality in newborn calves (200/208). PCP was found in the tissue of these cows as well as in several other Michigan herds with poor performance. The levels in the blood and in the livers ranged from 10 to 1000 ppb. Earlier, a high rate of stillbirths and increased incidence of death in young pigs had been reported when sows were placed in wooden facilities that had received PCP treatment. The above findings indicate the possible effect of PCP on the reproductive performance of animals. The contamination of food-producing animals by PCP also raises questions concerning potential human health hazards. Consequently, the study of the effect of PCP on the reproductive performance in animals is needed.

LITERATURE REVIEW

Chemical and Physical Properties of Pentachlorophenol

Pentachlorophenol is a fully chlorinated phenol (figure 1). The pure product is white, needle-like crystals soluble in most organic solvents, oils and petroleum hydrocarbons with aromatic and olefinic content (Table 1). The solubility in water is very low. The physical properties of PCP are shown in (Table 2). It is relatively stable in heat up to the boiling point. However, under acidic conditions, there are considerable losses through volatility when it is heated. The volatility of PCP with steam at $100^{o}C$ is 0.167 gm of material per 100 gm of steam at standard atmospheric pressure.

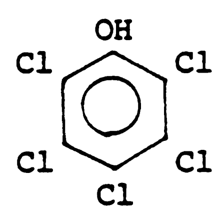


Figure 1 Chemical Structure of Pentachlorophenol

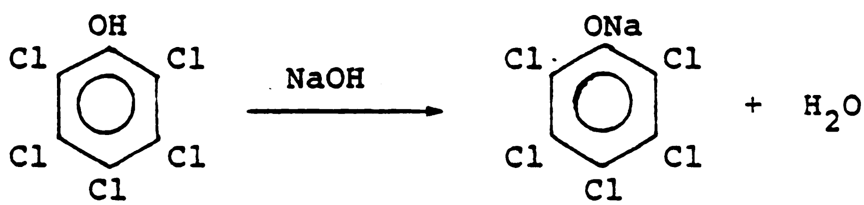
Table 1 Solubility (gm) of pentachlorophenol in 100 gm of solvent in the temperature range of $20^{\circ} - 30^{\circ}C$

water	$1.4 \times 10^{-3} - 1.9 \times 10^{-3}$
methanol	57 - 65
diethyl ether	53 - 60
ethanol	47 - 52
acetone	21 - 33
xylene	14 - 17
benzene	11 - 14
carbon tetrachloride	2 - 3

Table 2 Physical properties of pentachlorophenol

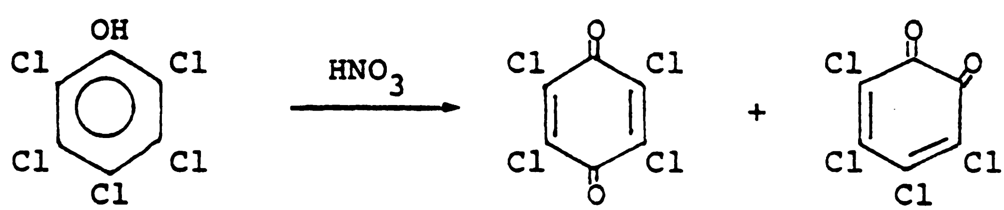
molecular weight	266.36
melting point	$190^{\circ}C$
boiling point	$293^{\circ}C$
density	1.85
vapor pressure ($20^{\circ} - 100^{\circ}C$)	$0.11 \times 10^{-3} - 0.12 \times 10^{-3}$ mm Hg
vapor pressure ($160^{\circ}C$)	5.5 mm Hg
ignition temperature (air)	$550^{\circ}C$ with formation of HCl

Pentachlorophenol is a relatively inert compound and not subject to coupling or substitution reaction common to most phenols. It dissociates with strong bases to yield the corresponding water-soluble salts (Reactionl). The metallic salt of PCP has very much the same efficacy of pesticide as PCP. Sodium pentachlorophenate is the most often specified substitution for PCP when high water-solubility is preferred.



Reaction 1 Formation of sodium pentachlorophenate

Pentachlorophenol is decomposed by strong oxidizing agents such as nitric acid, and converted to a mixture of tetrachloro-o- and p-quinones (Reaction 2).

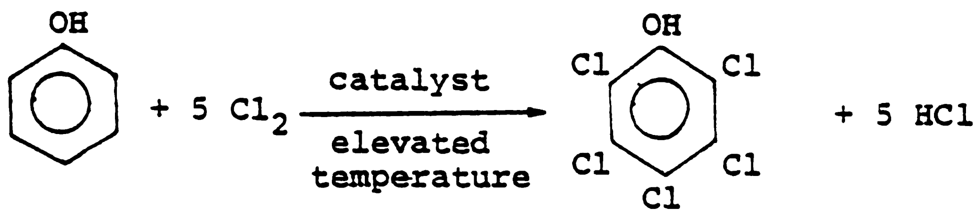


Reaction 2 Oxidation of pentachlorophenol by nitric acid

Pentachlorophenol can be readily converted to the ether derivative by reacting with diazomethane or diazoethene. This property is utilized for its analysis by gas chromatography.

Method of Industrial Production in U.S.A.

Pentachlorophenol is produced in the industry by chlorination of phenol (Reaction 3).



Reaction 3 Preparation of pentachlorophenol

The chlorination is performed in two steps. In the first step, the phenol and chlorine mixture is held at temperature 65^{o} - 130^{o}C until the melting point of the product is 95^{o}C, when most products are tri- or tetrachlorophenols. In the second step, reaction temperature is maintained at 10^{o}C above the melting point of the product until the PCP content in the mixture reaches the desired level. This process is performed substantially at atmospheric pressure, and 0.0075 mole of anhydrous aluminum chloride per mole of phenol is used as the catalyst. During this process, polychlorinated dibenzo-p-dioxins, dibenzofurans, and lower chlorinated phenols are formed. An example of the composition of industrial grade PCP is presented in (Table 3).

Toxicity Problems of Pentachlorophenol in Humans

Pentachlorophenol has been known to be a human toxicant for many years. It was used in the 1960's as a fungicide in some laundry detergents. Misuse of these commercial products containing PCP resulted in twenty poisonings and two deaths of newborn infants in a hospital nursery (1). Serum and urine analysis of one ill infant showed PCP levels of 118.0 and 64.6 ppm respectively (2). PCP residue in the contaminated linen ranged from 11.5 to 1950.0 ppm.

The use of PCP as a wood preserver in building material has also caused several toxicology problems. In one instance, a woman became ill and was diagnosed as being poisoned by PCP in her home (9). In another report, a man was poisoned by bath water contaminated by PCP leaking from pesticide mixtures used on roof timber (3). PCP was also used at one time to preserve soy sauce in Japan, although there was no official report of poisoning.

Many instances of occupational intoxication due to PCP have been reported between 1945 and 1965. In a West German plant, workers in the PCP production department complained of irritation of the eyes and the upper respiratory passages (4). Ten to seventeen of the workers developed similar skin disorders characterized by acne with pustular infection, furunculus, brown pigmentation, and some cicatrization.

Table 3 Composition of commercial grade and purified pentachlorophenol from The Dow Chemical Co., Midland, Michigan.[a]

	Pentachlorophenol	
	Commercial grade	Purified
Phenolics (%)[b]		
pentachlorophenol	88.4	98+
Tetrachlorophenol	4.4	0.27
Trichlorophenol	0.1	0.05
Higher chlorinated phenoxyphenols	6.2	0.5
Nonphenolics (ppm)[c]		
Dibenzo-p-dioxins		
2,3,7,8-tetrachlorodibenzo-p-dioxin	0.05	0.05
Hexachlorodibenzo-p-dioxin	4	0.5
Heptachlorodibenzo-p-dioxin	125	0.5
Octachlorodibenzo-p-dioxin	2500	1.0
Dibenzofurans (ppm)		
Hexachlorodibenzofuran	30	0.5
Heptachlorodibenzofuran	80	0.5
Octachlorodibenzofuran	80	0.5

[a] Analyzed by The Dow Chemical Co., (50).

[b] Determined by gas-liquid chromatography.

[c] Determined by use of an LKB 9000 gas chromatograph-mass spectrometer.

These symptoms were first noticed weeks or months after the start of PCP manufacturing. Serveral workers had neurologic pain of the lower extremities and others complained of bronchitis. All workers but one still showed extensive acne more than one year after the plant stopped PCP production. PCP levels in blood or urine were not determined.

In 1965 five cases of PCP poisoning were reported in Winninpeg, Manitoba, Canada (5). All victims worked in the same wood-processing plant. The first worker, who died five hours after being admitted to a hospital, had been employed by the plant for only one week. His job description was to dip wood into a preservative consisting of 4.1% PCP and 0.9% other chlorophenols.

The remaining four cases were nonfatal and had symptoms of excess weight loss, drenching sweating, polydipsia, evening fever, and elevated basal metabolic rate. One complained of anorexia and another vomited several times at work. Urine PCP analysis of these patients ranged from 2,400 to 175,000 ppb. Urine samples of other people working in the same general area ranged from 400 to 700 ppb. Blood samples were not collected.

More than fifty instances of occupational PCP poisoning have been reported during the early years of PCP mass production. Thirty cases have been fatal. In the last fifteen years, chemical and wood-processing plants have instituted proper precautions such as ventilating working areas and the use of protective gloves and aprons. Few poisoning cases have been reported since then.

There is still a need, however, to study the effects of chronic exposure to PCP in occupationally exposed people. Bevenue et al. (6) has done extensive studies in Hawaii where he found urine PCP levels in occupationally exposed individuals ranged from 0 to 1,840 ppb. Wylli et al. (7) reported on the case of six employees of a wood-processing plant in Idaho where samples of serum and urine were colleted and analyzed over a period of five months. PCP levels ranged from 348.4 to 3963.0 ppb in the serum and 42.0 to 760.0 ppb in the urine. Although no signs of toxicity were mentioned, PCP concentrations in several urine samples from this study were similar to those reported in other studies of PCP poisoning cases. Chromosomal aberrations were studied, but no significant differences were found compared to the normal population.

Toxicity of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans

Polychloronated dibenzo-p-dioxins (PCDDs) are a series of tricyclic aromatic compounds (Figure 2). Different isomers are formed during the synthesis of a number of chlorinated phenolic products. In the industrial grade PCP, octachlorodibenzo-p-dioxin (OCDD) and hexachlorodibenzo-p-dioxin (HCDD) are found in the highest levels, while 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is not detectable at the level of 0.05 ppm. Analysis of technical grade PCP by Dow Chemical Company is presented in Table 3.

There are few reports on the toxicity of PCDD isomers other than TCDD. But it is generally believed that all PCDDs have the same toxic effects and characteristics as TCDD. Because more is known about TCDD, this knowledge is often applied to the study of other PCDD isomers. However, TCDD is considered more toxic than the others, and is very often described as the most toxic synthetic chemical known.

The toxicity of TCDD has been associated with teratogenicity in mice, edema in chickens (8), chlorancne in man (10), and thymic atrophy and immunosuppression in several species (8, 11, 12, 13, 14, 15, 16).

Since thymic atrophy and immunosuppression are consistent findings in many species exposed to TCDD, researchers have focused much attention on the direct causes of these toxic effects and the relationship between these two.

Reduced production of thymic hormones by thymus epithilial cells can produce thymic atrophy in animals. Also zinc deficiency is proved to cause atrophy in several lymphoid organs (17, 18). With these theories in mind, Vos et al. (19) studied the cause of thymic atrophy in animals. After injection of thymosin to mice given TCDD, thymus weight and serum zinc concentrations were measured. Thymosin treatment did not alter the reduced weight of thymus, nor were the zinc levels different from controls.

In addition, phagocytosis of Lesteria Monocytogenes and the macrophage reduction of nitroblue tetrazolium were studied. The data indicated that immunosuppression is not due to a combined effect on both T-cells and macrophages; thus, an unknown effect of TCDD on T-lymphocytes is suggested.

Gross and microscopic observations showed liver lesions caused by TCDD in all the animals studied, including mice, rats and rabbits. Kociba et al. (22) noted multiple hepatocellular degenerative, inflammatory, and necrotic changes in the liver of rats given 0.1 or 0.001 ug/kg/day for two years. These animals had increased mortality and decreased weight gain compared with controls.

TCDD is also considered as a tumor initiator in rodents (23, 24). The dose of 0.1 ug/kg/day for two years increased the incidence of some types of neoplasms, but reduced the incidence of age-related lesions such as tumors of the pituitary, uterus, mammary gland, pancreas and adrenal glands which are usually encountered in the rats.

It is not known whether the toxicity of TCDD is the result of the action of the parent compound or some metabolites. However, elevated activity of certain drug metabolizing enzymes have been reported in TCDD dosed animals (25). Beatty et al. (26) observed an inverse relationship between the activity of the hepatic MFO enzyme systems and TCDD toxicity by measuring the intraperitoneal LD_{50} in rats. He proposed that the most logical pathway of TCDD metabolism be through the hepatic MFO enzyme system if TCDD is metabolized by mammals.

Kitchin and Woods (58) reported elevated activity of hepatic microsomal aryl hydrocarbon hydroxylase (AHH) in rats given TCDD. Administration of protein synthesis inhibitors, actinomycing D or cycloheximide, prevented TCDD induction of AHH completely; thus, the increased enzyme activity is explained by increased enzyme synthesis. AHH is well known to hydroxylate polycyclic aromatic hydrocarbons to yield cyclic phenols and quinones as well as carcinogenic compounds. However, scientists have not been able to demonstrate metabolites of TCDD either in vivo or in vitro (27).

Teratogenicity of TCDD has been a serious concern in environmental toxicology (30). Laboratory studies (31, 32) indicated that a single dose as little as 1 - 10 ug/kg is capable of triggering genital malformation in several research species. The most consistent effects are intestinal hemorrhage in rats, cleft palate in mice, and kidney abnormalities in both species. Chromosome mapping showed increased chromosome damage in the Vietnamese who worked in the American defoliation program during the Vietnam War (28, 29). At that time 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) was used as the defoliant in Vietnam. TCDD was present as an impurity in this product.

Polychlorinated dibenzofurans (PCDFs) are similar to TCDD in chemical structure (Figure 3). The nature of toxic responses of PCDFs is also similar to that associated with TCDD (31, 32, 33). The common toxicity symptoms are subcutaneous edema, thymic atrophy and liver necrosis.

The Fate of Pentachlorophenol in the Environment

Products from photolysis or microbial metabolism of PCP have been studied in the soil, aquatic environment, and flyash in the air. Price and Victor (33) observed a ten percent degradation of PCP in a waste holding pond; 2,3,5,6-tetrachlorophenol was the major product. Murthy et al. (34) reported the formation of lower chlorinated phenols from incubation of PCP-treated soil. Four products were found, 2,3,5,6-TCP, 2,3,4,5-TCP, pentachloroanisole, and 2,3,6-trichlorophenol (Tri-CP).

Two PCP contaminated rivers in West Germany were studied (35). Water samples were collected and analyzed for PCP and its possible products. Six lower chlorinated phenols were identified in the samples. The author explained that if PCP degradation to lower chlorinated phenols was extensive in the river, water samples taken progressively further away from the origin of the contamination should result in different ratios of PCP and degradation products. Because no changes of the ratios of different phenols were found among the samples taken from different regions of the river, it was concluded that there was no degradation of PCP to lower

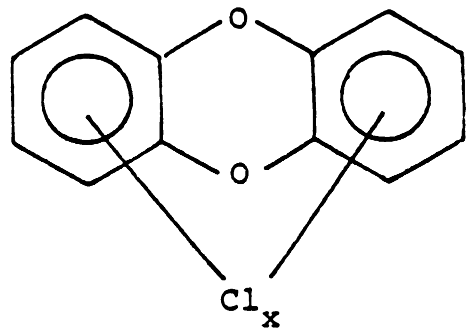


Figure 2 Chemical Structure of Polychlorinated dibenzo-p-dioxin

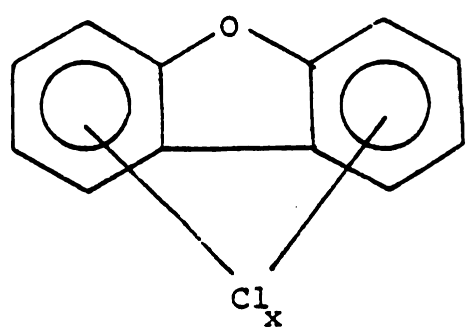


Figure 3 Chemical Structure of Polychlorinated dibenzofuran

chlorinated phenols in these rivers.

Chlorinated dibenzo-p-dioxins and dibenzofurans were also present in these rivers. Again, there was little difference in concentrations of these compounds in the samples collected from different areas.

A single axenic bacterial culture, KC-3, was shown to use PCP as growth substrate (59, 60). Reiner (36) investigated the mechanism of PCP degradation by studying metabolites accumulating in four mutant strains of KC-3. Tetrachlorohydroquinone (TCHQ), tetrachlorobenzoquinone (TCBQ), and trichlorohydrobenzoquinone (Tri-CHQB) were accumulated in three mutant cultures, while 2,6-dichlorohydroquinone (2,6-DCHQ) was accumulated in the other. In summary, the possible products for the biodegradation of PCP are lower chlorinated phenols, chloroanisoles, chlorohydroquinones, chlorobenzoquinones, and polychlorinated dibenzo-p-dioxins and furans.

Pharmacokinetics and Metabolism of Pentachlorophenol in Humans and Laboratory Animals

Metabolites of pentachlorophenol

The metabolism of PCP in rats was studied by Braun et al. (37). After a single administration of 100 mg of C^{14} PCP per kg of body weight orally, urine and blood samples were collected for eight days. Forty-eight percent of the dose administered was excreted as unchanged PCP, 10% as tetrachlorohydroquinone (TCH), and 6% as PCP-glucuronide.

Measurement of PCP and metabolites in blood plasma showed no detectable levels of TCH, whereas more than 80% of the radioactivity was due to unchanged PCP. A small fraction was present as PCP-glucuronide. The comparison of TCH and PCP-glucuronide concentrations between urine and blood plasma indicated rapid clearance of these compounds in the plasma through the kidney.

Metabolism of PCP in humans has been studied in four male volunteers (38). A single dose of 0.1 mg PCP per kg of body weight was given, and urine and fecal samples were collected for seven days.

The total amount of PCP and PCP-glucuronide in urine accounted for 74% and 12% of the dose ingested respectively. Four percent of the dose was recovered as PCP and PCP-glucuronide in the feces. This is similar to the pattern of PCP excreted in rats except that no detectable level of TCH was reported. The unchanged PCP has a half-life of 30 hours in human blood plasma.

Elimination of pentachlorophenol

The patterns of PCP excretion in animals is an important source of information for understanding the mechanism of PCP toxicity in biological systems. Results of different investigators have been somewhat variable. Bevenue et al. (6) observed PCP concentration in blood and urine of occupationally exposed people. After a rapid excretion in the first ten days after exposure, PCP concentration in blood did not decrease more than 60 - 80% for a long period of time. Thus, he suggested second compartmental control in human tissue. Braun et al. (38) could not demonstrate the two-compartment system model in study of volunteers. Instead, a one-compartment open-system model was described. In the study of pharmacokinetics in rats, differences between sexes and dose levels have been reported (37). Male rats receiving 10 mg per kg of body weight showed a two-compartment open-system model of excretion, whereas data from the female rats receiving the high dose, 100 mg per kg of body weight, suggested a one-compartment open-system.

Protein binding of pentachlorophenol in the blood

Protein binding in the blood has been suggested to increase the retention time of PCP in animals. Hobin et al. (39) studied the binding of PCP to albumin of three species: man, rat, and cow. The data of PCP/albumin was nearly the same. However, the amount of albumin binding in the plasma of humans was significantly higher than it was in the plasma of rats. An unknown factor present in the plasma must play a role in the albumin binding of PCP. This may explain the longer retention time and high blood values of PCP in humans as compared with the data from rats (40, 41).

Hot weather has long been associated with the toxicity of PCP in humans and animals (5, 42). It is believed that high environmental temperature can trigger PCP poisoning in occupationally exposed people. Studying the PCP binding in plasma or rats, Hoben et al. (39) found a reversed relationship between temperature and the amount of bound PCP. The increased toxicity of PCP in hot weather may be explained by decreased PCP-albumin binding thereby increasing the amount of free PCP in plasma. It is suspected that PCP in the free form rather than albumin bound PCP causes toxic effects in the biological system.

Effects of Pentachlorophenol on Oxidative Phosphorylation

Pentachlorophenol is an uncoupler of oxidative phosphorylation with the characteristics of biological effect similar to 2,4-dinitrophenol (43). Incubation of rat-liver mitochondria with PCP showed increased activity of adenosine triphophatase (ATP-ase) and increased oxidation of glutamic acid. However, PCP has no effect on oxygen uptake by the mitochondria except when high concentrations of PCP were incubated with the liver mitochondria and the process of oxidative phosphorylation is completely inhibited.

The radical uncoupling of oxidative phosphorylation in the tissue is responsible for the increased basal metabolic rate and hyperpyrexia common in PCP poisoned individuals. This may also explain why PCP poisoning was usually associated with environmental temperatures above 80° - 90°F.

Effects of Pentachlorophenol on Glycolysis and the Tricarboxylic Acid Cycle

The uncoupling effect of PCP in animals interrupts the link between energy supply and energy utilization. When fish were exposed to PCP, ATP concentration was decreased and therefore mitochondrial respiration was stimulated (44, 45, 46). Greater mitochondrial respiration demands more substrate; thus, the activity of enzymes in the tricarboxylic acid (TCA) cycle are expected to increase. Kruger et al. (47) and Bostrom and Johansson (46) studied PCP poisoning in fish and showed that PCP increased activity of isocitrate dehydrogenase and fumarase in the liver.

To meet the requirements of substrate for the high rate of TCA cycle, one would also expect the increased rate in the glycolysis in PCP poisoned fish. However, Bostrom and Johansson (46) observed decreased activity of pyruvate kinase and lactate dehydrogenase when PCP poisoned fish were starved for 4 days. Their study indicated the possible decrease in the rate of liver glycolysis. In the starved animals the rate of liver glycolysis was depressed due to the unavailability of glucose. Therefore, the rate of oxidation of body fat may be increased to supply substrates for TCA cycle. In normally fed animals one should observe increased rate of glycolysis to meet substrate requirements for a high rate of flux through the TCA cycle as explained in the previous paragraph. The present study was designed to measure activity of hepatic pyruvate kinase as an indication for the rate of glycolysis in nomally fed rats treated with PCP.

Teratogenicity and Fetotoxicity Study of Pentachlorophenol

There is a paucity of information on reproductive performance affected by PCP exposure. To our knowledge only two full papers have been published. They are one by Schwetz et al. (50) from the Dow Chemical Company, and the other by Larsen et al. (51) from Scott and White Clinic. Additionally, there are two abstracts, one by Hinkle (48) from the Environmental Protection Agency, and another by Schwetz and Gehring (49) from the Dow Chemical Company.

Hyperthermia is known to have a teratogenic effect on developing rat fetuses on day 9 through day 14 of gestation (52). Poisoning by PCP had always shown the elevated body temperature as mentioned previously. With these two facts in mind, Larsen et al. (51) studied placental transfer and teratogenicity of PCP in rats. When an oral dose of 60 mg per kg of body weight was given to rats, 0.05 to 0.1% of the administered dose was found per gram of fetal tissue. Body temperature increased about 0.5 to 0.8^{o}C in the animals receiving PCP; however, these were considerable lower than the temperature known to produce teratogenic effects. No significant increasing incidence of resorption and malformation were observed by giving a single oral dose of 60 mg/kg at day 8, 9, 10, 11, 12 or 13 of gestation. The compound used was 99% pure PCP.

Schwetz and Gehring (49) reported the evidence of fetal toxicity effects by giving daily PCP treatment from day 6 through 15 of gestation. Subcutaneous edema, dilated ureters and minor anomalies of the skull, ribs, vertebrae and sternebrae in pups were observed at an incidence which increased with increasing dose levels. The no effect level was 5 mg/kg/day.

Hinkel (48) gave 1.25 mg/kg or 20 mg/kg of PCP daily to hamsters from day 5 to 10 of gestation. Fetal deaths and/or resorptions were observed in 3 of 6 test groups. Concentration in entire fetuses was closely correlated to the concentration of PCP in maternal blood.

To determine the possibility that the nonphenolics in the commercial preparation of pentachlorophenol might be the cause of fetotoxic effects, Schwetz et al. (50) studied the comparison of toxic signs produced by purified and commercial grade pentachlorophenol in rats (see Table 3 for composition of purified PCP used). Maternal weight gain was decreased among dams treated with 30 or 50 mg/kg/day of either technical or purified PCP. Fetus resorptions increased from 4.2% in the controls to 27% in 37.7 mg/kg of commercial PCP treated rats and to 50% in 50 mg/kg treated rats. Purified PCP increased the resorptions to a greater extent; 100% in the 30 mg/kg and 97% in the 50 mg/kg treated rats. The ratios of male fetuses to female fetuses were increased with increasing amounts of PCP. Fetotoxic signs observed were similar to the study reported previously

(49). Purified pentachlorophenol caused the same forms of anomaly, but showed slightly more toxic effects than the commercial grade. The no-effect dose level of commercial grade PCP was 5 mg/kg/day; purified pentachlorophenol at the same level showed a significant increase of delayed ossification of skull bones.

In addition to the lack of information on the effects of PCP on the reproductive performance, none of the studies published have observed the postnatal function in the offspring from the dams receiving PCP treatments. The influences of the xenobiotics may not be limited in their effect to intrauterine life, but also exert their effects after birth. It is well known that in humans, only 10% of the abnormalities may be observed in neonates, while others will be discovered in the later developmental span. We also feel that the impairments of biological functions of the animals are just as important as the structure anomalies. An individual may survive well and appear normal in the postnatal period but belong to the categories of functional deficit in later life. Therefore, it is necessary to determine postnatal performance.

OBJECTIVES

(1) Compare the effects of technical grade PCP and purified PCP on rat reproductive performance.

(2) Study the mortality, growth rate and reproductive performance of the offspring from the dams treated with PCP.

(3) Study the effects of PCP on the activity of hepatic pyruvate kinase in pregnant rats, lactating rats and offspring.

MATERIALS AND METHODS

Reagents

The technical grade PCP and purified PCP were gifts from Monsanto Industrial Chemical Co., St. Louis, Missouri in 1977 (Table 4). The technical grade PCP was a composite of industrial grade pentachlorophenol from Reichhold, Vulcan, and Monsanto chemical companies. The composite was produced from the same lots as composites for Dr. David Firestone and Dr. John Moore of NIEHS, but not at the same time. It was also part of a composite prepared for the National Cancer Institute. Analytical data was provided for the two industrial composites prepared for Drs. Moore and Firestone.

Adenosine diphosphate (ADP), lactic dehydrogenase, phosphoenolpyruvic acid (crystalline, trisodium salt), NADH, and triethanolamine - HCl buffer (pH = 7.5) were purchased from Sigma Chemical Co., St. Louis, Missouri. Monobasic sodium phosphate, dibasic sodium phosphate, potasium chloride and magnesium sulfate were purchased from Mallinckrodt, Inc., St. Louis, Missouri. Sodium chloride was purchased from Mallinckrodt, Inc., Paris, Kentucky. Ethyl ether was purchased from J.T. Baker Chemical Co., Phillipsburg, N.J.. Corn oil was manufactured by International Inc., Clinton, Iowa.

Table 4 Analyses of the Moore and Firestone composites, and Monsanto purified pentachlorophenol (ppm)[a].

	Composite PCP		Purified PCP
	Dr. Moore	Dr. Firestone	Monsanto
Hexachlorobenzene	30	80	4.3
Octachlorodibanzo-p-dioxin	1410	1020	2.9
Octachlorodibenzofuran	182	54	0.6
Heptachlorodibenzo-p-dioxin and Heptachlorodibenzofuran	621	167	2.3
Hexachlorodibenzo-p-dioxin and Hexachlorodibenzofuran	16.2	12.4	0.72
Pentachlorodibenzo-p-dioxin and Pentachlorodibenzofuran	1.1	0.9	—[b]
Tetrachlorodibenzo-p-dioxin and Tetrachlorodibenzofuran	0.7	0.2	—[b]
Trichlorodibenzo-p-dioxin and Trichlorodibenzofuran	0.02	< 0.05	—[b]

[a] Analyzed by Monsanto Industrial Chemicals Co.

[b] Not measured

Animals

Eighty Sprague-Dawley rats from the Lab Animal Care Service of Michigan State University were used in this study. Five female rats, weighing approximately 300 gm, were caged together with a male breeder. Copulation was checked every day at 8:00 a.m.. The day on which sperm was first seen in a vaginal smear was considered day 0 of gestation. After breeding the animals were then housed individually in the animal laboratory which was controlled for temperature (74^{o} - 76^{o}F), and light cycle. Water and commerical laboratory rat chow were provided ad libitum throughout the study.

Experimental Design (FIGURE 4)

This experiment is divided into three phases. Phase I was the study of reproductive performance of the pregnant rats treated with either purified PCP or technical grade PCP. Thirty-nine bred rats were assigned randomly into seven groups. Rats in each group were treated intraperitoneally with 1 ml of corn oil containing different levels of either purified or technical grade PCP on day 3, 6, 9, 12 and 15 of gestation. The rats in the control group received only corn oil. Dose levels are 0.4, 4 or 40 mg of technical grade PCP per kg of body weight and 0.34, 3.4 or 34 mg of purified PCP per kg of body weight. These levels are designated as low, medium and high in this paper. All the rats were sacrificed on day 20 of gestation and investigated for the activity of hepatic pyruvate kinase. Data were also collected

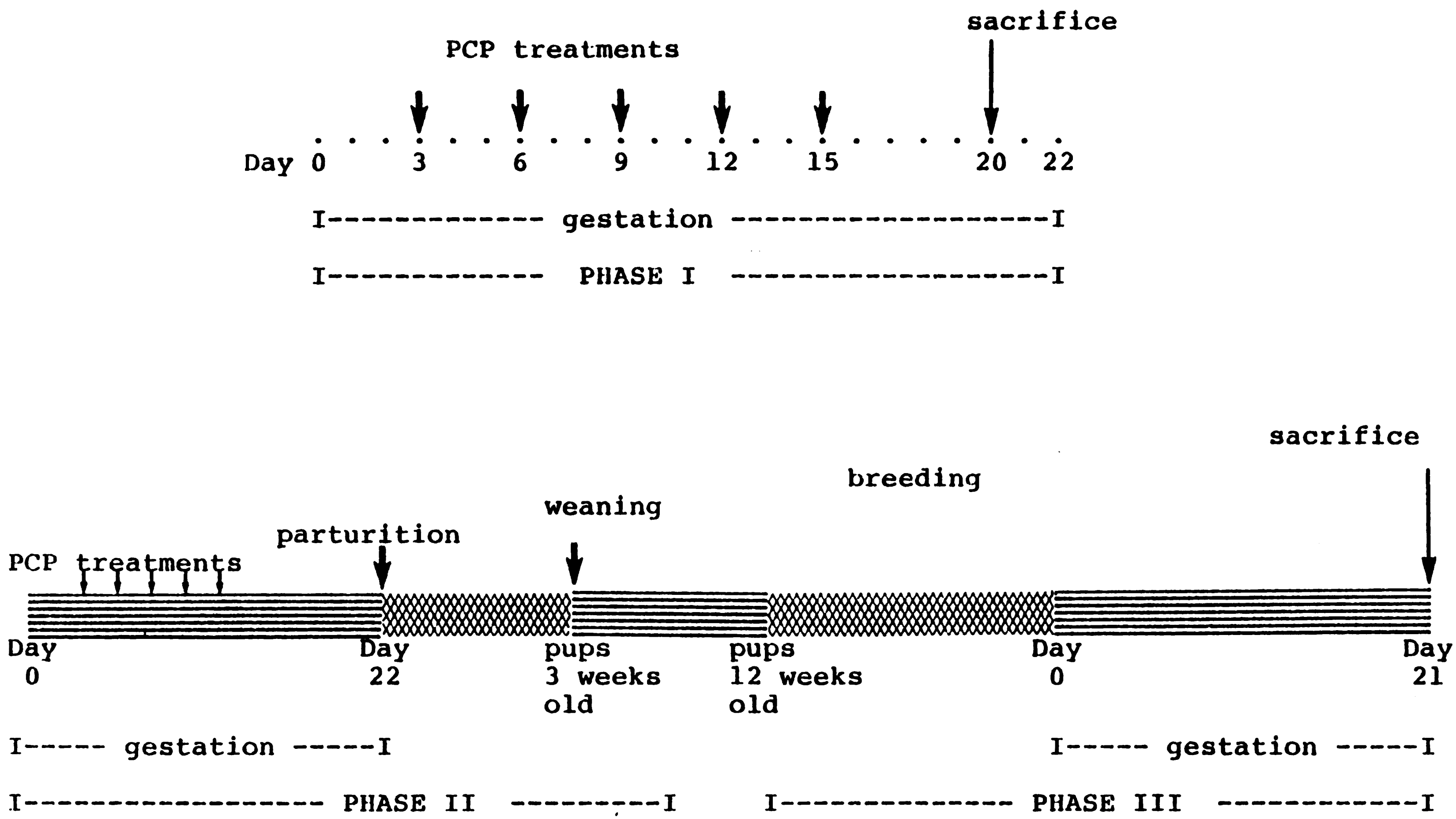


Figure 4 Experimental Design

on the fetuses at this time.

Phase II of this experiment was the study of the development of the pups from the PCP treated dams. Thirty-seven bred rats were assigned randomly into seven treatment groups and received the same treatments as decribed in Phase I, except that the animals were allowed to give birth and nurse the pups until weaning.

In Phase III, the reproductive performance of the offspring from dams treated with PCP was studied. Two male and two female offspring from Phase II experiment were selected randomly from each litter at 12 weeks of age. Copulation was allowed by caging one female with one male from the same treatment but from a different litter. Then the animals were sacrificed and the fetuses were recovered.

Parameters Measured

In Phase I, animals were observed daily for signs of toxicity. Body weight and feed intake were recorded at 3-day intervals. On day 20 of gestation, animals were sacrificed and the activity of hepatic pyruvate kinase was investigated in the dams. The uterus was opened for recording the number of fetuses (litter size), position of live, dead, and resorbed fetuses, sex ratios, weight, and external anomalies of the fetuses. In this study, sex ratio was defined as the number of males relative to the total number of fetuses.

In Phase II, the number of pups born alive or dead and data for 3-day, 1-week, 2-week and 3-week survivals were recorded. Body weight of the pups was taken at 7-day intervals from birth to 8 weeks old. Feed intake was taken from weaning (3 weeks old) to 8 weeks old at 3-day intervals. The activity of hepatic pyruvate kinase was investigated for the dams at 3 weeks postpartum and for the pups at 8 weeks.

In Phase III, copulation was taken as an indication of a complete estrual cycle for female rats; therefore, data were recorded showing the number of days the female and male were caged together until the sperm was first seen in vaginal smear. This information was also applied as the indication of the libido for the male rats.

After the female rats were bred, body weight was recorded at 7-day intervals from day 0 to day 21 of gestation. Then the animals were sacrificed and the fetuses were observed for sex ratios, weight, and external fetal anormalies. The number of corpora lutea was recorded as an indication for ovulation.

Enzyme Assay

Activity of hepatic pyruvate kinase was determined using the method of Bucher and Pfleiderer (20). In this method the total amount of pyruvate kinase is measured including the activity of the enzyme in both forms, active and inactive.

Protein Determination

Protein was determined by the method of Lowry et al. (21) using a Coleman Junior Spectrophotometer.

Statistical Analysis

The differences between control and test values for each of the parameters measured was determined using analysis of variance followed by the Dunnett-test. The specific comparisons of the effects of technical grade PCP with that of purified PCP were analyzed using designed non-orthogonal contracts and Bonferroni t-test.

RESULTS

Phase I:

Effect on Feed Intake and Body Weight Gain during Gestation (Table 5)

Total feed intake was not changed by PCP injection in any treatment groups. However, animals receiving high doses of purified PCP gained less weight than the controls. For the other treatment groups there was no difference of weight gain during gestation.

Number of Fetuses, Resorption Sites, Sex Ratios (Table 6) and Weight of Fetuses (Table 7)

A mean of 4.8 fetuses per litter was recovered from the dams receiving the high dose level of purified PCP, while 12.8 fetuses were recovered from the controls. These two values ar statistically different at the level of 0.01. There was not enough evidence to tell that either the medium or low dose level of purified PCP treated groups had different litter sizes from the controls. None of the technical grade PCP treatments affected litter size.

The number of fetuses per litter for the three purified PCP treated groups was pooled to compare with the pooled data of technical grade PCP treated groups. Treatment with purified PCP resulted in smaller litter size than with technical grade PCP ($p < 0.05$). Decreased litter size may

Table 5 Effect of pentachlorophenol on maternal feed intake and body weight gain from breeding to day 20 of gestation.

Test material[a] and dose (mg/kg/day)	No. of animals	Feed intake (gm)		Weight gain (gm)		Final body weight (gm)	
		$\overline{X}$	S.E.	$\overline{X}$	S.E.	$\overline{X}$	S.E.
Control	5	454.2	25.2	152.4	10.4	396.0	10.2
Pentachlorophenol, purified							
0.34	4	478.2	24.3	151.0	10.8	417.2	13.6
3.40	4	476.5	22.0	148.0	9.7	417.0	18.2
34.00	5	442.6	16.6	74.7[b]	14.0	338.2[c]	10.7
technical grade							
0.40	1	438.0	0.0	141.0	0.0	411.0	0.0
4.00	4	480.8	15.9	171.0	10.7	429.5	13.0
40.00	5	453.8	18.6	132.2	9.3	398.8	9.1

[a] Administered IP in corn oil on days 3, 6, 9, 12, 15 of gestation.

[b] Significantly different from the control value, $p < 0.01$.

[c] Significantly different from the control value, $p < 0.05$.

Table 6 Effect of pentachlorophenol on number of fetuses, resorption sites, and sex ratio.

Test material and dose (mg/kg/day)	No. of litters	No. of fetuses		No. of resorption sites		Sex ratio	
		$\overline{X}$	S.E.	$\overline{X}$	S.E.	$\overline{X}$	S.E.
Control	5	12.8	0.7	0.2	0.2	0.57	0.17
Pentachlorophenol, purified							
0.34	4	11.5[b]	1.0	1.2	1.0	0.53	0.23
3.40	4	11.3[b]	0.9	0.2	0.2	0.57	0.16
34.00	5	4.8[ab]	1.6	5.0[d]	2.0	0.82	0.17[d]
technical grade							
0.40	1	13.0[c]	0.0	0.0	0.0	0.69	0.00
4.00	4	13.5[c]	0.9	0.2	0.2	0.51	0.14
40.00	5	11.6[c]	1.2	1.4	0.7	0.59	0.17

[a] Significantly different from the control value, $p < 0.01$.

[bc] Pooled data from the animals treated with purified PCP is different than the pooled data from the animals treated with technical grade PCP, $p < 0.05$.

[d] Significantly different from the control value, $p < 0.05$.

Table 7 Effect of pentachlorophenol on the body weight of fetuses at day 20 of gestation.

Test material and dose (mg/kg/day)	No. of litters	Body weight of fetuses male (gm)		Body weight of fetuses female (gm)	
		$\bar{X}$	S.E.	$\bar{X}$	S.E.
Control	5	5.8	0.1	5.7[d]	0.1
Pentachlorophenol, purified					
0.34	4	5.8	0.2	5.6	0.2
3.40	4	6.0	0.1	5.8	0.2
34.00	4	5.2[c]	0.2	4.4[ab]	0.2
technical grade					
0.40	1	5.9	0.0	5.5	0.0
4.00	4	5.9	0.0	5.6	0.1
40.00	5	5.7	0.1	5.2	0.2

[a] Only two litters were observed for the female fetus body weight, because the other two litter in this group had all male fetuses.

[b] Significantly different from the control value, $p < 0.05$.

[c] Significantly different from the control value, $p < 0.1$.

[d] Significantly different from the pooled data of female fetuses from all six PCP treated groups $p < 0.1$.

explain the decreased maternal body weight gain in the same treatment group.

The sex ratio of the fetuses was higher among the dams treated with the high dose level of purified PCP compared with controls. This is not statistically significant because of the high resorption rate in this group and the small number of observations. The number of resorption sites in the high dose of purified PCP treated group (mean = 5.0) was larger than that of controls (mean = 0.2), $p < 0.05$. However, the evidence of increased resorption sites in the other treatment groups was not very strong.

Both female and male fetuses in the group treated with the high dose level of purified PCP had decreased body weight. However, the evidence of decreased body weight for the male fetuses ($p < 0.1$) was not as strong as it was for the female fetuses ($p < 0.05$). The body weight of the male fetuses in the other groups was not changes by the PCP treatment, while pooled information of the female fetuses from six PCP treated groups showed decreased body weight compared to the controls ($p < 0.1$).

Liver Weight (Table 8), Protein Content and Activity of Hepatic Pyruvate Kinase in the Prepartum Rats (Table 9)

Liver weight was not affected by any of the PCP treatments. No changes of liver protein content of specific activity of pyruvate kinase were observed in any of the

Table 8 Effect of pentachlorophenol on the liver weight of dams on day 20 of gestation.

Test material and dose (mg/kg/day)	Liver weight (gm)	
	$\overline{X}$	S.E.
Control	14.7	1.0
Pentachlorophenol, purified		
0.34	17.5	1.4
3.40	15.7	0.6
34.00	14.6	0.5
technical grade		
0.40	15.9[a]	0.0
4.00	16.2	0.9
40.00	17.0	1.0

[a] One observation in this group.

Table 9 Effect of pentachlorophenol on hepatic protein content and pyruvate kinase (PK) activity in rats at day 20 of gestation.

Test material and dose (mg/kg/day)	Protein[a] content (mg/ml)		Specific[c] activity of PK		Units of PK[d] per gram of liver	
	$\overline{X}$	S.E.	$\overline{X}$	S.E.	$\overline{X}$	S.E.
Control	35.8	1.4	22.15	0.01	12.8	0.2
Pentachlorophenol, purified						
0.34	35.3	3.0	21.07	0.02	13.4	1.2
3.40	38.4	2.4	22.20	0.01	13.1	0.8
34.00	—		—		—	
technical grade						
0.40	26.8[b]	0.0	25.20[b]	0.00	8.2[b]	0.0
4.00	33.0	3.4	32.98	0.08	16.8	2.0
40.00	37.4	3.1	24.90	0.02	15.8	1.6

[a] mg per ml of liver homogenates.

[b] One observation in this group.

[c] u mole per min per gm of protein.

[d] u mole per min per gm of wet tissue.

animals that received different levels of technical grade PCP or in those rats that received the medium or low dose levels of purified PCP. The protein content and activity of pyruvate kinase were not determined in the liver homogenates of the rats treated with the high level of purified PCP.

Pyruvate kinase activity per gram of liver was not significantly different among all the treatment groups.

Phase II:

Postnatal Performance of the Offspring from Birth to Eight-Weeks Old

The mortality (Table 10) of pups from dams treated with purified PCP tended to be higher than the pups from the dams treated with technical grade PCP from birth to day 3 after birth ($p < 0.12$). The mortality of pups from day 3 to day 7 and from day 8 to day 14 after birth was higher among the groups receiving the low dose level of technical grade PCP and the high dose level of purified PCP. However, the statistical evidence is not strong, because the high mortality was observed only in one of the five or six litters in each treatment group.

The number of male pups surviving per litter at weaning (Table 11) was not affected by either chemical, but the number of female pups surviving in the group of dams receiving high doses of purified PCP was significantly decreased ($p < 0.01$). The pooled number of female pups surviving from the three groups treated with purified PCP was less than that

Table 10 Effect of pentachlorophenol on the mortality of neonatal pups.

Test material and dose (mg/kg/day)	No. of litters	No. of Death[a]					
		birth to day 3		day 4 to 7		day 8 to 14	
		$\overline{X}$	S.E.	$\overline{X}$	S.E.	$\overline{X}$	S.E.
Control	6	0		0		0	
Pentachlorophenol purified							
0.34	5	0^c		0		0	
3.40	5	1.6^c	1.2				
34.00	6	1.0^c	0.4	0.2^b	0.2	0.3^b	0.3
technical grade							
0.40	5	0^d		1.6^b	1.6	0.2^b	0.3
4.00	4	0^d		0		0	
40.00	6	0^d		0		0	

[a] Average of the number of death per litter.

[b] Death of pups observed in one of the litters in the same treatment.

[cd] Pooled data from group of purified PCP is different than the pooled data from group of technical grade PCP, $p < 0.12$.

Table 11 Effect of sex on survival of the pups from dams treated with pentachlorophenol during gestation.

Test material and dose (mg/kg/day)	No. of pups surviving at weaning: male		female		Sex ratio[a]	
	$\overline{X}$	S.E.	$\overline{X}$	S.E.	$\overline{X}$	S.E.
Control	5.0	0.4	6.2	0.6	0.45	0.01
Pentachlorophenol, purified						
0.34	7.0	1.6	5.8[c]	1.0	0.53	0 04
3.40	6.2	0.7	4.6[c]	1.0	0.59	0.03
34.00	6.3	1.0	1.8[bc]	0.8	0.82[b]	0.03
technical grade						
0.40	4.6	0.7	5.0[d]	1.0	0.49	0.00
4.00	5.8	1.8	7.8[d]	1.0	0.42	0.04
40.00	6.7	0.8	4.2[d]	0.3	0.60	0.02

[a] Number of males relative to the total number of pups.

[b] Significantly different from the controls, $p < 0.01$.

[cd] Pooled data from groups of purified PCP is different than the pooled data from groups of technical grade PCP, $p < 0.01$.

from the three groups treated with technical grade PCP ($p < 0.12$).

The sex ratio of the pups at weaning was significantly higher for the high dose level of purified PCP treated group than the other groups.

The combined body weight of male and female pups at birth and at weaning showed no significant differences among all the groups (Table 12).

The total feed intake of the male or female pups from weaning to eight weeks old showed no significant differences from the control value (Table 13). However, the feed intake of the female pups from the high dose level of purified PCP treated group was significantly less than that from the high dose level of technical grade PCP. The body weight of the pups taken weekly from four weeks to eight weeks old was the same among all the groups for either sex (Table 14).

<u>Liver Weight (Table 15), Protein Content and Activity of Hepatic Pyruvate Kinase in the Lactating Rats (Table 16)</u>

Liver weight was not changed by any PCP treatments. Pooled data of protein concentration and unit of pyruvate kinase per gram of liver were higher from the purified PCP treated animals than that from the technical treated ones ($p < 0.12$ and $p < 0.05$, respectively). However, none of the data for individual treatment was statistically significantly different from the control values. The specific activity of pyruvate kinase was similar in all of treatment groups.

Table 12 Effect of exposure to pentachlorophenol during gestation on the birth weight and weaning weight of the pups.

Test material and dose (mg/kg/day)	Birth weight[ab] (gm)		weight at weaning[ab] (gm)	
	$\bar{X}$	S.E.	$\bar{X}$	S.E.
Control	6.93	0.15	44.3	3.4
Pentachlorophenol, purified				
0.34	6.88	0.12	41.8	1.1
3.40	6.48	0.19	46.7	2.3
34.00	6.48	0.10	46.7	4.2
technical grade				
0.40	6.58	0.32	42.6	2.8
4.00	6.83	0.12	39.5	1.6
40.00	6.65	0.12	46.3	1.7

[a] Data is reported as the average of the combined body weight of male and female pups per litter.

[b] No statistical significance was observed.

Table 13 Effect of exposure to pentachlorophenol during gestation on the feed intake of the offspring.

Test material and dose (mg/kg/day)	Feed intake (gm) from 3 weeks to 8 weeks old			
	male		female	
	$\bar{X}$	S.E.	$\bar{X}$	S.E.
Control	638	14	520	5
Pentachlorophenol, purified				
0.34	612	16	510	7
3.40	625	10	503	10
34.00	609	24	471[a]	41
technical grade				
0.40	607	15	508	10
4.00	624	14	511	29
40.00	637	14	538[b]	14

[ab] Significantly different from each other, $p < 0.1$.

Table 14 Effect of exposure to pentachlorophenol during gestation on the growth of offspring.

Test material and dose (mg/kg/day)	Body weight at 4 weeks old (gm)				Body weight at 8 weeks old (gm)			
	male[a]		female[a]		male[a]		female[a]	
	$\overline{X}$	S.E.	$\overline{X}$	S.E.	$\overline{X}$	S.E.	$\overline{X}$	S.E.
Control	84.4	9.7	78.3	3.0	283.5	8.4	206.3	4.4
Pentachlorophenol, purified								
0.34	79.5	2.9	71.5	1.3	271.2	7.9	192.6	4.9
3.40	83.2	7.7	81.0	2.2	279.4	3.4	199.0	5.8
34.00	85.8	11.0	76.8	3.8	290.5	10.1	205.6	4.7
technical grade								
0.40	80.2	8.7	74.4	4.0	269.4	15.1	197.2	4.5
4.00	74.2	5.9	70.8	3.6	267.8	11.0	187.5	7.4
40.00	82.8	3.9	75.3	1.5	285.5	6.4	199.3	6.1

[a] There is no statistical significance among all the treatment groups.

Table 15 Effect of pentachlorophenol on the liver weight of lactating rats three weeks postpartum[a].

Test material and dose (mg/kg/day)	Liver weight (gm)	
	$\bar{X}$	S.E.
Control	20.7	1.4
Pentachlorophenol, purified		
0.34	19.1	0.5
3.40	19.3	1.0
34.00	21.5	1.9
technical grade		
0.40	18.9	1.4
4.00	21.4	1.1
40.00	20.3	1.2

[a] There is no statistical difference among the treatments.

Table 16 Effect of pentachlorophenol on hepatic protein content and pyruvate kinase (PK) activity in lactating rats three weeks postpartum.

Test material and dose (mg/kg/day)	Protein[a] content (mg/ml)		Specific[b] activity of PK		Units of PK[g]			
					per gm of liver		per liver	
	$\bar{X}$	S.E.	$\bar{X}$	S.E.	$\bar{X}$	S.E.	$\bar{X}$	S.E.
Control	30.0	1.9	20.90	0.02	9.36	1.56	225	22
Pentachlorophenol, purified								
0.34	39.0[c]	2.0	20.76	0.01	12.24[e]	1.68	266[e]	17
3.40	36.4[c]	1.5	23.98	0.01	14.64[e]	0.60	281[e]	51
34.00	35.0[c]	3.0	24.18	0.03	13.32[e]	0.72	288[e]	31
technical grade								
0.40	26.7[d]	1.3	25.60	0.03	10.80[f]	1.32	206[f]	31
4.00	30.7[d]	2.3	22.10	0.03	11.28[f]	1.08	244[f]	29
40.00	35.6[d]	5.4	18.80	0.02	9.84[f]	0.84	199[f]	16

a mg of protein per ml of liver homogenates.
b u mole per min per gm of protein.
cd Pooled data from animals treated with purified PCP is different than the pooled data from animals treated with technical grade PCP, $p < 0.12$.
ef Pooled data from animals treated with purified PCP is different than the pooled data from animals treated with technical grade PCP, $p < 0.05$.
g u mole of substrates utilized per min.

Liver Weight (Table 17), Protein Concentration, and Activity of Pyruvate Kinase in the Pups (Table 18)

Pups from seven treatment groups all had similar liver weight. Protein concentration was lower in the pups from dams treated with technical grade PCP than those treated with purified PCP. Pups of the dams receiving low doses of technical grade PCP showed the greatest decrease in protein concentration among all three groups treated with technical PCP. The mean (28.1 mg/ml) was significantly different from the control (34.1 mg/ml) at the level of 0.05. Units of pyruvate kinase per gram of liver or per liver was similar among all the groups. The specific activity of pyruvate kinase tended to be higher in the pups from the technical grade PCP treated groups than the purified PCP treated groups ($p < 0.1$).

Phase III:

Reproductive Performance of the Offspring

All but one of the female rats were bred within the first estrual cycle. The exception was from the high dose level of technical grade PCP treated dams, and was bred on day 10 after being paired. A few bred rats were found not to be pregnant on day 21 of gestation when the rats were sacrificed. The number of non-pregnant rats relative to the number of observations in each treatment is presented in Table 19. No statistical significance was observed.

Table 17 Liver weight of the female offspring[a] from the dams receiving PCP treatments during gestation.[b]

Test material and dose (mg/kg/day)	Liver weight (gm)	
	$\overline{X}$	S.E.
Control	10.7	0.4
Pentachlorophenol, purified		
0.34	10.3	0.2
3.40	10.2	0.4
34.00	10.7	0.3
technical grade		
0.40	11.0	0.2
4.00	9.9	0.2
40.00	11.1	0.3

[a] Data was collected when the offspring were eight weeks old.

[b] There is no statistical difference among the treatments.

Table 18 Hepatic protein content and pyruvate kinase (PK) activity in the female offspring from the dams receiving PCP treatments during gestation.[a]

Test material and dose (mg/kg/day)	Protein[b] content (mg/ml)		Specific activity of PK (units per gm of protein)		Units of PK (u mole/min)			
					per gm of liver[g]		per liver	
	$\bar{X}$	S.E.	$\bar{X}$	S.E.	$\bar{X}$	S.E.	$\bar{X}$	S.E.
Control	34.1	0.6	24,78	0.02	14.4	0.6	154	11
Pentachlorophenol, purified								
0.34	34.1[e]	0.9	21.89[c]	0.02	12.4	1.1	128	11
3.40	32.9[e]	2.5	24.61[c]	0.01	13.7	1.2	141	10
34.00	34.2[e]	2.1	22.80[c]	0.02	12,7	0.8	138	10
technical grade								
0.40	28.1[af]	1.3	30.22[d]	0.02	13.9	0.5	154	8
4.00	29.4[f]	16	25.92[d]	0.04	12.5	1.1	122	11
40.00	31.0[f]	1.3	26.61[d]	0.03	14.6	1.0	163	14

a Data was collected when the offspring were 8 weeks old.
b mg of protein per ml of liver homogenates.
cd Pooled data from groups of purified PCP is different than pooled data from groups of technical grade PCP, $p < 0.1$.
ef Pooled data from groups of purified PCP is different than pooled data from groups of technical grade PCP, $p < 0.02$.
g No significant differences were observed.

Table 19 Fertility of the offspring from the dams treated with pentachlorophenol.

Test material and dose (mg/kg/day)	No. of non-pregnant rats	No. of bred rats
Control	0	12
Pentachlorophenol, purified		
0.34	1	9
3.40	1	7
34.00	1	3
technical grade		
0.40	1	8
4.00	0	8
40.00	0	12

No significant changes were observed in terms of maternal body weight gain, litter size, sex ratio of fetuses (Table 20), and implantation rate (Table 21). However, the number of resorptions was lower for the offspring from the PCP treated groups than that from the control group (Table 20). Rats from the dams receiving low dose levels of technical grade PCP showed the lowest number of resorption sites which was significantly different from the control value, $p < 0.01$.

All of the fetuses that were recovered had survived for longer than fifteen minutes.

Table 20 Effect of exposure to pentachlorophenol during gestation on the reproductive performance of the offspring.[c]

Test material and dose (mg/kg/day)	Maternal[d] weight gain (gm)		Litter size		No. of resorption sites		Sex ratio[a]	
	$\overline{X}$	S.E.	$\overline{X}$	S.E.	$\overline{X}$	S.E.	$\overline{X}$	S.E.
Control	114	22	13.3	3.2	1.3	0.5	0.59	0.08
Pentachlorophenol, purified								
0.34	116	21	14.3	1.9	0.9	0.5	0.48	0.09
3.40	112	20	13.8	1.8	0.5	0.3	0.50	0.11
34.00	129[b]	0	12.5	2.1	1.0	1.0	0.59	0.07
technical grade								
0.40	156	22	14.7	1.0	0.2	0.2	0.55	0.21
4.00	96	19	13.4	1.6	0.7	0.4	0.54	0.12
40.00	119	14	14.7	1.3	0.2	0.1	0.49	0.11

[a] The No. of male fetuses relative to the no. of total fetuses.

[b] One observation for this value.

[c] No statistical significances were observed for the parameters in this table.

[d] From day 0 to day 21 of gestation.

Table 21 Implantation rate of the bred rats from the dams treated with pentachlorophenol during gestation[a].

Test material and dose (mg/kg/day)	Implantation rate[b]	
	$\bar{X}$	S.E.
Control	83	7
Pentachlorophenol, purified		
0.34	88	4
3.40	75	8
34.00	74	0
technical grade		
0.40	79	7
4.00	79	2
40.00	84	6

[a] No significant difference were observed.

[b] $\frac{\text{No. of Fetuses + No. of Resorption Sites}}{\text{No. of Corpora lutea}} \times 100\%$

DISCUSSION

Purified PCP had more effect on the reproductive performance in the rats than the technical grade PCP, namely, maternal body weight gain, number of fetuses carried through gestation, number of resorption sites, weight of fetuses, and sex ratio. Mortality of newborn pups and activity of pyruvate kinase in the liver of lactating rats were also affected more by the purified PCP than the technical grade PCP.

Because of the concern for the toxicity of dioxins and furans, it has been suggested that more pure product should be manufactured by the chemical companies than is now supplied by most manufacturers. However, data from the present study does not encourage this suggestion.

The experimental results from our study and from Dow Chemical Company's study (50) are summarized in Table 22. Larger numbers of replication for each treatment in Dow Chemical Company's study than in ours probably contributes to the higher degree of confidence in reporting the toxic effects of both chemicals. Impurity content of the products used in these two studies were somewhat different, although in both studies the purified PCP had far less nonphenolics than the technical grade PCP. Compared with the chemicals used in the present study, the technical grade PCP used in Dow's study had a higher content of octachlorodibenzo-p-dioxin while the purified PCP had less content of all the

Table 22 Summary of the experimental results from Dow Chemical Company and Michigan State University[ab].

Effect of PCP treatments	DOW		M.S.U.	
	Technical grade	Purified	Technical grade	Purified
Decreased maternal body weight gain	+	+[c]	-	+
Maternal feed intake during gestation	not measured		-	-
Increased resorption sites	+	+[c]	-	+
Decreased litter size	+	+[c]	-	+
Decreased fetal body weight	+	+[c]	-	+
Decreased crown-rump length	-	+	not measured	
Increased sex ratio of fetuses	+	+	-	+
Fetal external anomalies	-	-	-	-

Table 22 (cont'd)

Effect of PCP Treatments	DOW		M.S.U.	
	Technical grade	Purified	Technical grade	Purified
Soft tissure anomalies of fetuses:				
subcutaneous edema	+	+	not measured	
dilated ureters	+	-	not measured	
Skeletal anomalies:				
skull (delayed ossification)	-	+[c]	not measured	
lumbar spurs	+[d]	+	not measured	
ribs (supernumerary, lumbar or fused)	+	+[c]	not measured	
vertebrae (supernumerary, abnormal shape, delayed ossification)	+	+[c]	not measured	
sternebrae (supernumerary, delayed or unfused centers of ossification, fused or staggered)	+	+[c]	not measured	

Table 22 (cont'd)

Effect of PCP treatments	DOW		M.S.U.	
	Technical grade	Purified	Technical grade	Purified
Liver weight of pregnant rats	not measured		-	-
Hepatic protein content of the pregnant rats	not measured		-	-
Hepatic pyruvate kinase activity of the pregnant rats	not measured		-	-
Liver weight of lactating rats	not measured		-	-
Hepatic protein content of the lactating rats	not measured		-	+
Hepatic pyruvate kinase activity of the lactating rats	not measured		-	+
Postnatal performance of the first generation from the dams treated with PCP during gestation:				
increased mortality from birth to two weeks old	not measured		-	+

Table 22 (cont'd)

Effect of PCP Treatments	DOW		M.S.U.	
	Technical grade	Purified	Technical grade	Purified
increased sex ratio at weaning	not measured		-	+
body weight at weaning	not measured		-	-
feed intake from weaning to eight weeks old	not measured		-	-
body weight from weaning to eight weeks old	not measured		-	-
liver weight at eight weeks old	not measured		-	-
decreased hepatic protein content at eight weeks old	not measured		+	-
hepatic pyruvate kinase activity at eight weeks old	not measured		-	-
fertility at eight weeks old	not measured		-	-
body weight gain during gestation	not measured		-	-

Table 22 (cont'd)

Effect of PCP Treatments	DOW		M.S.U.	
	Technical grade	Purified	Technical grade	Purified
implantation rate	not measured		-	-

[a] Symbol "+" indicates that significant change was observed.

[b] Symbol "-" indicates that no statistically significant effect was observed.

[c] Purified PCP had greater effect on the parameter measured than the technical grade PCP.

[d] Technical grade PCP had greater effect on the parameter measured than the purified PCP.

nonphenolics determined. The route of dose administration was different in the two studies; oral in Dow's study and intraperitoneal in the present study. However, both studies showed higher toxic effects of purified PCP on fetal development than that of technical grade PCP.

Compared with technical grade PCP, purified PCP has lower concentrations of nonphenolics. In the purified PCP, there are no impurities present other than what has been reported in the technical grade PCP. It seems that the high concentration of the impurities present in the technical grade PCP minimizes the toxicity of pentachlorophenol. There was no evidence supporting either theory that the toxic effects of PCP were caused by the parent compound or by its metabolites; therefore, it is difficult to propose the mechanism through which the nonphenolics cause their detoxification effects.

Ahlborg and Thunberg (53) reported that dechlorination of PCP in rats was enhanced by pretreatment with 2,3,7,8-TCDD. The dechlorination was found to be mediated by liver microsomal enzymes which metabolize PCP to form products less toxic or more water soluble. Although 2,3,7,8-TCDD was not detected in any of the PCP samples, it is generally believed that all other isomers of PCDD have the same effects on the drug metabolism system as 2,3,7,8-TCDD.

Although Larsen et al. (51) has concluded that the amount of placental transfer of PCP is negligible in rats, the present study shows that PCP greatly affects the weight and survival of fetuses and newborn pups from dams treated with high doses of purified PCP during gestation. Four possible interpretations are given for this phenomenon.

(1) In Larsen's study doses were given orally while in the present study doses were given intraperitoneally. Larger quantities of PCP might have reached the placenta when it was administered intraperitoneally rather than orally.

(2) The compound might be extensively accumulated in the fetuses after the mutiple-treatment was given to the dams.

(3) It may not be necessary for PCP to act directly on the fetuses to cause toxic effects. A non-detectable level of toxic chemical in the fetuses gives no assurance of non-effected offspring by the chemical.

(4) A very small amount of PCP exposure may cause extensive effects on the fetal and neonatal performance.

In the treatment groups receiving high dose levels of purified PCP, sex ratios of the fetuses recovered from cesarean section and of newborn pups surviving to weaning were higher than those in the control group. Fewer female fetuses and pups survived than males. Body weight of the female fetuses in the same treatment group decreased 20%, while the male's weight was not affected as much. Both parameters, sex ratios and fetal weight, indicated that during the early developmental stages the female offspring are more susceptable to the purified PCP than the male offspring.

Body weight of the newborn pups was taken as the average per litter; therefore, body weight of female pups was not available until after weaning when pups of different sex were separated. If the decreased weight of near-term fetuses is an indication of the decreased birth weight of pups, female pups from the high dose level of purified PCP treatment group should be born with less body weight than the others. However, by the 28th day when the first value of body weight was available for the female pups, no differences were observed among any treatment groups. The catch-up of body weight by the female pups must have occured during their first twenty-eight days of life.

The uptake and release of nutrients by the placenta is an energy dependent process (54). There is abundant evidence for the active transport of nutrient materials from maternal to fetal circulation; the substances which have been proved include iron, amino acids, lipids, cobalamin and calcium (55). Metabolic uncouplers decrease the uptake and release of some amino acids in the placenta (55). It would not be surprising if future research were to reveal that uncouplers also suppress active transport of other materials.

Pentachlorophenol is a powerful uncoupler of oxidative phosphorylation; hence, the intrauterine growth retarded offspring mentioned priviously may be caused by decreased nutritional supply from the maternal tissue. The catch-up growing pattern for growth retarded animals is a typical model of moderate malnutrition during the developing period. Impaired nutrient transport function may also be the cause of high resorption rates and small litter size in the same kind of treatment groups.

Pentachlorophenol as an uncoupler of oxidative phosphorylation increases mitochondrial respiration and fumarase activity in animals (27). Under this condition glycolysis is expected to increase to supply substrates for the TCA cycle. In this study increased glycolysis is reflected in the increased activity of hepatic pyruvate kinase in the lactating rats receiving the treatment of purified PCP. The increased activity of pyruvate kinase was also observed in the pregnant rats receiving the medium and the low doses of purified PCP and the high and the medium doses of technical grade PCP, although the statistical evidence was not very strong.

Pyruvate kinase is normally found in the liver in both its active and inactive form. Unless the animal has a need for a high rate of glycolysis, the demand for moderate increase in activity of pyruvate kinase can be filled by conversion of the inactive to the active form. Since the assay measures total amount of pyruvate kinase including both the active and inactive forms, it cannot detect the conversion between the inactive and active forms in vivo.

Based on the results from this study, it is proposed that PCP increases the synthesis of hepatic pyruvate kinase in lactating rats. However, the data are not as convincing for the pregnant rats. According to Romsos et al. (57), the energy requirements for lactating rats is three times that for pregnant rats. Also, the hepatic fatty acid synthesis in the lactating rats is 3.8 times that for the pregnant rats. Consequently lactating rats have a higher rate of hepatic glycolysis than pregnant rats. In pregnant rats treated with PCP, conversion of pyruvate kinase from the inactive to the active form may be adequate to stimulate glycolysis to meet energy needs. However, for lactating rats the energy demands are normally so much greater that an increase in total pyruvate kinase is necessary to support a high rate of glycolysis, particularly when oxidative phosphorylation is partially uncoupled by PCP. This may explain the difference in the amount of hepatic pyruvate kinase observed between pregnant rats and lactating rats after being exposed to PCP.

If there is increased hepatic pyruvate kinase activity in lactating animals environmentally exposed to low levels of PCP, then this may be of major concern to dairymen. In ruminant animals a very low amount of glucose is absorbed from the intestines. The liver is the major organ to supply glucose for metabolism, pregnancy, and lactation. Therefore, for rapid gluconeogenesis it is important to keep the activity of hepatic pyruvate kinase low so that phosphoenol pyruvate will be available for gluconeogenesis rather than oxidation.

It is generally believed that glucose availability is important in regulating milk synthesis. The possible increase in activity of hepatic pyruvate kinase in dairy cows exposed to PCP would decrease gluconeogenesis in the liver and consequently decrease milk production.

However, not much is known about the mechanism that keeps the activity of hepatic pyruvate kinase low in the dairy cow. PCP exposure may not increase the activity of pyruvate kinase if there is a different control mechanism in ruminants than in rats. Therefore, the high demand of substrate for increased rate of TCA cycle caused by uncoupled oxidative phosphorylation in the cow would probably be met by increased oxidation of propionate. This would decrease propionate availability for gluconeogenesis resulting in low blood glucose and low milk production.

CONCLUSIONS

(1) Pentachlorophenol at high levels did affect reproduction in rats.

(2) More toxic effects on reproduction were observed in rats treated with purified PCP than in rats treated with technical grade PCP.

(3) Prenatal PCP exposure had no effect on the reproductive performance of first generation.

(4) Further study of PCP effects on dairy cows should emphasize glucose metabolism, enzyme activity in carbohydrate metabolism, milk production, birth weight of calves and survival rate of calves.

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