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A TOXICOLOGIC ASSESSMENT OF PENTACHLOROPHENOL
IN LACTATING DAIRY CATTLE

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Animal Science

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Date 6/24/82

A TOXICOLOGIC ASSESSMENT OF PENTACHLOROPHENOL IN LACTATING DAIRY CATTLE

By

John Harold Kinzell

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Animal Science

1981

ABSTRACT

A TOXICOLOGIC ASSESSMENT OF PENTACHLOROPHENOL
IN LACTATING DAIRY CATTLE

By

John Harold Kinzell

Pentachlorophenol (PCP) is a registered antimicrobial agent widely used for the preservation of wood. Two studies were conducted in lactating dairy cattle. The first study examined the effect of subchronic oral administration of technical PCP (penta) on performance and health in adult cattle. Eight lactating Holstein dairy cattle were allotted in pairs to a control or treatment group and were fed penta from 6 ± 1 week post partum for about 135 days (0.2 mg/kg body wt/day for 75 - 84 days followed by 2 mg/kg body wt/day for 56 - 60 days). Feed intake and milk production were measured daily. All cattle were weighed bimonthly and observed daily for health effects. Blood samples were collected twice monthly and subjected to standard hematologic and clinical chemistry measurements. At two weeks and one week prior to necropsy, urine was collected from each cow by urethral catheter and subjected to standard urinalysis. One week prior to necropsy all cattle were examined by a veterinarian. At necropsy all cattle were subjected to gross pathologic examination and selected tissues were examined histopathologically. In vitro kidney function tests were conducted on tissue slices collected at necropsy. Penta fed subchronically had no significant effect on performance as indicated by feed intake, body weight or milk production. Periodic somatic cell counts of milk samples indicated that treatment did not increase the incidence

of mastitis. Generally, all cattle appeared normal throughout the entire period of exposure. Periodically, two of the four treated cattle had an elevated body temperature, mild anorexia and decreased milk production. A physical examination revealed no abnormalities. There were no biologically significant differences in hematologic measurements, clinical chemistry or urinalysis due to treatment. Organ to body weight ratios of liver, lungs, kidney and adrenals were significantly greater in the treated cattle. In three of the four treated cattle the kidneys had a pale white appearance. All other organs appeared normal. Microscopic examination of the discolored kidneys revealed a chronic, interstitial nephritis with mild hyperemia. Also the urinary bladders of all treated cattle had subacute urocystitis. The kidneys in the treated cattle were functionally impaired as demonstrated in vitro by significantly decreased uptake of para-aminohippurate, tetraethylammonium and amino isobutyrate. At the time of necropsy the kidneys may have been in a functional state of impairment, but not severe enough to cause significant changes in routine clinical laboratory tests, general health or performance of the animals. The interrelationship of the urocystitis and administration of penta is unclear.

The results of PCP analysis of tissues from penta-fed cattle revealed that the liver and kidney had the highest PCP concentrations (13.6 and 9.6 ppm) and brain and thyroid the lowest (0.7 and 0.5 ppm). At the 0.2 and 2 mg/kg doses, average milk PCP (total) concentrations were 0.4 and 3.1 ppm. The primary excretory route for PCP was urine, followed by feces and milk. Chlorodioxin (CDD) analysis of liver tissue from penta-fed cattle showed average concentrations (ppm) of octa-CDD, hepta-CDD, hexa-CDD and tetra-CDD to be 125, 14.8, 3.8 and non-detectable, respectively.

The pharmacokinetic profile of ^{14}C-PCP in a lactating dairy cow was studied in a second experiment. A mature Holstein cow fed 0.2 mg/kg body wt/day of technical pentachlorophenol for 95 days was given a gelatin capsule containing ^{14}C-PCP on alpha-cellulose. The subchronic oral administration of penta was continued throughout the duration of the experiment. Concentrations of ^{14}C were monitored in serum, urine, feces and milk through 76 hours post-administration. Maximum plasma concentrations were attained in about 10 hours. Both absorption into and elimination from serum followed first-order kinetics with corresponding half-lives of 4.28 and 42.8 hours. Approximately 75% of the dose was excreted in the urine with 5% each excreted in the feces and milk. Liver and kidney had the highest ^{14}C concentrations with brain, adipose tissue and spinal fluid having the lowest. Acid hydrolysis of the urine indicated that considerable quantities of PCP are excreted in a conjugated form. Fractionation of milk showed 62.2, 24.4 and 13.3% of the label in the whey, casein and fat, respectively.

ACKNOWLEDGEMENTS

I would especially like to express my gratitude to my wife Ann for her support and patience during the course of my Ph.D. program. Also, I thank my parents for initally encouraging me to seek a university education.

Sincere appreciation is extended to Dr. Harold Hafs and the Department of Animal Science for their financial assistance during my program. I would also like to acknowledge the assistance of my graduate committee, Drs. Bill Thomas and Mel Yokoyama of Animal Science, Dr. Stuart Sleight of Pathology and Dr. Robert Roth of Pharmacology and Toxicology. The technical assistance of Barbara Olson and my fellow graduate students is also acknowledged and appreciated.

To Dr. Peter Cheeke I feel a deep sense of appreciation for giving me the initial opportunity to pursue a graduate degree. Finally, I would like to thank Dr. Lee Shull for his friendship, support and guidance during my graduate training.

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INTRODUCTION

Pentachlorophenol is an antimicrobial and fungicidal agent used in the preservation of wood and other materials. It has also occasionally been used as a herbicide, insecticide and molluscacide.

As a clarification of terminology, pentachlorophenol or analytical pentachlorophenol (both abbreviated to PCP) refers to the chemically pure compound (99% purity). Penta and technical pentachlorphenol are terms used for the commercially-produced pentachlorophenol which varies from 85-93% in purity and contains less than 1% of dibenzo-p-dioxins (no 2,3,7,8-TCDD) and dibenzofurans as contaminants.

Penta's main use is in preservation of wood. According to Cirelli (1978) about 54 million pounds of penta were produced in 1974. Small quantities are converted to the alkali salts for use as broad-spectrum biocides, but more than 80% is used in the wood-preserving industry (Arsenault, 1976). Penta's popularity as a wood preservative stems from its superior ability to effectively control mold, termite infestation, powder post beetles and other wood-boring insects all of which decrease the structural longevity of wood (Monsanto, 1958; Dow, 1962; Carswell and Nason, 1938).

Penta-treated wood has come into relatively widespread use in the construction of housing and feeding facilities on farms. A recent study has shown that 50% of Grade A dairy farms in Michigan have used penta-treated wood in the construction of animal housing and feeding facilities (Foss et al., 1980). As is the case with many uses of efficacious

pesticides, misuse often accompanies use. In the case of construction on dairy farms, penta treated wood has been improperly and unnecessarily used in locations where moisture is not a critical factor in the structural longevity of the wood. Examples include: rafters, door jambs, fencing and splash boards well above ground level; all locations in which continual exposure to manure and moisture is not a significant problem.

In recent years, numerous pesticides which have been in commerce for many years and have not been considered to pose significant health or environmental problems have come under close scrutiny of the regulatory agencies. Some have subsequently been withdrawn from the market permanently or until sufficient data were made available to answer questions regarding their safety. Penta is no exception. Until the late 1960's, few questions were raised concerning the safety of penta, other than it might pose a possible residue problem. However, during the 1970's several situations occurred which created more concern about penta. As mentioned previously, most commercially-synthesized pentachlorophenol is contaminated to a level of about 1% with several chlorinated dibenzo-p-dioxin and chlorinated dibenzofuran congeners. Although none of the much-publicized 2,3,7,8-tetrachlorodioxin (TCDD) has ever been detected in any commercial penta, the mere presence of any dioxin congener was more than enough to bring penta into the political and scientific limelight.

Early in 1977 the state of Michigan was just recovering from the consequences of a commercial fire retardant (polybrominated biphenyls) being accidentally mixed into livestock feed and the resulting animal health problems and contamination of the food chain. The burden on the dairy industry included removal of contaminated herds, decontamination of animal facilities and purchase of replacement stock. People in the

animal industry became keenly aware of the potential health and residue problems that can result from a chemical in an animal's environment. Consequently, in 1977 when herd health and production problems were observed on several Michigan dairy farms that had previously decontaminated their facilities of polybrominated biphenyls and replaced their stock, a chemical was the suspected cause. Penta was suspected as a cause because of the prevalence of penta-treated wood on several of these farms. Attempts were made to quantitate penta in tissues of these cattle. Shortly thereafter, milk samples from one of the herds were found to contain both PCP and dibenzochlorodioxins. In these investigations, the blood of cattle in 13 herds was found to contain some PCP. These herds were subsequently quarantined by the Michigan Department of Agriculture. Penta has since lost its anonymity and status as a safe chemical. Currently, regulatory agencies are concerned about penta's possible effects on the human population resulting from residues of dioxins, furans and PCP in the food chain. Moreover, questions concerning the effects of PCP and its contaminants on the health and productivity of domestic animals have also been raised.

Prior to the initiation of the studies described herein, there were no reports which directly addressed the question of what effects subchronic exposure to penta had in lactating dairy cattle. Exposure to penta in the barn environment can occur through any one or all of three routes: oral, dermal or pulmonary. Clearly, an experiment examining all three routes would be a monumental task with considerable cost. Thus, to provide a starting point, these studies set out to address two questions. First, what are the effects of subchronic oral exposure to penta in lactat-

ing dairy cattle, and second, what is the fate of a single oral dose of ^{14}C-PCP in a lactating dairy cow previously exposed to penta subchronically?

LITERATURE REVIEW

Historical Perspective

The history of pentachlorophenol as a wood preservative dates back to 1930 when some chlorinated phenols were produced for wood preserving experiments (EPA, 1980). Interestingly, the first experiments were carried out using stakes treated with tetrachlorophenol. The stakes were installed on Barro Colorado Island in the Panama Canal Zone in 1931 as part of a study carried out by the U.S. Forest Products Laboratory and the U.S. Bureau of Entomology and Plant Quarantine. Similar studies carried out about two years thereafter with pentachlorophenol showed it to have superior wood preserving capabilities (EPA, 1980).

Usage of penta increased to the point where in 1947 about seven million pounds were reportedly used by commercial wood preservers (Hunt and Garratt, 1953). Recently, the plant capacities of the four major U.S. manufacturers were listed as follows: Dow Chemical (17 million pounds), Monsanto Company (26 million pounds), Reichhold Chemical (20 million pounds) and Vulcan Materials Company (16 million pounds) (EPA, 1980).

Pentachlorophenol has come to have many more uses than just as a wood preservative. These include use as a fungicide and/or bactericide in the processing of starches, adhesives, proteins, leather, oils, rubber and paints. It has also been incorporated into rug shampoos and textiles for mildew control. In food processing plants in pulp and paper mills PCP has been used for slime control (Bevenue and Beckman,

1967). Agricultural uses have been as a herbicide for control of weeds and as a preharvest desiccant on pasture land (Grigsby and Farwell, 1950), on pineapple and sugarcane fields (Gordon, 1956; Hilton, 1966) and rice fields in Japan (Goto et al., 1963).

Sodium pentachlorophenate, the sodium salt of PCP has been used as a molluscacide for control of snails which serve as an intermediate host of human schistosomes (Barry et al., 1950).

Agricultural applications of penta treated wood includes: treated posts and boards in the construction of fences, pens, feed troughs and bunk silos (Foss et al., 1980). Depending on the condition of the treated wood, its use in feed troughs presents a potential route for oral exposure to PCP. Similarly, applications such as on ceiling rafters and wall studs and boards provide a potential for inhalation of PCP.

As with many other useful pesticides, PCP has also been misused. One such case was the inclusion of PCP in soy sauce as a preservative by a Japanese manufacturer (Narahu et al., 1965).

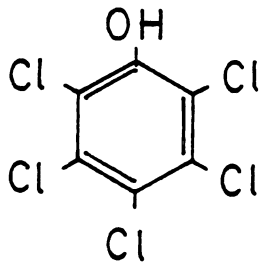
A pesticide with such wide and extensive use presents a potential risk to the environment. It was because of this suspected risk that pentachlorophenol was placed on notice of "Rebuttable Presumption Against Registration" (RPAR) by the Office of Pesticide Programs of the U.S. Environmental Protection Agency (Federal Register, 1978). Such a notice is issued when the EPA has determined there is evidence of sufficient risk in the use of a given pesticide to warrant a review of the advisability of its continued use and registration. Risk criteria are concerned with the following areas: acute toxicity; chronic toxicity (oncogenic and mutagenic); chronic effects such as reproductive (fetotoxicity,

teratogenicity, spermatogenicity and testicular effects); significant reduction in wildlife, endangered species or nontarget species; and finally lack of an emergency antidote (Chemical Regulation Reporter, 1980). There are several RPAR categories: Pre-RPAR involves an investigational phase where all the toxicological information on a particular chemical is gathered and reviewed. This phase produces a preliminary regulatory position on the potential risks of a chemical. The document is referred to as a Position Document One (PD1) and is published in the Federal Register with a formal Notice of Presumption Against Registration. The second stage or category involves issuance of the RPAR. This is a public process whereby interested parties are allowed to present rebuttals on the presumptions against registration. The period is usually about 105 days and if all risks are successfully rebutted, the chemical is returned to registration with the RPAR terminated for some or all of the uses. A second Position Document (PD2) is published in the Federal Register and becomes the terminal document of the process. When the rebuttal is not successful, the rebuttal assessment, a risk-benefit analysis and a proposed regulatory position are published in a Position Document 2/3 (PD2/3). Such a document has been released for penta (Federal Register, 1981). Review of the PD2/3 document by various agencies and panels will produce a PD4 document which announces the final regulatory action to be taken on a given chemical.

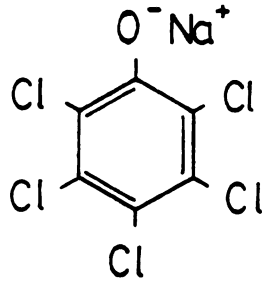
Chemical and Physical Properties of Pentachlorophenol

Pure PCP is a white, solid, aromatic organic compound with needle-like crystals (Bevenue and Beckman, 1967). Structurally it consists of five chlorine atoms (Figure 1). PCP is a weak acid with a pka of 4.74 (Table 1). As such, it reacts with strong alkali bases such as sodium or potassium

Figure 1. The Phenol and Sodium Salt of Pentachlorophenol



Phenol



Sodium Salt

TABLE 1

PHYSICAL CHARACTERISTICS OF PENTACHLOROPHENOL

Molecular weight	266.36
Melting point	190° C
Boiling point	293° C
Density	1.85 g/cm^3
Vapor Pressure (20°-100° C)	0.00011 - 0.12 mm Hg
Solubility in water (20°-30° C)	14 - 19 ppm
pKa	4.74 (EPA, 1980)

Bevenue and Beckman (1967)

TABLE 2

SOLUBILITY OF PENTACHLOROPHENOL IN VARIOUS ORGANIC SOLVENTS

Solvent	g PCP/100g solvent (20-30° C)
Methanol	57-65
Diethyl ether	53-60
Ethanol	47-52
Acetone	21-33
Xylene	14-17
Benzene	11-14
Carbon Tetrachloride	2-3

Bevenue and Beckman (1967)

hydroxide to give the corresponding water-soluble salts (the sodium salt is shown in Figure 1). The salts are highly water-soluble. At pH 8.0 the solubility is greater than 4,000 ppm (Myeling and Pitchford, 1966). As expected, the alkali salts are highly insoluble in most organic solvents. On the other hand, the phenol or protonated form is extremely soluble in most organic solvents (Table 2). Therefore, pH is a primary determinant of the dynamics and reactivity of PCP in a given environment. Moreover, pH is critical to the separation and analysis of PCP.

Synthesis of Pentachlorophenol

Pentachlorophenol is synthesized through the catalytic chlorination of phenol (Figure 2). Either ferric or aluminum chloride is the catalyst of choice. Analytical grade PCP (99% purity) can be produced in a chemistry laboratory. Synthesis of pentachlorophenol is a two-stage process which involves the catalytic chlorination of molten phenol. The temperature of the first stage is 105° C and isomers of tri- and tetrachlorophenol are formed. The second stage involves an increase in reaction temperature and further chlorination of the tri-and tetrachlorophenols. This reaction is not quantitative. Tetrachlorophenols persist and are carried through into the end product (Cirelli, 1978). It is during this stage that the various dioxins and dibenzofuran congeners found in penta are formed from reaction intermediates.

In an attempt to reduce the chlorodioxin and dibenzofuran contaminants in commercial pentachlorophenol, Dow Chemical developed a process whereby significant amounts of the dioxin impurities are removed (Johnson et al., 1973). The process not only results in decreased contaminant levels, but also increased pentachlorophenol concentration (Table 3).

Figure 2. Synthesis of Pentachlorophenol

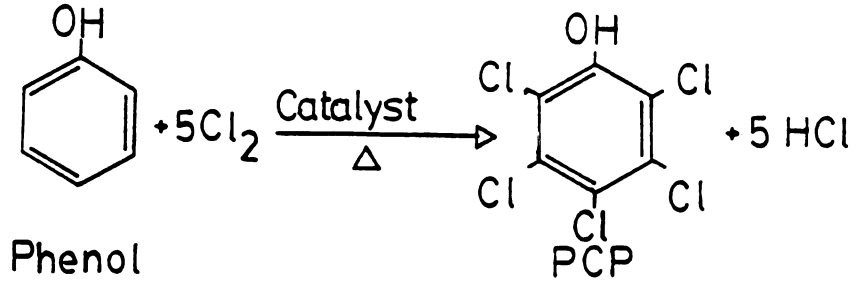


TABLE 3

PENTACHLOROPHENOL AND DIOXIN CONTENT OF
DOW'S IMPROVED PENTACHLOROPHENOL
(DOWICIDE EC-7R)

	Commercial PCP	Improved Dow PCP
Pentachlorophenol	85-90%	88-93%
Tetrachlorophenol	4 - 8%	7-12%
Trichlorophenol	0.1%	0.1%
Higher Chlorophenols	2 - 6%	0.1%
Chlorinated dioxins		
Octa	575-2510 ppm	30 ppm (max)
Hexa	9-27 ppm	1.0 ppm (max)
Tetra	0 ppm	0 ppm

Johnson et al. (1973).

Depending on the manufacturer, penta is packaged in various physical forms including pellets, prills or one-half to one ton blocks. Penta is received at pressure-treating plants in bags, bulk and solid blocks The various forms are then dissolved in petroleum solvent to form a 5-7% solution which is then used in the pressure-treatment process (EPA, 1980).

The Wood Preservation Process

Some 38 million pounds or 78% of the penta produced in the U.S. is shipped to wood preservation plants. About 35% of this is used in treating lumber and fence posts, 62% is used on utility poles and cross arms and 3% used in treating miscellaneous types of wood products (Von Rumker et al., 1975).

Penta is purchased from the chemical manufacturer and formulated into a 5% solution with solvents such as kerosene, mineral spirits or Number 2 fuel oil (Cirelli, 1978). Methylene chloride has been used when the availability of other solvents was restricted.

Wood to be treated with penta is debarked; cut into posts, poles or dimension lumber; conditioned, usually by kiln drying and then pressure-treated. Pressure treatment of hard wood species may be preceded by incising, a process in which the wood is pierced by knives to increase the penetration of the penta solution into the wood (EPA, 1980).

Preservation methods can be categorized as either pressure or non-pressure processes. Briefly, the pressure significantly increases the penetration of the preservative into the wood. The non-pressure processes include diffusion, brush, dip, thermal and cold-soak methods. Approximately 95% of all treated wood is pressure treated (EPA, 1980).

Two types of pressure-treatment systems are used, the "full cell"

Bethell Process and the "empty cell" Rueping or Lowry processes (EPA, 1980). The full cell process involves placing the dried wood in a cylinder in which it is subjected to a 22 inch vacuum for approximately 30 minutes. Then the penta solution is introduced under pressures of 50-259 psi and temperatures of 82-104^{o} C. After release of the pressure, the vacuum is applied to minimize bleeding or dripping. Bleeding may result when the low volatility solvents slowly migrate out and, in the process, serve as a carrier for a certain amount of penta. The empty cell process differs only in that no vacuum is applied initially. Rather, two to four atmospheres of pressure are applied, followed by the release of a heated penta solution into the chamber from above so that there is no drop in pressure. Like the full cell process, a vacuum is applied as a final step to minimize bleeding (EPA, 1980). Additional treatments to reduce bleeding include an expansion bath and/or a steam flash.

An alternative process is the Cellon method. It essentially involves replacement of the petroleum solvent carriers with liquefied petroleum gas (LPG). The advantage is that the LPG can be evaporated and recovered in a final step leaving only penta residue in the wood. As a result, the wood does not "bleed" because no solvent residue remains and the wood can be easily painted (EPA, 1980). Typical retentions achieved by the full cell process range from 20 to 30 pounds penta per cubic foot for most species, whereas the empty cell process results in retentions of 6 to 12 pounds penta per cubic foot (EPA, 1980).

The Thermal Process is the most widely used of non-pressure processes. This method involves soaking the kiln-dried wood in a hot penta solution. In effect, a natural vacuum process is utilized which is created by the

expansion of the air in the wood cells in the hot penta solution and contraction when the wood is quickly transferred into a cold penta solution, consequently, the cold penta solution is drawn into the wood (EPA, 1980).

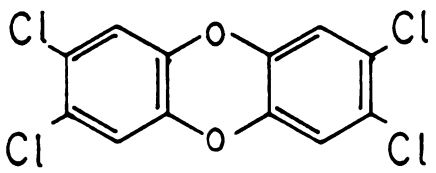
The pressure-type processes commonly utilize what are classified as low-volatility solvents: mineral spirits, kerosene or No. 2 fuel oil. Residues of the solvents remain in the wood at the conclusion of the treating cycle. A final cleanup step consisting of application of vacuum, expansion bath, or steam flash may be used. However, pressure processes often result in wood which may bleed considerably. Bleeding refers to the spontaneous migration of preservative onto the surface of the wood. Basically, it is a reversal of the pressure process. Consequently, when penta-treated wood is used in the construction of animal facilities on farms, the degree of exposure of animals by either the oral, dermal or pulmonary routes is directly related to the degree to which the surrounding wood is bleeding.

Chlorinated Dioxin and Furan Contaminants in Penta

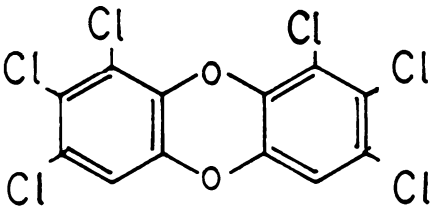
A discussion of the various aspects of penta would not be complete without mention of its controversial contaminants. These contaminants are the primary reason penta is under scrutiny by the regulatory agencies.

Much of the concern of the regulatory agencies stems from studies with 2,3,7,8-tetrachlorodibenzo-p-dioxin or 2,3,7,8-TCDD (Arsenault, 1976). However, 2,3,7,8-TCDD has never been found in any commercial PCP in the U.S. (Plimmer et al., 1973; Firestone, 1973; Villanueva et al., 1973). Therefore, the task is to evaluate the hazard of the higher-chlorinated dioxins found in penta which are hexa-, hepta- and octachlorodioxin (OCDD) as shown in Figure 3.

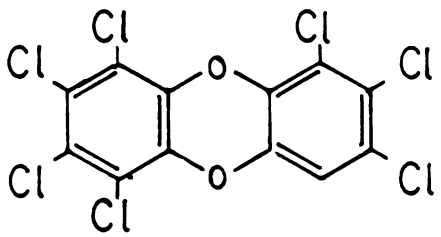
Figure 3. Common Dioxin Congeners



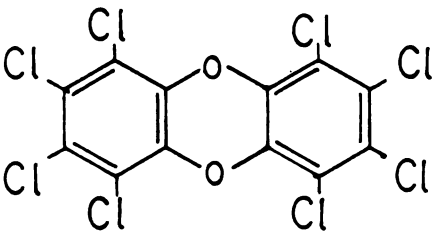
2,3,7,8 TCDD



1,2,3,7,8,9 HCDD



1,2,3,4,7,8,9 Hepta CDD



OCDD

The amounts of the various dioxin congeners in a given sample of penta vary significantly with the analytical technique. A case in point is the industrial composite used in the current study which was analyzed by two separate laboratories for dioxins (Table 4). There are marked differences in the quantities of the various dioxin congeners.

One reason for the difference in content of the congeners in a given sample can be found in the analysis itself. A critical step in the analysis of penta for dioxins involves an ion-exchange cleanup of the dioxin extract of the penta sample. Arsenault (1976) reported that the initial analysis of a sample using gas chromatography showed it to contain 2800 ppm OCDD. However, after using an ion-exchange cleanup step, which removes such minor contaminants as hydroxychlorodiphenyl ethers, octachlorodibenzofurans and hexachlorocyclohexanedione which co-chromatograph with OCDD, 1100 ppm OCDD was measured.

Another aspect of dioxin analysis is the actual generation of dioxins during gas chromatography. The same high temperature conditions which generated dioxins during the synthesis of the penta are similar to those found in the injection port and column of the GC. Typically, both the injection port and the column oven operate at temperatures greater than 200^{o} C. Jensen and Renberg (1972) demonstrated that two PCP molecules can react in a two-step reaction under alkaline conditions to form OCDD (Figure 4). The intermediate product, hydroxynonachloro-diphenyl, is termed a "predioxin". These authors developed a procedure to block conversion of the predioxin to the dioxin and in so doing, found that a sample which would have normally analyzed at 1100 ppm OCDD contained only 50 ppm OCDD. Others have also shown that the temperature

TABLE 4

DIOXIN AND CHLOROPHENOL ANALYSIS OF INDUSTRIAL COMPOSITE, TECHNICAL PCP AND DOWICIDE EC-7R

Dioxin Congener	Dowicide[a] EC-7 (ppm)	Technical[a] PCP (ppm)	Industrial[a] Composite (ppm)	Industrial[b] Composite (ppm)
Octa	16.7	340	2450	1000
Hepta	1.26	37.1	340	378
Hexa	0.08	1.8	16.7	173
Tetra	-	-	-	0.035[c]

Chlorophenols[d]	(%)
Pentachlorophenol	85-90
Tetrachlorophenols	4-8
Trichlorophenols	0.1
Other	2.6

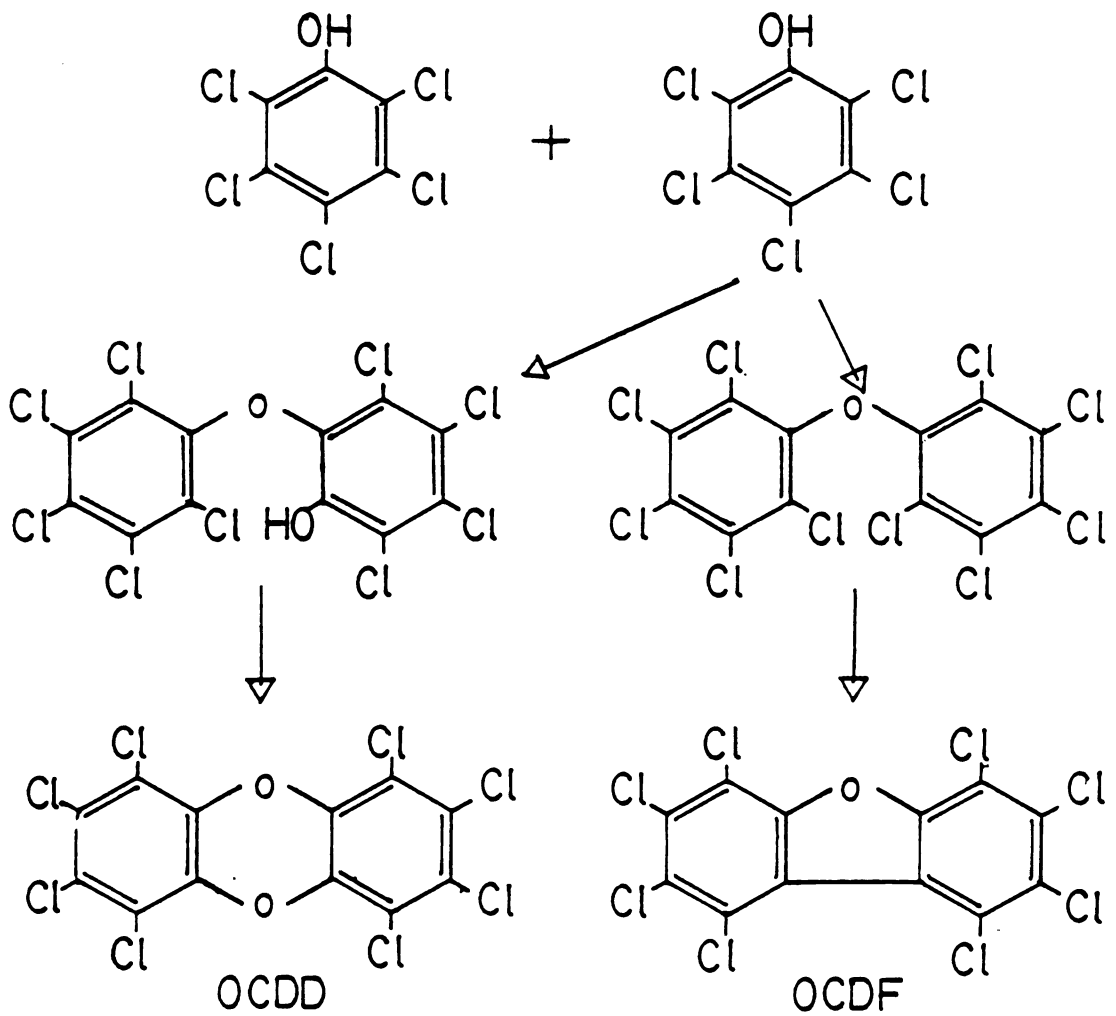
[a] Analysis courtesy of Agriculture Canada, Ottawa, Ontario

[b] Analysis courtesy of Pesticide Research Center, Michigan State University. Determined by HPLC with confirmation by GC/MS.

[c] Does not include 2,3,7,8-TCDD

[d] Reported by supplier (Roman, 1978).

Figure 4. Synthesis of Octachlorodibenzo-p-dioxin (OCDD) and Octachlorodibenzofuran (OCDF)



in the heated injection port of the gas chromatograph can contribute significantly to the overestimation of OCDD in penta. Therefore, it is important to know the method by which a sample of penta was analyzed for dioxins before judging its quality, especially if the concentration of OCDD is suspiciously high.

Analytical data available on dibenzofurans in penta are limited. However, it is apparent that similar quantitation problems like those found with dioxins also exist. In other words, the same composite analyzed by different laboratories may differ markedly in concentration of the various dibenzofuran congeners.

Toxicology of Chlorodioxins (CDD) and Furans (CDF) in Penta

The toxicity of CDD and CDF found in penta is probably best brought into perspective by comparing them to the toxicity of pentachlorophenol itself. The acute oral LD_{50} of pentachlorophenol in rats is reported to be 27-80 mg/kg depending on the solvent used (Dow, 1965). Hexachlorodioxin (HCDD) is somewhat less toxic with an acute oral LD_{50} of 100 mg/kg (Schwetz et al., 1971). There is no established LD_{50} for octachlorodioxin (OCDD). However, oral doses of 1000 mg/kg in male mice did not result in death (Schwetz et al., 1971). These data on OCDD are reasonable since it is poorly absorbed. In rats fed OCDD at the rate of 100 mg/kg, 95% of the compound was accounted for in the feces, 4% in the urine and 1% each in the fat, liver and skin (Norback et al., 1973). No acute toxicity data are available on the heptachlorodioxins, however, one would expect this group to be less toxic than HCDD. Therefore, on the basis of acute oral LD_{50}, HCDD is the most toxic dioxin in penta.

HCDD has been shown to be acnegenic (i.e. causes acne-like

dermatitis) and teratogenic (Schwetz et al., 1971). Teratogenicity was noted in pregnant female rats when fed from days 6 through 15 of gestation at the rate of 100 mg/kg/day. For a pregnant female rat weighing 0.2 kg this would amount to 20 mg of HCDD. If a value of 200 mg/kg is used as the level of HCDD in penta, the female would have to consume 100 mg of penta or 500 mg/kg body weight to produce terata in her offspring. This level of exposure is highly unlikely. Therefore, unless substantial subchronic preconception exposure to dioxins in penta occurred, the most likely outcome would be PCP intoxication.

There is the possibility that some HCDD congeners may be carcinogenic. The National Cancer Institute recently reported the results of an oral dosing study in rats and mice and a dermal study in mice (Chemical Regulation Reporter, 1980). Both studies were conducted over a period of 104 weeks. HCDD caused increased incidence of hepatocellular carcinomas in female rats and increased hepatocellular carcinomas and adenomas in both male and female mice. However, HCDD was not demonstrated to be carcinogenic in male rats. Similarly, HCDD was not carcinogenic in dermal bioassays in either female or male mice.

An additional concern regarding CDD and CDF is the translocation of residues into produce from domestic animals which are exposed to penta. Firestone et al. (1979) found three CDD isomers in milk, blood and adipose tissue of lactating cattle fed penta; these were 1,2,3,6,7,8-hexa-CDD; 1,2,3,4,6,7,8-hepta CDD and octa-CDD. In another study, yearling Holstein heifers were fed various mixtures of PCP and penta ranging from 100% analytical or pure PCP to 100% technical PCP at a dose level of 15 mg/kg body wt. per day (Parker et al., 1980). These authors showed that CDD and CDF concentrations in liver and adipose tissue were correlated with the

concentration of technical PCP in the diet. Moreover, the liver was shown to contain much higher levels of CDD's than adipose tissue.

Some caution should probably be used when comparing the risks of dioxin and the dibenzofuran contaminants in penta. However the concentrations of the octa- and heptachlorinated dibenzofurans are similar to the concentrations of the octa- and hepta-chlorodioxins. Moreover, they appear to cause toxic effects similar to chlorodioxins, but with a somewhat lower relative potency (WHO/IARC, 1978).

Analysis of Pentachlorophenol

Separation

There are several key considerations when determining an acceptable method for analysis of a given compound. One of these is the method by which the compound of interest is removed from a biological matrix such as blood, urine or tissue. Clearly, the optimal separation method is uncomplicated, rapid and recovers the maximum amount of the compound. Because pentachlorophenol is a weak acid (pKa 4.74) manipulation of pH provides a valuable tool for separating pentachlorophenol from a biological tissue or fluid. For instance, at pH 8.0 the solubility of the sodium salt of PCP is greater than 4,000 ppm in water (Myeling and Pitchford, 1966) and it is essentially insoluble in an organic solvent such as benzene or hexane. Conversely, at pH 2 essentially all of the PCP will be in the phenolic form and, as such, is virtually insoluble in any aqueous phase. Therefore, in extracting PCP from serum, the pH of an appropriate volume (1-2 ml) is adjusted to pH 2 and then the acidified aqueous phase can be extracted with an organic solvent. Because PCP is essentially insoluble in the aqueous phase at this pH, it will very efficiently partition into the benzene (upper) phase. A portion of the benzene

phase can then be analyzed directly by gas chromatography. If the concentrations of PCP are high enough, the PCP can be partitioned back into an aqueous phase which can then be analyzed by high performance liquid chromatography.

Manipulation of the partitioning characteristics of PCP through changing the pH becomes even more useful in analyses of lipid-containing tissues. Few chromatography systems are compatible with analysis of extracts containing lipid. Therefore, an efficient method to remove the lipid prior to the final analysis of the solvent extract of the tissue is necessary.

Traditionally, some kind of stationary phase has been used to trap the lipid, leaving the solvent extract lipid-free and ready for analysis. The alternative to this is to make a homogenate of the matrix of interest, then alkalinize it with weak base solution, such as NaOH, to a pH of approximately 10. The lipid in the alkalinized homogenate can then be extracted with a solvent such as hexane. Once the lipid is extracted, the aqueous phase can be acidified and the PCP extracted back into an organic solvent which can then be analyzed on the gas chromatograph. The main advantage of such a clean up method is that it is rapid and economical because both the clean up step and final extraction are carried out in the same test tube.

Detection

Once pentachlorophenol has been extracted into an organic solvent, the problem becomes one of how to determine the concentration using the appropriate detection method or analytical instrument. (Bevenue and Beckman (1967) reviewed the various methods for detection of PCP;

colorimetry, spectrophotometry, and gas chromatography (GC). Basically, the colorimetric methods lack the sensitivity of the gas chromatographic methods and, as such, require large samples for analysis. Moreover, other chlorinated phenols and substances inherent to the various matrices cause significant interference problems. GC methods, however, do not have these problems. For samples that have been sufficiently cleaned up, current column packing (solid phase) technology provides effective separation of PCP from other phenols (Supelco, 1978).

The detector of choice is ^{63}Nickel electron capture (ECD). This detector has a high sensitivity to halogenated aromatics with detection limits of 3-10 ppb. Cheng and Kilgore (1966) reported a GC method in which PCP was converted to the O-methylated derivative (pentachloroanisole) using diazomethane. The pentachloroanisole was then detected using electron capture, with a lower detection limit of 10 ppb. Using the same derivatization method and detector, but with a different column packing Bevenue et al. (1966) reported an even lower limit of 3 ppb. Other detectors have been used such as flame ionization (Barry et al., 1962; Koloff et al., 1963; Smith et al., 1964), hot wire (Kanazawa, 1963), microcoulometer (Yip, 1964) and thermal conductivity (Narahu, 1965).

Until recently, derivatization of PCP with diazomethane has been the most desirable method to prepare PCP for gas chromatographic analysis. Derivatization of PCP as described by Bevenue et al. (1966) and Cheng and Kilgore (1966) using diazomethane prepared by the method of Aldrich Chemical Co. (1954) is still routinely used in the preparation of PCP extracts for GC-ECD analysis. However, this derivatization step has several drawbacks. First, both preparation and use of the diazomethane carry with it the risk of explosion. Second, the glass diazomethane generation kit is relatively expensive and requires

extensive care when being handled and cleaned. Dust particles or minor surface scratches provide sites for cystallization of diazomethane and the resulting potential for explosion. Finally, the derivatization requires a second set of glassware in which to carry out the methylation reaction.

Because of the problems associated with derivatization using diazomethane, successful efforts have been made toward developing other derivatives. Chau and Colburn (1974) have reported a method where PCP was extracted from sewage water into benzene. The PCP was then extracted from the benzene into potassium carbonate and subsequently acetylated with acetic anhydride. The PCP acetate was then extracted from the potassium carbonate solution into hexane and analyzed using GC-ECD. The authors reported a minimum detection limit of 0.177 ppb.

More recently, a nonderivatized method has been reported (Supelco, 1978). The column packing used has had the "active sites" on the column support deactivated. Most column packings used in the separation of pesticides by gas chromatography are composed of a support, which is often silica coated with an organic compound. The coating process is not complete and as such, leaves some silica exposed. Because PCP readily binds to silica, the result is some loss of the sample of the column before it reaches the detector. The new packing developed by Supelco (Bellfonte, PA) is the result of a process whereby the exposed silica or "active sites" have been deactivated chemically. Consequently, PCP can be efficiently separated using this column packing with a minimal "on column loss" from binding to the silica support.

Toxicology of Pentachlorophenol

Exposure

PCP from penta-treated wood can enter animals through one or all of the following routes: oral, dermal of pulmonary. Unfortunately, relatively little is known about exposure of domestic animals to PCP through any of the routes.

Studies on pulmonary exposure are essentially non-existent. However, a recent study examined the effect of the type of solvent used to treat wood with penta on subsequent air concentrations (Thompson, 1978). Wood treated with penta dissolved in either heavy oil, liquefied petroleum gas (LPG) or methylene chloride was placed on a 30 liter test chamber at 30^{o} C with an air flow rate of one liter per minute. The air levels of PCP associated with the various solvents were 0.02, 0.048, 0.76 mg/m^3 for heavy oil, LPG and methylene chloride, respectively. The vapor density for PCP at 20^{o} C is 0.00011 mm Hg (Bevenue and Beckman, 1967) which translates to an equilibrium vapor density of 0.0017 mg/liter or 1.7 4 mg/m^3. Although it must be kept in mind that this value is for analytical PCP and not penta which is 85-94% PCP, both the solvent and the wood appear to have a significant retarding influence on the volatilization of PCP. Most wood is treated with solvents such as kerosene, mineral spirits or No. 2 fuel oil, which are similar to heavy oil in terms of solvent polarity. Although no information exists on pulmonary exposure in domestic animals, one can estimate the pulmonary exposure of a cow. In a barn partially constructed from wood treated with penta in methylene chloride, the expected air level of PCP would be about 0.076 mg/m^3 (Thompson, 1978). If a 514 kg cow breathes at the rate of 109 liters per minute, (the average of 104 L/min lying down and 114 L/m standing)(Respiration and

Circulation, 1971), in 24 hours she would have inhaled 150,960 liters of air or 156.9 m^3. This translates to a dose of 0.006 mg/kg. Factors such as ambient temperature, barn ventilation, proximity of the animal to the wood and extent of bleeding of the wood could significantly affect pulmonary exposure. Exposure to dioxins or furans in penta via this route would be insignificant due to the fact their vapor pressures are quite low (Anonymous, 1978).

Dermal exposure is probably more of a problem in swine than in cattle because of lack of a substantial hair coat and usage of penta-treated wood for flooring which swine lie on. Documented cases of health problems in swine have occurred when sows were confined in farrowing crates constructed of wood recently treated with penta (Walters, 1952; Schipper, 1961). The new-born piglets were found to be especially sensitive, but when the treated wood was covered with plywood or bedding, the problem disappeared.

Gastric exposure is a potential route of entry for both cattle and swine. Feed bunks and bunk silos, especially when they are constructed of recently treated or bleeding penta lumber, present an opportune site for oral exposure for cattle. Exposure can occur by either eating contaminated food from the bunk silo or directly by licking the penta lumber in the feed bunk. Swine, on the other hand, could be exposed orally by eating out of penta-treated troughs, chewing on pen boards treated with penta, or rooting on pen floors treated with penta. Schipper (1961) reported high piglet mortality when sows and their litters were exposed to farrowing pens constructed of penta lumber. In this case, there was a strong indication that the piglets refused to nurse. This is in agreement with Deichmann et al., (1942) who reported that when PCP was

included in the diet of rats their feed intake was reduced. Similarly, cats refused to eat salmon when it contained PCP (Deichmann et al., 1942). Also, cattle refused to graze pasture which had been sprayed with an emulsion of diesel fuel, water and penta (Grigsby and Farwell, 1950). However, it was not determined in this study whether the diesel fuel or the PCP was the reason for the avoidance.

In summary, most studies have examined oral exposure of domestic animals to penta. Therefore, much remains to be done regarding the pulmonary and dermal routes of exposure and the toxic effects of the various solvents.

Toxicokinetics of Pentachlorophenol

Absorption

Radiotracer studies, which have examined the kinetics of carbon-14 pentachlorophenol (^{14}C-PCP), demonstrated that absorption of PCP, regardless of the route, is both rapid and extensive. Jakobsen and Yllner (1971) injected mice either subcutaneously or intraperitoneally with ^{14}C-PCP and were able to account for 85-89% of the dose in the urine, feces and various tissues. In another study, male and female rats were given a single oral dose of either 10 or 1000 mg/kg (Braun et al., 1977). Again, by analysis of urine, feces and tissues these authors accounted for 99.8 and 97.6% of the dose in rats given 10 and 100 mg/kg ^{14}C-PCP, respectively. Furthermore, plasma levels of ^{14}C increased rapidly after ingestion and peaked within the first six hours. A sex difference was noted in that female rats absorbed PCP at a slower rate, but had higher ^{14}C concentrations in the plasma and almost every tissue at any given time, than did male rats. In a similar study with male and female Rhesus monkeys given a single oral dose of ^{14}C-PCP, the

^{14}C concentrations in the plasma also increased rapidly but required 12 to 24 hours to reach maximum concentration (Braun and Sauerhoff, 1976). A sex difference was also observed, however, in contrast to rats, the female monkeys absorbed PCP at a faster rate, but the ^{14}C concentrations in the plasma peaked at a lower concentration compared to males. Male monkeys absorbed PCP at a somewhat slower rate and the peak ^{14}C concentrations were higher than in the females.

The rapid appearance of ^{14}C in the blood and the high degree of absorption of pentachlorophenol are understandable in terms of gastric absorption. Most compounds cross both membranes through simple diffusion (Casarett and Doull, 1975). The current concept (Cohn, 1971) is that the cell membrane is a bimolecular layer of lipid molecules coated on each side with a protein layer. Therefore, movement across this structure is largely determined by the lipid-water partition coefficient of a given compound and secondly by the concentration gradient across the membrane (Casarett and Doull, 1975). Furthermore, many compounds are classified as weak acids or bases and as such can exist in solution in ionic and nonionic forms. For instance, the amount of a weak acid in the un-ionized form is dependent on its disassociation constant (Ka) which is the negative logarithm of the acidic disassociation constant (Ka). Pentachlorophenol is classified as a weak acid with a pKa 4.74 (EPA, 1980). Consequently, at a low pH PCP is very lipid soluble (Table 2). Assuming that the pH of a Rhesus monkey's stomach is close to that of a human (pH 2) and applying the Henderson-Hasselbalch equation (pH = pKa - log unionized/ionized), one can calculate that the ratio of the unionized

or protonated form of PCP to the ionized form is 501 to 1. Conversely, if the pH of the intestine is approximately pH 6, the ratio of the ionized to unionized forms is 20 to 1. Thus, under the latter condition much less PCP will be absorbed. Clearly, the absorption of PCP is favored in the more acidic environment of the stomach vs the environment of the intestine.

The gastrointestinal tract of the ruminant presents a somewhat different situation in terms of absorption of PCP. Unlike the monogastric or simple-stomach animal, the ruminant has four stomachs or compartments prior to the intestine: the rumen, reticulum, omasum and abomasum. All differ in pH, the most basic being the rumen at pH 6.5 and the most acidic being the abomasum at pH 3 (Phillipson, 1977). Thus, the abomasum would be the most favorable environment in which PCP could be absorbed and the rumen the least favorable.

Distribution

There are two important aspects associated with the distribution of PCP: its affinity for binding to serum proteins and the pattern of distribution in the various tissues of the body.

Blood is the means of distribution for most compounds absorbed from the stomach or intestines. Depending on its chemical characterisitcs, a compound may become associated with one or several components of the blood. The proteins in the blood, particularly albumin, can act as a storage depot for many foreign compounds (Casarett and Doull, 1977). The first study to examine the binding relationship of PCP and plasma proteins utilized an ultrafiltration membrane system (Braun et al., 1977). The procedure involved adding 5 ml of rat plasma to the untrafiltration membrane, which retained the protein components, plus

PCP at 1 or 100 mg/ml. When the loaded system was washed with phosphate buffer, the ultrafiltrate contained no ^{14}C. The authors concluded that the retention of the ^{14}C-PCP on the membrane was most likely due to PCP binding to plasma proteins. At 900 mg PCP/ml 94% of the ^{14}C-PCP was retained, indicating that even at very high concentrations of PCP its binding is extensive. Through similar types of experiments these workers also characterized the binding to be of a heterogeneous nature and the molar binding affinity constants in bovine serum albumin (BSA) were found to be 0.66 x 10^6 per mole and 2.1 x 10^6 per mole for K_1 and K_2, respectively. It has been demonstrated, in studies examining the effects of the molar binding affinities on the properties of drugs, that protein binding becomes a significant property of a given drug when its affinity constant(s) is 10^4 or greater (Keen, 1971).

The effects of temperature and pH on PCP binding to BSA have also been studied (Hoben et al., 1976a). When a dialysis bag system was used in conjunction with increasing concentrations of PCP against a fixed amount of BSA, these authors found that a linear relationship exists between the PCP concentration and the amount of PCP bound per milligram of BSA. Moreover, by varying the BSA concentration with a fixed concentration of PCP they found that as the protein concentration increased the amount of PCP binding decreased. The authors also suggested these results indicate that PCP binding to BSA is of a heterogeneous type. The number of binding sites for PCP on BSA was determined to be thirteen. An inverse relationship between pH and binding of PCP to BSA was demonstrated. Two explanations were suggested, both supporting the idea that the unionized species is the one involved in binding. First, as the pH approaches the pKa of PCP (4.74) the proportion of unionized

species increases, as does binding. Similarly, as the pH increases so does the net negative charge of the protein and the amount of ionized PCP, resulting in a repulsion by the negative charges of each other and a concomitant decrease in binding. Moreover, binding was directly related to temperature, i.e. as the temperature was increased from $5^{o}C$-$40^{o}C$ so did the amount of PCP bound.

The amount of PCP bound to albumin is nearly the same in the cow, rat and man (Hoben et al., 1976a). However, in a comparison of rat and human plasma, both of which can be about 50% albumin, the amount of PCP bound in human plasma was twice that in the rat. The authors suggested that other factors, in addition to albumin, affect retention of PCP in plasma. These results agree with earlier observations that at the same dose, humans had significantly higher blood PCP concentrations than did rats (Casarett et al., 1969; Hoben et al., 1976a). Studies examining the distribution of PCP in the various body tissues and fluids of different species generally show the same pattern. In a study carried out with mice, the highest concentrations of ^{14}C activity were found in the liver and the intestines, followed by the kidney, heart and lungs. Whole body autoradiograms taken four hours after injection showed the greatest ^{14}C concentration in the fundus wall of the stomach and in the intestinal contents (Jakobsen and Yllner, 1971). The high ^{14}C content in the wall of the stomach would tend to suggest that this is a major site of absorption for PCP. However, these workers suggested that this is also evidence of secretion of PCP in the gastric juices. Larsen et al. (1972) dosed rats

with ^{14}C-PCP and found the highest concentrations of ^{14}C in the liver followed by the kidney, blood, stomach, adrenal and lung. Adipose tissue and muscle had the lowest, and nearly equal, concentrations of ^{14}C. Similar results were obtained when male and female rats were dosed with ^{14}C-PCP (Braun et al., 1977). They found ^{14}C primarily distributed, in decreasing magnitude, in the liver, kidney and plasma, with brain and adipose tissue having the least. The preferential retention of ^{14}C in the plasma was suggested to be primarily related to the strong tendency of PCP to bind to plasma protein. Almost every tissue, as well as the plasma, in female rats had a greater concentration of ^{14}C than did the male rats. In contrast, Larsen et al. (1972) reported no observable sex differences, in terms of tissue or fluid concentrations of ^{14}C, in rats. In a study in which male and female Rhesus monkeys were given a single oral dose of 10 mg/kg ^{14}C-PCP, the percentage distribution of ^{14}C was: liver, 1.1%; small and large intestine, 7.6%; and the remainder of the tissues, 2.76% (Braun and Sauerhoff, 1976). Carbon-14 in the urine and feces accounted for 70 and 18% of the dose, respectively.

The only study examining PCP residues in milk, to date, involved lactating dairy cattle which were fed 10 mg penta per kg body weight per day for 60 days (Firestone et al., 1979). The PCP concentration measured in the milk, at steady state, was 4 µg/ml (ppm).

Metabolism

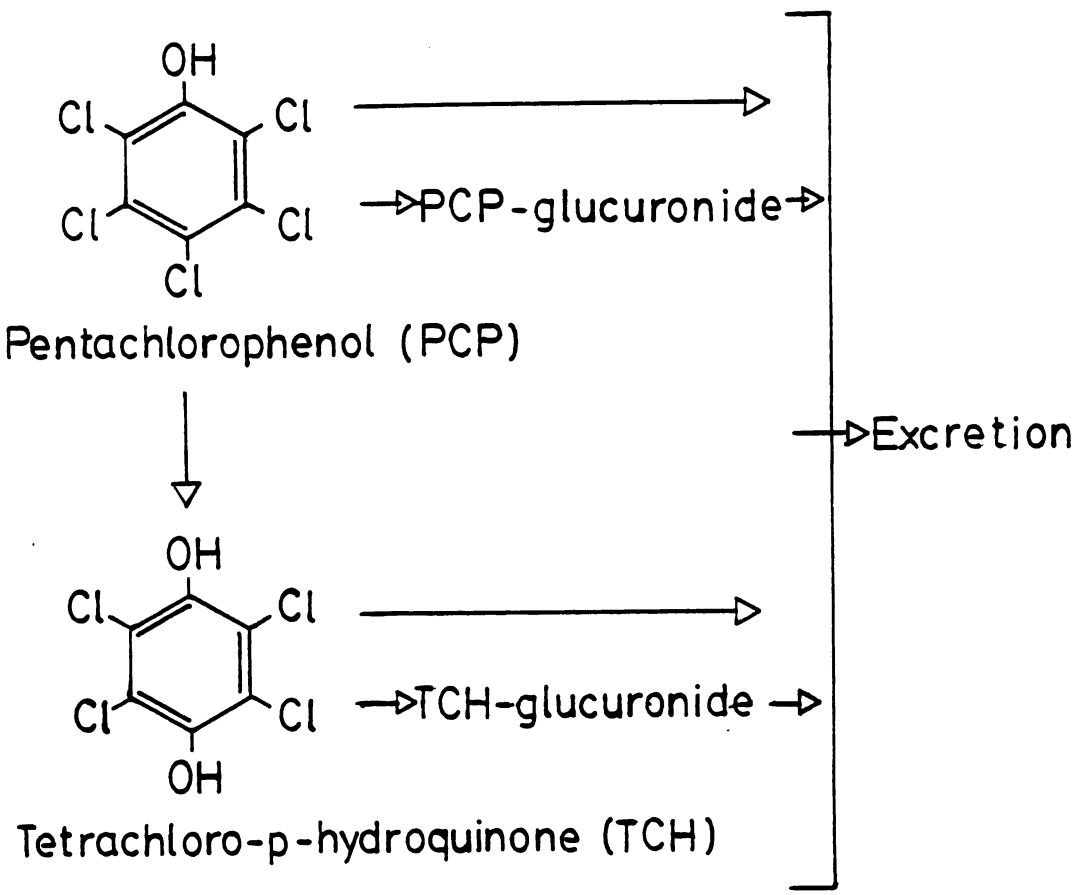
The metabolism of PCP has been relatively well defined in several mammalian species. With the exception of the mouse, most studies show PCP is excreted largely unmetabolized in the urine, with the metabolites

being tetrachlorohydroquinone (TCH) and the glucuronide conjugate of TCH and PCP.

In a study examining ^{14}C-PCP metabolism in mice, about 30% of the dose was excreted unchanged in the urine as PCP (Jakobsen and Yllner, 1971). Upon acid hydrolysis, this value increased by about 8% indicating the presence of some type of conjugate. Approximately 21% of the ^{14}C in the urine was identified as tetrachlorohydroquinone (TCH). An accurate estimate of the amount of conjugated TCH was not available, due to lack of sample. After an acid hydrolysis step, they found the ^{14}C-TCH and ^{14}C-PCP accounted for all of the ^{14}C present. Similarly, Braun et al. (1977) reported that most of the ^{14}C in the plasma of rats administered ^{14}C-PCP was unchanged PCP, with only a small amount present as the glucuronide conjugate. These authors further determined that ^{14}C-PCP and ^{14}C-tetrachlorohydroquinone accounted for 75% and 15% of the administered dose in the urine, respectively. The remainder of the ^{14}C in the urine (9.4%) after treatment with glucuronidase was shown to be ^{14}C-PCP glucuronide (Figure 5). These authors reported if the urine were not extracted to remove both PCP and TCH, they would inhibit the glucuronidase hydrolysis of the conjugate. Ahlborg et al. (1974) also reported that TCH inhibited hydrolysis of the PCP-glucuronide by glucuronidase. Braun and Sauerhoff (1976) found that in male and female monkeys given a single oral dose of ^{14}C-PCP, all of the ^{14}C in the urine was PCP.

Ahlborg et al. (1978) further characterized the metabolism of ^{14}C-PCP and its metabolites in urine. They reported the percentage distribution of the ^{14}C-PCP dose in the urine to be 60% PCP; 9-16% PCP-glucuronide and 16-22% TCH-glucuronide. The

Figure 5. Metabolism of PCP in the Rat



liver enzymes involved in metabolism of PCP to TCH are inducible with phenobarbital (Ahlborg et al., 1978). These workers characterized the effects of phenobarbital, a known inducer of hepatic microsomal enzymes (Conney, 1967), on the metabolism of PCP. The effects of SKF 525-A, a known inhibitor of several microsomal enzymes (Anders, 1971), were also examined. Rats pretreated with phenobarbital showed a 6-fold increase in conversion of PCP to TCH in the first 24 hours, whereas rats pretreated with SKF 525-A once or twice showed 3- and 2-fold increases, respectively. Rats treated repeatedly every 6 hours for 24 hours with SKF 525-A did not show an increased conversion of PCP to TCH. Further in vitro studies using liver microsomes from phenobarbital-pretreated rats showed an approximate 3-fold increase in conversion of PCP to TCH. Conversely, when ^{14}C-PCP was incubated with microsomes from SKF 525-A pretreated rats, there was a marked inhibition of conversion of PCP to TCH.

Penta contains up to 12% 2,3,4,6 tetrachlorophenol (Johnson et al., 1973). Ahlborg and Larsson (1978) have shown that this isomer is excreted essentially unchanged in rats.

Excretion

The main route of excretion of pentachlorophenol appears to be urinary, with the remainder being fecal. Jakobson and Yllner (1971) reported results of an experiment where ^{14}C-PCP was administered subcutaneously and intraperitoneally to mice. They found that 73 to 83% of the dose was excreted in the urine within 4 days and 8 to 21% in the feces. Only traces (0.05%) were expired in the air. In another study, female mice were given oral doses of ^{14}C-PCP (37 to 41 mg/kg); 68% and

9-14% were recovered in the urine and feces, respectively, after ten days (Larsen et al., 1972). Again, less than 0.04% of the dose was detected in the respired air. They concluded that the urinary excretion of pentachlorophenol is biphasic, the first phase having a half-life ($t_{\frac{1}{2}}$) of 10 hours and the second, a $t_{\frac{1}{2}}$ of 102 days. These values were obtained by plotting body-burden over time. The body-burden was determined by subtracting the cumulative amount excreted in the urine from the ^{14}C dose initially administered. Apparently, there are problems associated with this method in that it may cause errors in estimation of elimination rate unless one can account for nearly all of the dose originally administered. Thus, the 102 day half-life for the second component of elimination reported by these authors is suspect.

Ahlborg et al. (1974) found approximately 80% and 50% of the ^{14}C activity in the urine of rats, 24 hours following intraperitoneal and oral administration, respectively. The metabolism and excretion of ^{14}C-PCP were evaluated in female and male rats using single oral doses of either 10 mg/kg and 100 mg/kg (Braun et al., 1977). Males given 10 or 100 mg/kg and females given 10 mg/kg excreted about the same percentage in the urine (72-80%) and feces (19-24%). However, females given 100 mg/kg excreted significantly less in the urine (54%) and more in the feces (43%). These authors proposed a two-compartment open model to describe the elimination of ^{14}C-PCP in rats. The model is comprised of a central, or fast-exchange, compartment from which there is rapid elimination. The half life of elimination ($t_{\frac{1}{2}}$) in males given 10 mg/kg ^{14}C-PCP was 17 hours, and 13 hours in females given 10 mg/kg, or males given 100 mg/kg. A slow-exchange compartment has a slow rate of elimination, with a $t_{\frac{1}{2}}$ of 40 hours for males given 10 or 100 mg/kg

^{14}C-PCP, 121 hours for females given 10 mg/kg and 33 hours for females given 100 mg/kg. They suggested two possible reasons for the existence of the second, or slow-exchange compartment: 1) radioactivity is retained in the liver, where most of the radioactivity in the body remains at later times after administration, and 2) binding of PCP to plasma proteins. These two factors, in combination, may also be the cause of the long half life of PCP in urine, where small amounts are excreted over long periods. The influence of plasma-protein binding on the elimination rate is evidenced by a renal clearance rate of 0.138 ml per minute (3% of the glomerular filtration rate in a 200g rat) (Renken and Gilmore, 1973). They suggested this indicates that either the ^{14}C-PCP is extensively reabsorbed or a large fraction is strongly bound to plasma protein and not filtered.

In another study, 3 male and 3 female Rhesus monkeys were each given a single oral dose of 10 mg/kg ^{14}C-PCP (Braun and Sauerhoff, 1976). Male monkeys excreted 75% in the urine and 12% in the feces whereas, in female monkeys, urinary excretion was 70% and fecal excretion was 18 percent. These authors suggested that although there was no direct evidence of biliary elimination of the ^{14}C, that the ^{14}C in the feces most likely represented unchanged PCP or metabolites secreted into the bile. Moreover, they also proposed that biliary elimination is supported by a slow, but steady elimination of ^{14}C in the feces. In contrast, unabsorbed PCP would have been eliminated as a bolus in the first 24 hours. These authors also demonstrated that excretion of PCP by the kidneys is a first-order process, i.e. the rate of excretion is proportional to the concentration of PCP in the blood. Renal clearance was shown to be 14.5 ml/min which according to the authors suggests that

PCP is not actively transported into the tubular filtrate, nor reabsorbed at the same site, but is filtered by the glomerulus. Furthermore, they could not find any evidence of saturation of the excretion mechanism.

Toxicodynamics of Pentachlorophenol

Mechanism of Action

Pentachlorophenol's toxicity to a wide variety of organisms ranging from bacteria to mammals is based on its ability to interfere with the biochemical energetics of the cell. Experiments aimed at uncovering the enzymatic basis for PCP's molluscacidal activity led to the demonstration that minute concentrations of PCP completely inhibit the coupling of oxidation to phosphorylation in both rat and snail mitochondrial preparations (Weinbach, 1954, 1956). Substrate-level phosphorylation (glycolysis) was shown not be be interrupted by the same PCP concentrations which uncoupled oxidative phosphorylation. This author was the first to suggest that PCP's effect on oxidative phosphorylation in vitro was the basis for the increased respiration and glycolysis observed in living snails. Subsequent in vitro studies on rat liver mitochondria demonstrated that PCP is tightly bound to mitochondrial proteins (Weinbach and Garbus, 1965). These authors proposed that the protein binding was a major factor in the uncoupling phenomenon. They showed that oxidative phosphorylation, when interrupted by PCP in mitochondrial preparations could be effectively restored by addition of albumin to the incubation medium. The ability of PCP to strongly bind to albumin has been documented by other workers (Hoben et al., 1976a ; Braun et al., 1977).

PCP has also been shown to be a selective inhibitor of P-450, the terminal oxygenation enzyme in liver microsomes (Arrhenius et al., 1977). These authors suggest that the inhibition is due either to a specific attack on the P-450 enzyme itself or to a disturbance of the transfer of electrons from the flavin enzyme (NAD) to the P-450 enzyme.

Studies on Domestic Animals

The first attempt to examine the toxicity of pentachlorophenol in livestock involved the spraying of a diesel fuel-water emulsion containing penta on pasture (Grigsby and Farwell, 1950). They found that cattle avoided the sprayed areas. However, since the penta was applied at 2-4 times the recommended amount and in diesel fuel, it is difficult to conclude whether diesel fuel or penta caused the avoidance behavior. In another study, swine were dosed with 35 grams of a solution containing 5% PCP, 5% diacetone alcohol and 95% mineral spirits (Walters, 1952). This single dose resulted in blood levels of 42 ppm. PCP-concentrations in urine and fecal samples were 184, 6.0 ppm (24-h) and 212, 30.6 ppm (48-h), respectively. Tissue samples, taken at necropsy had the following PCP concentrations: 0.5 ppm (fat), 6 ppm (kidney), 5.8 ppm (liver) and 0.5 ppm (muscle). No observable deleterious effects were noted in these animals. In two other experiments, the sows and their piglets were confined to either farrowing houses treated with PCP on the outside or, in a second experiment, farrowing houses in which both the outside and inside were treated with a 5% solution of PCP in mineral spirits (Walters, 1952). The newborn piglets were in the farrowing house from 2-8 weeks of age. None of the animals showed any detrimental effects, with respect to health or growth. However, there was no mention of the amount of ventilation and no blood PCP measurements were made. In contrast, Schipper (1961) observed increased mortality of fetuses and newborn piglets and birth

of weak piglets when pregnant sows were confined in penta-treated farrowing pens. Moreover, nursing was decreased, possibly because of contamination of the udder with preservative, resulting in undernourished piglets. This agrees with the finding of Deichmann et al., (1942) and others who have observed that both the odor and taste of penta discouraged eating. Schipper (1961) also found that, in some cases, piglets consumed enough PCP to result in severe toxicosis. Lesions in the liver, spleen, stomach, intestinal and respiratory tracts, kidneys and urinary bladder were seen at necropsy. In general, toxicosis was most severe with insufficiently dried lumber treated with penta. Also, the mortality rate of piglets at birth closely coincided with the length of time the sow was confined in the penta-treated farrowing pen prior to farrowing. Moreover, the age of the piglets, at the time of exposure was also inversely related to the degree of toxicity (i.e. the older the animal, the less severe the effects). Inclusion of bedding in the farrowing pen prevented the problem. Unfortunately PCP concentrations in the serum were not reported.

In a clinical investigation reported by Blevins (1965), the owners of a newly-constructed farrowing house had overtreated the floor with a solution of PCP in used engine oil. Exposure to the preservative, in combination with poor ventilation and no bedding resulted in the death of nine newborn piglets. The author suggested that the piglets were potentially exposed via all three routes: oral, dermal and pulmonary.

Harrison (1959) measured PCP concentrations in the blood of sheep after they were force-fed sawdust impregnated with pentachlorophenol. Results indicated that PCP was rapidly absorbed from the gut, since maximum PCP concentrations blood were attained within 3-6 hours after dosing. This author observed a wide variability in blood concentrations

and individual animal responses resulting from various doses. When two animals were given a dose of 139 mg/kg, one lived for 24 days while the other died in 12 hours.

Several studies examining the effects of penta and analytical pentachlorophenol on mixed cultures of rumen microbes indicate that both preparations are effective inhibitors of cellulose digestion (Shull et al., 1977; Shull and McCarthy, 1978). These authors also demonstrated that the in vitro inhibitory effect was due to pentachlorophenol and not the impurities in penta. The significance of this in vivo is not yet clear. However, during the course of the subchronic study reported by this author, the digestibility in vivo of several feedstuffs was measured using an intraruminal nylon bag technique.

McConnell et al. (1980) fed four groups of yearling female Holstein cattle either analytical PCP, penta (technical PCP) or various mixtures of the two for 160 days. The objectives were to determine the toxic effects of long term exposure to analytical PCP and the influence of the dioxin contaminants in penta. The initial dose regime of 20 mg/kg body weight per day was decreased to 15 mg/kg per day after 42 days because of body weight loss in all cattle fed pentachlorophenol. The treatments were: 100% analytical PCP, 10% technical PCP (90% analytical), 35% technical PCP (65% analytical), and 100% technical PCP (penta). These authors observed a dose-related decrease in body weight, decreased feed efficiency and progressive anemia. These same cattle had dose-related increases in liver and lung weights and decreased thymus weights. Pathological findings included a marked villous hyperplasia of the urinary bladder mucosa in animals exposed to 100% technical PCP and hyperplasia of the gall bladder

and bile duct in some animals. The group exposed to 100% analytical PCP was essentially comparable to control animals.

In summary, there is little information regarding effects due to low-level exposure to PCP, chronically or subchronically, in domestic animals where accurate daily dosing was maintained and blood and urine PCP concentrations measured. Also, the effects of such exposure on production and health have not been documented.

McConnell et al. (1980) also reported a decrease in triiodothyroxine and thyroxin (T_3, T_4) concentration in all animals exposed to both analytical and technical PCP. They proposed no definitive explanation; however, the results suggest that PCP itself is the causative agent. Hepatic mixed funtion oxidase activities were measured and included: aryl hydrocarbon hydroxylase (AHH), aminopyrine N-demethylase and cytochrome P-450. AHH activity was increased 3-fold in the analytical PCP-fed group over the control group whereas, the group given technical PCP had an AHH activity 5-fold greater than the controls. Only the group fed 100% technical PCP had a small, but significant increase in aminopyrine-N-demethylase activity over that of the control group. Immunologically, there was a demonstrable dose-related enhancement in the lymphoproliferative response, an in vitro correlate for cell-mediated immunity. Furthermore, a hyperkeratotic lesion of the lining of the Meibomian glands of the eyelids was also found to be dose related. The results of this study led the authors to propose that the toxic effects associated with chronic exposure to technical PCP are primarily associated with the dioxin or dibenzofuran contaminants. A noteworthy aspect of this study was the method used for administering the penta dose. A premix containing penta

was mixed with the basic diet so that each animal received 1 kg of feed per 32 kg body weight. The weight of the feed not consumed was recorded daily. However, there was no mention in the report as to whether there were rejections. Consequently, whether the animals were consistently exposed to 15 mg/kg per day of pentachlorophenol is questionable.

Studies in Other Species

Information in the literature on the effects of low dose, chronic or subchronic exposure via any of the three routes of exposure in experimental animals is limited. In the case of oral exposure,the reason is that PCP contaminated food for most animals is very repellent (Deichmann et al., 1942). Consequently, the feed intake decreases with a concomitant decrease in body weight. Kehoe et al.(1939) reported that dermal application of PCP, at levels low enough to avoid gross skin damage produced no chronic systemic effects in rabbits. However, the information available on low dose, chronic exposure through any or all of the possible routes of exposure indicates there are no readily definable health effects.

Plakhova (1966) reported several toxic responses of animals (species not stated) exposed via the pulmonary route to PCP (23 mg/m^3 of air). for 4 hours per day for four months. The observed responses included: decreased body weight, hemoglobin content, and erythrocyte count. Also, an increased number of eosinophils and leucocytes was noted. However, a concentration of 7.44 mg PCP/m^3 resulted in unstable, but insignificant changes in these same hematologic measurements. The results of an acute inhalation study with PCP in rats showed the LD_{50} to be 11.7 mg/kg body weight (Hoben et al., 1976b). This is considerably less than

the acute oral LD_{50} in rats of 150 mg/kg (Schwetz et al., 1973).

Knudsen (1974) administered PCP to female and male rats for a period of 12 weeks at doses of 9, 25, 50 and 200 mg/kg in their feed. Histopathologic examination of the kidneys indicated a dose-related decrease in calcium deposits. This author suggested that this was possibly due to a lowering of blood calcium through an indirect action of PCP on calcium metabolism. Laborers in Japan chronically exposed to PCP had elevated levels of total bilirubin and creatinine phosphokinase (Takahashi et al., 1975). However, the authors noted that the values were within the normal limits. An epidemiological survey of occupationally-exposed workers showed them to have higher than normal serum levels of the liver enzymes, aspartate aminotransterase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LH) (Klemmer, 1972). However, the author did not speculate as to the clinical significance of these findings. Arsenault (1976) reviewed the results of an epidemiological study conducted by the University of Hawaii's Biomedical Research Center at Monoa which found no readily apparent long term chronic effects from exposure to pentachlorophenol.

Considering the wide variety of uses of pentachlorophenol, one would expect some degree of chronic exposure in the general population. Survey data accumulated by Cranmer and Freal (1970) tend to support this expectation. They found urinary levels in the general population ranging from 6 to 11 parts per billion. Casarett et al. (1969) suggested that pulmonary exposure is a reasonable explanation for the existence of measurable PCP levels in the general population. Much of the survey performed by this group was conducted in Hawaii where penta-treated wood is commonly used to prevent termite infestation.

Goldstein et al., (1977) examined the hepatic effects of technical grade and analytical grade PCP in rats. Technical PCP fed at 20 ppm in the diet produced hepatic porphyria and increased the following parameters: hepatic aryl hydrocarbon hydroxylase activity, liver weight, cytochrome P-450 activity, and microsomal heme content. These workers suggested that these effects were due to the contaminants. Analytical PCP fed at 550 ppm in the diet resulted in decreased body weight gains, a slight increase in liver glucuronyl transferase activity and pigmentation of the liver.

RESEARCH OBJECTIVES

Two experiments were undertaken to examine the effects of technical pentachlorophenol in lactating dairy cattle for the following reasons: 1) Information from the dairy industry indicated that production and health problems in several dairy herds were related to exposure to penta-treated wood. 2) Milk samples from some of these herds had measurable PCP concentrations. 3) A perusal of the literature indicated that little was known about the effects of subchronic penta exposure in lactating dairy cattle.

In an effort to provide information with regards to possible production and health problems, in addition to tissue and milk residues of PCP, two experiments were undertaken with the following objectives in mind:

Experiment 1: To determine the effects on performance and health in lactating dairy cattle fed technical PCP subchronically.

Experiment 2: To determine the fate of a single dose of ^{14}C-PCP in a lactating dairy cow previously fed technical PCP subchronically.

RESEARCH METHODS AND PROCEDURES

Animals and Diets

Eight mature Holstein dairy cattle were randomly allotted as pairs to either a control or treatment group. Cows were paired on the basis of stage of lactation. Rumen cannulas (10.2 cm, i.d.) were surgically installed in two of the four pairs, 5-6 weeks pre-partum. Each pair of animals was started on experiment, 6 ± 1 weeks postpartum. All cattle were housed in portable tie stalls located in the toxicology wing of the Michigan State University Dairy Cattle Teaching and Research Center. In addition, each animal had water available on an ad libitum basis. The basal diet consisted of alfalfa haylage and high moisture corn, supplemented with 38% protein concentrate added to a rate of one pound per three pounds of milk production. The total ration was maintained at 14.7% protein, 47% dry matter, 1.85 Mcal net energy/lb dry matter, 0.5% Ca and 0.39% P.

Composition of Technical Pentachlorophenol

The penta was a composite sample from three manufacturers of technical pentachlorophenol. The dioxin content of the composite penta was determined by Dr. M.J. Zabik using high-performance liquid chromatography (HPLC) according to the method of Pfeiffer (1976) and is given in Table 4. The method involved dissolving 4 grams of penta in 50 ml of 1N NaOH which was then combined with 10 ml of benzene in a 100 ml separatory funnel and the contents shaken for 20 minutes. The aqueous phase was re-extracted with another 10 ml of benzene. The extracts

were combined and placed on an anion-exchange column (Bio-Rad AG-21R, hydroxyl form; 17.8 mm x 16 cm glass column) and eluted with 150 ml of benzene-methanol (1:1). The column effluent was evaporated to 10 ml and washed 3 times in a separatory funnel using 1N NaOH. The 10 ml of solution was then brought to 100 ml with hexane. The dioxin congeners in the solution were quantitated using a high-pressure liquid chromatograph (Altex pumps, Rheodyne valve, Hitachi variable wave length UV detector and Altex microprocessor). Congeners were separated by using two Du Pont RP-18 columns in series. The solvent system was absolute methanol with a flow rate of 1 ml/min. Chlorodioxin standards included 100 mg/liter solutions of Octa-CDD; 1,2,3,4,6,7,8-hepta-CDD; 1,2,3,4,6, 7,9-hepta-CDD; 1,2,3,6,7,9-hexa-CDD; 1,2,3,6,7,8-hexa-CDD; 1,2,3,4,6, 8-hexa-CDD, and 2,3,7,8-tetra CDD. Except for tetra-CDD, quantitative results were based on peak areas for each CDD congener in comparison with standard curves for that congener. The quantitation of tetra-CDD peaks was based on the assumption that the relationship of detector response to concentration for tetra-CDD congeners is the same as that of the 2,3,7,8-CDD standard. Gas chromatography coupled with mass spectrometry (GC-MS) was subsequently used to confirm all HPLC identifications. The chlorophenol content was reported by the supplier (Roman, 1978). Included in Table 4 is the chlorodioxin analysis on the same industrial composite conducted by another laboratory using a different method (Scott, 1980). This clearly indicates that the method by which chlorodioxin is analyzed can significantly affect the results. Further evidence of this is shown in Table 5. The analysis conducted by Monsanto represents two composite samples of penta. Composite No. 2 was used in a study conducted by FDA (Firestone et al., 1979). The results given for composite No. 3 are

Table 5. Dioxin and Furan Analysis of Industrial Composite of Penta by Monsanto, FDA, and NIEHS

	Monsanto[a]		FDA[b]	NIEHS[c]
	Composite No.			
Dibenzodioxin	1	2	3	4
Octa	1410	1020	690	1500
Hepta	505	130	280	410
Hexa	9.4	5.3	20.5	15
Penta	0.03	0.1	0.50	1.0
Tetra	0.01	0.05	0.10	
Tri	0.01	0.05		
Dibenzofurans				
Octa	182	54	130	90
Hepta	116	37	120	130
Hexa	6.8	7.1	32	37
Penta	1.1	0.9	4.0	2.0
Tetra	0.7	0.2	0.9	
Tri	0.02	0.05		
Hexachlorobenzene	30	80		
Pentachlorophenol	125			
PCB's	1	2		

[a] Personal communication from Dr. Dan Roman (Monsanto Chemical Co.) composite no. 1 used by McConnell *et al.* (1980), no. 2. used by Firestone *et al.* (1979).

[b] Analysis reported by Firestone *et al.* (1979). Composite no. 2 and 3 believed to be subsamples of the same composite.

[c] Analysis reported by McConnell *et al.* (1980). Composite no. 1 and 4 believed to be the same.

those reported by the authors. Similarly, composite No. 1 was used in a study in heifers reported by McConnell et al., 1980. Also, the results given for composite No. 4 were reported in the study. The chlorophenol content in the current study was reported by the supplier (Roman, 1978) and are shown in Table 4.

Preparation of the Pentachlorophenol Dose

Treated cows were fed a penta-containing ration prepared as follows: A premix was prepared by mixing penta, previously solubilized in acetone, with a 38% protein concentrate (containing 3% molasses), after which the acetone was evaporated. The premix was then mixed with the daily allotment of protein concentrate and fed separately from the rest of the ration to insure total ingestion of the dose. All treated cattle were given a dose of 0.2 mg penta/kg body weight/day for 75-84 days and then 2 mg penta/kg body weight/day for a subsequent 56-62 days. The 0.2 mg/kg dose was derived from the estimated exposure of cattle on some Michigan dairy farms where penta-treated wood was heavily used (Van Gelder, 1977). The 2 mg/kg dose was selected to represent a level of exposure not likely to be exceeded on a dairy farm, even one with extensive use of treated wood in livestock facilities.

Animal Care and Daily Observation

All cattle were fed and milked at 12 hour intervals (7:00 am and 7:00 pm), at which time individual milk weights and feed intakes were recorded. The allotment of concentrate was periodically adjusted to the level of milk production of each cow. Similarly, the amount of haylage-high moisture corn was adjusted so that approximately 5% was routinely weighed back. All cattle were observed daily for clinical

signs including: general appearance; vigor; stool consistency; stance; mastitis; and discharges from the eyes, nose and genito-urinary tract. One week prior to necropsy, all animals were given a complete physical examination by Dr. Kent Ames (Department of Large Animal Surgery and Medicine, Michigan State University) which included measurement of: pulse rate, rectal temperature and respiratory rate and examination of: skin, ears, eyes, and mucous membranes.

Collection of Blood and Urine for Clinical Analysis

Approximately 20 ml of blood was collected from the jugular vein of each cow at two week intervals. All clinical analyses were done, courtesy of the Michigan State University Veterinary Medicine Clinical Laboratory, under the supervision of Dr. J. Krehbiel. Red and white blood cell counts and hemoglobin concentrations were measured with a Coulter Counter^{R} (Model S, Coulter Electronics, Inc., Hialeah, FL). Hematocrit values were determined by a standard micro-hematocrit centrifugation. Total plasma protein was estimated with a Goldberg refractometer. A differential WBC count was performed by enumerating the relative numbers of segmented and non-segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Platelet numbers were estimated from a peripheral blood smear. Total serum protein, albumin, globulin, blood urea nitrogen (BUN), creatinine, glucose, aspartate aminotransferase (AST) and sorbitol dehydrogenase (SH) activity were determined using a Gemsaec^{R} centrifugal analyzer (Electro-Nucleonics, Inc., Fairfield, NY) using standard methods. During the two weeks before necropsy, a representative sample of urine was collected weekly from each cow by urethral catheter for urinalysis. Evaluation included: subjective evaluation of color and turbidity, specific gravity determination by refractometry, urobilinogen,

pH, protein, glucose, ketone bodies and occult blood by the use of Ames Multistix^{R} (Ames Co., Elkhart, IN) and red and white blood cells and epithelial cells and bacteria by direct microscopic examination of urinary sediment.

Collection and Analysis of Milk for Fat And Somatic Cells

Milk fat percentage and somatic cell counts were measured by a DHIA operated milk-o-tester (N. Foss Electric, Denmark) and a Fossomatic somatic cell counter (N. Foss Electric, Denmark), respectively.

Necropsy Methods, Collection of Tissues and Histopathologic Techniques

Between 138 and 141 days of exposure all cattle were killed by the use of captive bolt pistol. Post-mortem and histopathologic examinations were performed by Dr. S.D. Sleight, Department of Pathology, Michigan State University. Liver, lungs, kidneys, spleen, heart, thyroid and adrenals were removed intact, trimmed of excess fat and connective tissue, and weighed. Representative tissue samples selected for histopathologic examination included: lung, liver, kidney, heart, spleen, adrenal, mesenteric lymph node, small intestine, gall bladder, urinary bladder, skeletal muscle, trachea, thymus, thyroid gland, salivary gland, pancreas, brain, rumen, reticulum, omasum, colon, ovary, uterus and skin. All tissue samples were fixed in buffered 10% formalin. Paraffin-embedded sections of 6 micron thickness were routinely prepared and stained with hematoxylin and eosin for histologic examination under a light microscope.

Kidney Function Tests

A portion of each kidney was placed in ice-cold saline and taken to the laboratory for <u>in vitro</u> function tests which included measuring

the accumulation of p-aminohippurate (PAH), tetraethylammonium (TEA), and amino isobutyrate (AIB) in renal slices. Ammoniagenesis was also measured. These tests and data interpretation were conducted by C. Kuo, Department of Pharmacology and Toxicology, Michigan State University. Cortical slices (150 to 200 mg) were incubated in a phosphate-buffered medium (Cross et al., 1950) containing 7.4×10^{-5}M PAH and 1×10^{-5}M ^{14}C-TEA under an atmosphere of 100% O_2 at 37°C for 90 minutes. After incubation the slices were removed, blotted, weighed and homogenized in 3 ml of 10% TEA, and brought to a final volume of 10 ml with distilled H_2O. A 2 ml aliquot of medium was treated similarly. The samples were centrifuged and the supernatant was used to determine the concentrations of PAH (Smith et al., 1945) and the radioactivity of ^{14}C-TEA. The data were expressed as the concentration in slice to the concentration in medium (S/M) ratio.

Amino isobutyrate was assayed by the method of Rosenberg et al. (1961). Cortical slices (100-150 mg) were incubated in a Krebs-bicarbonate buffer containing 1.2×10^{-5}M AIB under 95%/5% (O_2/CO_2) for 90 minutes at 37°C. After incubation, slices were treated as before and the radioactivity in slice and medium was measured. The data were expressed as slice to medium ratio.

Production of ammonia was determined by the method of Roobol and Alleye (1974). Approximately 100 mg of tissue was incubated in a Krebs-bicarbonate medium containing 2 mM glutamate. The flask was gassed with 95%-5% (O_2/CO_2), capped tightly, and incubated for 60 minutes at 37°C. The tissues were then removed and the dry weight was determined. The incubation medium was then added, with 0.5 ml of 10% perchloric acid, and centrifuged. Ammonia in the supernatant was assayed by the method of

Kaplan (1965).

Analysis of Pentachlorophenol

Extraction from Serum (for total PCP):

1. Replicate 1 ml subsamples of the serum to be analyzed were placed in 16 x 125 screw cap (teflon lined) culture tubes (Corning #982616X).
2. A 0.22 ml aliquot of reagent sulfuric acid (18M) was added to each tube and vortexed at slow speed for 5 sec (Vortex-Genie, Model K-550-G, Scientific Industries, Inc., Bohemia, N.Y.).
3. All tubes were then placed in a water bath (85^{o} C) for three hours to assure complete hydrolysis and release of any conjugated or protein-bound PCP.
4. The tubes were allowed to cool to room temperature, 1 ml of 30% saline solution added (to break emulsions), followed by 3 ml of glass distilled benzene , containing 0.5 µg lindane/ml benzene as an internal standard.
5. Tubes were placed on a Fischer Rotorack^{R} and allowed to extract for 2 hours at 70 rpm, after which they were allowed to set at room temperature for an additional 8 hours.
6. The contents of all tubes were then centrifuged for 15 minutes at full speed (4250 x g) in an IEC centrifuge (Model 25179P).
7. Following the centrifugation, about 1.6 ml of the benzene (upper) phase was transferred with a Pasteur pipette into a 2 ml screw cap GC auto sampler vial with a teflon septum (Varian Instruments, Walnut Creek, CA).

Extraction from Serum (for free PCP):

1. Replicate 1 ml subsamples of the serum to be analyzed were placed in 16 x 125 disposable test tubes.
2. One ml of 30% saline (w/v) and 3 ml of benzene (with a 0.5 μg/ml lindane internal standard) was added to each tube.
3. A 0.22 ml volume of 4N sulfuric acid was added to each set of replicate samples. The replicates were then vortexed together at a speed setting of one. The next set of replicates in the test tube rack was treated in a similar manner and so on until all sets of replicates had been vortexed once. The speed setting on the vortex mixer was increased to five and the process was repeated. A third vortexing was done at a speed setting of ten.
4. The samples were then allowed to set for 15 minutes, after which they were centrifuged in an IEC clinical centrifuge for 15 minutes at 4250 x g.
5. Following centrifugation, about 1.6 ml of the benzene phase was transferred, using a Pasteur pipette into a 2 ml screw cap GC auto sampler vial with a teflon septum.

Extraction from Urine (for total PCP):

Replicate 1 ml subsamples of the urine were analyzed using the same procedure as that for total serum PCP with the exception that in the hydrolysis step, 0.1 ml of 4N H_2SO_4 was used instead of 0.22 ml. The 0.22 ml of reagent H_2SO_4 (18 M) used in the total serum PCP extraction procedure was found to produce peaks which interfered with quantitation of the peaks of interest. Note, bile PCP was extracted using this method.

Extraction from Urine (for free PCP):

Replicate 1 ml subsamples of urine were analyzed using the same

procedures as for extraction of free PCP from serum, with the exception that 1 ml of 30% saline (w/v) was not added to the extraction system.

Extraction from Feces (for total PCP):

PCP was extracted from fecal samples using the following procedure:

1. A homogenate (1:3, feces: deionized, double-distilled H_2O, w/v) of the sample was made using a Polytron^R homogenizer/sonicator. The sample was homogenized until 1 ml samples could be easily pipetted using a 1-5 ml Finn Pipette.
2. Then 3-20 ml samples were aliquotted into pre-weighed foil pans while the homogenized sample was being stirred (Polytron^R on speed setting three). The pans were weighed and then placed in a convection drying oven at 60^o C for two days. At the end of this time, the pans were removed, allowed to cool in a desiccator to room temperature and then weighed.
3. Once the samples for dry matter were removed, replicate 1 ml samples of the homogenate were placed in 16 x 125 mm screw cap (teflon-lined) culture tubes with 1 ml of 30% saline, 0.3 ml of 1 N NaOH and 10 ml of glass distilled hexane.
4. The samples were then extracted on a Fisher Rotorack^R (70 rpm, 15 min) followed by centrifugation (4250 xg, 15 min.).
5. The hexane layer was then discarded. The hexane extraction step was repeated two additional times.
6. After removal of the 3rd hexane extract, the samples were then analyzed using the same method to measure total PCP in serum from the point of addition of 0.22 ml of 18 M H_2SO_4.

Extraction from Rumen Fluid (for total PCP):

Initial attempts at developing this protocol showed that more than 90% of the PCP was associated with solids; i.e., high speed centrifugation of whole rumen fluid produced an essentially PCP-free supernatant. Therefore, rumen fluid was collected during the experiment by squeezing rumen contents through six layers of cheese cloth into glass jars (foil-lined lids) and analyzed later for PCP in the following manner:

1. The sample to be analyzed was homogenized using a Willeims Polytron^{R} homogenizer/sonicator (Lucerne, Switzerland). The sample was subjected to this treatment until 1 ml samples could be easily pipetted using a 1-5 ml Finn pipette.
2. While the sample was being stirred, using a speed setting of 3 on the Polytron^{R}, triplicate samples (20 ml) were removed into preweighed foil pans. The pans were then placed in a convection drying oven for two days at 60^{o} C. At the end of this time, the pans were removed, weighed and sample dry matter calculated.
3. Once the samples for dry matter analysis were removed, replicate 1 ml samples of homogenized rumen fluid were aliquoted into 16 x 125 mm screw cap culture tubes.
4. From this point on, the samples were subjected to the same extraction procedure as the serum samples, with the exception that the concentrations of PCP were adjusted to a dry matter basis.

Extraction of Tissues and Whole Milk (for total PCP):

A major problem in development of a usable procedure for analysis of tissues was that lipid in certain tissues significantly shortened

the GC column life. Thus, a procedure was developed which included extracting the lipid into hexane under alkaline conditions while retaining PCP in the aqueous phase for later extraction into benzene under acidic conditions. Therefore, PCP was extracted from all tissues except body fat, using the following procedure:

1. Tissues to be analyzed were removed from the freezer and were placed in a water bath (approximately 25^{o} C) to thaw.
2. Once thawed, the tissue was removed from its Whirl-Pack^{R} plastic bag and blotted dry.
3. A representative 2 gram sample was minced in a Coulter cup followed by the addition of 6 ml of double-distilled/deionized water.
4. The contents of the cup were then homogenized, using a Polytron^{R} homogenizer/sonicator, until the homogenate was easily pipetted using a 1-5 ml Finn pipette.
5. Replicate 1 ml aliquots of the homogenate were then placed into 16 x 125 mm screw cap culture tubes. Note, replicate 1 ml samples of milk were analyzed for total PCP using steps 5 through 14.
6. To facilitate extraction of the fat, 1 ml of 30% saline, 0.3 ml 1 N NaOH and 10 ml of glass-distilled hexane were added to each tube.
7. Tubes were then placed on a Fisher Rotorack^{R} at half speed for 30 minutes, after which they were capped and centrifuged at 4250 xg in an IEC clinical centrifuge for 15 minutes.
8. Then, the hexane (upper phase) was aspirated and discarded.
9. This extraction process was repeated 2 additional times.
10. To the remaining aqueous layer was added 0.22 ml of reagent sulfuric acid (18 M) followed by vortexing a low speed.

11. After the addition of the acid all tubes were placed in a water bath at 80° C for three hours.
12. At the end of three hours the tubes were allowed to cool to room temperature and 3 ml of benzene (0.5 µg lindane/ml benzene internal standard) were added.
13. The tubes were then placed on a Fisher Rotorack® for two hours at full speed (70 rpm), allowed to set for eight hours, and then centrifuged with caps on (4250 xg, 15 minutes).
14. Approximately 1.6 ml of benzene (upper) phase were analyzed under the same conditions as the serum, urine and rumen, with the exception that the PCP concentration (in tissues, but not milk) was adjusted upward because of the four-fold dilution in the homogenate.

Gas Chromatographic Analysis

All benzene extracts of various fluids and tissues were analyzed using a Varian 3700 gas chromatograph equipped with a ^{63}Ni pulsed electron capture detector (ECD) and an autosampler. A Varian CDS-111 microprocessor coupled to the GC was used to compute PCP concentrations. A Houston Instruments Omniscribe® strip chart recorder was used to record peaks. The column used was a 1.8 m x 2 mm id coiled glass column (Supelco Inc., Bellefonte, PA) packed with 1% SP-1240DA on 100/120 Supelcoport (Supelco Inc.). The injection volume was 0.5 µl. Instrument settings were: injector temperature 200° C; column temperature 160° C; detector temperature 350° C; carrier gas (N_2 - 99% purity) flow rate 40 ml/min; GC and recorder attenuation were ten and sixteen. The column temperature and carrier gas flow rate were adjusted slightly from column to column to achieve the same separation.

Analysis of Chlorodioxins (CDD) in Liver

Samples of liver from the penta-fed cattle were analyzed for chlorodioxins (CDD) by Dr. M.J. Zabik using the method developed by Dow Chemical (method no. ML - AM 7309). A modification of the procedure involved digestion of the samples with concentrated HCl, followed by extraction with hexane, instead of a concentrated base digestion followed by extraction with a hexane-benzene mixture. Quantitation of CDD congeners was by electron capture gas chromatography (ECD - GC). GC conditions were as follows: column packing - 3% OV-101 on 100/120 mesh chromosorb W-HP; column 1.8 m x 4 mm id; injector temperature was 250^{o} C, column temperature was 200^{o} C, detector temperature was 300^{o} C and carrier gas was N_2 at a flow rate of 40 ml/min. CDD peaks were confirmed using gas chromatography-mass spectrometry (Dupont Model 320 GC-MS).

Purification of ^{14}C - Pentachlorophenol

Five millicuries (mCi) of uniformly labelled ^{14}C-PCP with a specific activity of 19 mCi/mg were obtained from New England Nuclear, Labelled Chemical Division (Boston, Mass). An initial purity check was done using thin-layer chromatography in combination with radioautography. The thin-layer plates were 20 x 20 cm silica gel GF (No. 6-601A Fisher Scientific, Pittsburgh, PA). The solvent mixture used to elute the plate was toluene: acetone: ammonium hydroxide (50:40:1, v/v/v). The plate was initially developed in the same solvent system and allowed to dry. A 10 mg/ml methanol solution of the original stock compound was diluted to obtain 10%, 1% and 0.1% solutions. Then 1 µl of each of the five solutions was spotted at about 2 cm intervals across the origin of the plate, allowed to dry, and then the plate was developed in the previously

described solvent system in a preequilibrated chromatography tank. When the solvent had migrated approximately 90% of the length of the plate, the solvent front was marked, the plate was removed, and it was allowed to dry. Then in a dark room a 20.3 cm x 25.4 cm sheet of x-ray film (X-OMat, R-Film, XR-5, Eastman Kodak, Rochester, NY) was exposed to the plate for 108 hours. The film was then developed using Kodak liquid x-ray developer for five minutes, followed by fixation with Kodak Rapid Fixer for eight minutes. The results indicated that the radiolabelled compound contained approximately 6% impurities rather than the 1% claimed by the manufacturer.

Thus, a high-performance liquid chromatography (HPLC) method was developed to purify the ^{14}C-PCP. The HPLC system was Waters Associates equipment (Milford, Mass) and consisted of two M6000-A solvent delivery pumps, one Model 660 Programmer, one Model 400 Absorbance Detector and a reverse phase, C_{18} μ Bondapack column (3.9 mm x 30 cm). The separation system developed was as follows: the running solvent was methanol: water (72:28 v/v) with 0.5 M acetic acid added to the respective solvents; the flow rate was 1 ml/min; the detector sensitivity was 2 AUFS. Repeated 0.1 to 0.9 ml injections of a 3 mg/ml solution of ^{14}C-PCP (in 65:35, methanol-water, 0.5 M acetic acid) were made. The fraction corresponding to PCP was collected from the effluent outlet after each injection. The ^{14}C-PCP fractions were combined and the purity was checked by repeating the TLC procedure at dilutions of 100, 10, 1 and 0.1%, respectively. The plate was eluted using the previously described solvent mixture, allowed to dry and then placed in contact with a sheet of x-ray film for 68 hours. The film was then developed as before. The purity of the ^{14}C-PCP was found to be 99%.

Preparation of the ^{14}C-Pentachlorophenol Dose

Immediately prior to the start of the radiotracer study, exactly 814.65 μCi of ^{14}C-PCP was dissolved in methanol (5.4 ml) and then deposited in alpha-cellulose contained in a gelatin capsule. The concentration of the PCP was shown to be 7.94 mg/ml which translates to a dose of 42.9 mg of PCP. The specific activity, as reported by the supplier, was 19 mCi/mg.

Preparation of the Lactating Dairy Cow for the ^{14}C-PCP Radiotracer Study

Prior to the initiation of Experiment 2 and during the course of Experiment 1, a pregnant Holstein cow weighing approximately 400 kg was allowed to calve. Thereafter, she was administered, in a manner identical with the cows in Experiment 1, 0.2 mg of penta/kg body weight daily for 95 days. At this time, this cow was fitted with a jugular cannula and an indwelling urethral catheter for serial collection of blood and urine samples. Just prior to the administration of the ^{14}C-PCP the cow was placed in a radioactivity containment stall. The containment was total with the exception of respired gases. No effort was made to account for these, since previous studies in rats and monkeys had shown that $\leq$ 0.05% of the dose was accounted for as respired $^{14}CO_2$ (Braun and Sauerhoff, 1976; Braun et al., 1977). During the experiment, water was available on an ad libitum basis. The schedule for feeding and administration of penta (0.2 mg/kg) was the same as that used for the 95 days prior to administration of ^{14}C-PCP.

Collection of Blood, Urine, Milk, Feces and Selected Tissues During the Radiotracer Study

Prior to the administration of the single dose of ^{14}C-PCP, the following samples were collected at various time intervals: blood (20 ml), urine

(1 L), milk (10 L), and feces (10 kg) which had been produced over the previous 12 hour period. Blood samples collected during the course of the study were allowed to clot, taken to the laboratory and centrifuged to obtain the serum. The ^{14}C-concentration was measured in each sample by liquid scintillation counting (LSC). Two 0.1 ml aliquots from each sample were counted using 5 ml of aqueous counting cocktail. All samples were counted for 10 minutes and the ^{14}C-concentration calculated using the external standard channels ratio method with quench correction. The cow was milked at 4-hour intervals using an automatic milking machine. The total volume was recorded and samples were placed in plastic milk containers and frozen immediately. Liquid scintillation counting was used to measure the ^{14}C-concentration in replicate 0.2 ml milk samples, after addition of 5 ml of aqueous counting cocktail. Urine samples were also collected and the samples frozen in plastic milk containers. Again, two, 0.2 ml aliquots were mixed with 5 ml of aqueous counting cocktail and counted. The total weight of fecal samples collected every 4 hours was recorded and the total sample was placed in double plastic bags and subsequently frozen.

Seventy-six hours after administration of radiolabelled PCP the cow was killed using the same methods as those used in the previous 8-cow study (Experiment 1). Selected tissues were removed, trimmed of excess fat and weighed.

Liquid Scintillation Counting Techniques

Carbon-14 concentrations in serum, urine and milk collected during the course of Experiment 2 were measured by the following procedure:

1. 0.1 ml samples of serum, urine or milk were pipetted into 10 ml

glass scintillation vials.

2. To each vial was added 0.3 ml of deionized, double-distilled H_2O and 5 ml of aqueous counting cocktail.
3. All samples were counted on an Isocap 300 liquid scintillation counter using the external standard channels ratio method for quench correction.

PCP Residue Analysis of ^{14}C-Tissues and Fluids

Tissues and fluids from the radiotracer experiment were analyzed for PCP residues, using the same methods as for those tissues and fluids collected and analyzed from Experiment 1. In addition, 0.2 ml aliquots of the benzene extracts were counted in 10 ml glass scintillation vials with 5 ml of aqueous counting cocktail. This allowed for quantitation of the ^{14}C in the tissues and fluids.

Milk Fractionation Procedure

1. Milk samples were allowed to thaw at room temperature.
2. The samples were then placed in a 37^{o} C water bath for about 30 minutes.
3. The contents of each container were shaken and approximately 250 ml of milk were decanted into a French glass bottle. The remainder of the sample was refrozen.
4. The contents of the French glass bottle were then mixed using a Polytron^{R} homogenizer/sonicator operating at a speed setting of three. While the contents were being mixed, 40 ml aliquots of milk were transferred into each of two 50 ml polycarbonate centrifuge tubes.
5. The tubes were weighed before and after addition of the milk

samples.

6. The samples were then centrifuged for two hours at 17,000 rpm in a Sorvall RC2-B centrifuge fitted with a Sorvall SA-600 rotor.
7. After centrifugation, the fat pad on each sample was carefully circumscribed, using a spatula, and removed from the centrifuge tube. Excess whey was carefully blotted off.
8. The fat pad was subsequently placed in a preweighed 10 ml glass scintillation vial , weighed and the weight recorded.
9. Two, 0.1 g samples of the milk fat were weighed into 10 ml scintillation vials; 5 ml of aqueous counting cocktail (Amersham, Chicago, IL) was then added to each vial and the samples were then counted using liquid scintillation counting (LSC) (Isocap 300, Tracor Analytic Inc., Elk Grove Village, IL).
10. The centrifuge tube was then reweighed.
11. The whey (supernatant) was poured off into a preweighed graduate cylinder and the subsequent volume and weight recorded.
12. Two, 0.4 ml aliquots of the whey were counted using 5 ml of aqueous counting cocktail. Two, 20 ml samples of whey were frozen in plastic vials for later use.
13. The centrifuge tube which now contained the casein pellet was reweighed.
14. The surface of the casein pellet was carefully rinsed twice with 10 ml of deionized, double-distilled H_2O. Two, 0.4 ml aliquots of each rinse were counted using 5 ml of aqueous counting cocktail.
15. The casein was then washed by adding 30 ml of deionized, double-distilled H_2O, followed by homogenization using the Polytron^R (about 1 minute at setting no. 5).

16. The casein homogenate was then centrifuged for one hour at 17,000 rpm in a Sorval RC2-B centrifuge fitted with a SA-600 rotor.
17. Two 0.4 ml aliquots of the supernatant were counted using 5 ml of aqueous counting cocktail. The remainder was discarded.
18. The casein pellet was carefully circumscribed, using a spatula, and removed into a preweighed 10 ml glass scintillation vial. The vial was weighed and the weight recorded.

Analysis of Milk Fractions for PCP and ^{14}C

Whey

This fraction was analyzed for conjugated and unconjugated PCP by using the same methods as those used for serum. An additional step involved counting two 0.2 ml aliquots of the benzene extract with aqueous counting cocktail. This step, in conjunction with the original count of the whey during the fractionation procedures, allowed the calculation of a percentage recovery value for ^{14}C.

Casein

1. A 1:4 homogenate (w/v) of the casein pellet was made (1 g casein plus 3 g deionized double-distilled H_2O) using a Polytron^R.
2. Two 0.2 ml aliquots of the homogenate were then counted using 5 ml of aqueous counting cocktail.
3. Replicate 1 ml aliquots of the casein homogenate were then analyzed for total and free PCP using the same method as that used for serum.
4. An additional step involved counting two 0.2 ml aliquots of the benzene extract with 5 ml of aqueous counting cocktail. This step in conjunction with a count of the casein homogenate allowed for calculation of a percentage recovery value for ^{14}C.

Milk Fat

Replicate 0.5 g samples of milk fat were counted with 5 ml of aqueous counting cocktail using LSC.

Quantitation of ^{14}C in Tissue Using a Total Oxidation Method

1. Tissues were allowed to thaw at room temperature.
2. A representative sample of each tissue was minced into a Coulter cup, the weight recorded and 50 ml of deionized, double-distilled H_2O added.
3. The contents of the cup were then homogenized using a Polytron^R homogenizer/sonicator, until the contents were easily pipetted using a 1-5 ml Finn Pipette. For some tissues, additional water had to be added to achieve the necessary consistency. The total volume was recorded.
4. The Coulter cup and contents were then reweighed.
5. Duplicate samples of the homogenate with the volume recorded for each, were oxidized for 4 minutes using a biological oxidizer (Harvey Co., Model OX-200).
6. The evolved $^{14}CO_2$ from each sample was collected directly into glass scintillation vials containing 5 ml of liquid scintillant (Permafluor V: Carbo-Sorb II, 2:1, Packard Chemicals).
7. All samples were then counted for 10 minutes each. The ^{14}C-concentration was then calculated by the external standard channels ratio method.

Statistical Analysis

Treatment means of the following measurements from Experiment 1 were compared using Student's t-test (Gill, 1978): feed intake, daily milk

production, body weights, milk fat, feed efficiency and somatic cell counts. Chi-Square analysis and Student's t-test were used to compare group means of blood chemistry, hematologic measurements, urinalysis and *in vitro* kidney function measurements.

RESULTS

Experiment 1. Subchronic Oral Administration of Penta to Lactating Dairy Cattle

Effects on Performance

The initial mean body weight of the treated cattle was less than that of control cattle (Figure 6). However, penta had no effect on body weight during the course of the two exposure periods. Although control cattle gained slightly more weight, the difference was not significant ($P > .05$).

All cattle in the treatment group readily consumed the penta concentrate. Although treated cattle in this study consumed less total feed than did control cattle (Figure 7a), when the data were expressed on a body weight basis the amount of feed consumed per day by each group was equivalent (Figure 7b).

The cattle fed penta tended toward a more efficient conversion of feed to milk, i.e. they produced more fat-corrected milk (FCM) per Mcal (NE_L) of feed consumed (Figure 7c). However, this effect was significant ($P < .05$) only through 14-28 days in the 2 mg/kg dose period.

Less total milk was produced by treated cattle, but the difference was not significant ($P > .05$) (Figure 8a). Results of somatic cell measurements on milk collected during the last week of the second treatment period indicated that the penta-fed cattle did not have cell counts (number of cells/ml ± SE) different from that of control cattle (control, 180,917 ± 8,374; treated 20,042 ± 121,784). There was no significant difference ($P > .05$) in milk fat production between control and penta fed cattle

Figure 6. Time Course Status of Body Weight of Cattle Fed 0.2 Mg Penta/Kg Body Weight/Day for 75 to 84 Days Followed By 2 Mg Penta/Kg Body Weight/Day for 56 to 60 Days.

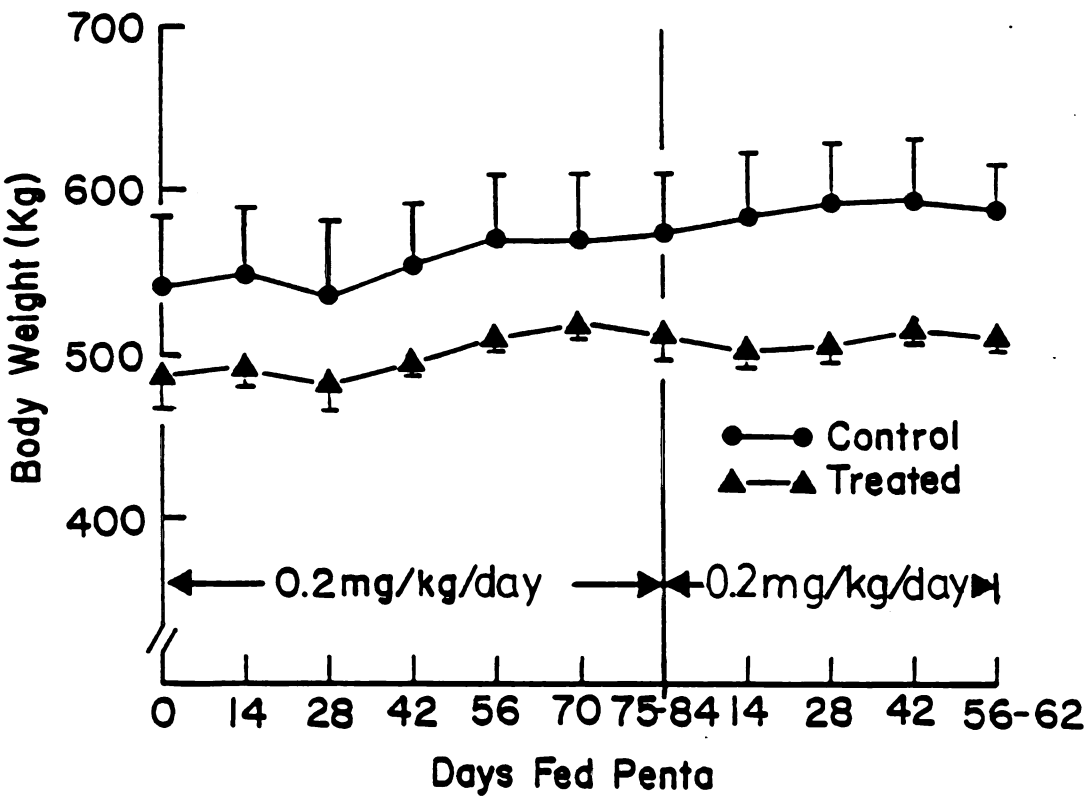

700
600
500
400
Body Weight (Kg)
Control
Treated
0.2mg/kg/day
0.2mg/kg/day
0
14
28
42
56
70
75-84
14
28
42
56-62
Days Fed Penta

Figure 7. Time Course Status of Daily Feed Intake of an As-Fed Basis (a), Ratio of Intake to Body Weight (b), and Ratio of Fat Corrected Milk Produced to Megacalories Consumed (c) in Cattle Fed 0.2 Mg Penta/Kg Body Weight/Day for 75 to 84 Days Followed by 2 Mg Penta/Kg Body Weight/Day for 56 to 60 Days. Each Graphed Point Represents the Mean of All Data Collected During the 14 Day Period Preceding the Specified Number of Days Indicated.

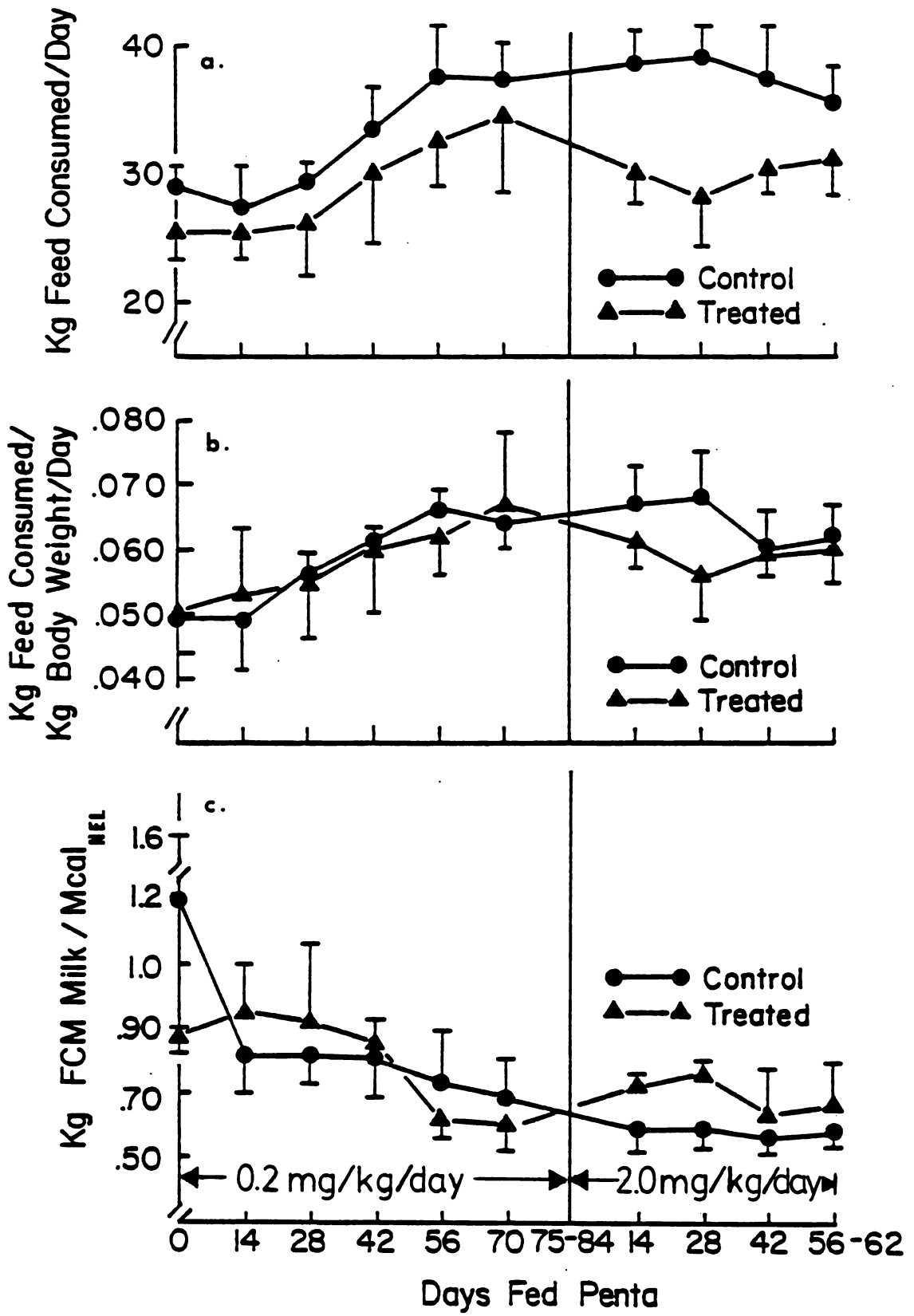
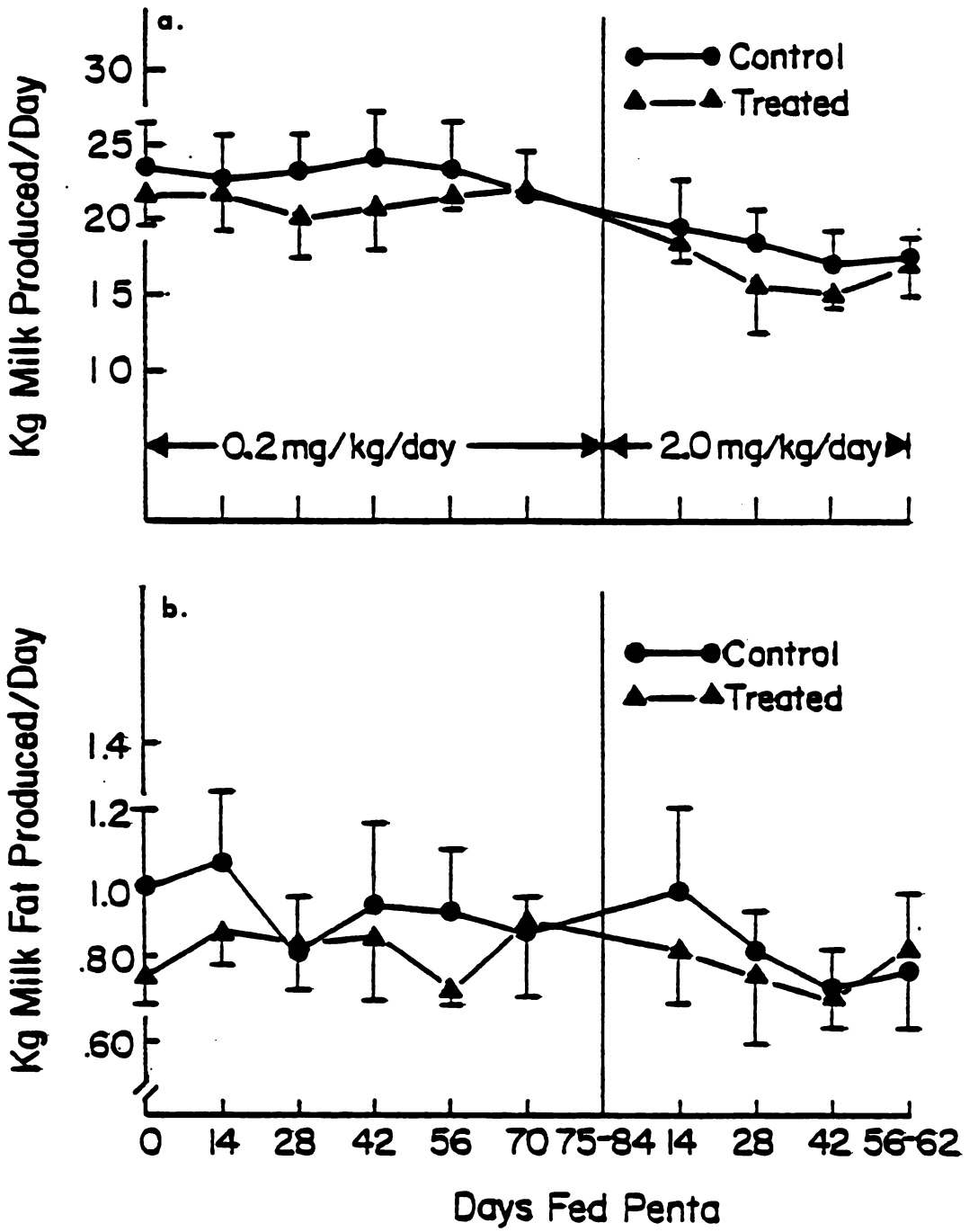

a.
Kg Feed Consumed/Day
40
30
20
Control
Treated
b.
Kg Feed Consumed/
Kg Body Weight/Day
.080
.070
.060
.050
.040
Control
Treated
c.
Kg FCM Milk / Mcal NEL
1.6
1.2
1.0
.90
.70
.50
Control
Treated
0.2 mg/kg/day
2.0mg/kg/day
0
14
28
42
56
70
75-84
14
28
42
56-62
Days Fed Penta

Figure 8. Time Course Status of Total Milk Production (a) and Milk Fat Production (b) in Dairy Cattle Fed 0.2 mg Penta/kg Body Weight/Day For 75 to 84 Days Followed by 2 mg Penta/kg Body Weight/Day for 56 to 60 Days. Each Graphed Point Represents the Mean of all Data Collected During the 14 Day Period Preceding the Specified Number of Days Indicated.


a.
Kg Milk Produced/Day
30
25
20
15
10
Control
Treated
0.2 mg/kg/day
2.0 mg/kg/day
b.
Kg Milk Fat Produced/Day
1.4
1.2
1.0
.80
.60
Control
Treated
0
14
28
42
56
70
75-84
14
28
42
56-62
Days Fed Penta

(Figure 8b).

Effects on Appearance and General Health

In general, all cattle appeared normal throughout the entire period of exposure. However, some treated cattle had problems, not observed in control cattle, but they were not believed by the investigators to be treatment related. One treated cow contracted mastitis early in the experiment and eventually milk production ceased in two quarters even though antibiotic therapy was administered. Also, two treated cattle periodically contracted a health condition characterized by elevated body temperature, mild anorexia and decreased milk production for one or two days. The cause of the condition was never diagnosed. The physical examination conducted one week prior to necropsy revealed no abnormalities in any cattle, other than that the papillae in the buccal cavity of two of the treated cattle were noticeably eroded.

Effects on Clinical Chemistry, Hematology and Urinalyses

There were no biologically significant differences in the results of hematologic and blood chemical measurements that could be attributed to the subchronic administration of penta (Appendix, pg 141). Also, no statistically significant differences between groups were found in the urinalyses obtained on subsamples from samples collected one or two weeks before necropsy (Appendix, pg 145).

Histologic Examination of Tissues

The liver, lungs, kidneys and adrenals represented a significantly greater percentage of the body weight in penta-fed cattle than in control cattle (Table 6). Macroscopic examination of these and other organs

Table 6. Organ Weights of Control and Penta-Treated Cattle Expressed as a Percent of Body Weight[a].

Organ	Control $\bar{X}$ ± SE		Treated $\bar{X}$ ± SE	
liver	0.63	0.03	0.86	0.07[b]
lungs	0.31	0.01	0.40	0.07[b]
kidneys	0.106	0.004	0.140	0.009[b]
spleen	0.082	0.005	0.094	0.004
heart	0.196	0.011	0.219	0.007
thyroid	0.003	0.001	0.003	0.001
adrenals	0.003	0.0004	0.004	0.0001[b]

[a] Organs were trimmed of extraneous body fat before weighing

[b] Different from control mean ($P < .05$), n = 4

revealed certain distinct changes in the treated cattle. The wall of the urinary bladder was thickened in all four treated cattle. The kidneys in three of the treated cattle were pale in appearance. The discoloration was localized primarily in the cortical areas but occasionally, extended into the medullary areas. All other organs in the treated cattle appeared normal. No abnormalities were observed in any organs of control cattle, except for multiple nodular abscesses in the liver of one control cow. Histologic examination of this liver resulted in a diagnosis of chronic focal suppurative hepatitis.

Microscopic examination revealed pathologic changes in several organs of treated cattle, particularly in the kidneys and urinary bladder. The kidneys of three treated cattle had a chronic, diffuse, interstitial nephritis and were mildly hyperemic. In all four treated cattle, some glomeruli were swollen, while others were atrophied or had disappeared and been replaced by connective tissue. Also, the Bowman's capsules were noticeably thickened. In one treated cow, the subcapsular space of many capsules was obliterated and the parenchyma replaced by connective tissue. Some of the tubules in three treated cattle were dilated and connective tissue had proliferated into the interstitial areas. In two treated cattle, there were foci of lymphocytic infiltration in the medulla and cortex. The basement membrane of some renal tubules in one treated cow had undergone hyaline degeneration and deposits of a homogeneous eosinophilic hyalinoid substance were seen in the tubular lumens in two treated cattle.

The urinary bladder of all four treated cattle had a subacute urocystitis, characterized by edema and diffuse infiltration of inflammatory cells, primarily lymphocytes. In one treated cow, the bladder had undergone epithelial desquamation and there was a prominent neutrophilic infiltration

into the transitional epithelial lining. The ureters of one treated cow had a mild ureteritis with some infiltration of inflammatory cells. The kidneys, urinary bladder, and ureters in all control cattle were considered to be histologically normal.

Organ enlargement was the only significant effect noted in the liver. However, a relationship of enlargement to treatment is not conclusive. Furthermore, there was a general enlargement, although not as dramatic, in several organs.

The adrenal glands of one treated cow were abnormal. The capsule was thickened by connective tissue, foci of neutrophils were seen in the zona fasciculata, and small hemorrhages were evident. Except for the one instance of suppurative hepatitis described above, all other tissues of control cattle examined were considered histologically normal.

Effect of Kidney Function

The kidneys of treated cattle were functionally impaired (Table 7). This was demonstrated by the significantly decreased uptake of para-aminohippurate (PAH), tetraethylammonium (TEA) and amino isobutyrate (AIB) in renal cortical slices ($P < .05$). Conversely, the rate of ammoniagenesis was not significantly different ($P > .05$) from that of the control cattle.

Pentachlorophenol Residue Analysis

Serum PCP-Concentrations

Analysis of serum from the penta-fed cattle for PCP during the low dose period showed concentrations to have essentially reached a steady state by three days and averaged 2802 ng/ml (ppb) PCP during the next 65 days (Figure 9). For reasons which cannot be fully explained, there was a slight decline in serum PCP-concentration at the 2, 4 and 6 week sampling times. However, this trend

Table 7. *In Vitro* Renal Function in Penta-Treated and Control Cattle.

Renal Function	Control	Treated
	$\bar{X}$ ± SE	$\bar{X}$ ± SE
PAH S/M[a]	5.32 0.05	3.47[e] 0.27
TEA S/M[b]	3.01 0.16	2.35[e] 0.07
AIB S/M[c]	1.90 0.10	1.57[e] 0.03
Ammoniagenesis[d]	121.00 22.00	153.00 22.00

[a]p-Aminohippurate, slice to medium ratio

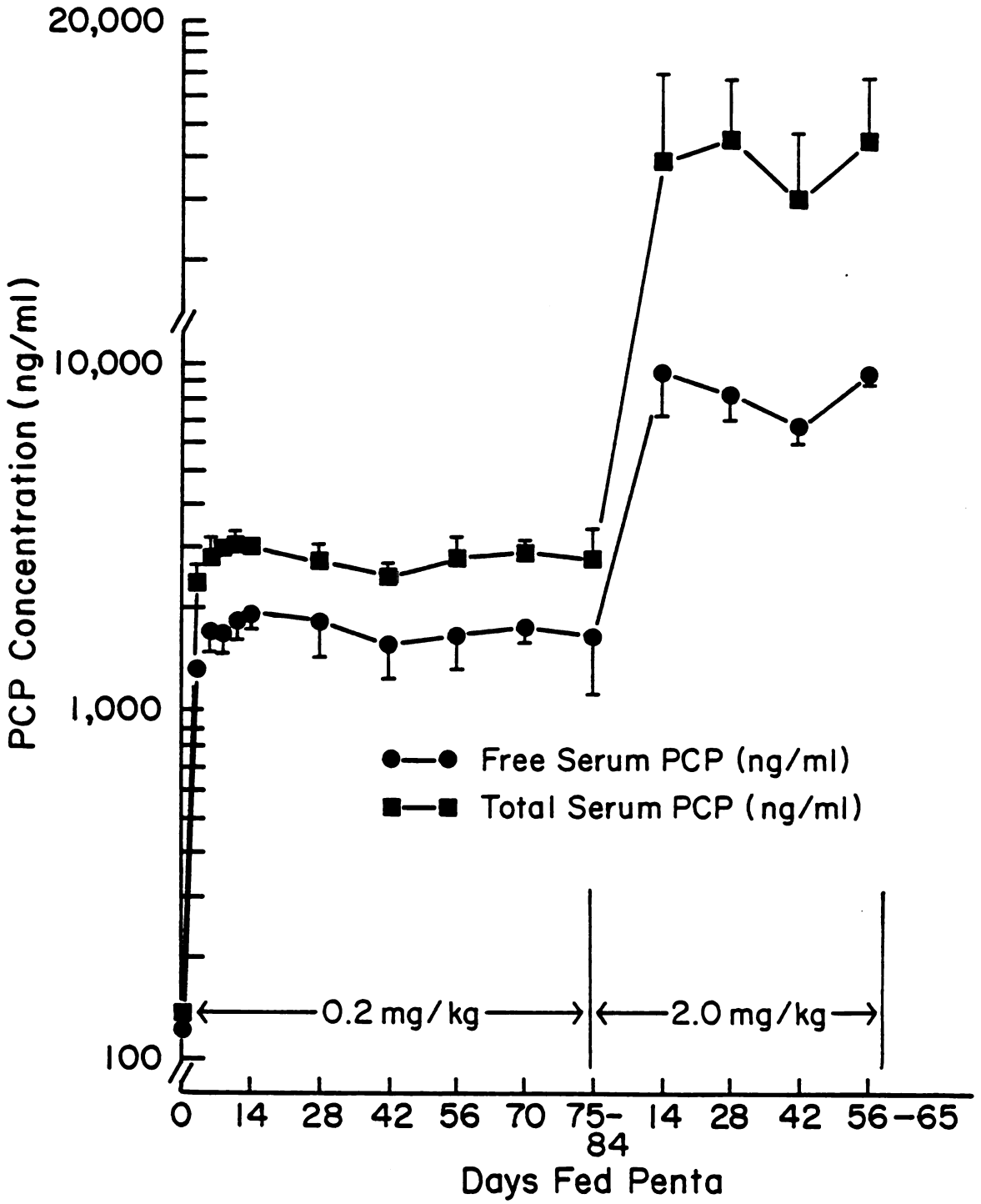
[b]Tetraethylammonium, slice to medium ratio

[c] Amino isobutyrate slice to medium ratio

[d]Mole NH_4 produced per gram dry tissue per hr

[e]Different from control mean ($P < .05$), $n = 4$

Figure 9. Concentrations of Total and Free Serum PCP in Cattle Fed Penta Subchronically.


20,000
10,000
1,000
100
PCP Concentration (ng/ml)
Free Serum PCP (ng/ml)
Total Serum PCP (ng/ml)
0.2 mg/kg
2.0 mg/kg
0
14
28
42
56
70
75-84
14
28
42
56-65
Days Fed Penta

was reversed at the 8 and 10 week sampling periods.

The PCP extracted under very mild acidic conditions is classified as free or unconjugated PCP. Such PCP is not covalently bound to any blood component, but is bound electrostatically or hydrophobically to serum proteins or is not associated with any component of the blood. Total PCP is the amount that is extracted after rigorous acid hydrolysis and consists of both the free PCP and any PCP that is not acid extractable such as conjugated PCP. Therefore, the difference between total and free PCP is expressed as a percent of the total and probably consists of conjugated PCP ((total - free/total) x 100 = % conjugated PCP). Further, 100% - % conjugated should approximately represent that percentage of PCP which is not associated with any serum component other than water or that which is protein bound. The average free serum PCP concentration during the low dose period was 1714 ng/ml. The corresponding values for unconjugated and conjugated PCP are 61% (1714/2082 x 100) and 39% (100% - 61%). Similarly, the average free PCP concentration during the high dose period was 9364 ng/ml and the average total PCP concentration was 14,726 ng/ml. The calculated values for the amounts of unconjugated and conjugated PCP are 64% and 34%.

In a manner similar to that observed during the low dose period there was a slight decline in serum PCP concentrations after the fourth week.

PCP Concentrations in Tissues and Fluids

The body tissues and fluids analyzed for total PCP are shown in Table 8, in order of decreasing concentration. Of the tissues analyzed, liver and kidney contained the highest concentration of PCP, with brain and thyroid having the lowest concentration.

Table 8. Total Pentachlorophenol Concentration in Tissue (ng/gm of wet tissue) or Fluid (ng/ml) Collected at Necropsy from Cattle Fed Penta.

Tissue	PCP Concentration			Number of Samples Analyzed
	$\bar{X}$	±	SE	
liver	13,587		1867	4
kidney	9,579		706	4
spleen	3,437		266	4
lung	3,376		955	4
bile	2,829		734	4
thymus	2,218		166	4
muscle	2,725		168	4
mammary	2,058		247	4
adrenal	1,886		0	1
thyroid	754		78	3
brain	504		0	1
serum	14,520		2289	4

Total PCP concentrations were also measured in rumen fluid, milk, urine and feces after 10 weeks of feeding 0.2 mg penta/kg and after four weeks of feeding 2 mg/kg (Table 9). Total PCP analysis of serum also collected at these times is included for purposes of comparison. At both sampling times, feces and milk had significantly lower PCP concentrations than did urine. After four weeks of being fed 2 mg penta/kg in their diet, the four penta-fed cows had average total PCP concentrations of 14,520 ng/ml in serum and 3,141 ng/ml in milk.

As expected, rumen fluid contained significant quantities of PCP. Interestingly, the initial attempts to analyze a sample of rumen fluid known to contain PCP proved fruitless. Only after fractionating the rumen fluid and analysis of the supernatant and pellet was it determined that the PCP was associated with the particulate portion. As a result of this finding, subsequent analyses were carried out on a homogenate of the whole rumen fluid with the final PCP concentration expressed on a dry matter basis.

Dioxin Residue Analysis of Liver

The livers of the four treated cattle were analyzed for several dioxin isomers. The average concentrations ± SE were: 125 ± 32 ppb (octa), 14.8 ± 2.6 ppb (hepta), and 3.8 ± 0.8 ppb (hexa).

Experiment 2. The Fate of a Single Dose of ^{14}C-Pentachlorophenol in a Lactating Cow

Absorption of ^{14}C-PCP

The main purpose of this study was to examine the fate of a single dose of ^{14}C-PCP in a lactating cow after subchronic administration of

Table 9. Total Pentachlorophenol Concentration in Urine, Serum, Rumen Fluid and Milk.

Sample	Dose					
	0.2 mg/kg/day			2 mg/kg/day		
	$\bar{x}$	±	SE	$\bar{x}$	±	SE
Rumen Fluid[a]	4171[b] ng/ml		1188	30,688[c]		1705
Milk (fat free)[d]	422 ng/ml		86	3,141		1159
Urine[e]	1473 ng/ml		197	25,533		7615
Serum[f]	2917 ng/ml		238	14,520		2289
Feces[a,e]	1916[c] ng/gm		394	4,613		798

[a] Fecal and rumen fluid PCP concentrations expressed on a dry matter basis.

[b] PCP determinations made on samples collected after feeding 0.2 mg penta/kg per day for 10 weeks.

[c] PCP determinations on samples collected after feeding 2 mg penta/kg per day for 4 weeks.

[d] Milk samples were representative samples from the am and pm milkings.

[e] Fecal and urine samples were representative samples from a 12 hour collection (8 am - 8 pm).

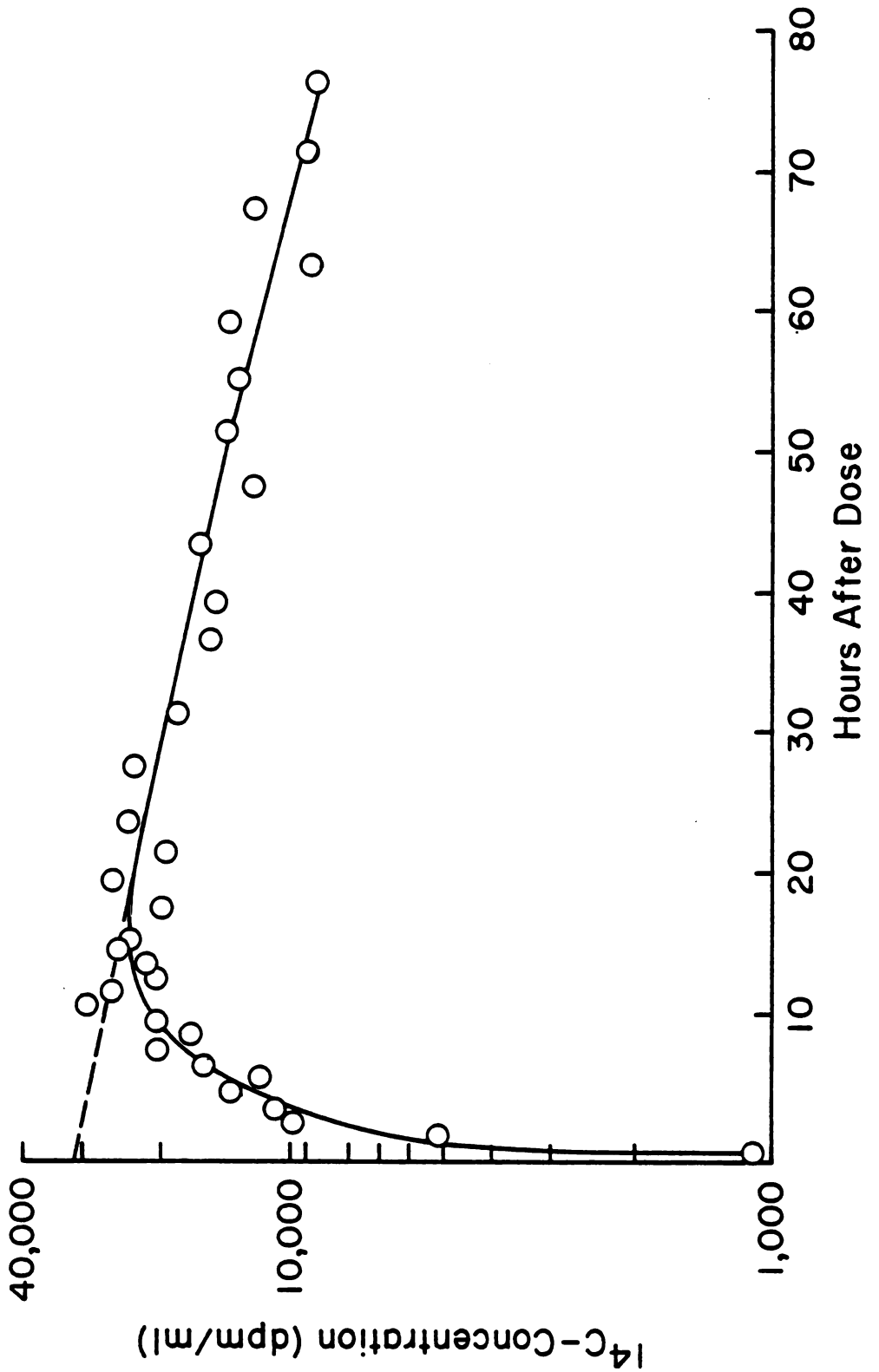
[f] Serum samples collected about one hour before the am dosing.

(0.2 mg/kg per day) of technical PCP. By doing so, the pharmacokinetics of PCP in an animal exposed on a daily basis can be approximated. The amounts of ^{14}C in serum during the 76 hour period ^{14}C administration are shown in Figure 10. The time taken for the ^{14}C to reach its maximum concentration was about 10 hours.

A logarithmic plot of the ^{14}C-concentration in serum over time allows certain assumptions and statements to be made regarding the type of pharmacokinetic model for PCP. Pharmacokinetic modeling involves the use of data, such as the serum data previously described to estimate the number and types of theoretical compartments with which a chemical is associated over time. A compartment can refer to all organs, tissues and cells for which the rates of uptake and subsequent clearance of a chemical are sufficiently similar to preclude kinetic resolution (Gehring et al., 1976). An excellent example of a rapidly equilibrating compartment would be all of those tissues with an abundant blood supply. A slowly equilibrating compartment would be one with less blood flow and examples would be tissues such as fat and bone. The results of the current study (Figure 10) indicates a one compartment open model. The concentration of ^{14}C in the plasma can apparently be described by first order kinetics. The one compartment open model is suggested by the shape of the terminal portion of the curve (Figure 10) which is essentially a straight line. If the line descended and then tended to plateau, one would suspect a two-compartment open model, wherein PCP was eliminated from a rapidly-equilibrating first compartment followed by slower release from a second compartment.

It was assumed that elimination and absorption followed first-order kinetics and that the model is a one-compartment open type. The methods described by Gehring et al.(1976) for estimating the absorption (K_a) and

Figure 10. ^{14}C-Concentration in Serum Measured at Selected Intervals after ^{14}C-PCP Administration.


40,000
10,000
1,000
^{14}C-Concentration (dpm/ml)
10
20
30
40
50
60
70
80
Hours After Dose

elimination (K_e) rate constants were utilized (See Appendix, pg 135). In this case, the K_a = 0.162 hr^{-1} (i.e., about 16% of the dose was absorbed per hour). The time required for the plasma concentration to increase by one-half ($t_{\frac{1}{2}}$) is approximately 4.28 hours. The elimination rate constant (K_e) and associated $t_{\frac{1}{2}}$ are 0.0162 hr^{-1} and 42.8 hours respectively.

Because of the limited amounts of serum available, analysis for free and total PCP was not possible. The quantities available were reserved for the analysis of metabolites, which will not be addressed in this thesis.

Distribution of ^{14}C-PCP

^{14}C- concentration was measured in various tissues and fluids collected at necropsy (Table 10). Liver and kidney had the highest ^{14}C-concentration of the tissues analyzed. Conversely, brain, adipose tissue and spinal cord had the lowest, and nearly equal, ^{14}C-concentrations. Serum and lymph had the highest, and similar, ^{14}C-concentrations of the fluids examined, whereas aqueous humor had the lowest concentration.

Interestingly, the endocrine organs in which ^{14}C was quantified contained significant concentrations of the label.

Metabolism of ^{14}C-PCP

Due to the lack of information on the metabolites in the serum and urine, little can be said at this point regarding the extent to which the ^{14}C-PCP was metabolized. However, because of the large quantity of urine available, analysis for free and total pentachlorophenol by gas chromatography was conducted (Table 11). Rigorous acid treatment of urine is one method by which conjugates can be hydrolyzed. In this study approximately 85% of the PCP was conjugated in the urine; % conjugated = ((total - free)/

Table 10. ^{14}C-Concentration and Corresponding Pentachlorophenol Concentration in Various Tissues and Fluids.

Tissue/Fluid	^{14}C[a] Concentration (tissues, dpm/g) (fluids, dpm/ml)	^{14}C[b] Concentration	PCP[c] Concentration (tissues, ng/g) (fluids, ng/ml)
Serum	9240	NA[d]	NA[d]
Lymph	8873	NA[d]	NA[d]
Liver	8837	7964	2052
Kidney	5758	4941	2149
Bile (total)	5756	--	1072
Bile (free)	3831	--	475
Gall Bladder	4236	4047	1427
Lymph Node	3920	2499	1784
Lung	3138	3562	1100
Ovary	2760	1693	1354
Adrenal	2759	2878	1064
Mammary Tissue	2752	2753	732
Pancreas	2712	1550	887
Heart Muscle	1812	1000	980
Thyroid	1554	--	1424
Duodenal contents	1400	8755	368
Spleen	1052	964	984
Round Muscle	741	844	354
Adipose Tissue	368	353	--
Spinal Cord	349	210	--
Brain	268	343	--
Spinal Fluid	256	--	--
Rumen Fluid	No Activity	--	--
Aqueous humor	No Activity	--	--

[a] ^{14}C-concentration measured by liquid scintillation counting.

[b] ^{14}C-concentration measured by oxidation to $^{14}CO_2$ and liquid scintillation counting.

[c] Total PCP concentration measured by gas chromatography.

[d] NA = not available

Table 11. Gas Chromatographic Analysis of Urine for Total and Free Pentachlorophenol.

Hours After Dose	Free PCP (ng/ml)	Unconjugated %	Total PCP (ng/ml)	Conjugated %
0	860	18	4864	82
4	995	18	5469	82
8	689	12	5916	88
12	874	15	6002	85
16	682	15	4650	85
20	682	13	5244	87
24	873	14	6063	86
28	712	16	4506	84
32	697	13	5311	87
36	732	14	5161	86
40	675	14	4696	86
44	546	14	3886	86
48	698	15	4650	85
52	754	16	4654	84
56	572	13	4401	87
60	493	11	4514	89
64	574	16	3641	84
68	596	15	4016	85
72	966	20	4931	80
76	872	17	4992	84
	Mean	14.95		85.05
	SE	0.49		0.49

total) x 100. The solvent extracts of the free and total urine analysis were quantified by liquid scintillation counting (Table 12). The amounts of ^{14}C classified as % unconjugated (15%) and % conjugated (85%) closely reflect the concentrations measured by GC analysis.

Excretion of ^{14}C-PCP

The fact that approximately 75% of the original dose was excreted in the urine clearly indicates that this is the primary route of PCP excretion (Figure 11). Feces provided only a minor route for excretion of PCP and/or its metabolites. In the current study, approximately 5% of the dose was accounted for in the feces (Figure 12).

Milk was a minor excretory route, only 4.8% of the original dose being accounted for in the milk (Figure 13). The distribution of PCP in milk was investigated. A 40 ml aliquot from each four-hour collection was centrifuged and the amount of ^{14}C in the milk-fat, casein and whey fractions quantified (Table 13). The approximate percentage distribution was 62.2, 24.4 and 13.3 for the whey, casein and fat fractions, respectively. Except for one instance, this distribution remained quite constant over the 76 hours. The 36 hour sample was thawed and frozen several times prior to analysis. Such treatment appeared to significantly alter the relationship between ^{14}C and the whey fraction such that it released and partitions into the casein fraction.

In an attempt to characterize the nature of ^{14}C and PCP in the casein and whey fractions, these fractions were treated in an identical manner to serum. A 1:4 homogenate of the casein was made with deionized, double-distilled water then duplicate 1 ml aliquots of both the whey and casein homogenate were analyzed for free and total

Table 12. Solvent Extracts of Urine Analyzed for Total and Free ^{14}C-Concentration Using Liquid Scintillation Counting.

Hours After Dose	Free ^{14}C-Concen.[a]	Unconjugated %	Total ^{14}C-Concen.[a]	Conjugated %
0	--	--	--	--
4	1487	12	11,933	88
8	3527	12	29,027	88
12	5650	14	40,935	86
16	5539	16	34,395	84
20	6016	14	42,368	86
24	7537	15	49,134	85
28	5601	16	35,224	84
32	5709	14	40,093	86
36	6053	16	36,917	84
40	4800	15	31,580	85
44	3779	15	25,602	85
48	4311	15	26,643	85
52	4258	16	26,326	84
56	3247	14	23,783	86
60	2642	12	22,537	88
64	2671	17	16,160	83
68	2631	14	16,160	83
72	4040	21	19,015	79
76	3134	17	18,074	83
		$\bar{X}$ 15.0		85.0
		SE 0.48		0.48

[a] ^{14}C-concentration in dpm/ml.

Figure 11. ^{14}C-Concentrations in Urine and Cumulative Percentage of Dose Excreted into Urine Measured at 4-Hr Intervals After Administration.

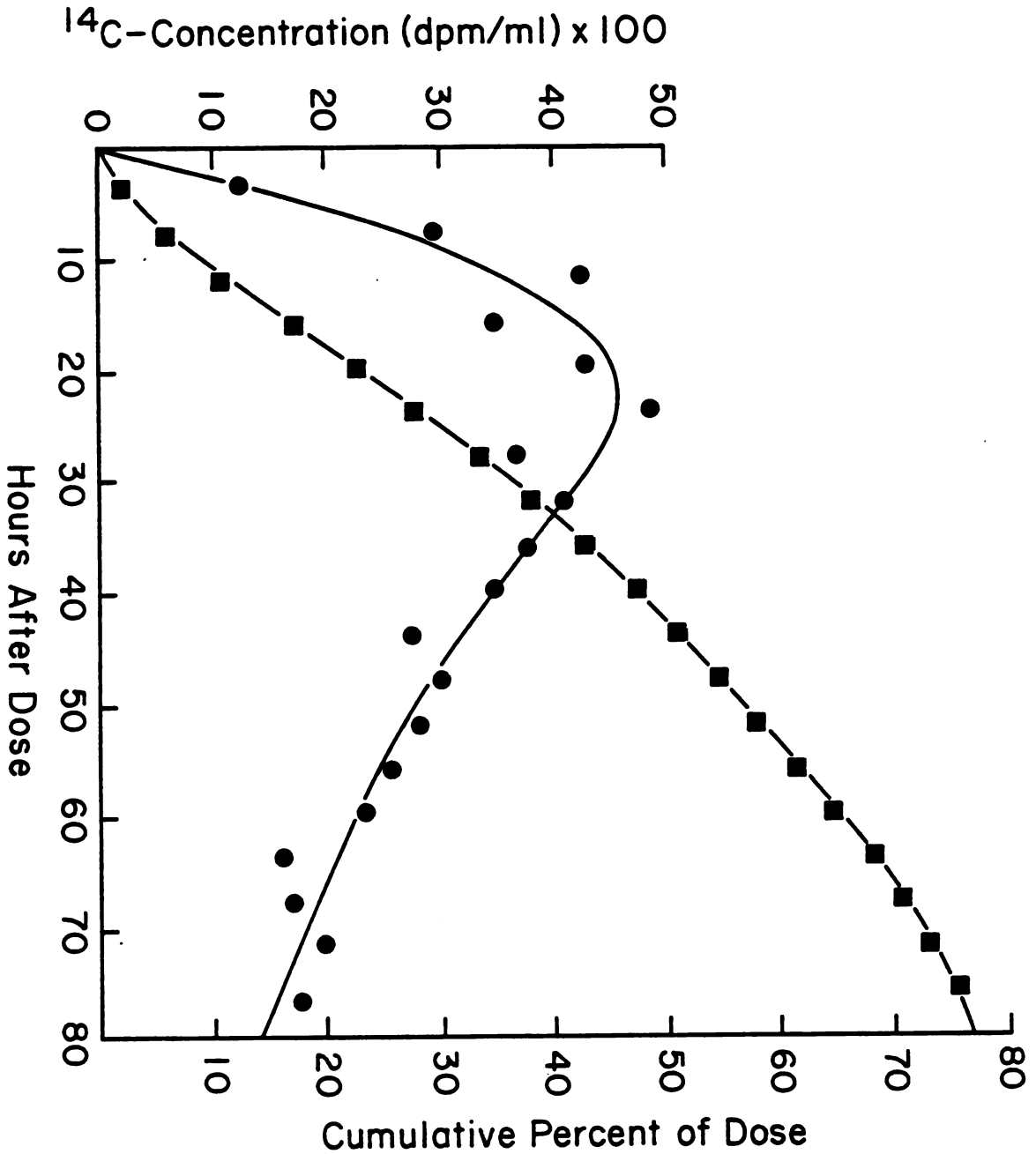

^{14}C-Concentration (dpm/ml) x 100
0
10
20
30
40
50
Hours After Dose
10
20
30
40
50
60
70
80
Cumulative Percent of Dose
10
20
30
40
50
60
70
80

Figure 12. ^{14}C-Concentrations and Cumulative Percentage of Dose Excreted into Feces Measured at 4-Hr Intervals After Administration.

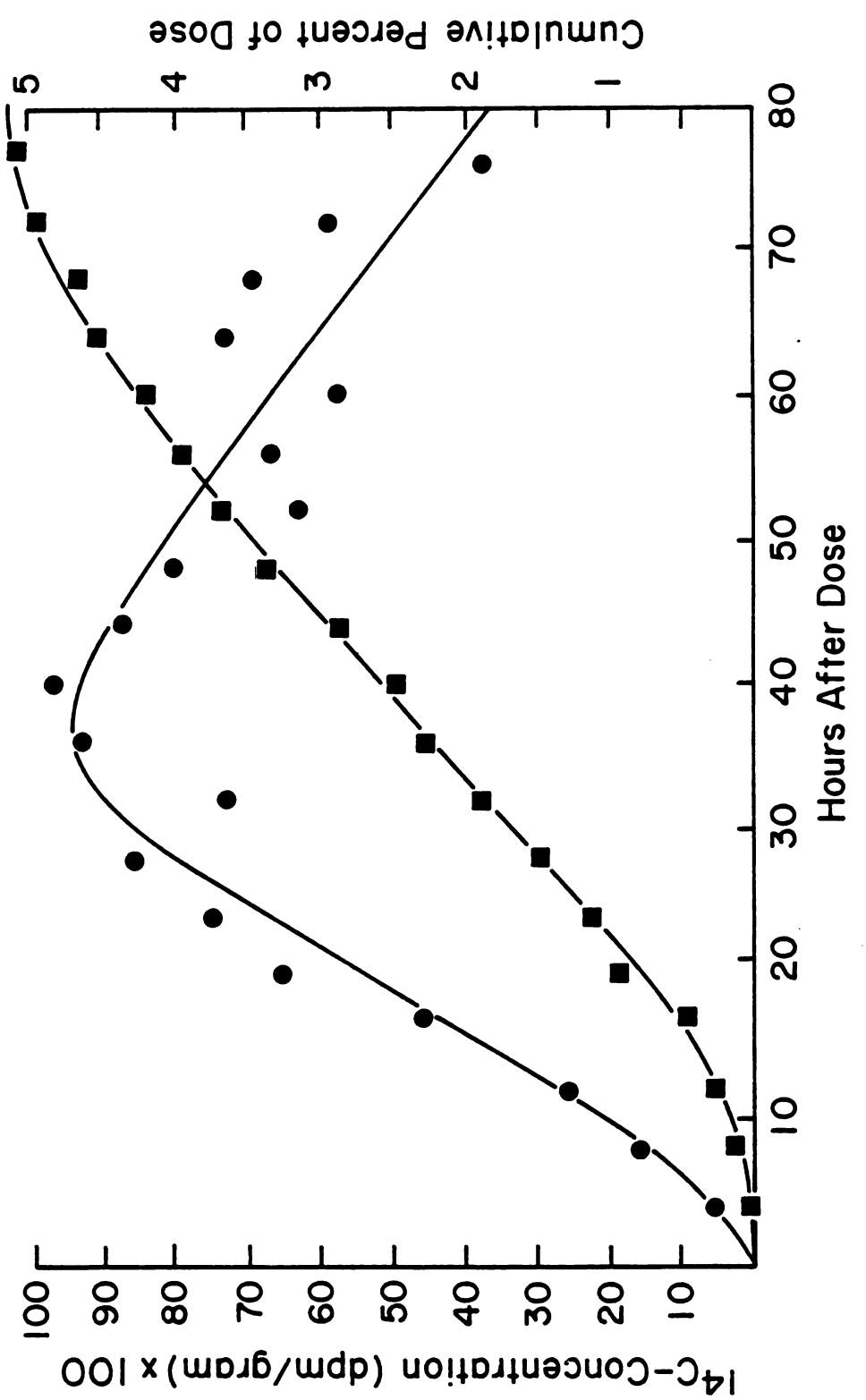

^{14}C-Concentration (dpm/gram) x 100
Cumulative Percent of Dose
Hours After Dose
10
20
30
40
50
60
70
80
90
100
1
2
3
4
5

Figure 13. ^{14}C-Concentration in Milk and Cumulative Percentage Secreted into Milk, Measured at 4-Hr Intervals After Administration.

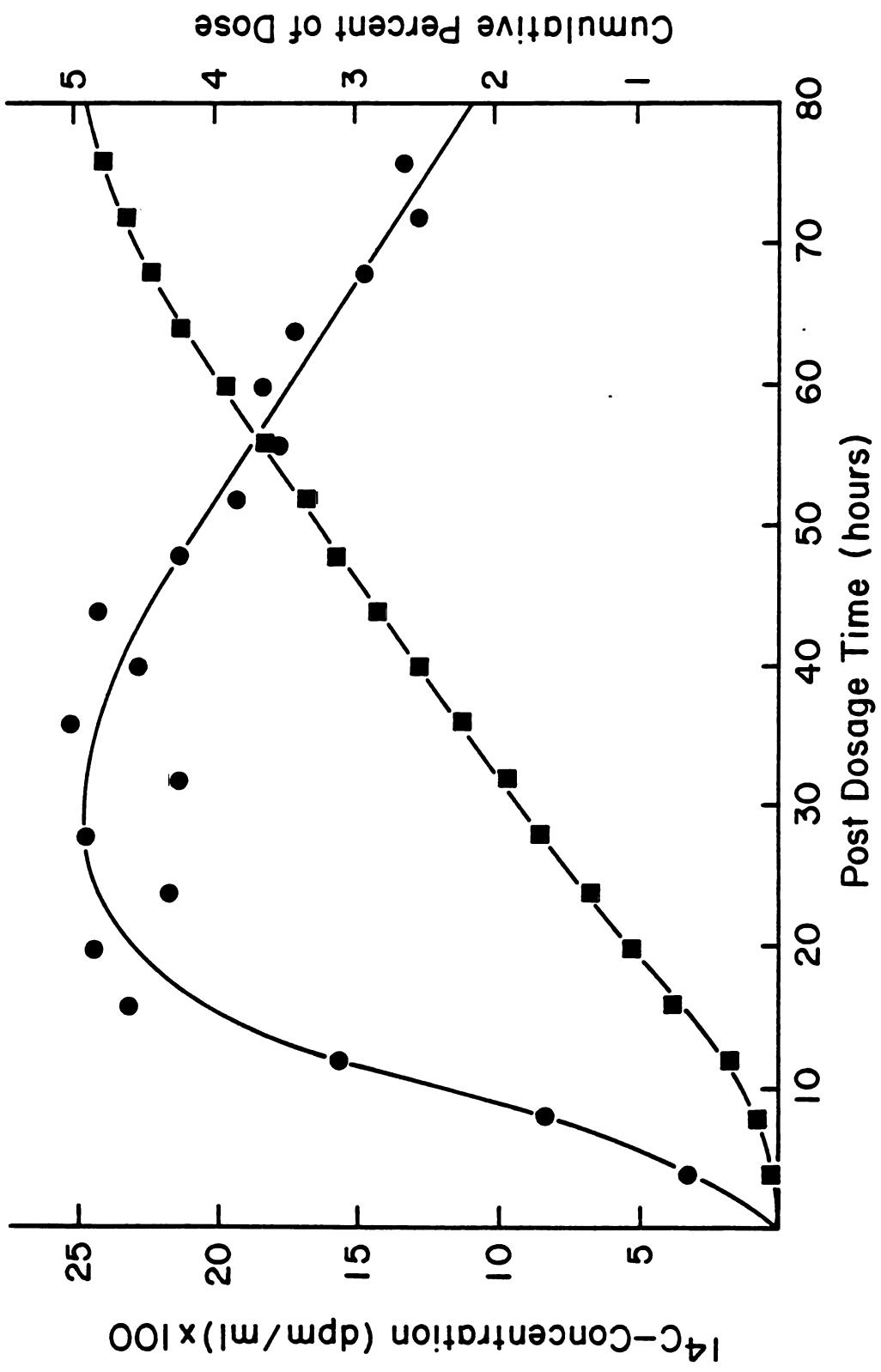

^{14}C–Concentration (dpm/ml) x 100
25
20
15
10
5
Cumulative Percent of Dose
5
4
3
2
1
10
20
30
40
50
60
70
80
Post Dosage Time (hours)

Table 13. Percentage Distribution of ^{14}C in Milk Fractions at 4-Hour Intervals After Administration.

Hours After Dose	Milk Fat % of Total	Whey % of Total	Casein % of Total
0	--	--	--
4	14.8	63.8	21.4
8	15.3	60.7	24.1
12	13.0	61.8	25.3
16	11.1	64.6	24.3
20	17.1	56.9	25.9
24	14.1	60.8	24.7
28	10.6	61.0	28.2
32	18.2	54.7	27.1
36	14.2	40.1	45.7
40	11.2	64.6	24.2
44	14.6	64.6	20.7
48	12.3	67.5	20.2
52	15.8	61.0	23.1
56	11.5	66.5	21.6
60	10.0	67.4	22.6
64	8.5	63.2	28.2
68	15.3	56.1	28.6
72	20.1	50.3	30.0
76	12.5	62.8	24.7
Overall Means [a]	13.3	62.2	24.4
SE	0.6	0.4	0.4

[a]Percentages for the various components for 36 and 72 hours are not included in the overall mean. See text for explanation.

PCP by gas chromatography (GC). The solvent extracts analyzed by GC were also counted (^{14}C) using liquid scintillation counting (LSC). The ^{14}C and PCP distribution in the casein fraction are outlined in Table 14. GC analysis indicated about 66% of the PCP was conjugated in casein, whereas 34% was unconjugated. LSC analysis showed that approximately 28% of the ^{14}C was unconjugated and 72% was apparently conjugated. In contrast, by GC analysis of the whey fractions, nearly equal amounts of PCP were unconjugated and conjugated (Table 15). LSC analysis of the solvent extracts showed that about 58% of the ^{14}C was unconjugated and about 42% was conjugated.

Percentage recovery of the ^{14}C label from casein was calculated after analysis of the casein homogenates. Replicate samples of the casein homogenates were counted using LSC prior to their analysis. Following their analysis for total ^{14}C, two aliquots (.01 ml) of each of the benzene extracts from replicate samples analyzed were counted using LSC. Thus, knowing how much ^{14}C was initially in the casein homogenate and how much ^{14}C was extracted into the benzene allowed for calculation of recovery values. The average percentage recovery value (± SE) for replicate analysis of the casein homogenates each prepared from the casein fraction of 19 milk samples was 97.6 ± 1.4%. The same procedure was followed for calculation of an average percentage recovery value for analysis of replicate samples of whey prepared from the 19 milk samples. In this case the average percentage recovery (± SE) was 100.4 ± 1.1%.

Table 14. Percentage Distribution of Conjugated and Unconjugated ^{14}C and Pentachlorophenol (PCP) in Casein, as Measured by Liquid Scintillation Counting (LSC) and Gas Chromatography (GC) at 4-Hour Intervals After Administration.

Hours After Dose	LSC Analysis		GC Analysis	
	% of Total Unconjugated ^{14}C	% of Total Conjugated ^{14}C	% of Total Unconjugated ^{14}C	% of Total Conjugated ^{14}C
0	--	--	42.9	57.1
4	40.0	60.0	32.0	68.0
8	36.8	63.2	38.2	61.8
12	27.5	72.5	33.0	67.0
16	26.4	73.6	28.1	71.9
20	27.6	72.4	38.7	61.3
24	20.3	79.7	36.3	63.7
28	29.1	70.9	37.6	62.4
32	21.5	78.5	37.2	62.8
36	17.3	82.7	40.2	59.8
40	27.4	72.6	28.2	71.8
44	22.6	77.4	32.8	67.2
48	22.6	77.4	35.8	64.2
52	24.8	75.2	30.6	69.4
56	27.2	72.8	32.7	67.3
60	27.2	72.8	33.1	66.9
64	27.2	72.8	33.1	66.9
68	26.0	74.0	35.3	64.7
72	26.9	93.1	27.7	72.3
76	32.3	67.7	24.5	75.3
Overall Means	27.8	72.2	34.3	65.7
SE	1.3	1.3	1.1	1.1

[a]Percentages for the various components for 26 and 72 hours are not included in the overall mean. See text for explanation.

Table 15. Percentage Distribution of Conjugated and Unconjugated ^{14}C and Pentachlorophenol (PCP) in Milk Whey as Measured by Liquid Scintillation Counting (LSC) and Gas Chromatography (GC) at 4-Hr Intervals After Administration.

	LSC Analysis		GC Analysis	
Hours After Dose	% of Total Unconjugated ^{14}C	% of Total Conjugated ^{14}C	% of Total Unconjugated ^{14}C	% of Total Conjugated PCP
0	--	--	63.0	37.0
4	60.0	34.6	52.6	47.4
8	65.3	34.6	53.2	46.8
12	63.4	36.6	65.6	34.4
16	60.2	39.8	56.8	43.2
20	69.7	30.3	56.0	44.0
24	51.3	48.7	46.3	53.7
28	56.1	43.9	37.2	62.8
32	55.7	44.3	53.2	46.8
36	68.7	31.3	52.9	47.1
40	52.9	47.1	48.1	51.9
44	66.4	33.6	65.7	34.3
48	52.9	40.8	58.4	41.6
52	56.3	43.7	55.2	44.8
56	64.9	35.1	56.6	43.4
60	52.4	47.6	53.1	46.9
64	49.5	50.5	49.9	50.1
68	60.2	39.8	50.9	49.1
72	48.2	51.8	54.4	45.6
76	54.7	45.3	58.4	41.6
Overall Means[a]	58.4	41.6	54.5	45.5
SE	1.4	1.4	1.6	1.6

[a]Percentage for the various components for 37 and 72 hrs are not included in the overall mean. See text for explanation.

DISCUSSION

Toxicodynamics of Technical Pentachlorophenol

The dosage levels of penta used in this experiment were selected to approximate the daily exposure of lactating dairy cattle on farms where treated wood may be extensively used in structural components in livestock facilities. However, it should be noted that oral administration may not be completely representative of actual farm exposure. As mentioned earlier in a discussion of its chemical and physical properties, pentachlorophenol has a small, but substantial vapor pressure (Bevenue and Beckman, 1967). Also, due to conditions such as overuse of penta-treated wood, poor ventilation and high ambient temperature, there is a potential for considerable inhalation of vapors in livestock barns (Shull, 1977). This possibility is supported by studies of Casarett et al. (1969) and Thompson (1978). Furthermore, dermal absorption is another possible route of exposure. Studies by Schipper (1961) have shown high mortality rates in piglets confined in farrowing crates constructed of wood freshly treated with penta. However, cattle are rarely confined on wood and their hair coat provides some degree of protection. The findings presented here are representative of cattle subchronically exposed to penta through the oral route. Consequently, there is a need for toxicologic evaluation of penta administered to cattle via the dermal and pulmonary routes.

Performance, Appearance, General Health, and Kidney Function

Exposure of lactating dairy cattle to penta had no significant effect on body weight ($P > .05$). McConnell et al. (1980) reported that heifers fed 15 mg/kg body weight per day of penta had reduced body weight

gains. However, this effect was due to the dioxin or dibenzofuran impurities in penta, rather than PCP itself. Moreover, these same animals also had a significantly poorer feed conversion ratio in conjunction with a decreased rate of gain. In contrast, the penta-fed cattle in this study maintained their body weight throughout both treatment periods. In studies reported by McConnell et al. (1978b) the toxicity of several dioxins was compared in several species. Weight changes were observed which could not be entirely attributed to decreased feed intake.

The fact that all cattle in the treatment group in our study readily consumed the penta-containing concentrate suggests that the levels of penta were below the taste threshold or the molasses added to the ration effectively masked the reported repellant properties of penta (Deichmann et al., 1942). McConnell et al. (1980) successfully fed 15 mg/kg body weight of both analytical and technical PCP. In our study, when feed intake was related to body weight over the test period, the amount of feed consumed by the penta fed-cattle was not different ($P > .05$) from the control cattle (Figure 7b). This suggests that penta had no effect on metabolic processes.

The observation that the penta-fed cattle tended to be more efficient in the conversion of feed to milk during the later part of the high-dose period is not readily explainable. This tends not to agree with studies that showed PCP to interfere with cellulose digestion when it was added to mixed cultures of rumen microorganisms (Shull et al., 1977; Shull and McCarthy, 1978). However, the lack of an effect of penta on body weight, milk production and efficiency suggests that penta does not have an in vivo effect on cellulose digestion. This may, in part, be explained by the fact that the rumen is a dynamic system where PCP is removed from the rumen

rapidly, whereas the in vitro digestion method involves a static system.

The lack of an adverse effect on milk production does not coincide with the field observations of Thomas et al. (1977) which included decreased milk production in penta exposed animals. However, there is the suggestion that expression of the toxic effects of penta may be a time-dependent phenomenon. Firestone et al. (1979) found that dioxins accumulate in the bodies of cattle fed penta chronically, but require a significant period of time to reach a steady-state concentration. In Firestone's cattle, the dioxin content had not yet reached a steady-state concentration after 70 days of continuous exposure. This may be, in part, due to the fact that dioxins are poorly absorbed from the gastrointestinal tract (Schwetz et al., 1971). Accordingly, MCconnell et al. (1980) showed that growth suppression, in heifers fed penta is both dose and time-dependent. Therefore, the performance of the penta-fed cattle in this study may have been impaired had penta administration continued for a longer period of time.

Somatic cell measurement in milk provides a means of monitoring the presence of mastitis in lactating dairy cattle. Somatic cell measurements in milk from control and penta-fed cattle showed treated cattle to have cell counts not significantly different ($P>.05$) from those of control cattle. These results do not conform to the findings of Thomas et al. (1977) where lactating cattle housed in penta-treated barns had an increased incidence of mastitis.

The lack of an effect of feeding penta on milk fat production coincides well with other measurements of performance such as feed intake, milk production and feed efficiency.

The generally normal appearance of the treated cattle throughout the

entire period of exposure tends to support other findings with respect to the performance of these cattle. As for the health problem which periodically plagued two of the treated cattle, the inconsistency of its appearance and lack of correlation with dose suggest that it is not treatment related. The findings of the physical examination generally support the lack of any treatment effects on performance. In fact, there were no abnormal observations and a generally normal appearance in all cattle. The presence of eroded papillae in two of the four treated cattle may have been a local effect of penta. However, because the papillae were not evaluated prior to exposure, a cause-effect relationship cannot be established.

Clinical Chemistry, Hematology and Urinalyses

There were no biologically significant differences in the results of hematologic examinations, blood chemistry evaluations or urinalyses which could be attributed to the subchronic administration of penta. Minor variations in blood measurements, routinely performed throughout both treatment periods were inconsistent between sampling periods and failed to follow any definitive trends or relationships with dose. In contrast, McConnell et al. (1980) found a dose-related decrease in packed cell volume (PCV), accompanied by a comparable decrease in the hemoglobin concentration. It should be remembered that these findings were in heifers fed 15 mg penta/kg per day. In our study, no statistically significant differences ($P > .05$) were found between groups in urinalyses obtained one or two weeks before necropsy. McConnell et al. (1980) also found no statistically significant differences in the results of urinalyses in heifers fed penta subchronically for 160 days.

Pathologic Findings

The microscopic findings in the kidneys coincide with the gross pathologic findings. However, the observed lesions were apparently not severe enough to be reflected by significant changes in routine clinical laboratory tests. In general, more than half of the original renal parenchyma must be destroyed before hematologic, clinical chemistry and urinalysis values are altered (Kaneko and Cornelius, 1971). Thus, at the time of necropsy the kidneys may have been in an early stage of functional impairment. Even though there was no similar kidney damage in control cattle, there is not sufficient evidence to state definitively that the observed effects were in fact caused by penta exposure. A similar situation was encountered by McConnell et al. (1980). They observed interstitial nephritis in heifers fed penta chronically, but concluded that the effect was not treatment related on the basis that it was seen with equal frequency in both control and treated cattle.

The pathologic condition of the urinary bladders in the treated cattle in our study was classified as urocystitis. McConnell et al. (1980) described a thickening of the bladder wall in heifers fed 15 mg penta per kg body weight. These authors classified the thickening of the bladder as epithelial hyperplasia rather than urocystitis. Moreover, in their study the effect was directly related to the amount of penta fed, which totalled more than ten times the amount fed in the present study. Also, they observed concurrent hyperplasia of renal papillae, which was not detected in the penta-fed cattle in our study. The nature of the interrelationship among bladder wall hyperplasia, urocystitis and the administration of penta are unclear. The effect could be related to the use of urethral catheters for urine collections, i.e., catheterization may have been responsible for the direct introduction of an infectious microorganism

into the urinary bladder which caused the urocystitis. However, control cattle were also catheterized using an identical procedure and equivalent time frame, without ill effect. Because urine is the primary excretory route of PCP in cattle, an increased susceptibility of the bladder to infection may have developed. However, the status of the immune system in these cattle was not impaired by the penta treatment. (Forsell et al., 1980).

Organ enlargement coupled with liver monooxygenase induction was reported by McConnell et al. (1980) in heifers fed either penta or purified PCP. Microscopic examination showed mild vacuolation of hepatocytes in addition to the presence of foci of lymphocytic infiltration and capillary dilation in the lobules. Hepatocytic vacuolization had also been observed in cattle environmentally contaminated with penta (Thomas et al., 1977). However, in the absence of specific serum chemistry changes this histologic abnormality is not a sufficient basis for establishing a cause-and-effect relationship with treatment. In view of the fact that the liver is a primary site of dioxin accumulation, the lack of severe hepatic injury is somewhat surprising. McConnell et al. (1980) reported other pathologic changes associated with the liver, namely bile duct hyperplasia and dilation of bile canaliculi. Neither of these effects was observed in the present study.

The relative lung weights, like those of the liver were significantly increased, an effect also noted by McConnell et al. (1980) in heifers fed penta. Enlargement was accompanied by interstitial pneumonia in two of four treated cattle. Microscopic examination showed mild proliferation of connective tissue and infiltration of lymphocytes. The relationship of this lesion to treatment is not clear, as a similar effect was not observed

in heifers fed 15 mg/kg per day penta for approximately the same duration (McConnell et al., 1980).

The results of the in vitro kidney function tests tend to support the histologic findings in the kidneys. The significantly decreased uptake of para-aminohippurate (PAH), tetraethylammonium (TEA), and amino-isobutyrate (AIB) in renal cortical slices indicate some degree of renal impairment, but not to such a degree that the animal's general health was overtly impaired.

One possible explanation for the decreased uptake of these prototype substances is decreased active transport, which is an ATP-requiring process, in the proximal tubule. PCP is known to interfere with the synthesis of ATP.

Toxicokinetics of Technical Pentachlorophenol

Absorption and Serum Concentrations

In the interest of integrating the information on residue analysis from the lactating cattle fed penta subchronically (Experiment 1) and the one lactating cow given a single dose of ^{14}C-PCP (Experiment 2) the results of both will be discussed in this section.

A relatively short period of time was required for PCP concentrations in serum to reach steady state in the cattle fed penta subchronically. By the third day, steady state was clearly attained. In the cow dosed with ^{14}C-PCP the serum ^{14}C-concentration peaked in approximately 10 hours. This is a somewhat longer time than in rats, but is a shorter time than in monkeys given a single dose of ^{14}C-PCP. Braun et al.(1977) gave single doses of ^{14}C-PCP to rats and observed that plasma ^{14}C-concentrations peaked in about four hours, whereas Rhesus monkeys given a single dose of ^{14}C-PCP

attained maximum plasma ^{14}C-concentrations in 12-24 hours (Braun and Sauerhoff, 1976). Apparently the female monkeys absorbed the PCP more slowly than did the males, but reached a greater ^{14}C-concentration in the blood. The difference in the rate of absorption between the bovine vs monogastric species (rat, monkey) may be because the ^{14}C-PCP was administered in a gelatin capsule with alpha-cellulose as a carrier. The rate absorption constant (ka) of 0.162 hr^{-1} in our study is similar to the ka of 0.215 hr^{-1} found in male Rhesus monkeys dosed orally with ^{14}C-PCP (Braun and Sauerhoff, 1976). The absorption $t_{\frac{1}{2}}$ of 4.28 hours in the cow is also similar to the absorption $t_{\frac{1}{2}}$ of 3.64 hours in male monkeys. Both male and female rats have significantly larger absorption rate constants of 1.95 and 1.52 hr^{-1} respectively (Braun et al., 1977). These convert to absorption $t_{\frac{1}{2}}$ of 0.355 and 0.456 hours, respectively.

The deviations in linearity in the serum data in Figure 10 may be partially explained by the possible enterohepatic recirculation of PCP. This would involve elimination of PCP in the bile and storage of bile containing PCP in the gall bladder. Then, upon ingestion of food, biliary secretion into the small intestine would result, followed by the subsequent reabsorption of PCP.

The amounts of PCP classified as unconjugated (i.e., bound to serum proteins) and conjugated, in cattle fed penta subchronically, appears to remain relatively constant regardless of dose. Interestingly, the serum analysis data from these cattle suggest that a considerable amount of PCP is present in a conjugated form. In contrast, Braun et al. (1977) showed that the majority of ^{14}C found in the serum of rats given ^{14}C-PCP was unchanged PCP, with only a small amount present as the glucuronide conjugate. However, since these rats were not previously exposed sub-

chronically to penta, it is possible the conjugating enzymes, such as glucuronyl transferase were not induced by previous exposure to dioxins and furans.

Distribution

Liver and kidney contained the greatest concentrations of total PCP in cattle fed penta subchronically. Analysis of liver and kidney tissue, for total ^{14}C, from the cow given a single dose of ^{14}C-PCP produced similar results. In terms of compartments, these data are reasonable, as both tissues represent rapidly equilibrating compartments because of their profuse blood supply. In the ^{14}C study, brain and adipose tissue contained the lowest ^{14}C-concentrations. Adipose tissue represents a slowly equilibrating compartment. It is likely in the case of the brain that although it is highly vascularized, it has a high phospholipid content into which PCP does not readily partition at physiologic pH. The low levels of ^{14}C in the spinal fluid, in addition to the low concentration in the brain clearly demonstrate that PCP or its metabolites have a limited distribution to the central nervous system. Braun et al. (1977) also found the highest concentrations of ^{14}C, in decreasing magnitude, in the liver, kidney and plasma of rats dosed with ^{14}C-PCP. Brain and adipose tissue contained the lowest concentrations of ^{14}C. Similarly, Larsen et al. (1972) found that after dosing rats with ^{14}C-PCP, the liver contained the highest ^{14}C-concentration followed by the kidney, blood, stomach, adrenals and lungs. These authors found adipose tissue and muscle to have the lowest, and nearly equal concentrations of ^{14}C. In the present subchronic study the spleen, lungs and muscle had relatively high concentration of PCP. One explanation for this may be the presence of residual blood in these tissues.

A significant ^{14}C-concentration was found in the gall bladder and lymph nodes. This may be partially explained by the fact that they were both in contact with fluids which contained high concentrations of ^{14}C. Considerable ^{14}C-concentration in the kidney, lung and liver may be due to their extensive vascularization in conjunction with their high protein content. Barbiturates behave in the blood in a manner not unlike PCP, i.e., they are highly serum protein bound (Harvey, 1975). It appears that the ability of tissues to concentrate barbiturates is largely dependent on their protein binding capacity for barbiturates. Further, the tissue binding capacity of the various barbiturates is closely related to their plasma protein binding capacity. Thus, both liver and kidney tend to have higher concentrations of barbiturates than do other tissues. In contrast, several of the short acting barbiturates should be able to concentrate easily in the body fat, but because of the plasma protein binding, slow rate of blood flow and low surface-to-volume ratio of adipocytes, uptake is slow. This may also explain the significant concentrations of ^{14}C measured in the endocrine organs.

Rumen contents contained no ^{14}C-activity, which suggests that no PCP or its metabolites are secreted back into the rumen or in the saliva. Note, however, that GC analysis of rumen fluid shows it to have about 800 ppb PCP, on a dry matter basis. Unfortunately, neither samples of abomasal tissue ("true stomach") nor its contents were collected at necropsy. Although the stomach in monogastrics is probably the major site of PCP absorption because of its low pH, there is one report which suggests that PCP may be secreted back into this compartment (Jakobson and Yllner, 1971). In contrast to rumen fluid, duodenal contents contain a considerable concentration of ^{14}C. Given that the duodenum preceded the area of the small intestine into which bile is released, this may be

indirect evidence that PCP was secreted into the abomasum. However, it is also possible that the glands of Brunner, which are the major contributor to secretion in the upper duodenum (Knobel, 1971) are the source of the ^{14}C appearing in the duodenal contents. This is reasonable, given that the cow had been given half of her 0.2 mg/kg daily dose of penta five hours prior to necropsy. The rumen fluid samples measured for total PCP from the cows fed penta subchronically averaged about 4 ppb PCP during the 0.2 mg/kg dose period. The marked difference in PCP concentrations can be partially explained by the fact that rumen fluid sampling in the cattle fed penta subchronically was done about one hour after dosing. The significance of PCP being associated with rumen particulates and the resulting effect on digestion of feed by rumen bacteria is unknown.

Serum and lymph had the highest ^{14}C-concentrations of the fluids examined. This is understandable in that they share common proteins (Knobel, 1971). Lymph contains 1-4 times less protein than serum. However, albumin is present in significant quantities in lymph, due to its small size and ability to cross capillary walls. Braun et al. (1977) showed that PCP can bind both tenaciously and in extremely large quantities to serum albumin. These authors suggested that this relationship is the primary reason for the preferential retention of ^{14}C in the plasma. Probably a more obvious reason for the similar ^{14}C concentrations in serum and lymph is that lymph is derived from serum.

Examination of the distribution of ^{14}C in milk from the cow given a single dose of ^{14}C-PCP showed that the majority of ^{14}C in milk is associated with the whey fraction (62.2%) with lesser amounts associated with the casein (24.4%) and fat (13.3%) fractions. This can be partially explained

by considering the gross composition of milk which is about 82% whey, 15% casein and 3.0% fat. Although the ^{14}C-concentration is higher in the casein than in the whey, the latter still represents the largest fraction in milk. Analysis of the casein and whey fractions of the ^{14}C milk suggested that significant quantities of conjugated PCP are associated with these fractions.

Metabolism

The urine from the lactating cow given a single dose of ^{14}C-PCP was analyzed using two methods to determine the extent of metabolism of PCP. The first involved extraction of urine under mildly acidic conditions with benzene. The benzene extract was subsequently analyzed using LSC and GC to quantify ^{14}C and PCP respectively. This method was assumed to extract PCP and/or its metabolites which were not conjugated. The second method involved treatment of urine with strong acid and heat followed by extraction with benzene and subsequent analysis of the benzene extract using LSC and GC.

Use of strong acid and heat constitute conditions which are known to hydrolyze the chemical bond between PCP or tetrachlorohydroquinone and glucuronic acid (Ahlborg et al., 1978). The additional ^{14}C released by this method and analyzed by liquid scintillation counting was believed to be ^{14}C-PCP and/or its metabolites which are conjugated. Further, the additional PCP released by hydrolysis and measured by gas chromatography of the benzene extract was conjugated PCP. The percentage of conjugated PCP or ^{14}C was determined by the calculation: ((total - free)/total)x 100. Similarly, the percentage of unconjugated PCP was determined by the calculation: 100% - % conjugated. It should be noted that the values produced by LSC analysis of the benzene extracts of the ^{14}C urine most

likely represent ^{14}C-PCP and its metabolites. In contrast, GC analysis of the benzene extracts represents PCP only. The results (Table 14, 15) strongly suggest through indirect evidence, that about 85% of the ^{14}C-PCP dose was excreted as a glucuronide conjugate. In contrast, Rhesus monkeys excreted all of the administered ^{14}C-PCP in the urine as unchanged PCP (Braun and Sauerhoff, 1976).

However, rats metabolize PCP to a moderate degree. When rats were given ^{14}C-PCP orally, the ^{14}C in the urine was characterized as PCP (60%), PCP-glucuronide (9-16%), tetrachlorohydroquinone (TCH) (7%) and TCH-glucuronide (16-22%) (Ahlborg et al., 1978). Similar results were obtained by Braun et al. (1977) when rats were given 100 mg/kg ^{14}C-PCP. In this study ^{14}C was excreted in the urine as PCP (75%), TCH (15%) and as PCP-glucuronide (9%). However, the rats and monkeys in these studies had not been previously exposed to PCP orally unlike the present study. The rationale for the previous subchronic exposure of the experimental cow is that cattle are typically exposed chronically to penta on farms. Therefore, a single dose pharmacokinetic study with an animal having had no previous exposure may not give an accurate assessment of the fate of PCP in animals exposed to penta under typical environmental conditions. It is likely that with previous exposure, the degree of conjugation and metabolism would be somewhat different, particularly in light of the fact that dioxin contaminants are potent inducers (Goldstein et al., 1977).

PCP analysis of serum from cattle fed 0.2 mg/kg/day suggests that a significant amount of the PCP in the serum was not conjugated, whereas 39% was in a conjugated form. This appears reasonable since one would expect that a glucuronide conjugate produced in liver would be transported in the blood and subsequently excreted via the kidney.

Elimination

The terms elimination and excretion are pharmacokinetic terms which are often used in a manner which would suggest they are interchangeable. Elimination typically describes the clearance of a chemical from a compartment, such as serum or plasma, whereas excretion is the process whereby the chemical is actually discharged from the body, such as by way of the urine, milk, feces or lungs.

In Experiment 2, a single dose of ^{14}C-PCP was given to a lactating cow and the concentration of ^{14}C in serum was subsequently monitored over the next 76 hours. Applying the methods described by Gehring et al. (1976) which are shown in the Appendix (pg 135), the rate constant of elimination (ke) and its associated half-life ($t_{\frac{1}{2}}$) were calculated to be 0.016 hr^{-1} and 42.8 hrs, respectively. Because studies with orally administered ^{14}C-PCP in other species were done in animals which were not previously exposed to PCP, a direct comparison of values for ke and $t_{\frac{1}{2}}$ may not be valid. However, some general comparisons can be made. The plot of serum ^{14}C-concentrations in the cow shows that absorption and clearance follows first-order kinetics. Absorption and elimination of ^{14}C-PCP also followed first-order kinetics in Rhesus monkeys (Braun and Sauerhoff, 1976). However, this is not the case in rats. Although absorption appears to follow first-order kinetics, elimination is biphasic and requires description with two elimination constants and their corresponding half-lives (Braun et al., 1977). These authors suggested that the secondary, or slow elimination phase was related to heterogeneous plasma protein binding and a small, but strongly protein-bound PCP in the liver and kidneys, which they categorized as "deep" (slowly-equilibrating) compartments in the rat.

Excretion

The results of the analysis of urine collected from the cow given a single dose of ^{14}C-PCP demonstrate that urine provides the main excretory route of PCP. When mice were administered ^{14}C-PCP subcutaneously (s.c.) or intraperitoneally (i.p.),73-83% of the dose was excreted into the urine (Jakobson and Yllner, 1971). Similarly, when mice were given ^{14}C-PCP orally, about 86% of the label was recovered in the urine (Larsen et al., 1972). Rats dosed with ^{14}C-PCP either orally or i.p. excreted 50 and 80% of the dose, respectively, in the urine (Ahlborg et al., 1974). Male Rhesus monkeys given ^{14}C-PCP orally excreted 75% of the dose in the urine whereas female Rhesus monkeys similarly exposed excreted 70% of the dose in the urine (Braun and Sauerhoff, 1976).

In contrast, the results of analysis of feces from the ^{14}C-PCP cow (Experiment 2) and cattle fed penta subchronically (Experiment 1) showed that feces provides a minor excretory route. Similar findings have been reported in rats and monkeys. Braun et al.(1977) found that male rats given a single oral dose of ^{14}C-PCP excreted 19-24% of the dose in the feces. Female rats given the same dose excreted significantly more in the feces (43%). Further, male Rhesus monkeys given ^{14}C-PCP orally excreted about 12% of the dose in the feces (Braun and Sauerhoff, 1976). Female Rhesus monkeys excreted about 18% of an oral dose of ^{14}C-PCP in the feces.

Milk also provides a minor route for excretion of PCP. In the cow dosed with ^{14}C-PCP, only 5% of the dose was accounted for in the milk. This is also shown in a comparison of milk and urine PCP levels from the cattle fed penta subchronically (Figure 11). The serum and milk PCP levels in these cattle when fed 2 mg penta/kg per day were 14,520 and 3,141 ng/ml, respectively. Interestingly, when Firestone et al. (1979) fed cattle 10 mg

technical pentachlorophenol per kg per day, they found 40,000 ng PCP/ml in serum and 4,000 ng PCP/ml in milk. However, when heifers were fed 15 mg penta per kg, blood PCP concentrations averaged 32,800 ng/ml (Parker et al., 1980). Development of the serum analysis used in the present study clearly showed that acid hydrolysis (acid & heat) of serum for a period of three hours, significantly increased the PCP yield. In contrast, extraction of the serum after mild acidification followed by solvent extraction resulted in significantly lower PCP concentrations. The acid hydrolysis step was subsequently included in the milk PCP analysis. Since Firestone et al. (1979) did include a hydrolysis step (acid & heat) in their blood analysis, the PCP levels reported are probably accurate. Whereas, a partial explanation for the lower blood PCP concentration in the study reported by Parker et al. (1980) is the lack of a hydrolysis step in their blood PCP analysis procedure. Our serum analyses suggest approximately 38% of the PCP in the blood of penta-fed cattle is conjugated. Thus, applying such a factor to the Parker et al. data (i.e. 32,800 ng/ml/.62) would result in a blood PCP level of 54,903 ng/ml which is more reasonable considering the cattle were receiving 5 mg more penta per kg than cattle in the Firestone et al. study.

Firestone et al.(1979) did use concentrated acid in their PCP milk analysis, but did not expose the milk & acid mixture to heat as was done in their blood PCP analysis. Lack of additional heat for an extended period of time appears to have resulted in only partial release of the bound PCP in the milk samples. Such an explanation would account for the average milk PCP concentration of 4,000 ng/ml in cattle fed 10 mg

penta/kg per day. In contrast the cattle in our study fed 2 mg penta/kg per day had an average milk PCP concentration of 3141 ng/ml.

SUMMARY

Experiment 1 indicated that technical PCP or penta fed subchronically to lactating dairy cattle does not significantly affect feed intake, milk production or body weight gains at the levels administered. If there is any subchronic effect from oral exposure, it is possibly an effect on the kidney. Histopathological changes were present in the kidneys of the treated animals, but not in the control cattle. Furthermore, kidney function tests tended to support the histopathologic observations. The impaired in vitro kidney function may have manifested itself in other ways, had the exposure continued for a longer time. Generally, the results of this study do not support the findings of a fact-finding task force, in that penta exposure does not seem to result in decreased milk production or increased incidence of mastitis.

It should be pointed out that the cattle in our study would be considered low to medium producers with respect to their level of milk production. It is possible that cattle producing 80 to over 100 lbs of milk per day, under a high metabolic stress and also exposed subchronically to penta may have exhibited some or all of the production and health problems observed by the fact-finding task force in penta-exposed dairy herds.

The pharmacokinetic profile of PCP in lactating cattle shows that PCP is both rapidly and extensively absorbed. In addition, the results of tissue residue analyses agree with the findings of other studies in that the liver and kidneys are major sites of PCP deposition, whereas brain and adipose tissue are minor ones. In terms of metabolism, hydrolysis of both urine and serum samples indicated that PCP is extensively conjugated.

Analysis of fecal, urine and milk samples showed that urine is the major excretory route for PCP in cattle. In contrast, both milk and feces represent only minor routes by which PCP is eliminated from the body.

FUTURE WORK

A review of the current toxicological data base on pentachlorophenol, as it relates to penta, suggests several possible studies which would significantly increase our knowledge of the effects of this chemical on domestic animals. One essentially unknown component is the contribution that inhalation of PCP makes to an animal's overall exposure. As compared to the assessment of dermal exposure, the assessment of inhalation of xenobiotics is a difficult task. However, practicality suggests that some of the most meaningful information might easily be obtained by field studies. Such studies might involve monitoring serum, urine and milk PCP residues in cattle, previously unexposed to penta, before and after being moved into a facility containing penta-treated wood. Clearly, one requirement would be that oral exposure be at a minimum or nonexistent. A study of this kind would eliminate the costs and intricate considerations associated with inhalation chambers and the need to extrapolate to actual conditions.

To date, the studies in cattle have involved limited numbers of yearling heifers (McConnell et al., 1980) and the lactating cattle in the current study. Thus, it would seem that the definitive study yet to be done is one whereby heifers exposed to penta-treated wood are bred, carried through one complete lactation and then bred once more. Such a study would involve making many of the same kinds of measurements made in the current study, plus it would provide data on the effects of penta on such aspects as fertility, gestation and teratogenicity, which are of critical interest in dairy production. Clearly, to provide both statistically and biologically meaningful information, this study would have to involve sufficient numbers

of cattle, and more importantly, it would be very costly. However, until such a study is conducted, significant questions will remain as to the effects of chronic exposure to penta on lactating dairy cattle.

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APPENDIX

Estimation of the Rate Constants of Absorption (ka) and Elimination (ke) and Their Associated Half-Lives ($t_{\frac{1}{2}}$)

The rate constant for elimination (ke) can be estimated by taking a straight edge and extrapolating back to the point where the line intercepts the y-axis (Figure 10). The line is described by the equation:

$$\ln C = \text{in } C_o - \frac{ke}{t}$$

where:

C_o = concentration at the point where the extrapolated line intercepts the y-axis

C = concentration at time t

ke = rate constant for elimination

Solving for ke, the equation becomes:

$$ke = \frac{\ln C - \ln C_o}{t}$$

Using the following data: C = 9300 dpm/ml, C_o = dpm/ml, and t = 76 hrs, then:

$$ke = \frac{\ln 32{,}000 - \ln 9{,}300}{76}$$

or,

$$ke = 0.0162 \text{ hr}^{-1}$$

Thus, the half-life for elimination is:

$$t_{\frac{1}{2}} = \frac{0.693}{ke} = \frac{0.693}{0.0162} = 42.8 \text{ hours}$$

The rate constant for absorption (ka) is obtained by a method called "curve stripping" or "feathering". This involves determining the differences between the experimental values from the absorption phase of the curve and the corresponding values on the line which was previously extrapolated back to an intercept on the y-axis (Figure 14). These values are then plotted vs their respective times. The values for C, C_o, and t can then be used in an identical manner as shown above, to determine ka as follows:

$$ka = \frac{\ln C - \ln C_o}{t}$$

using the following data: 30,408 dpm/ml, C_o = 7,029 dpm/ml, and t = 9 hr, then:

$$ka = \frac{\ln 30{,}408 - \ln 7{,}029}{9}$$

or,

$$ka = 0.162 \text{ hr}^{-1}$$

Thus, the half-life for elimination is:

$$t_{\frac{1}{2}} = \frac{0.693}{ka} = \frac{0.693}{0.162} = 4.28 \text{ hr.}$$

Figure 14. Use of "Curve Stripping" to Estimate the Rate Absorption Constant (ka).

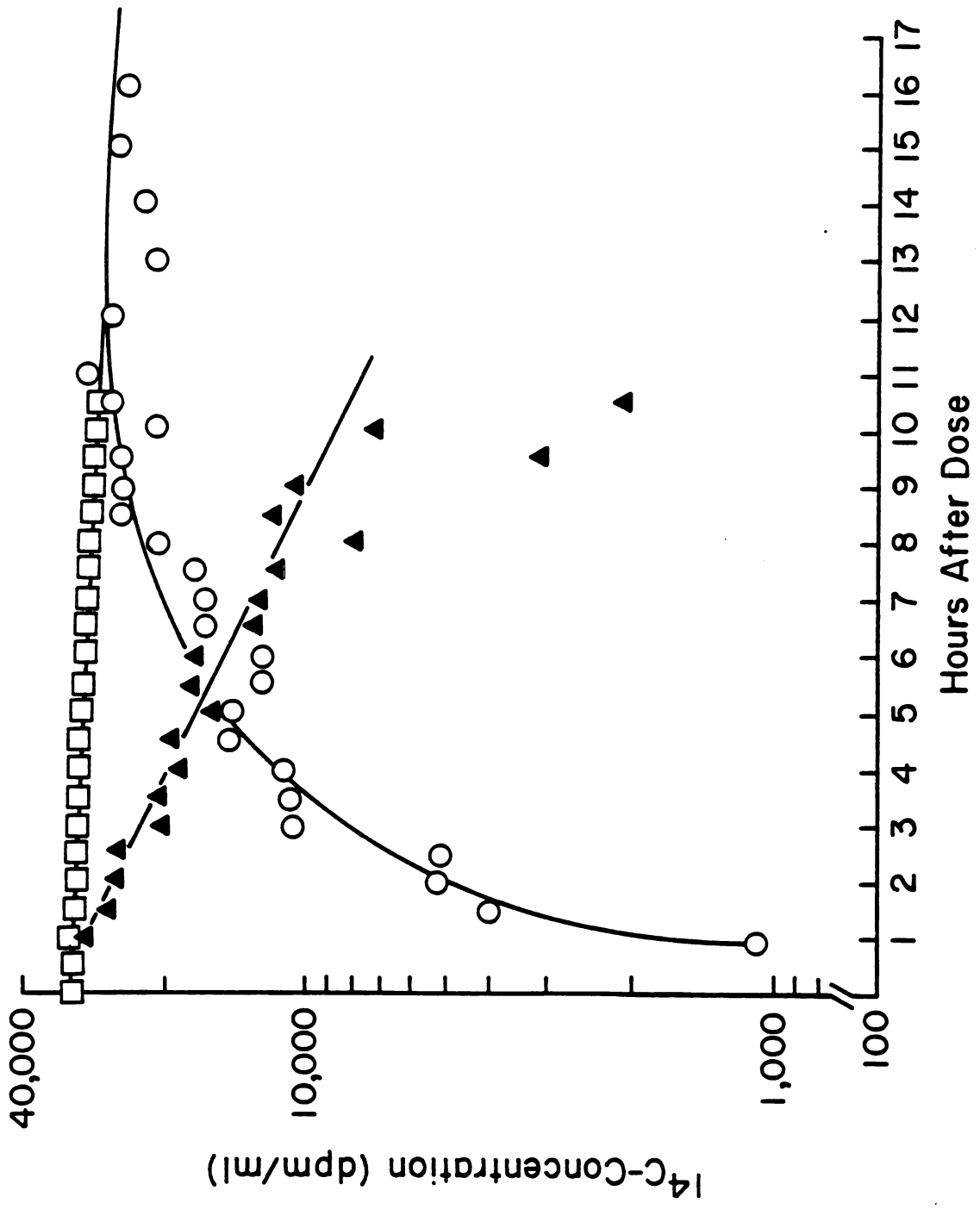
40,000
10,000
1,000
100
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Hours After Dose
14C-Concentration (dpm/ml)

Table 16. Values Generated by the 'Curve Stripping' Method.

Hours After Dose	Extrapolated (E_1) Serum ^{14}C Conc.	Experimental (E_2) Serum ^{14}C Conc.	E_1-E_2
1.0	31,500	1,092	30,408
1.5	31,000	4,028	26,972
2.0	31,000	5,140	25,860
2.5	30,800	5,093	25,707
3.0	30,500	10,411	20,089
3.5	30,500	10,085	20,415
4.0	30,300	11,341	18,959
4.5	30,000	10,450	19,550
5.0	30,000	14,008	15,992
5.5	29,700	12,131	17,569
6.0	29,500	12,272	17,228
6.5	29,000	16,187	12,813
7.0	29,000	16,360	12,640
7.5	28,800	17,335	11,465
8.0	28,500	20,551	7,989
8.5	28,300	16,654	11,646
9.0	28,000	17,443	10,557
9.5	28,000	24,881	3,119
10.0	27,800	20,771	7,029

Table 17. ^{14}C Concentration in Serum over Time in a Cow Given a Single Dose of ^{14}C-PCP.

Time After Dose (Hours)	^{14}C-Concentration	Time After Dose (Hours)	^{14}C-Concentration
0.25	0[a]	10.0	20,771
0.50	0[a]	10.5	25,463
0.45	0[a]	11.0	29,073
1.0	1,092	12.0	25,400
1.25	2,492	13.0	20,351
1.50	4,028	14.0	21,271
1.75	4,146	15.0	24,278
2.0	5,140	16.0	23,058
2.25	5,212	18.0	19,889
2.50	5,093	20.0	25,912
2.75	9,354	22.0	19,032
3.0	10,411	24.0	23,982
3.25	10,160	28.0	22,240
3.5	10,085	32.0	18,205
4.0	11,341	36.0	15,873
4.5	10,450	40.0	15,459
5.0	14,008	44.0	16,084
5.5	12,131	48.0	12,598
6.0	12,272	52.0	14,272
6.5	16,187	56.0	13,864
7.0	16,360	60.0	14,010
7.5	17,335	64.0	9,671
8.0	20,551	68.0	11,678
8.5	16,654	72.0	9,739
9.0	17,443	76.0	9,240
9.5	24,881		

[a]Not distinguishable from background

Table 18a. Results of Hematologic Examination from Control and Penta-Fed Cattle (0.2 mg/kg/day Exposure Period)[1]

Measurement	Units	Pre-Exposure Control $\bar{X}$ ± SE		Pre-Exposure Treated $\bar{X}$ ± SE		Dose = 0.2 mg/kg body wt/day After 4 weeks Control $\bar{X}$ ± SE		After 4 weeks Treated $\bar{X}$ ± SE		After 8 weeks Control $\bar{X}$ ± SE		After 8 weeks Treated $\bar{X}$ ± SE	
Total protein (plasma)	g/dl	8.7	0.4	8.6	0.7	8.7	0.2	8.8	0.4	8.7	0.4	8.6	0.3
Packed cell volume (PCV)	%	28.8	1.4	27.4	1.2	30.7	1.4	27.4	2.6	29.8	0.7	27.8	2.8
Hemoglobin (Hb)	g/dl	10.9	0.3	10.2	1.2	11.2	0.6	10.0	0.9	10.6	0.8	10.2	5.1
Red blood cells (RBC)	$nx10^6$/ul	6.2	0.2	6.0	0.7	6.5	3.2	6.1	3.1	6.1	0.2	6.4	0.2
White blood cells (WBC)	n/ul	9.25	811	8600	324	8400	9.4	9125	792	7899	4.8	8325	630
Mean corpuscular volume (MCV)	fl	48.5	0.5	46.8	2.1	47.8	1.2	45.8	1.8	47.8	1.6	43.5	0.9
Mean corpuscular Hb (MCH)	pg	17.4	0.3	16.9	0.8	17.0	0.3	16.3	0.6	17.2	0.6	15.6	0.6
Mean corpuscular Hb conc. (MCHC)	%	36.4	0.4	36.3	0.5	36.1	0.8	36.1	0.8	36.9	0.6	37.1	1.9
Segmented neutrophils (seg.)	n/ul	4040	318	3710	604	3108[a]	373	4973[a]	497	31558	363	3536	645
Lymphocytes	n/ul	3835	590	3395	646	4158[a]	416	3331[a]	575	3937	663	3400	443
Monocytes	n/ul	82	31	132	81	210	50	201	27	112	45	333	150
Eosinophils	n/ul	1112	465	1341	268	857	92	529	73	572	167	866	244
Basophils	n/ul	56	--	21	--	67	42	137	55	40	23	101	75
Platelets[2]		1.2	0.2	1.0	0.01	2.4	0.5	2.4	0.5	--	--	--	--

[1] Means with different superscripts (a,b) in the same line within a specific time period are significantly different ($P < .05$)

[2] Numbers of platelets in a smear were estimated (and given rating) as decreased = 1, adequate = 2, slightly increased = 2.5, increased = 3.

Table 18b. Results of Hematologic Examination from Control and Penta-Fed Cattle (2.0 mg/kg/day Exposure Period)[1]

Measurement	Units	Dose = 2.0 mg/kg body wt/day											
		Pre-high dose exposure				After 4 weeks				After 8 weeks			
		Control		Treated		Control		Treated		Control		Treated	
		$\bar{X}$	± SE	$\bar{X}$	± SE	$\bar{X}$	± SE	$\bar{X}$	± SE	$\bar{X}$	± SE	$\bar{X}$	± SE
Total protein (plasma)	g/dl	8.9	0.3	9.3	0.8	8.1	0.2	8.9	0.5	8.8	0.2	9.0	0.3
Packed cell volume (PCV)	%	30.3	0.6	26.3	0.4	27.6	1.3	26.8	0.5	30.4	0.8	29.8	1.1
Hemoglobin (Hb)	g/dl	11.3	0.2	9.9	0.2	10.3	0.4	10.1	0.2	11.6	0.4	11.3	0.3
Red blood cells (RBC)	$nx10^6$/ul	6.4	0.2	6.1	0.3	5.8	0.3	6.2	0.3	6.5	0.4	7.2	0.7
White blood cells (WBC)	n/ul	9000	473	8666	1278	8625	492	9175	2449	8400	702	10,800	2306
Mean corpuscular volume (MCV)	fl	48	1.5	45.0	2.6	48.3	0.8	44.0	1.4	47.5	0.5	43.0	2.0
Mean corpuscular Hb (MCH)	pg	17.3	0.3	16.2	1.4	17.7	0.3	16.2	0.3	17.4	0.4	15.7	1.1
Mean corpuscular Hb conc. (MCHC)	%	37.1	0.5	37.1	0.5	37.1	0.1	37.7	1.1	37.8	0.2	37.6	0.05
Segmented neutrophils (seg.)	n/ul	3532	1056	4658	898	4061	629	5472	209	3794	519	5960	1118
Lumphocytes	n/ul	4125	804	2892	678	3898	780	2479	427	4063	1218	3525	1063
Monocytes	n/ul	357	95	44	44	316	171	323	159	154	154	3525	1063
Eosinophils	n/ul	903	345	647	214	378	165	756	224	343	112	494	101
Basophils	n/ul	83	83	22	22	47	27	60.5	35.5	45	--	42	--
Platelets[2]		2.0	0.0	2.0	0.0	--	--	--	--	2.5	0.5	2.5	0.3

[1] No significant differences found ($P > .05$)

[2] Numbers of platelets in a smear were estimated (and given rating) as decreased = 1, adequate = 2, slightly increased = 2.5, increased = 3.

Table 19a. Results of Clinical Chemistry Analyses from Control and Penta-Fed Cattle (0.2 mg/kg/day Exposure Period).

		Dose = 0.2 mg/kg/day											
		Pre-exposure				After 4 weeks				After 8 weeks			
		Control		Treated		Control		Treated		Control		Treated	
		$\bar{X}$	± SE	$\bar{X}$	± SE	$\bar{X}$	± SE	$\bar{X}$	± SE	$\bar{X}$	± SE	$\bar{X}$	± SE
Blood urea nitrogen (BUN)	mg/dl	5.5	0.6	4.8	0.8	8.0	0.7	10.0	3.9	7.5	1.2	4.0	2.0
Glucose	mg/dl	59.0[a]	3.4[1]	66.2[b]	8.5	39.5	7.1	59.2	5.7	56.3	5.5	63.3	3.0
Total protein	g/dl	8.8	0.5	8.9	0.3	8.2	0.3	8.6	0.4	7.9	0.3	8.2	0.4
Albumin	g/dl	3.4	0.2	3.1	0.3	3.4	0.1	2.8	0.2	3.6[a]	0.2	2.7[b]	0.2
Globulin	g/dl	5.3	0.4	5.8	0.6	4.8	0.3	5.3	0.6	4.3[a]	0.3	5.4[b]	0.6
Calcium (Ca)	mg/dl	9.8	0.3	9.1	0.2	9.6	0.2	9.2	0.4	10.2	6.3	9.8	0.6
Phosphorous (P)	mg/dl	4.2	0.4	4.7	0.6	3.4	0.2	3.7	0.1	3.6	0.4	3.4	0.4
Magnesium (Mg)	mg/dl	2.4	0.2	2.2	0.2	2.1	0.0	1.9	0.2	2.2	0.2	2.3	0.2
Aspartate amino-transferase (AST)	IU/ml	40.3	2.4	38.5	3.3	38.2	3.2	43.8	3.2	43.6	3.1	41.2	4.5
Sorbitol dehydrogenase (SDH)	IU/ml	9.7	1.8	10.2	1.1	11.5	1.0	11.2	1.9	14.0	1.7	10.3	2.9
Creatine phosphokinase (CPK)	IU/ml	22.7	0.9	26.5	2.8	27.0	2.7	19.2	2.9	30.2	6.4	20.5	3.2

[1]Means with different superscripts (a,b) on the same line within a specific time period are significantly different ($P < .05$)

Table 19b. Results of Clinical Chemistry Analyses from Control and Penta-Fed Cattle (2.0 mg/kg/day Exposure Period).

Measurement	Units	Pre-high dose exposure				Dose = 2.0 mg/kg body wt/day							
						4 weeks				8 weeks			
		Control		Treated		Control		Treated		Control		Treated	
		$\bar{X}$	± SE	$\bar{X}$	± SE	$\bar{X}$	± SE	$\bar{X}$	± SE	$\bar{X}$	± SE	$\bar{X}$	± SE
Blood Urea nitrogen (BUN)	mg/dl	10.7	2.3	12.3	3.4	12.2	2.5	14.5	3.3	9.0	0.1	9.5	1.5
Glucose	mg/dl	72.5	2.5	68.0	15.0	71.5	4.7	75.5	7.7	7.4	2.0	75.5	4.5
Total protein	g/dl	8.1	0.03	8.6	0.9	7.6	0.2	8.4	0.8	8.2	0.4	8.6	0.3
Albumin	g/dl	3.7	0.2	2.8	0.3	3.5	0.1	2.8	0.3	3.1	0.01	3.0	0.6
Globulin	g/dl	4.4	0.2	5.9	0.0	4.1	0.3	5.6	0.9	4.8	0.1	5.6	0.9
Calcium (Ca)	mg/dl	9.9	0.5	9.0	0.6	9.7	0.2	8.8	0.7	7.4	2.0	7.6	2.6
Phosphorous (P)	mg/dl	4.0	0.7	3.3	0.2	3.9	0.6	3.3	0.2	3.8	1.1	2.6	0.5
Magnesium (Mg)	mg/dl	2.5	0.4	2.3	0.3	2.6	0.2	2.7	0.3	5.8	3.4	5.8	3.3
Aspartate amino-transferase (AST)	IU/ml	47.3	0.7	42.7	5.2	44.0	5.2	39.5	3.8	66.5	27.6	47.5	10.5
Sorbitol dehydrogenase (SH)	IU/ml	18.0	0.0	15.0	0.0	14.0	2.0	1.7	1.0	14.0	0.01	28.0	0.01
Creatine phosphokinase (CPK)	IU/ml	33.3	5.2	23.3	3.9	24.5	5.5	33.5	6.5	30.0	10.1	20.0	1.0

No significant differences found ($P > .05$)

Table 20. Results of Urinalyses[1] from Control and Penta-Fed Cattle

Indices	2-Week Pre-Necropsy				1-Week Pre-Necropsy			
	Control		Treated		Control		Treated	
	Mean	± SE	Mean	± SE	Mean	± SE	Mean	± SE
Color[2]	3.0	0.0	3.0	0.0	2.0	0.6	2.0	0.6
Turbidity[3]	0.0	0.0	0.8	0.5	0.0	0.0	0.0	0.0
Specific gravity	1.025	0.003	1.021	0.003	1.027	0.001	1.021	0.002
pH	8.0	0.4	7.2	0.2	8.2	0.2	8.0	0.4
Protein[4]	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.3
Glucose[5]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ketones[6]	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Bilirubin[7]	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Urobilinogen[7]	0.05	0.03	0.05	0.03	0.8	0.2	0.8	0.2
Occult blood[8]	0.2	0.2	0.5	0.3	0.0	0.0	0.2	0.2
White blood cells[8]	11.9	11.1	34.6	16.2	9.5	4.1	16.4	9.9
Red blood cells[8]	13.1	6.5	12.4	6.2	2.1	1.0	5.6	3.2
Epithelial cells[9]	1.0	0.4	1.0	0.4	1.0	0.0	0.8	0.2
Casts[8,10]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bacteria[10]	0.5	0.2	1.2	0.2	0.8	0.2	1.6	0.4

[1] Urinalyses performed by Multistix (dip-and read test). This method provides clinical not quantative data

[2] Clear = 0, straw = 1, pale yellow = 2, yellow = 3, dark yellow = 4, other = 5.

[3] Clear = 0, slight haze = 1, moderate haze = 2, extreme haze = 3.

[4] 0 = negative, 1 = trace, 2 = ca 30 mg %, 3 = ca 100 mg %.

[5] 0 = negative, 1 = 0.1 g%.

[6] beige = 0, pale purple = 1, moderate purple = 2.

[7] Ehrlich units/dl

[8] number/high power field

[9] number/low field

[10] none 0, few 1, moderate 2, many 3.

Table 21. Recovery of a ^{14}C-PCP Spike in Various Tissues and Fluids of Cattle Fed Penta

Tissue	Percentage Recovery of ^{14}C-PCP Spike[a]		
	$\bar{x}$	±	SE
liver	91.8		1.1
kidney	90.8		2.2
spleen	89.8		1.6
lung	96.0		1.4
bile	86.6		0.6
thymus	90.2		2.2
muscle	93.2		1.9
mammary	95.0		1.6
adrenal	98.3		2.5
thyroid	79.0		2.4
brain	90.0		0.2

[a] average percentage recovery ^{14}C-PCP from replicate spiked samples of tissue homogenate per animal

Table 22. Recovery of a ^{14}C-PCP Spike from Urine, Serum, Rumen Fluid and Milk

Sample	Percentage Recovery of a ^{14}C-PCP Spike[a]		
	$\bar{x}$	±	SE
Rumen Fluid	98.4		0.64
Milk	90.2		1.46
Urine	99.0		-
Serum	99.0		0.38
Feces	94.0		1.20

[a] average percentage recovery of ^{14}C-PCP from replicate spiked samples

BIOGRAPHICAL SKETCH OF J.H. KINZELL

The author of this thesis was born in Calgary, Alberta on April 14, 1950. Shortly thereafter, he moved with his family to a mixed farm 30 miles south of Calgary at High River. He attended school at High River and in his free time, assisted his father in managing their beef cattle operation and participated in school athletics and 4H programs.

After graduating from high school in 1969, he attended junior college in Everett, Washington and thereafter, obtained an Associate Science Degree in Biology. He subsequently transferred to Oregon State University and two years later received a Bachelor's of Science Degree in Zoology. His farm background led him to choose a graduate program in animal nutrition at Oregon State University under Dr. Peter R. Cheeke. Thesis research for this degree involved experiments which examined the nutritional and toxicological aspects of novel protein sources for monogastric animals.

In the fall of 1976, he began his doctoral studies and research at Michigan State University in the Department of Animal Science with Dr. Lee Shull. As Dr. Shull's first graduate student, the author assisted his supervisor in developing what is now the Food Animal Toxicology program as an integral part of the Center for Environmental Toxicology at Michigan State University.

At the present time the author is employed as a toxicologist in the Toxicological Evaluation Division of the Health Protection Branch (Health and Welfare Canada) in Ottawa, Ontario. His duties as a toxicologist

include examining toxicological data supporting the safety-in-use of agricultural chemicals and supplying consultative services regarding these chemicals, to other governmental agencies.