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WITHDRAWAL OF POLYCHLORINATED BIPHENYL (PCB) AND POLYBROMINATED BIPHENYL (PBB) RESIDUES FROM RATS USING FEED RESTRICTION AND/OR MINERAL OIL IN THE DIET

By

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ABSTRACT

WITHDRAWAL OF POLYCHLORINATED BIPHENYL (PCB) AND POLYBROMINATED BIPHENYL (PBB) RESIDUES FROM RATS USING FEED RESTRICTION AND/OR MINERAL OIL IN THE DIET

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Rats were fed diets containing 10 mg Aroclor 1254/kg diet or 10 mg fireMaster® FF-1/kg diet for 14 days followed by 21 days of withdrawal treatment involving 50% feed restriction, 5% mineral oil, or a combination of the two treatments. PCB and PBB residues in ground whole-body rat samples were determined using gas chromatography. Body burdens of PCBs and PBBs on day 0 withdrawal were 1505 and 181 ug/rat, respectively. Feed restriction or mineral oil alone significantly ($p< 0.5$) reduced PCB body burdens by approximately 27%. The combination of feed restriction and mineral oil enhanced withdrawal of PCB and PBB body burdens significantly ($p< 0.5$), with losses of 49.8% and 45.4%, respectively.

In memory of my grandfather,
Alfred R. Stanley, PhD,
whose search for knowledge I share.

ACKNOWLEDGEMENTS

I would like to thank the members of my guidance committee, Dr. Donald Polin, Dr. Steven Bursian, and Dr. Patricia O'Handley, for their assistance during the preparation of this manuscript. Special thanks goes to my major professor, Dr. Polin, for his encouragement, patience, and faith in my ability to complete this degree.

I would also like to thank Dr. Richard Leavitt, Bob Kon, Bob Schuetz, and Leister Geissel of the Pesticide Research Center for their valuable assistance in teaching me the preparation and method of analysis of samples for PBB and PCB residues.

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INTRODUCTION

In July 1973, 10-20 50# bags of fireMaster® FF-1, a fire-retardant containing polybrominated biphenyls (PBBs), was accidentally mixed into Michigan Farm Bureau Service's (Battle Creek, MI) dairy pellets instead of Nutrimaster®, a magnesium oxide supplement. Both fireMaster® FF-1 and Nutrimaster® were made by Michigan Chemical Company (St. Louis, MI) and packaging was identical, except for the name. The mix-up and the nine month delay in determining the cause of the toxicity observed resulted in widespread PBB contamination in Michigan. The result was the destruction of approximately 30,000 cattle, 5,900 swine, 1,470 sheep, and 1,500,000 chickens (FDA, 1975; Carter, 1976). The quarantine, destruction of animals, disposal of milk, eggs, and feed, and the cleanup resulted in losses of $75-100 million or greater (Carter, 1976). Several thousand farm families and their neighbors had consumed meat, eggs, and milk contaminated with PBBs. The level of exposure of the general public was less due to the mixing of milk (Carter, 1976).

At about the same time, the toxic effects of polychlorinated biphenyls (PCBs) were being examined. PCBs

were used in industry worldwide and had become widely distributed in the environment. PCB residues had been found in sediments, fish, wildlife, domestic animals and humans (Hutzinger et al., 1979; Kimbrough, 1980; Safe, 1984).

Both PCBs and PBBs are very stable, resist breakdown, and therefore persist in the environment. They are both lipophilic, bioaccumulate in the adipose tissue, and remain in the body indefinitely. As PCBs and PBBs produce toxic effects and have been identified as possible carcinogens (Kimbrough, 1980; Safe, 1984), it would be advantageous to be able to remove them from the body. In addition, many high quality breeding animals and animals with low level PBB contamination had to be destroyed following the Michigan PBB accident. They may have been salvaged if some method of removal was available. Several studies have shown that the use of mineral oil in conjunction with feed restriction enhanced PBB and PCB elimination (70-80% eliminated in 21 days) in chickens (Polin and Leavitt, 1984; Polin et al., 1985; Polin et al., 1989). The objectives of this study were:

1) To quantify the PCB and PBB residues in ground whole-body rat samples obtained from rats fed diets containing 10 mg PCBs or PBBs/kg diet.

2) To determine if addition of 5% mineral oil to the diet during the 21 day withdrawal period would enhance elimination of the PCB or PBB residues from rats.

3) To ascertain if 50% feed restriction would enhance the elimination of PCB or PBB residues from rats over a 21 day period.

4) To determine if 50% feed restriction in combination with 10% mineral oil in the diet enhanced elimination of PCB or PBB residues from rats during a 21 day withdrawal period.

LITERATURE REVIEW

I. Polychlorinated Biphenyls

A. Chemical Properties of PCBs

Polychlorinated biphenyls (PCBs) are chemical compounds with the empirical formula $C_{12}H_{10-n}Cl_n$, where n=1-10. Theoretically, there are 209 possible PCB congeners, although at least 20 have not been found in any technical PCB mixture analyzed. Monsanto Chemical Company produced PCBs in the United States under the tradename, Aroclor. Aroclors contain a mixture of different PCBs, which are specific to a particular Aroclor. Production of these mixtures of PCBs is by chlorination of biphenyl with subsequent separation and purification of the desired chlorinated biphenyl fractions. Contaminants which include polychlorinated dibenzofurans and polychlorinated naphthalenes are occasionally present and vary from batch to batch. The first two numbers of the Aroclors, with the exception of 1016, indicate the number of carbons in the biphenyl molecule, and the last two numbers indicate the percent chlorination by weight. Individual chlorinated biphenyls are colorless crystals in their pure form, and commercial mixtures (i.e. Aroclors) are liquids due to

depression of the melting point occurring with mixing of PCBs. These commercial mixtures of PCBs have properties which include thermal stability, resistance to chemical and biological degradation, low water solubility, high dielectric constants, high electrical resistivity, stability to conditions of oxidation and hydrolysis encountered in industrial use, and low vapor pressures. Water solubility, vapor pressure and the ability to be degraded decrease as the chlorination of the compound increases.

Aroclor 1254, which was used in the present study, is a light-yellow viscous liquid. It is soluble in ethyl acetate and very soluble in toluene. Aroclor 1254 has the following properties:

Chlorine %	= 54%
Specific gravity	= 1.495-1.505 (65°/15.5°C)
Density	= 12.82 lb/gal at 25°C
Distillation range	= 365-390°C
Viscosity	= 1400-2500 sec at 37.8°C
Vapor pressure	= 1.8×10^{-4} mmHg at 20°C
Vaporization rate	= 0.053 mg/cm^2/hr
Pour point	= 10°C
Principal components	= Cl_4 - Cl_6
# of components reported	= 27-116
Flash and Fire points	= none to boiling
Dielectric constant	= 5.0 at 20°C, 4.3 at 100°C

Aroclor 1254 is primarily pentachlorobiphenyl and contains 54% chlorine. At least 85 components have been detected in Aroclor 1254 using high resolution capillary columns, although with gas chromatographic analysis using packed columns there are 12 to 15 peaks present on the

chromatogram. Webb and McCall (1973) described 13 peaks where the number of chlorines on the biphenyl for each peak was determined. Peaks 1-3 contained tetrachlorobiphenyls, peaks 4-5 contained 25% tetra- and 75% pentachlorobiphenyls, peaks 6-8 contained pentachlorobiphenyls, peaks 9-10 70% penta- and 30% hexachlorobiphenyls, peaks 11-12 contained hexachlorobiphenyls, and peak 13 contained heptachlorobiphenyls.

The preceding information was taken from Webb and McCall (1973), Mieure et al. (1976), Hutzinger et al. (1979), Kimbrough (1980), Richardson and Waid (1982), Safe (1984), Erickson (1986) and Alford-Stevens (1986).

B. Production and Uses of PCBs

PCBs were produced under the tradename Aroclor in the U.S. by Monsanto Chemical Company from 1929 through 1977. From 1971 to 1973 approximately 1 million pounds of PCB-based heat-transfer oil were manufactured by Geneva Industries (Houston, TX). In the period 1930 to 1975, total production of PCBs in the U.S. was 1400 million pounds. Imports equalled 3 million pounds, domestic sales were at 1253 million pounds, and exports equalled 150 million pounds. In April 1971, Monsanto voluntarily ceased PCB production that was for use in open-ended or nominally closed systems. Production and sales of PCBs were at the maximum in 1970 and by 1974 had declined to one-half that level. Monsanto ceased production of PCBs in mid-1977 and had shipped its last inventory by

October 1977.

Non-U.S. production of PCBs was 80-85 million pounds annually prior to 1971. In 1971, production was 100 million pounds. Production fell after 1971 to 43 million pounds in 1973 and to 30 million pounds in 1976. Japan was a major producer of PCBs sold under the tradename of Kanechlor, from 1954 to 1972. Other producers of PCBs were the German Federal Republic, France, Italy, and the USSR. Total world production through 1980 was 2.4 billion pounds.

PCBs were used as coolants and dielectric fluids in transformers and capacitors, heat transfer fluids, flame retardant coatings for wood products, components of carbonless paper, paints, inks, dust control agents, pesticides, hydraulic fluids, and lubricants, plasticizers in rubbers and resins, adhesives, and as wax extenders. By far the greatest single use of PCBs was in capacitors for fire protection and increased service life, although in 1968 to 1971, use as plasticizers was the largest.

In 1976, the manufacture, processing, distribution and use of PCBs, except in totally enclosed systems (transformers, capacitors, and electromagnets) was banned by Congress. This was in response to evidence that PCBs were possible promoters of cancer.

The information on production and uses of PCBs was compiled from Hutzinger et al. (1979), Kimbrough (1980),

Richardson and Waid (1982), Safe (1984), Erickson (1986), and Alford-Stevens (1986).

C. Environmental Distribution and Metabolism of PCBs

Due to the worldwide use and production of PCBs, they can be detected in nearly all niches of the global ecosystem. Residues have been found in highly industrial areas to remote areas like the Arctic. PCB residues have been detected in sediments, fish, wildlife, domestic animals, and humans. The highly lipophilic nature of PCBs is evident by high residue levels due to bioaccumulation in fat of carnivores. The resistance of PCBs to breakdown by acids, bases, heat, light, oxidizing and reducing agents contributes to their stability and environmental persistence. Uptake by plants does not readily occur. The most important method for the destruction of waste PCBs seems to be thermal degradation at temperatures of greater than 800°C, which results in the formation of organic compounds like CO, CO_2, HCl, and Cl_2 (Hutzinger et al., 1979; Kimbrough, 1980; Safe, 1984). Photolysis occurs under laboratory conditions with the primary reaction being reductive dechlorination. Chlorines in the meta positions are lost preferentially and dechlorination occurs more rapidly in polar solvents (Hutzinger et al., 1972; Ruzo and Zabik, 1975; Kimbrough, 1980).

D. Distribution, Metabolism, and Excretion in Animals

The distribution and metabolism of PCBs in animals has been studied by many researchers. When i.v. doses of 2,4,5,2′,4′,5′-hexachlorobiphenyl (6-HCB) were given to beagles, monkeys (Sipes et al., 1982; Ryerson et al., 1984), and rats (Birnbaum, 1986), it was found that 70-82% of 6-HCB in the blood was redistributed to the liver and muscle within 30 minutes to 2 hours. By 24 hours, 6-HCB was redistributed from the liver and muscle to fat, omentum and skin. The fat continued to accumulate 6-HCB over the course of the study (up to 90 days in the monkeys). In all three species, the major route of excretion was found to be through the feces, with a small amount being excreted in the urine. The dog excreted 66% of the total dose of 6-HCB in 15 days, the monkey 18% in 90 days, and the rat 2% in 21 days. The greater excretion of 6-HCB by the dog appeared to be due to a higher rate of biotransformation, as the quantity of metabolites found in the blood of dogs was 4-8 times that found in monkeys. In the dog, there was no significant enterohepatic circulation, whereas the monkey had more parent compound in the bile than was excreted in the feces, indicating that reabsorption occurred. In the rats, the fecal excretion was primarily of metabolites.

Other PCBs were also studied for distribution and metabolism in animals. Birnbaum (1986) injected rats i.v.

with 2,3,6,2′,3′,6′-hexachlorobiphenyl. Greater than 50% of radiolabeled 2,3,6,2′,3′,6′-HCB was metabolized and excreted via the bile into the feces within 2 days (Birnbaum, 1986). Yoshimura and Yamamoto (1975) injected rats which had the bile duct ligated with 2,4,3′,4′-tetrachlorobiphenyl (2,4,3′,4′-TCB) i.v. They found an average of 0.6% of the dose was excreted unchanged from the small intestine daily. No other parts of the gastrointestinal tract were found to be secreting the parent compound or metabolites, indicating that the small intestinal wall serves as a major site of secretion of unchanged 2,4,3′,4′-TCB.

Several studies were done to determine the role of lipoproteins in the mobilization and distribution of 6-HCB in the rat and human. It was found (Vomachka et al., 1983; Spindler-Vomachka et al., 1984; Rau and Vodicnik, 1986) that hyperlipidemic conditions in humans and rats, like those occurring during pregnancy, cause an increase in the release of 6-HCB from the liver in association with very low density lipoproteins (VLDL). The distribution of 6-HCB within the different fractions of plasma (VLDL, low density lipoproteins or LDL, high density lipoproteins or HDL, and the bottom fraction consisting of albumin and corticosterone- binding globulin) was found to be dependent on the content of triglyceride (TG) and protein in the plasma. Therefore, it appeared that 6-HCB was released from hepatocytes in

association with newly synthesized TG, and then distributed in the circulation based on the TG:protein ratio.

Kimbrough (1980) and Erickson (1986) reviewed the in vivo metabolism of individual PCB congeners and indicated that phenolic products were the major metabolites with lesser amounts of sulfur metabolites (methylsolfones), trans-dihydrodiols, polyhydroxylated PCBs and their methyl ether derivatives, and ring-degraded microbial oxidation products. Several rules appear to describe the metabolism of PCBs:

1. Hydroxylation is favored at the para position of the less chlorinated phenyl ring unless this site is hindered sterically (ie. 3,5-dichloro substituted congener).

2. The para position of both biphenyl rings and the carbon atoms, which are para to the chloro substituent in the lower chlorinated biphenyls are all readily hydroxylated.

3. Oxidative metabolism of the PCB substrate is enhanced by the availability of 2 neighboring unsubstituted carbon atoms (especially C_5-C_4 in the biphenyl nucleus), but this is not required for metabolism.

4. Increasing degree of chlorination on both phenyl rings decreases the rate of metabolite excretion. Therefore, lower chlorinated PCB isomers are preferentially eliminated.

5. Different species metabolize specific PCB congeners differently resulting in a large variation in metabolite distribution.

In summary, it appears that the distribution and metabolism of PCBs in animals is dependent on species, the congener of PCB involved, and the TG:protein ratio of plasma.

E. Toxicity of PCBs

1. Toxicity of PCBs in mammals

Many studies on the toxicity of PCBs to different mammals have been done to determine the effects of this widely distributed environmental contaminant. One of the earliest effects observed as a result of low level exposure to PCBs is hepatomegaly, which was seen in rats on 20 ppm dietary Aroclor 1254 after 4 days (Carter, 1983), and in rats fed 50 or 500 ppm Aroclor 1242, 1248, 1254 or 1260 for 4 weeks (Litterst et al., 1972), and in rats receiving 100 mg Aroclor 1242/kg body weight per os (p.o.) or intraperitoneal (i.p.) (Bruckner et al., 1973). In all three of the experiments, no changes in body weight or weight gain were seen as a result of PCB treatment.

Another commonly observed effect of low-level exposure to PCBs is the induction of hepatic microsomal enzymes. This induction of enzymes is seen early in the treated animals, and the specific enzymes induced are dependent on the particular PCB congeners to which the animal is exposed. Depending on the structure and thus the binding site of the particular PCB, there is phenobarbital-type induction and/or 3-methylcholanthrene (3-

MC) type induction of enzymes. The 3-MC type inducers are, in general, more toxic (Poland and Glover, 1977; Kimbrough, 1980; Parkinson et al., 1980). The most toxic PCBs are those which are sterioisomers of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). They bind to the cytosolic Ah receptor protein and elicit toxic responses similar to TCDD (ie. 3-MC type induction). The structure required is Cl substitution at both para positions and substitution on at least one of the meta positions on both phenol rings (Safe et al., 1982). Bruckner et al. (1973) injected rats with a single i.p. dose of 100 mg Aroclor 1242/kg body weight, and noted that N-demethylation activity, hydroxylation and cytoplasmic P-450 concentrations were all increased. Litterst et al. (1972) fed male rats four different Aroclors (1242, 1248, 1254, and 1260) for 4 weeks at doses of 0.5, 5, 50 or 500 ppm. They found increased microsomal cytochrome P-450 concentrations in the treated rats, which increased with increasing chlorine content of the compound fed. There was also a dose-related increase beginning at concentrations as low as 0.5 ppm in hydroxylation, demethylation, and nitroreductase activities with increasing chlorine content of the PCB fed. In addition to liver enzyme induction, there was also induction of intestinal and serum enzymes by individual PCB congeners (Walden et al., 1982).

Other effects of PCBs on the liver included decreased vitamin A content (Bitman et al., 1972), hepatic porphyria in female rats (Kimbrough, 1981), increased triglyceride concentration, increased serum glutamic oxaloacetic

transaminase (SGOT) activity, and hepatic histopathologic changes in rats at doses of 500 ppm Aroclor 1242, 1248, 1254 or 1260 (Litterst et al., 1972). Furthermore, hepatic toxicity was also detected after oral doses of 2.5 or 4.5 g Aroclor 1242/kg body weight (Bruckner et al., 1973). Histopathology of the livers in rats treated with PCBs, as above, involved pale foci, large cytoplasmic vacuoles, increased cytoplasmic volume, fatty deposits around the central veins, and widely scattered necrotic foci. Hepatocellular carcinomas resulting from PCB exposure have been reported (Kimbrough, 1981). The kidney was also affected by high doses of PCBs in rats (Bruckner et al., 1973), resulting in scattered foci of vacuolated tubular epithelial cells and proteinaceous casts.

At very high doses of PCBs (2.5 or 4.5 g/kg body weight) in rats, Bruckner et al. (1973) observed loose stools to profuse diarrhea, decreased exploratory activity, decreased response to painful stimuli, chromodacryorrhea, adipsia, oliguria, anorexia, unusual gait and stance, and eventually ataxia followed by coma and death in the highest dose group. They determined the minimum, lethal oral dose to be 2.5 g/kg body weight and the 14-day oral LD50 for rats to be 4.25 g/kg body weight. Rats given single oral doses of 2500 or 5000 mg Aroclor 1242/kg body weight, or multiple oral doses of 500 mg Aroclor 1242/kg body weight/d for 4 days lost weight and had greater than 50% mortality (Green et al., 1975). The study indicated that cumulative toxicity occurred with Aroclor 1242.

Green et al. (1975), in treating rats with Aroclors 1242 and 1254, found no effect on spermatogonial cells and no chromosomal abnormalities of the bone marrow with oral doses of up to 5000 mg/kg body weight (b.w.) or 500 mg/kg b.w./d for Aroclor 1242 and up to 300 mg/kg b.w./d for Aroclor 1254. Aroclor 1254 did inhibit bone marrow mitosis at 150 and 300 mg/kg b.w./d. Bitman et al. (1972) described increased estrogenic activity in rats with various PCBs.

The effect of PCBs on reproduction and on the fetus or nursing young has been studied by several researchers in mice (Vodicnik and Lech, 1980; Vodicnik et al., 1980) PCBs underwent transplacental and significant mammary transfer from dams to fetus or nursing offspring. The loss of PCBs through the mammary gland proved to be a major route of elimination in the lactating female. Liver enzyme induction in the offspring of PCB-treated mothers was noted.

Several studies on the immunotoxicity of PCBs were done which indicated that there was thymic atrophy, significant suppression of the humoral immune response, and alterations of cell mediated immunity in rats and mice dependent on the PCB involved. In the case of mice, the strain of mice seemed to be involved (Silkworth and Grabstein, 1982; Bleavins and Aulerich, 1983). Bleavins and Aulerich (1983) discussed the findings of decreased antibody production in guinea pigs and mice, the selective toxicity of Aroclor 1254 on immature B cells, and the decreased weight of the bursa of Fabricius in birds.

There was an immune stimulation at low level of exposure to PCBs, and in general, there was greater toxicity seen with the higher chlorinated PCBs.

Aulerich and Ringer (1977), Ringer et al. (1981), and Aulerich et al. (1987) found that mink are one of the most sensitive species to the toxicity of PCBs. Feeding Great Lake fish, contaminated with PCBs, caused many of the problems reported by commercial mink ranchers. Signs of toxicity in mink included anorexia, bloody stools, fatty liver, hepatomegaly, elongated nails, delayed molts, decreased thyroid hormone levels, kidney degeneration, hemorrhagic gastric ulcers, reproductive failure, and death. Doses of 3,4,5,3',4',5'-hexachlorobiphenyl as low as 0.5 mg/kg b.w./d in the diet produced 50% mortality. Levels of 2 mg Aroclor 1254/kg b.w./d in the diet impaired mink reproduction, and levels greater than 5 mg/kg b.w. were completely fetotoxic. Removal of PCB contaminated diets brought about a reversal of the reproductive complications.

2. Toxicity of PCBs in poultry

There have been many studies on the toxic effects of PCBs in poultry. The pentobarbital sleeping times in Japanese quail had been measured as an indication of the liver microsomal enzyme activity. As enzyme activity increased sleeping time decreased (Bitman et al., 1972; Cecil et al., 1975). Initially, with a single oral dose of different Aroclors at 100 mg/kg b.w., there was an increase in sleeping times. By 18-24 hours after dosing, the sleeping times in treated quail were less than or

equivalent to control sleeping times. With increasing chlorination, sleeping times decreased (Bitman et al., 1972; Cecil et al., 1975). Also observed were increased liver weights and liver lipid content, a large decrease in liver vitamin A content, and a decrease in egg production in quail on Aroclor 1242 at 100 mg/kg b.w. for 60 days. Female quail were more sensitive to Aroclors 1232, 1242 and 1248, and there was 20-36% mortality from anesthesia used 2 hours after dosing. Lillie et al. (1974) tested the toxicity of various Aroclors in White Leghorn pullets at 20 ppm for 9 weeks. They found significant decreases in egg production, feed consumption (except those fed Aroclor 1232), and hatchability with Aroclors 1232, 1242, 1248 and 1254. Progeny growth was decreased with all four Aroclors, but only Aroclor 1248 significantly increased mortality in progeny. No effects were noted on adult body weights, weight gain, livability, egg weight, eggshell thickness or fertility. Other signs seen in poultry include edema formation in chicks, hepatic porphyria, and immunosuppression (Vos, 1972; Kimbrough, 1981).

3. Toxicity in non-human primates and humans

Allen et al. (1973, 1974) studied the effects of PCBs in rhesus monkeys. At levels of 25 mg Aroclor 1248/kg diet fed for two months, the resulting toxicity included facial edema, alopecia, and acne, which were persistent 8 months after PCB treatment had discontinued. By the end of the experiment, the level of PCBs in the subcutaneous adipose tissue was 127

ug/g, and 8 months later it was 34 ug/g. In monkeys receiving doses of 300 mg Aroclor 1248/kg diet for 90 days there were the same signs as above, as well as liver hypertrophy, gastric mucosa hypertrophy and hyperplasia, weight loss, ascites, decreased hematocrit and lymphocyte numbers, decreased serum protein, and increased activity of hepatic microsomal enzymes.

The most characteristic signs of PCB toxicity in humans were acne and dermal lesions (Safe, 1984). Wasserman et al. (1982) found that there were high serum levels (128 ppb versus 19.2 ppb in controls) of PCBs in 8 of 17 women that had premature deliveries, indicating that more research needs to be done to determine if there is a cause-effect relationship.

II. Polybrominated Biphenyls

A. Chemical Properties of PBBs

Polybrominated biphenyls (PBBs) are chemical compounds with the empirical formula $C_{12}H_{10-n}Br_n$, where n=1-10. Theoretically, there are 209 possible PBB congeners, although only approximately 45 have been purified. In the United States, two companies produced PBBs. Michigan Chemical Corporation (St. Louis, MI) produced them under the tradename fireMaster®. All commercially available PBBs were highly brominated, with 76-85% Br in the octa- and decabromobiphenyl mixtures. Production of these mixtures of PBBs is by bromination of biphenyl with subsequent separation and purification of the desired brominated biphenyl fractions. Contaminants are occasionally present, vary

from batch to batch, and include pentabromonaphthalenes, hexabromonaphthalenes, brominated benzenes, and methyl brominated furans. Technical mixtures of PBBs are typically white, off-white, or beige powdered solids. Properties of the PBBs include thermal stability, resistance to breakdown by acids,bases,light, reducing and oxidizing agents, low water solubility, low vapor pressure, solubility in non-polar solvents such as toluene and benzene. Unlike PCBs, PBBs are readily degraded by UV light.

fireMaster® FF-1, which was used in this experiment, is fireMaster® BP-6 mixed with 2% calcium silicate, an anticaking agent. This mixture has been found to contain trace quantities of tetra-, penta-, and hexabromonaphthalenes as well as 23 other compounds. fireMaster® FF-1 has the following properties:

Bromine %	= 75%
Density	= 2.6 g/ml at room temp.
Melting point	= 72-?°C
Decomposition pt.	= 300-400°C
Wavelength-max	= 219 nm
Principal components	= Br_4 - Br_8

The main hexabromobiphenyl in fireMaster® FF-1 is 2,4,5,2',4',5'-hexabromobiphenyl (60-70%), which is peak #4 measured on chromatograms to determine the level of PBBs in samples as fireMaster®. The next most prevalent component is 2,3,4,5,2',4',5'-heptabromobiphenyl, which makes up 22-27% of FF-1 and is identified as peak #8. Other components include: 2,4,5,2',5'-pentabromobiphenyl (pentaBB, peak #1); 2,4,5,3',4'-pentaBB, peak #2; 2,3,6,2',4',5'-hexaBB, peak #3; 2,3,4,2',4',5'-hexaBB, peak #5; 2,4,5,3',4',5'-hexaBB, peak #6; 2,3,4,5,3',4'-

hexaBB, peak #7; 2,3,4,5,2',4',5'-heptaBB, peak #8; 2,3,4,5,2',3',4'-heptaBB, peak #9; 2,3,4,5,2',3',4',5'-octaBB, peak #12; and 2,3,4,5,6,2',3',4',5'-nonaBB, peak #13. Using high resolution capillary chromatography, at least 60 compounds were identified in FF-1 along with other more minor components.

The preceding information was taken from Sundstrom et al. (1976), Kay (1977), Di Carlo et al. (1978), Moore and Aust (1978), Moore et al. (1978a), Kimbrough (1980), Aust et al. (1981), Orti et al. (1983), and Safe (1984).

B. Production and Uses of PBBs

PBBs were produced in the United States by Michigan Chemical Corp. (St. Louis, MI), White Chemical Co. (Bayonne, NJ), and Hexcel Corp. (Sayreville, NJ). Michigan Chemical Corp. produced hexabromobiphenyl as BP-6, and the other two companies produced octa- and decabromobiphenyls. Commercial production of PBBs in the U.S. began in 1970 and ended in 1977. The total quantity of PBBs produced in the U.S. between 1970-1976 was 13.3 million pounds, and 11.2-11.8 million pounds of this was hexabromobiphenyl. In July 1973, fireMaster® was accidentally mixed into Michigan Farm Bureau Service dairy pellets instead of Nutrimaster® (magnesium oxide), which was also produced by Michigan Chemical Corporation. By April 1974, when PBBs (fireMaster® FF-1) were found to be the cause of livestock problems, the contamination in Michigan was widespread. Production of PBBs by Michigan Chemical Corp. was discontinued November 1974, and their inventory was gone by April 1975. The

other two companies discontinued manufacturing of PBBs in 1977. Since 1975-76, all PBBs produced in the U.S. have been exported and none have been imported. Other countries producing PBBs included the German Federal Republic, France, and the United Kingdom.

The primary use of PBBs was as flame retardants, since they were very heat resistant, economical, and did not affect the flexibility of the base compounds. Even at their peak usage, they represented only 1% or less of the total sales of the flame retardant chemicals. The major use of PBBs as a flame retardant was in the production of flame retardant resins of acrylonitrile, butadiene, and styrene for business machine and electrical housings. They were also used in coatings and lacquers and in polyurethane foam for automobile upholstery. All of these uses were discontinued in late 1974, and there are no known current users in the United States.

Data on production and uses of PBBs were obtained from Carter (1976), Kay (1977), Di Carlo et al. (1978), Kimbrough (1980), and Safe (1984).

C. Environmental Distribution and Metabolism of PBBs

Contamination of the environment with PBBs has occurred from pollution due to manufacturing, industrial use, and the PBB accident in Michigan. Because PBBs like PCBs, are very stable, they tend to remain in the environment for a long time. PBBs in the soil tend to stay there, tightly absorbed to clay and other soils, and are not absorbed by plants. They are nonvolatile, and

resist bacterial degradation. PBBs have been found in sediments in the rivers adjacent to the plants which manufactured them, as well as further downstream. Fish readily store PBBs and have been found to contain PBB residues in these rivers. Michigan soils have been contaminated with PBBs from the manure of contaminated animals and from the disposal of carcasses, feed, milk, etc. Laboratory research indicated that PBBs can be degraded by UV irradiation, but in fields contaminated with PBBs there was very little degradation of the PBBs, even in a year's time. This suggests that PBBs will remain in the environment for a very long time.

The information above is from Jacobs et al. (1976), Kay (1977), Di Carlo et al. (1978), Kimbrough (1980), Damstra et al. (1982), and Safe (1984).

D. Distribution, Metabolism, and Excretion in Animals

PBBs are absorbed rapidly, with about 90% being absorbed if given orally. The amount absorbed seems to decrease with increased bromination of the compound. Initially, PBBs are distributed throughout the body, but later are found primarily in the fat (Di Carlo et al., 1978; Damstra et al., 1982; Domino et al., 1982).

Metabolism of PBBs in animals appears to be similar to that of PCBs, with formation of hydroxylated degradation products. The individual PBB congeners in fireMaster® are eliminated from the body at different rates, which is due to both the structure (availability of two adjacent unsubstituted sites

on the biphenyl) and the bromine content of these compounds (Di Carlo et al., 1978; Kimbrough, 1980; Damstra et al., 1982). Studies on the in vitro metabolism of PBBs by Dannan et al. (1978a) show that of the twelve major peaks present in fireMaster®, only peaks #1 and #3 were lost following incubation with PBB-pretreated rat microsomes or the microsomes from phenobarbital-treated rats. There were no effects seen with incubation in microsomes from control or 3-MC treated rats.

The excretion of PBBs is primarily via the feces, although milk and eggs were the most significant routes of excretion in animals producing them (Fries and Morrow, 1975; Fries et al., 1976; Di Carlo, 1978; Kimbrough, 1980; Damstra et al., 1982). Matthews et al. (1977) concluded that less than 10% of the total dose of 2,4,5,2',4',5'-hexabromobiphenyl would ever be excreted from rats and that their adipose tissue levels would remain high.

E. Toxicity of PBBs

1. Toxicity of PBBs in mammals

Many studies on the toxicity of PBBs have been done since 1973 when the tragic mixing of PBBs into the feed supply in Michigan occurred. As with PCBs, PBBs with different structures and degrees of chlorination cause different magnitudes and even totally different signs of toxicity. One of the commonly observed effects of PBBs is hepatomegaly (Di Carlo et al., 1978; Damstra et al., 1982; Safe, 1984). Hepatomegaly was noted when feeding rats fireMaster® BP-6, 2,4,5,2',4',5'-

hexabromobiphenyl (HBB), and 3,4,5,3',4',5'-HBB at as little as 10 mg/kg diet, but not at 1 mg/kg diet, over a nine day period (Render et al., 1982). Gupta et al. (1981) also saw liver enlargement with fireMaster® FF-1 and 2,4,5,2',4',5'-HBB at doses of 30 mg/kg b.w. and 16.8 mg/kg b.w.(but not at 3.0 and 1.68), respectively, in rats and mice dosed p.o. 22 times in 30 days. In both studies, the affected livers contained diffusely swollen hepatocytes, dose related increase of lipid vacuoles, and proliferation of the smooth endoplasmic reticulum. Liver lesions were found to be more severe in the 3,4,5,3',4',5'-HBB-treated rats. Rough endoplasmic reticulum disorganization, myelin body formation, and bile duct hyperplasia were described, as well as the aforementioned pathologic changes. The liver lesions in the rats and mice were dose related and more severe in the FF-1 than the 2,4,5,2',4',5'-HBB-treated animals (Gupta et al., 1981). No distinction was made by Render et al. (1982) between the toxic effects of 2,4,5,2',4',5'-HBB and BP-6. Other researchers who found hepatomegaly in rats include Dent et al. (1976), Moore et al. (1978b), Dannan et al. (1978b), Moore et al. (1979), and Dannan et al. (1982).

PBBs induce hepatic microsomal enzymes comparable to PCBs, with individual PBBs acting differently depending on their structure (Di Carlo et al., 1978; Aust et al., 1981; Damstra et al., 1982; Safe, 1984). Much was done to determine which PBBs were causing a particular type of induction phenobarbital type or PB-type, 3-methyl cholanthrene type or 3-

MC type, or a combination of the two and how this related to their toxicity. PB-type induction included the induction of epoxide hydratase, NADPH-cytochrome P-450 reductase, aminopyrine-N-demethylase, and increased cytochrome P-450 with spectral maximum at 450 nm. In contrast, 3-MC type inducers increased the activities of benzo[a]pyrene hydroxylase, UDP glucuronyltransferase, and shifted the spectral maximum of cytochrome P-450 hemoproteins to 448 nm. fireMaster® BP-6 has been found to cause a mixed-type induction of hepatic microsomal enzymes in rats at levels as low as 4.7 ppm in the diet over a two week period (Dent et al., 1976; Moore et al., 1978b). Studies involving the major (60-70%) component of BP-6, 2,4,5,2',4',5'-HBB, have indicated it to be a strictly PB-type inducer (Moore et al., 1978b). The second most prevalent congener (22-27%) in BP-6, 2,3,4,5,2',4',5'-heptaBB, was observed to be a strictly PB-type inducer (Moore et al., 1979). Minor components of BP-6 were also strictly PB-type inducers, including 2,4,5,2',5'-pentaBB, and 2,3,4,5,2',3',4',5'-octaBB (Orti et al., 1983). Congeners making up a small percentage of BP-6 that are mixed type inducers include 2,4,5,3',4',5'-hexaBB (Dannan et al., 1978b), 2,4,5,3',4'-pentaBB (Dannan et al., 1982), 2,3,4,5,2',4'-hexaBB, and 2,3,4,5,3',4'-hexaBB (Orti et al., 1983). Minor PBB congeners in BP-6 with strictly 3-MC type induction and the only ones known to cause hyperkeratosis of rabbit ears include 3,4,3',4'-tetraBB, and 3,4,5,3',4'-tetraBB (Orti et al., 1983). Another congener studied was 2,2'-diBB (Moore et al., 1979) which

had little or no effect on any enzymes at 90 mg/kg b.w. dosed i.p. in rats.

The most toxic congener identified so far is 3,4,5,3',4',5'-HBB, a 3-MC type inducer, which is not present in fireMaster® BP-6 or FF-1 (Orti et al., 1983). Besides the toxicity seen with other PBBs, this compound also causes significant decreases in feed intake, thymic weight, splenic weight, and body weight at levels as low as 10 mg/kg diet and death of 2 of 6 rats fed 100 mg/kg diet for 9 days (Render et al., 1982). It causes hyperkeratosis of rabbit ears (Orti et al., 1983). It has also been shown to cause thyroid and pituitary vacuolation and other ultrastructural damage at doses as low as 10 mg/kg diet. The alterations are more severe than any changes observed from 100 mg/kg diet of BP-6 (Akoso et al., 1982). A component making up 1% of FF-1 and 4% of BP-6, 2,4,5,3',4'-pentaBB, was shown to be quite toxic. It caused significant weight loss, decreased thymic and splenic weights, impaired splenic T-helper and B cells, and the other PBBs signs (Dannan et al., 1982). Both of these compounds are primarily MC-type inducers and because they bind at the Ah receptor, induce toxicity similar to TCDD, as described for PCBs.

Other effects caused by PBBs include porphyria in female rats (McCormack et al., 1982a) and mice receiving 22 oral doses of FF-1 over 30 days (Gupta et al., 1981), marked increases in serum proteins in rats given FF-1 at doses as low as 3 mg/kg b.w. (Gupta et al., 1981), decreases in packed cell

volume, hemoglobin and other red blood cell values in male rats (Gupta et al., 1981), decreased body weights from FF-1 in rats (McCormack et al., 1982a, 1982b) and male mice, and increased body weights in female mice on FF-1 (Gupta et al., 1981; Tilson et al., 1978). Rats given 2,3,5,2',4',5'-HBB did not show porphyria, increased serum proteins, or decreased body weights as seen with FF-1 (McCormack et al., 1982a, 1982b). No change in feed consumption was observed in rats or mice given up to 30 mg FF-1 or 2,4,5,2',4',5'-HBB/kg b.w., orally (Gupta et al., 1981). Rats exposed to 100 mg PBBs/kg b.w. either pre- or post-natally showed decreased concentrations of hepatic vitamin A, and increased left atrial inotropic response to calcium. No effect was found on cardiac contractile function (McCormack et al., 1982a). Other microsomal enzymes are induced besides liver enzymes, including those in the lung (McCormack et al., 1982b), kidney (McCormack et al., 1978), and intestine (Manis and Kim, 1980). Iron absorption was found to be increased with a single oral dose of 200 mg FF-1/kg b.w. (Manis and Kim, 1980). Apparently, PBBs also cause decreased response times in discrimination tasks and neuromuscular function (CNS depression) at 6 mg/kg b.w., although at the low dose of 1 mg/kg b.w., hyperactivity was noted (Tilson et al., 1978, Geller et al., 1979). Immunosuppression, hepatic nodules and hepatocellular carcinomas occurred from high doses of PBBs in treated rats (Aust et al., 1981; Damstra et al., 1982; Safe, 1984).

PBBs pass the placental barrier and transfer to the young via milk. Young rats born to dams that were treated with PBBs at levels as low as 1 mg/kg diet, and young rats that nursed PBB-treated animals had hepatic and renal microsomal enzyme induction, decreased body weights, and increased liver weights (Moore et al., 1978c; Dent et al., 1978). Based on vaginal cytology, rats exposed to PBBs at 100 mg/kg diet had significantly increased estrous cycles (Johnston et al., 1980). Cattle contaminated with high levels of FF-1, due to mistaken incorporation of fireMaster® into cattle feed showed a significant decrease in milk production, possible early embryonic reabsorption, lengthened pregnancy by 2-4 weeks, dystocias, udders which did not develop, little or no milk production, metritis, larger calves, and calves born dead or that died soon after birth, and hydrops amni (Jackson and Halbert, 1974).

The Halbert dairy herd in Michigan received some of the most contaminated feed from the accidental mixing of fireMaster® FF-1 into the Farm Bureau's dairy ration. Some of these cows were estimated to have eaten as much as 227 grams of PBBs. His herd showed the reproductive problems described above as well as other signs. Those signs were weight loss, anorexia, hair loss, the formation of hematomas which later became abscesses, abnormal hoof development, lacrimation, runny noses, and wrinkled, thickened skin on the thorax, dorsal neck and shoulders. Twenty-four cows died out of his herd of 200. All died after removal of contaminated feed which was fed for only a

few weeks. Five of 12 calves, 6-18 months old, given the contaminated diet died within 6 weeks. They showed grating of teeth, prostration, coma and death. Necropsy revealed liver changes including fatty metamorphosis, fat vacuoles replacing liver cells, and amyloidosis. Changes in the kidney included pigment nephrosis, and acute, subacute and chronic interstitial nephritis. Some showed abomasal ulcers or hematomas and abscesses in the peritoneal and thoracic cavities (Carter, 1976; Jackson and Halbert, 1976; Kay, 1977). Due to the length of time it took to determine the cause of the problem and how it had occurred, the minimal period of exposure for most herds was 9 months. Halbert's herd was taken off contaminated feed after only several weeks. In March of 1975, the Michigan Department of Agriculture decided to do a herd health study to determine if low level PBBs in cattle not destroyed were causing adverse symptoms or if the problems being described were within normal occurrence. They compared exposed and non-exposed herds in Michigan to non-exposed herds in Wisconsin. They found there were no herd health problems that could be attributed to low level PBBs contamination (FDA, 1975).

Mink are one of the most sensitive species to PBBs, as well as PCBs (Aulerich and Ringer, 1979; Ringer et al., 1981). Diets containing 6.25 ppm, or greater, of fireMaster® FF-1 were lethal to adult mink within 10 months. Dietary levels as low as 1-2.5 ppm FF-1 fed for 9 months caused decreased litter size, lower birth weights of kits, and decreased kit survival.

Overall, FF-1 proved less fetotoxic than PCBs, but was lethal at a lower dietary concentration. Mink fed meat from contaminated cows or chickens that had metabolized the PBBs were more severely affected than those fed FF-1 in their diet. Other signs of PBB poisoning included food rejection, weight loss, unthrifty appearance, hepatomegaly, fatty livers, and increased kidney weight. The adipose tissue contained 60 times the concentration of PBB that was in the diet (Aulerich and Ringer, 1979; Ringer et al., 1981).

2. Toxicity of PBBs in poultry

Many studies on the toxicity of PBBs in poultry have been done. FF-1 or BP-6 fed to hens at 625 and 640 ppm, respectively, caused marked inanition, cessation of egg production, and eventually death (Cecil and Bitman, 1975; Polin and Ringer, 1978). A decrease in feed intake was seen in hens fed FF-1 at 125 mg/kg diet, and their egg production dropped from 66% to 48%. Egg production was found to be significantly decreased with levels of FF-1 greater than 30 ppm in the diet, and there was a decrease in hatchability and viability of offspring when PBBs were 45 ppm or greater (Polin and Ringer, 1978). Decreased egg production and hatchability were noted from feeding 20 ppm of BP-6 (Cecil and Bitman, 1975).

The half-life of PBBs in tissues was determined to be 17 days for muscle, 31 days for liver, and 17 days for eggs (Polin and Ringer, 1978). No change in PBB concentrations of the adipose tissue was seen in 56 days. Egg production resumed

to pretreatment levels within 2 weeks after withdrawal from the contaminated diet if hens had been fed 120 ppm or less, 3-4 weeks if fed 125 ppm, and 5-6 weeks if fed greater than 125 ppm (Polin and Ringer, 1978). Signs of toxicity included increased liver and thyroid weights, edema of abdominal and cervical regions in embryos and newly hatched chicks from treated hens, decreased feed intake, decreased body weight, decreased size of the comb, testes, spleen and bursa of Fabricius, hydropericardium and ascites, decreased hematological values (heart rate, PCV, hemoglobin, cardiac output) with decreased hematopoietin, decreased ECG voltage amplitude and mean electrical axis shift (Polin and Ringer, 1978; Ringer, 1978). Egg weights and egg shell thickness remained unchanged from PBB feeding. Japanese quail refused to eat diet containing 500 ppm PBBs and died (Babish et al., 1975). Males had higher levels of PBBs in their tissues than egg-laying females. Induction of liver microsomal enzymes occurred maximally at dietary levels of 20 ppm fed to males and 100 ppm fed to females. Other signs seen in treated birds included decreased egg production and 0% hatchability at 100 ppm. Liver weights were increased but there were no gross or microscopic lesions.

3. Toxicity in humans

Exposure of humans to PBBs may result in halogen- or brom-acne, irritant or allergic dermatitis, pigmentary changes, alopecia, nail dystrophy, folliculitis, and an increase in sweating (Chanda et al., 1982). The exact signs of toxicity

are hard to determine as much data are based on volunteers and are subjective.

III. Enhanced Withdrawal of Xenobiotics

The elimination of xenobiotics concentrated in the adipose tissue and remaining there indefinitely has been the subject of many research projects. Many compounds have been given to animals to try to enhance the withdrawal of these persistent xenobiotics. Cholestyramine, an anion exchange resin that binds to xenobiotics, has been used in the past to detoxify victims of chlordecone (kepone) poisoning. It increased the excretion of kepone 700% in humans (Anonymous, 1980). Rozman et al. (1982d) found that addition of 4% cholestyramine to the diet for 6 days increased elimination of pentachlorophenol from rhesus monkeys from 38% to 59%, but had little or no effect on the elimination of mirex from rhesus monkeys (Rozman et al., 1981b) or hexachlorobenzene from rats and monkeys (Rozman et al., 1981a). Rozman et al. (1982a) found that 4% cholestyramine in the diet of rhesus monkeys increased elimination (increased fecal and decreased urinary elimination) of 2,4,5,2',4',5'-hexabromobiphenyl by 50% and when used in combination with 5% mineral oil in the diet there was an additive effect on excretion. Mineral oil is another compound widely tested for its ability to enhance withdrawal of xenobiotics. It increased intestinal elimination of 2,4,5,2',4',5'-HBB by 50% 6-7 weeks after dosing, although it had little effect in the first two

weeks following dosing (Rozman et al., 1982a). Mineral oil at 5% in the diet has also been shown to increase elimination of hexachlorobenzene from sheep by a factor of 3 (Rozman et al., 1982b), increase fecal elimination of mirex from rhesus monkeys 50% in the 1st month and by 400% in six months (Rozman et al., 1981b), and cause a 6-9 fold increase in fecal excretion of hexachlorobenzene from rhesus monkeys (Rozman et al., 1981a). Using a bile duct bypass on rhesus monkeys, Rozman et al. (1983) determined that fecal excretion of hexachlorobenzene, stimulated by 5% mineral oil in the diet, was directly through the intestine with no loss via the bile duct bypass. The shunt of loss via the intestine came at the expense of urinary and biliary excretion of hexachlorobenzene metabolites by 20-60%. Liquid paraffin, which is similar to mineral oil in composition, was also studied for its ability to increase elimination of toxins from the body. Both are composed of aliphatic hydrocarbons of lengths C_{10}-C_{30}, and both contain hexadecane, which has also been tested for its ability to enhance elimination. Liquid paraffin in the diet at 8% was found to increase the elimination by rats of 2,4,6,2',4'-pentachlorobiphenyl (Richter et al., 1979) and hexachlorobenzene (Richter et al., 1977; Richter and Schafer, 1981). Hexadecane at 5% of the diet was found to enhance elimination of hexachlorobenzene by sheep 3-fold (Rozman et al., 1982b), and by rats and rhesus monkeys 4-13-fold (Rozman et al., 1981a; Rozman et al., 1982c). Hexadecane appears to act similarly to mineral oil in the elimination of xenobiotics

through the intestinal wall. In particular, the large intestine plays a major role in the excretion of hexachlorobenzene (Rozman et al., 1981a; Rozman et al., 1982c). Squalene (2,6,10,15,19,23 hexamethyltetracosan) at 8% in the diet leads to a five-fold increase in fecal elimination of 2,4,5,2',4',5'-hexachlorobiphenyl by rats (Richter et al., 1983).

Restriction of feed intake has been tried to mobilize lipid stores of xenobiotics, with the hope that this would cause elimination of those stores. Oishi et al. (1979) studied the effect of a 4 week food restriction on rats. They found depressed weight gains, lower organ weights, increased relative organ weights of brain and testes, decreased leukocyte counts based on level of restriction, lower concentrations of triglycerides and inorganic phosphorus, and decreased activities of glutamic pyruvic transaminase and alkaline phosphatase. Hematocrit also was increased in rats fed only 10-15 g/d (ad lib.= 22-23 g/d). Feeding 75% of the normal diet led to enhanced fecal excretion of 2,4,5,2',4',5'-hexachlorobiphenyl by rats, which eliminated 47.8% of the total dose (Matthews and Anderson, 1975). Wyss et al. (1982) restricted feed intake to 25% of control intake beginning 2 weeks after 2,4,5,2',4',5'-hexachlorobiphenyl (6-HCB) dosing. They found body weights decreased by 50% at the 4th week of feed restriction, after which weight stabilized with adipose tissue nearly absent. Levels of 6-HCB were increased in tissues (except adipose tissue) and blood up to the fourth week, after which levels declined (except in

skin) with half-lives of 8-13 days. Fecal excretion was 10 times that of control rats. Villeneuve (1975) and Villeneuve et al. (1977a) found that food deprivation (25% of normal) did not enhance the excretion of hexachlorobenzene (HCB) from rats, and in fact caused toxic signs to be seen in rats pretreated with 100 mg HCB/kg b.w..

The combination of feed restriction with colestipol was tried to determine if the two together would cause a greater increase in the amount of xenobiotic eliminated from the body. Polin and Leavitt (1984) compared 0, 0.5, or 2.5% (0,0.625, or 3.125% in restricted diets for the same total amount) colestipol hydrochloride, an anion exchange resin, in the diet with or without concurrent feed restriction to 80% of control intake, for 21 days following 14 days of feeding fireMaster® FF-1 to male White Leghorn chickens. They found that colestipol at 2.5% with or without restriction resulted in a 50% increase in excretion of FF-1 during the first 21 days of withdrawal. This was not seen with lower levels of colestipol or in animals on feed restriction alone. After 42 days of withdrawal, body burdens were decreased by 80% over control levels and 20% more than within the same treatment groups on day 21. At this level of treatment, there were also decreases in body weight gains and carcass lipid content. Polin et al. (1985) used mineral oil or colestipol alone or in combination with a 50% restriction in dietary intake to enhance elimination of fireMaster® FF-1 from egg- and meat-type chickens. In this experiment, they found that chickens

pretreated with 10 ppm fireMaster® in their diets had body burdens of PBBs reduced by 70% after 21 days of 50% feed restriction in combination with 10% mineral oil or colestipol in the diet. Feed restriction, mineral oil, or colestipol alone, or colestipol at 3.5% with feed restriction did not prove as effective or consistent as the feed restriction plus dietary mineral oil. Mineral oil at 5% in the diet did decrease body burdens by 25%. Two different levels of PBBs were fed in this study, and of these levels, there was a greater percentage of PBBs lost in the chickens fed 1 ppm versus those receiving 10 ppm indicating the elimination process may be saturable. Colestipol was found to decrease body weight gains and lipid concentration, while mineral oil did not have this effect. In another experiment, Polin et al. (1986) determined the withdrawal of hexachlorobenzene and pentachlorophenol from chickens using colestipol or mineral oil alone or in combination with a 50% feed restriction. When 5% mineral oil or colestipol was added to the diet, or if only feed restriction was applied, body burdens of hexachlorobenzene were reduced in 21 days by 63%. Chickens not treated had reductions of 37%. When a combination of 10% mineral oil or colestipol with feed restriction was used, body burdens decreased by 81%. Animals fed pentachlorophenol and then not treated had body burdens reduced 30% while those restricted in feed intake lost 35%. The combination of feed restriction and mineral oil removed all of the pentochlorophenol. Polin et al. (1989) studied the withdrawal of Aroclor 1254 from meat type chickens treated for 21

days with 5% mineral oil, colestipol, petroleum jelly, or propylene glycol in the diet, alone or at 10% of the diet in combination with 50% feed restriction. The combination of 50% feed restriction and mineral oil reduced PCB levels to 32% of those for nontreated chickens. Petroleum jelly, propylene glycol, and colestipol in combination with feed restriction reduced body burdens to 47, 57, and 77%, respectively, of control levels. Feed restriction alone had no significant effect on body burdens. When any of the compounds were used alone at 5% of the diet, they reduced body burdens to 67-90% of control levels. Colestipol and petroleum jelly were the least effective. From the data provided in the combination experiments above, it appears that the use of feed restriction in combination with bile-binding resins or lipotropic agents cause the greatest reductions in the body burdens of lipophilic xenobiotics that accumulate and persist in the body.

MATERIAL AND METHODS

I. Experimental Methods

A. Preparation of Experimental Diets

The residue build-up phase of this study used a PCB-contaminated diet, a PBB-contaminated diet and a non-contaminated diet. The non-contaminated diet used for all three diets was Purina Certified Rodent Chow #5002. The rodent chow, in large pellet form, was ground to a mash using a Hammermill feed grinder to facilitate mixing of PCBs or PBBs evenly throughout the diet. Non-contaminated diet consisted of ground rodent chow only. A PCB-contaminated diet was formulated using Arochlor 1254 (Monsanto Chemical Corp., St. Louis, MO), a commercial mixture of polychlorinated biphenyls. Incorporation of Aroclor 1254 into the ground rodent chow was accomplished by diluting a weighed amount of Arochlor 1254 into a measured volume of hexane. The volume needed to make 10 ppm in the diet was blended into a premix composed of finely ground rodent chow. The premix was then mixed into 4 kg ground rodent chow by tumbling for 5 minutes in a modified paint tumbling machine. fireMaster® FF-1 (Michigan Chemical Co., St. Louis, MI), a commercial mixture of polybrominated biphenyls, was the source of PBBs. The PBB material used was obtained from the original batch of PBBs

accidentally introduced into Michigan agriculture in the fall of 1973. The same mixing method was used to prepare the PBB-contaminated diet as was used to prepare the PCB-contaminated diet. The final concentration of PBBs in the diet was 10 ppm.

The withdrawal phase of this study required the preparation of non-contaminated diet with mineral oil at 0, 5 or 10% by weight. The mineral oil was obtained from the Veterinary Clinical Center at Michigan State University. It was mixed thoroughly into the diet by hand-stirring the mixture in a 20 gallon plastic container.

B. Husbandry

Sprague-Dawley male rats, 12 weeks of age and weighing 300-350 grams, were received through University Laboratory Animal Resources (ULAR) at Michigan State University from a commercial breeder. All the animals fed PCBs or PBBs were isolated in a room at a ULAR facility located in the MSU's Clinical Center. Rats were housed three per polypropylene cage, with wire tops, measuring 33 x 38 x 18 cm. Ground corn cobs were used as bedding which was changed two times per week. Artificial lighting was supplied on a schedule of 16 hours light : 8 hours dark. The temperature was maintained at 22 +/- 2°C. Water was provided ad libitum.

C. Schedule

Rats were received on November 12, 1985 and were allowed a week to adapt to their new environment and experimental conditions. The experimental design is presented in Table 1.

Table 1. Experimental design.

	Cage[1] Numbers	Contamination Diet (14 day period)	Withdrawal Treatment (21 day period)
Control rats:			
	7,11,15	Non-contaminated	None[2]
	1,2,3	Non-contaminated	None[3]
	4,5,6	Non-contaminated	50% feed restriction
	8,9,10	Non-contaminated	5% Mineral oil
	12,13,14	Non-contaminated	10% Mineral Oil @ 50% FR[4]
PBB rats:	22,26,30	10 ppm PBBs	None[2]
	16,17,18	10 ppm PBBs	None[3]
	19,20,21	10 ppm PBBs	50% feed restriction
	23,24,25	10 ppm PBBs	5% Mineral oil
	27,28,29	10 ppm PBBs	10% Mineral Oil @ 50% FR[4]
PCB rats:	34,38,42	10 ppm PCBs	None[2]
	31,32,33	10 ppm PCBs	None[3]
	35,36,37	10 ppm PCBs	50% feed restriction
	39, 40,41	10 ppm PCBs	5% Mineral oil
	43,44,45	10 ppm PCBs	10% Mineral Oil @ 50% FR[4]

1 Rats were housed three per cage.

2 The three rats in each of these cages were euthanized at the end of the 14 day contamination phase and analyzed for PCB and PBB residues.

3 Rats in these cages were fed ad libitum.

4 FR = feed restriction.

The contamination phase of the experiment lasted 14 days, during which time PCB- or PBB-contaminated diets were fed. The rats received feed ad libitum. Rats in fifteen cages (45 rats) were on each diet including the control diet at this time. Feed intake was determined two times per week and body weights were obtained weekly (see appendix A for data on feed consumption and body weights). On day 14 of the contamination phase, after an overnight fast, three rats from three different cages from each of the three treatment groups were randomly selected and euthanized bloodlessly with excess CO_2. The three euthanized rats from each cage were sealed in a plastic bag and frozen at -20°C. They were later prepared for analysis of PCBs, PBBs, lipid and water content as described in the section on tissue preparation. After completion of this phase of the experiment, the room was thoroughly cleaned to remove all PCB and PBB contamination.

During the 21 day withdrawal phase, the rats were fed non-contaminated diet containing 0, 5 or 10% (by weight) mineral oil. Three rats from three different cages previously fed each of the contamination diets and from three cages fed the non-contaminated diet were fed non-contaminated diet containing 0% mineral oil, ad libitum (Table 1). Nine rats from another three cages from each of the original three diet groups were fed non-contaminated diet at a 50% feed restriction. As indicated in Table 1, nine rats from three more cages from each group were fed 5% mineral oil diet ad libitum, while the nine rats in the final three cages from each group were fed a diet containing 10%

mineral oil at a 50% feed restriction. The 50% feed restriction was based on feed intake of ad libitum rats which were measured every other day. On day 21 of the withdrawal phase all remaining rats were euthanized with excess CO_2. The three rats from each cage were put in a plastic bag and frozen at -20°C. Later, preparation of the rats for analyses was conducted as described in the section on tissue preparation. Appendix B outlines the coding and treatment associated with each cage of rats.

D. Safety Methods and Contaminated Waste Disposal

Protective clothing was mandated for all personnel who entered the animal room at the ULAR facility. All cages and other equipment used for the experiments were rinsed with hexane prior to their removal from the room. Bedding, feces, and all inorganic and organic waste were sealed in 50 gallon plastic or steel barrels. Hexane used to rinse equipment and cages was collected and sealed in plastic jugs. The barrels and plastic jugs were disposed of by Michigan State University's Office of Radiation, Chemical and Biological Safety in accordance with state and federal regulations.

II. Lipid and Water Determination on Tissue Samples

A. Tissue Preparation

Plastic bags containing the three rats from each cage were removed from the freezer and thawed overnight at 4°C. Carcasses were then cut into several small pieces with a Hobart 5212 F electric saw, and ground to a hamburger-like consistency

in a Hobart 4732 SS electric meat grinder. Samples were put through the grinder five times to produce a homogeneous blend of the three rats from each cage. Grab samples were removed and two Nasco whirl-pak® bags were filled and labelled for each set of rats. Samples in whirl-paks® were refrozen and stored at -20°C.

B. Water Determination

The percent water in whole-body samples was determined by weighing out approximately 60 grams of the ground sample, to the nearest 0.1 gram, into a tared aluminum dish. The sample in the dish was covered with cheesecloth, to prevent sample loss. The labelled aluminum dishes containing samples were placed into a Virtis 25SRC freeze drier for 48 hours, to reach a constant weight. The weights of the dish and sample were taken immediately upon removal from the freeze drier to determine the weight lost as water. The percent water in the sample was then determined by dividing the lost weight by the initial carcass sample weight, and multiplying by 100%. See Appendix C for raw data on percent water.

C. Lipid Determination

Lipid was determined gravimetrically by soxhlet extraction of the sample using petroleum ether in a biosafety cabinet. Extraction thimbles were dried prior to use for 12 hours in a Precision Scientific Oven (either Model 19 or 26) at 80°C. A tare for each thimble was obtained by weighing them dry. Approximately five grams of freeze-dried sample were added to each weighed thimble. Each of the freeze-dried samples was

analyzed in duplicate. The thimbles containing samples were then dried in the oven for 12 hours at 80°C. Samples in thimbles were removed from the oven, cooled in a desiccator and then immediately weighed to obtain a pre-extraction dry weight. Thimbles were placed in the soxhlet apparatus where samples were repeatedly extracted with petroleum ether for 18 hours. Then, thimbles containing extracted samples were set in racks under the fumehood to allow evaporation of petroleum ether. Once the ether had evaporated, samples were oven-dried at 80°C for 12 hours, and a post-extraction dry weight obtained. The formula for percent crude lipid is as follows:

$$\text{Percent crude lipid} = \frac{\text{Pre-extraction dry weight} - \text{Post-extraction dry weight}}{\text{Pre-extraction dry weight}} \times 100\%$$

See Appendix C for raw data on percent lipid from whole-body ground rat samples.

III. Gas Chromatographic Analysis of PCB and PBB Residues in Rat Whole-Body Samples

A. Gas Chromatographic (GC) Conditions

Residues of PCBs and PBBs in whole body samples were determined with a Varian Aerograph 3700 Gas Chromatograph with a ^{63}Ni electron capture detector. Chromatograms were printed by a Varian 9176 Chromatogram Recorder. For analysis of both PCBs and PBBs, the gas chromatograph was equipped with a 6 meter x 2 millimeter i.d. glass column containing 3% OV-1 liquid phase on

100/120 mesh Chromosorb W-HP solid support. For PCB analysis, conditions were as follows:

Injector temperature = 220°C
Column temperature = 200°C
Detector temperature = 270°C
Carrier gas = 99.99% pure Nitrogen gas
Carrier flow rate = 40 ml/min.
Chart speed = 1.0 cm/min.
Attenuation = 32

For PBB analysis, conditions were as follows:

Injector temperature = 270°C
Column temperature = 250°C
Detector temperature = 270°C
Carrier gas = 99.99% pure Nitrogen gas
Carrier flow rate = 40 ml/min.
Chart speed(single peak) = 1.0 cm/min.
Chart speed(multiple peaks) = 0.5 in/min.
Attenuation(single peak) = 128
Attenuation(multiple peaks) = 16

To prevent leakage of air through septums damaged by multiple injections, they were changed weekly.

B. General Procedures for Preparing Samples for Gas Chromatographic Analysis

PCBs and PBBs were analyzed in duplicate ground whole-body samples (coded for identification - see Appendix B) by gas chromatography. They were extracted and the extracts clarified according to the following procedure:

1. Take a portion of thawed sample out of the whirl-pak^{R} and chop it with a razor blade to a fine consistency.

2. From the finely chopped sample, weigh out 2 grams into a 50 ml Erlenmeyer flask.

3. Homogenize for one minute with 25 ml of toluene/ethyl acetate (1:3) solvent using a Tekmar tissuemizer.

4. Decant fluid through a 5 cm Buchner funnel containing solvent wetted 5 cm Whatman glass fiber filter, under vacuum, into a 250 ml filter flask, leaving any solid portion of the sample in the Erlenmeyer flask.

5. Repeat steps 3 and 4 two more times. Combine all extracts in the filter flask. Rinse the Erlenmeyer flask and tissuemizer blades with toluene/ethyl acetate and collect that solvent as well.

6. Pour combined filtered solvent through a glass funnel containing a small plug of glass wool and 5 grams of granular anhydrous sodium sulfate (Na_2SO_4, Mallinckrodt , Analytical grade) into a 250 ml flat bottom flask with a 24/40 top. Rinse filter flask and funnel with three 5 ml portions of solvent.

7. Rinse joint of flat bottom flask and attach flask with a clip to a rotoevaporator unit. Rotoevaporate in a 350°C waterbath to about 5 ml and pour through a small funnel into a 10 ml volumetric flask. Rinse the 250 ml flask and funnel with several small portions of solvent and add to the volumetric flask. Fill the volumetric flask to the 10 ml line with solvent, cap, and seal with parafilm (American Can Company) and store refrigerated until further preparation.

8. Five ml of the 10 ml from each sample were processed through a ABC Lab Autoprep 1001 gel permeation chromatograph (GPC) using S-X3 Biobeads, 200/400 mesh packing, to remove high molecular weight lipids, etc. from the sample. The solvent used was toluene/ethyl acetate (1:3). GPC collection was made into a 250 ml flat bottom flask with a 24/40 top. GPC parameters were as follows:

   Dump = 21 minutes
   Collect = 15 minutes
   Wash = 5 minutes
   Flow rate = 5 ml/min.

These parameters were determined with standards prior to doing samples to ensure that all the PCBs and PBBs residues were recovered.

9. The GPC output was rotoevaporated to approximately 5 ml, and transferred quantitatively with rinsing through a small funnel into a 10 ml volumetric flask. Then, the solution in the volumetric flask was brought to 10 ml with toluene/ethyl acetate, and the contents transferred into a screw top tube that was stored in a refrigerator until analysis.

C. Preparation and Storage of PCB and PBB Standard Solutions and Spiked Samples

Stock solutions of PCBs were made using Arochlor 1254 diluted with toluene/ethyl acetate (1:3). PCB stock solutions contained 12, 20 and 598 ug PCBs/ml. Standard solutions were made from these stock solutions by diluting the stock solutions with toluene/ethyl acetate (1:3) to produce PCB standards of 0.24, 0.48, 0.84 and 1.2 ug/ml. Spiked samples were prepared from 1 ml of 12 ug PCB/ml stock, 7 ml of 1.2 ug PCB/ml solution, 4 ml of 1.2 ug PCB/ml solution and 2 ml of a 1.2 ug PCB/ml solution by adding these to 2 gram samples of ground non-contaminated whole body rat samples. After allowing the solutions to soak into the carcass sample for 10 minutes, they were processed as the regular samples to produce 1.2 ug/ml, 0.84 ug/ml, 0.48 ug/ml and 0.24 ug/ml, respectively, in the final 10 ml extract if 100% recovery occurred.

PBB stock solutions were prepared from fireMaster® diluted in toluene/ethyl acetate (1:3). Stock solutions contained 10, 100 and 1000 ug PBB/ml. Standard solutions of 0.2, 0.5, 0.8 and 1.0 ug/ml were made by diluting the stock solutions with toluene/ethyl acetate to produce the proper concentration of PBBs. Spiked samples were prepared similarly to the PCBs to produce spikes of 0.2, 0.5, 0.8 and 1.0 ug/ml in the final extraction volume of 10 ml.

All spiked sample extracts, stock and standard solutions were stored under refrigeration.

D. General Techniques for GC Injections

All injections into the GC were made using Hamilton microliter syringes. Syringes were rinsed 20 times with toluene/ethyl acetate (1:3) before filling with a sample or standard for injection. The amount injected was determined by reading a total volume of solution prior to injection as compared to the volume remaining after injection into the GC. All samples and standards were warmed to room temperature prior to injection. The time needed for a sample to completely pass through the column was determined as follows. A PCB-spiked sample and a PBB-spiked sample, prepared earlier, were injected separately to determine the point where no more peaks appeared on the chromatogram. The point in time where no more peaks appeared was chosen to be the minimum analysis time for each PCB and PBB residue.

E. Analysis of PCB Residues in Tissues

Using the GC conditions as described for PCBs, three injections of approximately 7 ul of 1.2 ug/ml standard solution were injected in rapid succession to load the column, decreasing daily variation in detector response to standards. Once the column had been loaded and peaks ceased to form on the chromatogram, standards were injected, followed by samples and then another set of standards. This procedure was followed each day, with no more than 10 samples being injected before standards were injected again. Standards for PCBs were 0.24, 0.48, 0.84 and 1.2 ug/ml. Quantification of PCB residues was performed by

measuring the peak heights of six major peaks (peaks #4-6,and 8,9 and 11) and peak areas for four smaller broad peaks (#12-15) that appeared on chromatograms for both samples and standards with retention times of two to ten minutes. The heights or areas for each peak of each standard injected on a particular day were used to determine a dose-response line for each peak. The equations for these dose-response lines were then used to quantitate the PCB residues in the final sample extracts by peak, after which the ug PCBs/ml for all peaks in the sample were totaled. The ug PCBs/ml calculated for final sample extracts was corrected for recovery based on the recovery of 84.8% determined from the spiked samples, which yielded the ug PCB/g tissue residues in the rat carcasses. Body burdens of PCBs were then determined as ug/rat by multiplying the ug PCBs/g tissue by the average weight of a single rat in each cage.

F. Analysis of PBB Residues in Tissues

Using the GC conditions as described for PBBs-single peak, three injections of approximately 7 ul of 1.0 ug/ml standard solution were injected in rapid succession to load the column. Once the column had been loaded and peaks had ceased to form on the chromatogram, standards were injected, followed by samples, and then another set of standards. This procedure was followed each day, with no more than 10 samples being injected before standards were injected again. Standards for PBBs were 0.2, 0.5, 0.8, and 1.0 ug/ml. Quantification of PBB residues was performed by measuring the peak area of one large peak (peak #4)

representing 2,4,5,2',4',5'-hexabromobiphenyl which is the major congener of fireMaster® FF-1. To be able to measure this peak, the samples had to be diluted, 1:2 with toluene/ethyl acetate (1:3). Peak areas for the standards run each day were used to form a daily dose-response curve, allowing quantitation of PBB levels in the diluted final sample extracts. The ug PBB/ml calculated for these sample extracts had to be corrected for the one-third dilution and for 88.4% recovery determined from spiked samples. Once the corrections had been made, PBB residues in ug/g was the end result. Body burdens, as ug/rat, were determined by multiplying the ug PBB residue/g tissue by the average weight of a single rat in the cage the sample represented.

The areas of 5 smaller peaks (#1-3,5, and 6) were measured without diluting the samples using the GC conditions described for PBB-multiple peaks. The same procedures for loading the column and order of injections were used as with the large peak analysis. Total peak areas for each standard were used to form a daily dose-response curve, with an equation allowing quantitation of PBB levels in final sample extracts. These values were corrected for recovery of 88.4%, to give ug/ml PBBs or residue of PBB in ug/g. Body burdens were calculated as above.

IV. Statistical Analysis

All statistical analyses were performed using the computer program Statview 512+, marketed by Brainpower Inc., 24009 Ventura Blvd. Suite 250, Calabasos, CA 91302.

RESULTS

I. Residues and Body Burdens of PCBs and PBBs

PBB residues and body burdens in this section are based on single peak measurement. Rats euthanized on day 0 of withdrawal, after 14 days feeding of diet containing 10 ppm PCBs (Aroclor 1254), or 10 ppm PBBs (fireMaster® FF-1), contained average residue concentrations of 4.123 ug/g tissue or 0.500 ug/g tissue, respectively (Table 2). Those rats receiving ad libitum diet during the 21 day withdrawal period showed no significant ($p > 0.05$) loss of residues from day 0 levels, with residues of PCBs and PBBs of 3.967 ug/g and 0.499 ug/g, respectively (Table 3). Residues in rats restricted in feed intake during the withdrawal period were 3.675 ug PCBs/g tissue and 0.471 ug PBBs/g tissue (Table 3), representing insignificant ($p > 0.05$) losses of 7.4% and 5.6%, respectively. Mineral oil added to the diet, resulted in residues of 2.943 ug PCBs/g tissue (significant at $p \leq 0.05$) and 0.424 ug/g (non-significant at $p > 0.05$) PBBs (Table 3), equivalent to losses of 25.8% and 15%, respectively, over levels in rats receiving no treatment. The combination of feed restriction and mineral oil resulted in residue reductions of 35.9% and 32% (both significant at $p \leq 0.05$), to 2.543 ug/g and 0.339 ug/g (Table 3), over nontreated rats for PCBs and PBBs,

Table 2. Residues and body burdens of PCBs and PBBs in rats fed non-contaminated diet or diets containing 10 ppm PCBs or PBBs for 14 days (day 0 withdrawal).

Pre-treatment Diet[1]	Cage #[2]	Residue of PBBs or PCBs (ug/g b.w.)[3]	Body Burden (ug/rat)[4]
Non-contaminated:	7	ND[5]	ND
	11	ND	ND
	15	ND	ND
Mean ± SE		ND	ND
10 ppm PBBs:	22	0.626	225
	26	0.444	165
	30	0.430	153
Mean ± SE		0.5 ± 0.063	181 ± 22
10 ppm PCBs:	34	4.253	1517
	38	3.891	1463
	42	4.225	1535
Mean ± SE		4.123 ± 0.116	1505 ± 22

1 Pre-treatment diets were fed for 14 days prior to withdrawal phase.

2 Rats were housed three per cage and were analyzed as a composite.

3 Values represent the average concentration of PBBs/PCBs in whole body rat samples, as detected by gas chromatography.

4 Body burden was calculated by multiplying body weight (g) by residue of PBBs/PCBs in the sample (ug/g body wt.).

5 ND = not detectable; no peaks on the chromatogram.

	Residues			Body Burdens		
Source:	df	MS	f	df	MS	f
PBBs:						
Between groups	1	0.375	62.73**	1	49357.2	66.4**
Within groups	4	5.978E-3		4	743.1	
PCBs:						
Between groups	1	25.499	1257.21**	1	3397541.5	4837.4**
Within groups	4	0.02		4	702.3	

* $p \leq 0.05$

** $p \leq 0.01$

Table 3. Residues and body burdens of PCBs and PBBs in rats fed non-contaminated diet or diets containing 10 ppm PCBs or PBBs for 14 days followed by a 21 day withdrawal period involving no treatment, 5% mineral oil (MO), 50% feed restriction (FR), or 10% MO + 50% FR (day 21 withdrawal).

	Controls			PBB fed rats			PCB fed rats		
Withdrawal Treatment	Cage[1]	Residue of PBB or PCB (ug/g b.w.)[2]	Body Burden (ug/rat)[3]	Cage[1]	Residue of PBBs (ug/g b.w.)[2]	Body Burden (ug/rat)[3]	Cage[1]	Residue of PCBs (ug/g b.w.)[2]	Body Burden (ug/rat)[3]
None[4]	1	ND[5]	ND	16	0.609	239	31	4.444	1772
None[4]	2	ND	ND	17	0.480	188	32	3.535	1423
None[4]	3	ND	ND	18	0.407	168	33	3.923	1572
Mean ± SE		ND	ND		0.499 ± 0.059	198 ± 21		3.967 ± 0.263	1589 ± 101
50% feed restriction	4	ND	ND	19	0.477	149	35	4.421	1397
50% feed restriction	5	ND	ND	20	0.542	175	36	3.276	1016
50% feed restriction	6	ND	ND	21	0.393	125	37	3.329	1034
Mean ± SE		ND	ND		0.471 ± 0.043	150 ± 15		3.675 ± 0.373	1149 ± 124
5% mineral oil	8	ND	ND	23	0.495	193	39	2.881	1133
5% mineral oil	9	ND	ND	24	0.374	152	40	2.947	1157
5% mineral oil	10	ND	ND	25	0.404	162	41	3.000	1170
Mean ± SE		ND	ND		0.424 ± 0.036	169 ± 12		2.943 ± 0.034	1153 ± 11
10% MO + 50% FR[6]	12	ND	ND	27	0.407	135	43	2.829	896
10% MO + 50% FR[6]	13	ND	ND	28	0.290	87	44	2.647	821
10% MO + 50% FR[6]	14	ND	ND	29	0.320	102	45	2.153	675
Mean ± SE		ND	ND		0.339 ± 0.031	108 ± 14		2.543 ± 0.202	797 ± 65

[1] Rats were housed three per cage and were analyzed as a composite.

[2] Values represent the average concentration of PBBs or PCBs in whole body rat samples, as detected by gas chromatography.

[3] Body burden was calculated by multiplying body weight (g) by residue of PBBs or PCBs in the sample (ug/g b.w.).

[4] Fed ad libitum.

[5] ND = non-detectable: no peaks on the chromatogram.

[6] MO = mineral oil; FR = feed restriction.

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Table 3 (cont'd)

Summary of ANOVA

Source:	Residues df	Residues MS	Residues f	Body Burden df	Body Burden MS	Body Burden f
PBBs:						
Between groups	3	0.015	2.471	3	4279.6	5.623*
Within groups	8	5.926E-3		8	761.1	
PCBs:						
Between groups	3	1.286	6.842*	3	315359.8	14.048**
Within groups	8	0.188		8	22448.8	

* $p \leq 0.05$
** $p \leq 0.01$

respectively. In summary, combination of 50% feed restriction and 10% mineral oil in the diet produced significant ($p \leq 0.05$) reduction in both PCB and PBB residues, while mineral oil at 5% in the diet produced significant ($p \leq 0.05$) reduction only in PCB residues.

Body burdens of rats on day 0 of withdrawal for PCBs were 1505 ug/rat and for PBBs 181 ug/rat (Table 2). Day 21 body burdens for rats receiving no withdrawal treatment were 1589 ug/rat and 198 ug/rat (Table 3) for PCBs and PBBs, respectively. These values did not represent a significant ($p > 0.05$) difference in PBB or PCB body burdens. Rats on 50% feed restriction during the withdrawal period had body burdens of 1149 ug PCBs/rat and 150 ug PBBs/rat (Table 3), which represent reductions of body burden greater than nontreated rats of 27.7% (significant at $p \leq 0.05$) and 24.2% (not significant at $p > 0.05$), respectively. Use of mineral oil alone resulted in body burdens of PCBs of 1153 ug/rat (reduction of 27.4% when compared to nontreated rats, significant at $p \leq 0.05$) and a reduction of PBBs to 169 ug/rat (less by 14.6% when compared to nontreated rats, not significant at $p > 0.05$) (Table 3). The combination of feed restriction and mineral oil resulted in significant ($p \leq 0.05$) reductions in body burdens of 49.8% and 45.4% or concentrations of 797 ug PCBs/rat and 108 ug PBBs/rat (Table 3), respectively. In summary, both PCB and PBB body burdens were significantly ($p \leq 0.05$) reduced by 50% feed restriction in conjunction with 10% mineral oil in the diet. Both feed restriction and mineral oil, alone, resulted in

comparable but significant ($p \leq 0.05$) reductions in PCB body burdens as compared to rats not receiving withdrawal treatment.

When multiple peaks were measured for PBBs, excluding the largest peak that had been measured previously, the values for PBB residues and body burdens were as follows (Table 4).

Table 4. PBB residues and body burdens in rats fed 10 mg/kg PBB in the diet for 14 days followed (on Day 0) by a 21 day withdrawal involving no treatment, 5% mineral oil (MO), 50% feed restriction (FR), or a combination of 10% MO and FR (FR + MO)-based on multiple peaks excluding peak #4.

Day killed & Treatment	Residue (ug/g)	Body Burden (ug/rat)
killed Day 0	0.36 ± 0.074 (100%)	132.4 ± 29.4 (100%)
killed Day 21		
None	0.32 ± 0.031 (89%)	125.8 ± 11.6 (95%)
MO	0.30 ± 0.036 (83%)	120.1 ± 14.7 (91%)
FR	0.30 ± 0.012 (83%)	94.9 ± 2.9 (72%)
MO + FR	0.29 ± 0.043 (81%)	91.4 ± 18.0 (69%)

PBBs were not lost from rats receiving no treatment during the withdrawal period, as residues and body burdens on day 0 and day 21 were not significantly ($p > 0.05$) different. Residues of PBBs were not significantly ($p > 0.05$) reduced by mineral oil or feed restriction treatments. Feed restriction alone, and in combination with mineral oil resulted in comparable significant ($p < 0.05$) reductions in body burdens of PBBs over nontreated and mineral oil treated rats.

When considering all the peaks in PBB samples, the total reduction occurring in body burdens with a combination of feed restriction and mineral oil was 46% of the day 0 burdens (Table 5). The combination of feed restriction and mineral oil decreased body residue concentrations significantly ($p \leq 0.05$) to 73% of day 0 values (a reduction of 27%) or 0.63 ug PBBs/g tissue

(Table 5). Body burdens of PBBs were significantly ($p \leq 0.05$) reduced by feed restriction alone or in combination with mineral oil (Table 5). Reductions in PBB body burdens were 22% for feed restriction alone and 36% for the combination of feed restriction and mineral oil.

Table 5. Total PBB residues and body burdens (based on all peaks) in rats fed PBBs in the diet at 10 mg/kg for 14 days followed (on day 0) by a 21 day withdrawal involving no treatment, 5% mineral oil (MO), 50% feed restriction (FR), or a combination of 10% MO and 50% FR (FR + MO).

Day killed	Treatment	Residue (ug/g)	Body Burden (ug/rat)
day 0	None	0.86 (100%)	313.4 (100%)
day 21	None	0.82 (95%)	323.8 (103%)
day 21	MO	0.72 (84%)	289.1 (92%)
day 21	FR	0.77 (90%)	244.9 (78%)
day 21	FR + MO	0.63 (73%)	199.4 (64%)

II. Gas Chromatographic Peaks for PCBs and PBBs

In analysis of PBBs, the one major peak present, representing 2,4,5,2',4',5'-hexabromobiphenyl, which is peak #4 (Figure 1) was measured. Figure 1 compares chromatograms for detecting peak #4 for a PBB standard solution made from fireMaster® FF-1, and the PBB extracted from whole body rat sample. The extract from the whole body rat was diluted to one-third to allow measurement of peak #4. The chromatogram is for a 0.1 ug PBB/ml standard and the sample extract is from rats killed on day 21 of withdrawal after receiving no withdrawal treatment. In comparing the two chromatograms there is a loss of the earlier eluting peaks (peaks 1 and 3). After injection of the sample into the gas chromatograph it takes longer for the whole-body extract to come back down to the baseline. At the

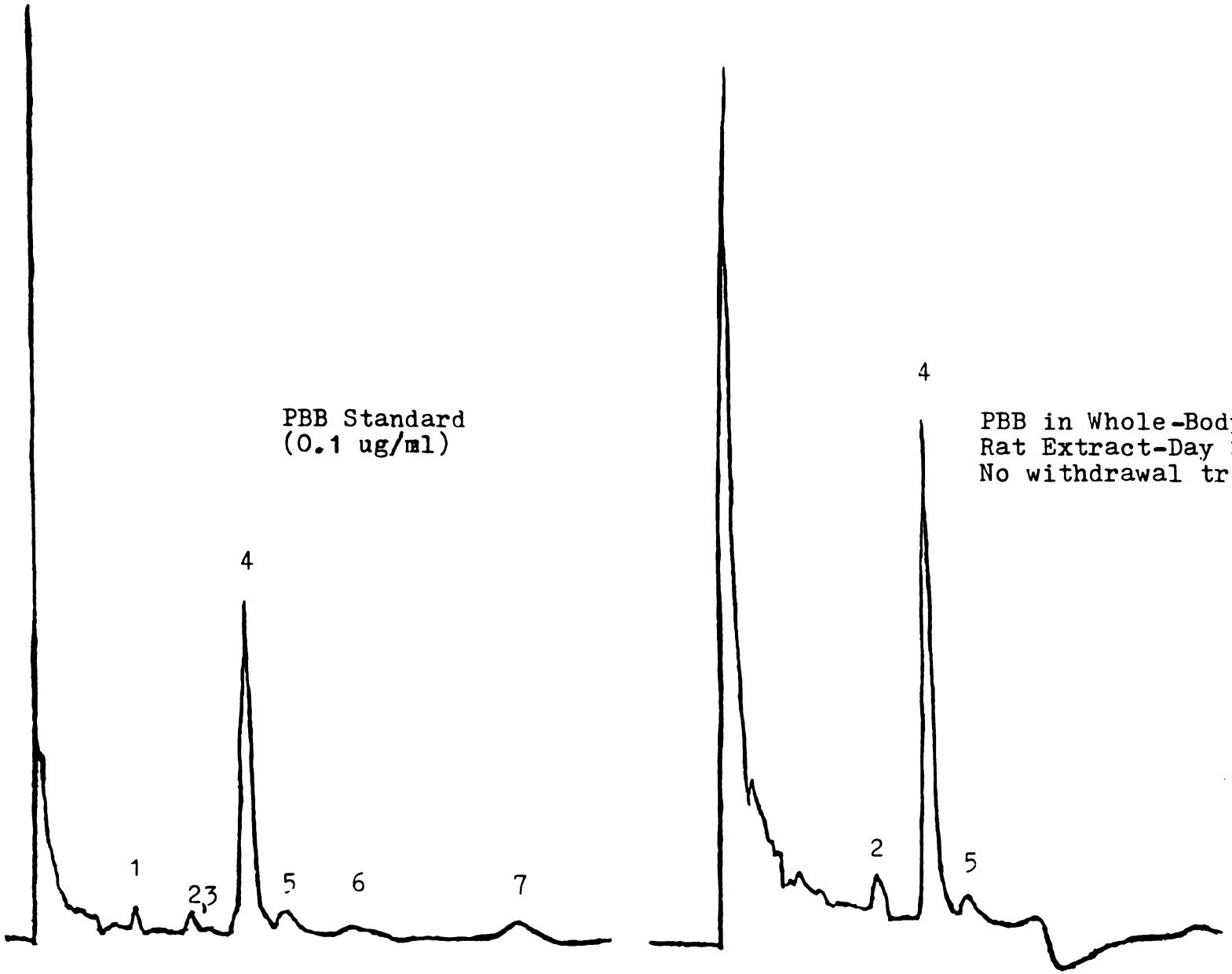

Figure 1. Gas chromatograms to measure peak #4 of a 0.1 ug PBB/ml standard and a rat whole-body extract (at 1/3 dilution), day 21-no withdrawal treatment.

tail end of the chromatogram, the two last peaks also disappear (peaks 6 and 7) which may be due to their small size. Figure 2 has chromatograms for a PBB standard solution, and an extract of a whole-body sample. The later represented an undiluted sample for measuring the smaller peaks seen previously in Figure 1. The standard is 0.1 ug/ml and the extract is from rats killed on day 21 withdrawal after receiving no withdrawal treatment. Again the earlier eluting peaks (peaks 1 and 3) are lost from the chromatogram.

The chromatogram for a PCB standard (1.2 ug/ml) made from Aroclor 1254 has 15 peaks (Figure 3). A chromatogram from an extracted whole-body PCB sample has only 9 peaks (Figure 3). The extracted sample was from a group of rats killed on day 21 withdrawal that had been treated with combined feed restriction and mineral oil. The peaks present in the standard and not in the sample are peaks # 1-3, 6, 7, and 10. As with the PBB samples, it takes longer for the extract sample to approach the baseline directly after injection into the gas chromatograph. Also present was a negative peak that was not seen in the standard chromatograms.

III. Feed Intake, Body Weights, and % Lipid

Feed intakes during the 14 day contamination phase were 19.3, 19.1, and 19 g/rat/d (Table 6) for rats being fed non-contaminated, PBB-contaminated, or PCB-contaminated diets, respectively. There were no significant ($p > 0.05$) differences

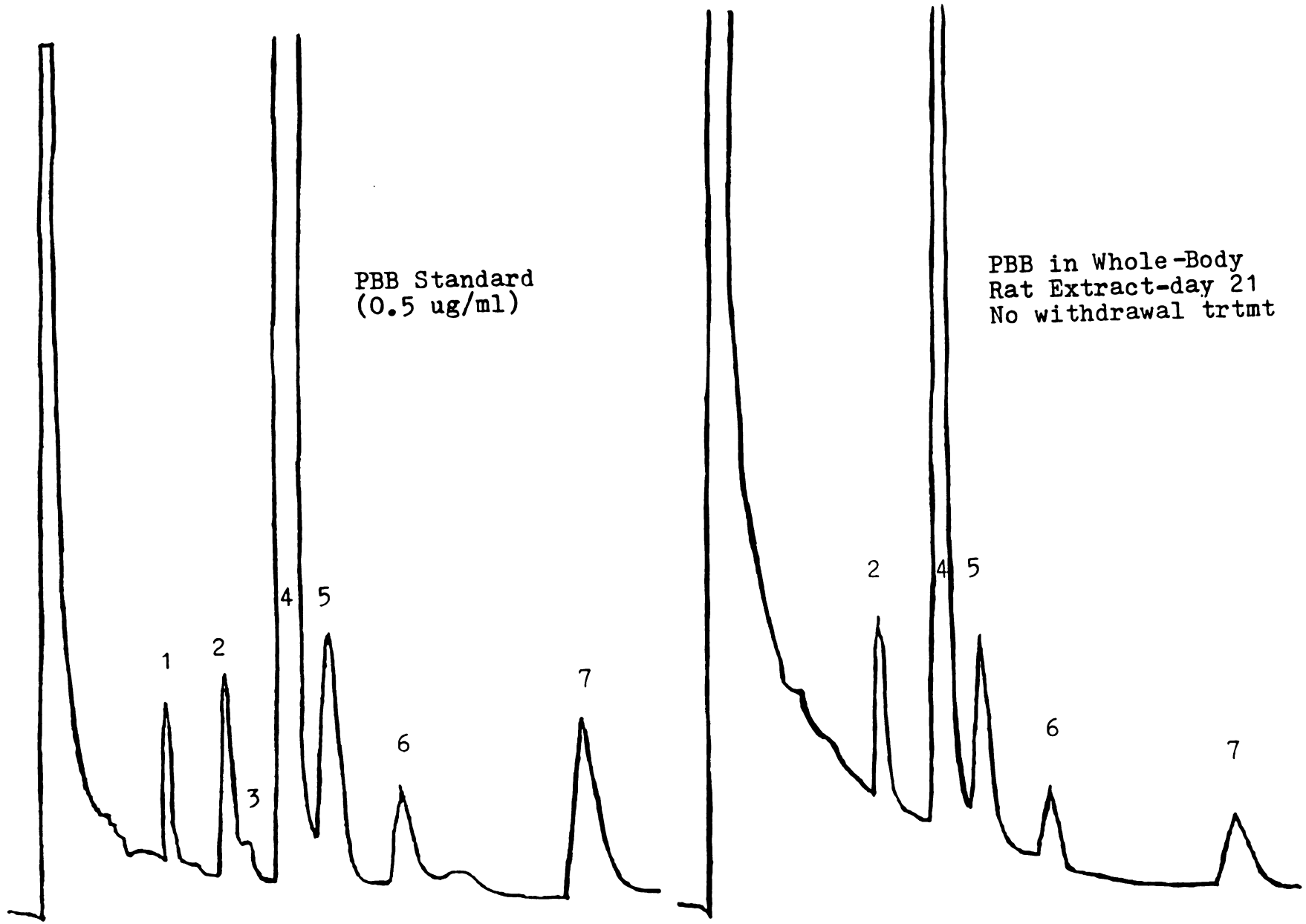

Figure 2. Gas chromatograms to measure multiple peaks (excluding peak #4) of a 0.5 ug PBB/ml standard and a rat whole-body extract, day 21-no withdrawal treatment.

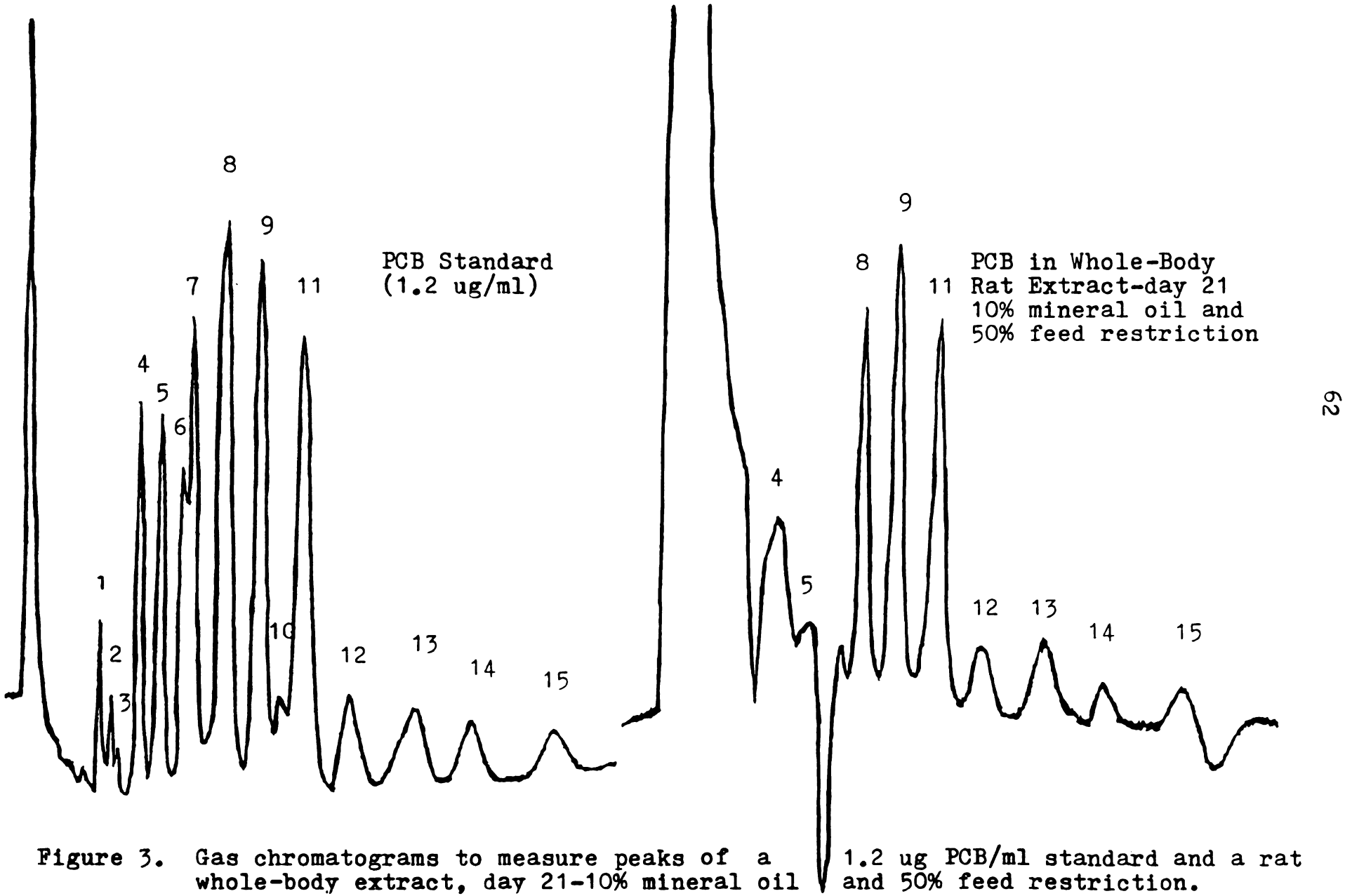

Figure 3. Gas chromatograms to measure peaks of a 1.2 ug PCB/ml standard and a rat whole-body extract, day 21-10% mineral oil and 50% feed restriction.

Table 6. Feed intake, body weights, and % lipid of rats fed non-contaminated diet or diets containing 10 ppm PCBs or PBBs for 14 days (day 0 withdrawal).

Pre-treatment Diet[1]	Cage[2]	Feed Intake (g/rat/day)	Body Wt (g/rat)	% lipid
Non-contaminated:	7		378	4.64
	11		377	5.86
	15		363	5.02
Mean ± SE		19.25[3]	372 ± 5	5.17 ± 0.36
10 ppm PBBs:	22		360	5.62
	26		373	6.14
	30		357	4.45
Mean ± SE		19.06[3]	363 ± 5	5.40 ± 0.50
10 ppm PCBs:	34		357	4.36
	38		376	4.85
	42		363	4.67
Mean ± SE		18.95[3]	365 ± 6	4.63 ± 0.14

[1] Contamination diets were fed for 14 days prior to withdrawal phase.

[2] Rats were housed three per cage and were analyzed as a composite.

[3] Average for the three cages.

Summary of ANOVA

Source:	Body Wt df	Body Wt MS	Body Wt f	% lipid df	% lipid MS	% lipid f
PBBs:						
Between groups	1	133.8	0.2463[NS]	1	0.079	0.728[NS]
Within groups	4	72.6		4	0.569	
PCBs:						
Between groups	1	78.2	0.3923[NS]	1	0.448	0.231[NS]
Within groups	4	85.2		4	0.226	

[NS] Not significant $p > 0.05$.

in feed intakes based on the diets fed during the contamination phase. During the 21 day withdrawal phase the feed intakes for the non-restricted rats were 20.6, 22.0, and 21.6 g/rat/d (Table 7) for rats receiving no treatment that had previously been fed non-, PBB-, and PCB-contaminated diet. There were no significant ($p > 0.05$) differences among feed intakes of rats not on feed restriction. Rats on 50% feed restriction received 11.1 g/rat/d whereas rats on the combination of mineral oil and feed restriction were fed 11.9 g/rat/d (Table 7).

Body weight of rats on day 0 withdrawal were 372, 363, and 365 g/rat (Table 6) for non-, PBB-, and PCB-contaminated diets, respectively. No significant ($p > 0.05$) differences among body weights due to content of the diet were detected on day 0 of withdrawal. On day 21 of withdrawal, the rats receiving no withdrawal treatment had average body weights of 384-401 g/rat, with no significant ($p > 0.05$) difference among them based on prior diet fed (Table 7). Body weights were found to be significantly ($p \leq 0.05$) decreased by feed restriction alone, or in combination with mineral oil as compared to nontreated and mineral oil treated rats. Average body weights for rats on feed restriction alone were 312-328 g/rat, compared to 313-318 g/rat when feed restriction and mineral oil were combined (Table 7). Body weights were not significantly different ($p > 0.05$) between feed restriction alone and in combination with mineral oil.

Table 7. Feed intake, body weights, and % lipid of rats fed non-contaminated diet or diets containing 10 ppm PCBs or PBBs for 14 days followed by a 21 day withdrawal period involving no treatment, 5% mineral oil (MO), 50% feed restriction (FR), or a combination of 10% MO and 50% FR (MO + FR).

	Controls				PBB treated				PCB treated			
Withdrawal Treatment	Cage [1]	Feed Intake[2] (g/rat/d)	Body Wt (g/rat)	% lipid	Cage	Feed Intake[2] (g/rat/d)	Body Wt (g/rat)	% lipid	Cage	Feed Intake[2] (g/rat/d)	Body Wt (g/rat)	% lipid
None[3]	1		372	4.71	16		393	5.42	31		399	5.11
None[3]	2		389	5.92	17		391	4.33	32		403	5.85
None[3]	3		391	5.69	18		413	4.83	33		401	5.11
Mean ± SE		20.6 ± 0.3	384 ± 6	5.4 ± 0.4		22.0 ± 0.1	399 ± 7	4.9 ± 0.3		21.6 ± 0.3	401 ± 1	5.4 ± 0.2
50% feed restriction	4		323	2.37	19		313	2.16	35		316	2.51
50% feed restriction	5		329	2.55	20		323	2.55	36		310	3.11
50% feed restriction	6		333	2.41	21		317	2.43	37		311	2.47
Mean ± SE		11.1 ± 0.1	328 ± 3	2.4 ± 0.1		11.1 ± 0.1	318 ± 3	2.4 ± 0.1		11.1 ± 0.1	312 ± 2	2.7 ± 0.2
5% mineral oil	8		403	6.68	23		390	6.11	39		393	5.35
5% mineral oil	9		387	4.91	24		406	7.53	40		393	5.43
5% mineral oil	10		385	5.86	25		402	5.92	41		390	5.35
Mean ± SE		22.4 ± 0.1	392 ± 6	5.8 ± 0.5		22.7 ± 0.5	399 ± 5	6.5 ±0.5		21.9 ± 0.4	392 ± 1	5.4 ± 0.0
10% MO @ 50% FR[4]	12		317	2.49	27		333	2.09	43		317	1.99
10% MO @ 50% FR[4]	13		320	2.12	28		301	1.88	44		310	1.66
10% MO @ 50% FR[4]	14		307	2.28	29		320	2.16	45		313	2.20
Mean ± SE		11.9 ± 0.2	314 ± 4	2.3 ± 0.1		11.9 ± 0.2	318 ± 9	2.0 ± 0.1		11.9 ± 0.2	313 ± 2	1.9 ± 0.2

[1] Rats were housed three per cage and were analyzed as a composite.

[2] Mean feed intake for a three week period.

[3] Fed ad libitum.

[4] MO = mineral oil; FR = feed restriction.

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Table 7 (cont'd)

Summary of ANOVA

Source:	Feed Intake df	Feed Intake MS	Feed Intake f	Body Wt df	Body Wt MS	Body Wt f	% lipid df	% lipid MS	% lipid f
PBBs:									
Between groups	3	119.257	1.0000E-4**	3	6615.16	1.0000E-4**	3	13.533	1.0000E-4**
Within groups	8	0.227		8	126.63		8	0.283	
PCBs:									
Between groups	3	105.699	1.0000E-4**	3	7019.704	1.0000E-4**	3	9.541	1.0000E-4**
Within groups	8	0.255		8	7.259		8	0.097	

* $p \leq 0.05$

** $p \leq 0.01$

The percent lipid in whole-body samples on day 0 of the withdrawal phase from rats fed noncontaminated diet was 5.17% (Table 6). The lipid content of rats on PBB and PCB contaminated diets were 5.40 and 4.63 %, respectively (Table 6). There was no significant ($p > 0.05$) difference in the % lipid on day 0 of withdrawal among the rats fed different diets. On day 21 of withdrawal, the % lipid values were 4.9 to 5.4 % for the rats receiving no withdrawal treatment (Table 7). A significant ($p \leq 0.05$) decrease in % lipid was caused by feed restriction either alone or in combination with mineral oil. With feed restriction alone, the values for % lipid were 2.4-2.7%, in comparison to 1.9-2.3% for feed restriction with mineral oil (Table 7). Only the rats that were initially fed PCBs had a significantly ($p \leq 0.05$) greater loss in % lipid with the combined treatment (1.9%) than with feed restriction alone (2.7%). In rats previously treated with PBBs, there was a significant ($p \leq 0.05$) increase in % lipid with mineral oil treatment as compared to nontreated rats.

DISCUSSION

The combination of 50% feed restriction and 10% mineral oil in the diet produced a marked reduction in body burdens of both PCBs and PBBs in rats during a 21 day withdrawal period. Feed restriction at 50% of ad libitum intake with addition of 10% mineral oil to the diet reduced rat body burdens of PCBs and PBBs by 49.8% and 45.4%, respectively. These results are in accordance with studies performed earlier with chickens (Polin et al., 1985; Polin et al., 1989), in which a combination of feed restriction and mineral oil resulted in approximately 70% reduction in body burdens of PCBs and PBBs. When considering residue concentrations in the carcass, the combined feed restriction and mineral oil treatment again proved to be the most effective in reducing PCBs and PBBs. The effectiveness of the combination of the two is presumably due to the nonbiliary intestinal secretion, as described by Yoshimura and Yamamoto (1975), which is increased as lipid stores of PCBs and PBBs are mobilized due to feed restriction.

As with previous studies in chickens (Polin et al., 1985), feed restriction or mineral oil used alone were not proven effective in reducing body burdens of PBBs in rats. Rat body burdens of PCBs were reduced with both feed restriction and

mineral oil independently, but to a lesser degree than when used in combination. Polin et al. (1989) reported that PCB body burdens were reduced with 5% mineral oil in the diet, but that 50% feed restriction alone did not significantly reduce burdens in chickens. Conversely, feed restriction at 25% of ad libitum intake had been found to enhance the elimination of 2,4,5,2',4',5'-HCB, a major congener in Aroclor 1254, by 50% from rats (Matthews and Anderson, 1975; Wyss et al., 1982). Feed restriction or mineral oil alone, either were not effective or were less effective than the combination of the two in their ability to remove PCBs and PBBs from the body. Each alone serves a function in increasing the loss of xenobiotic from the body, but together there is a additive effect on PCB elimination and a synergistic effect on PBB elimination. Feed restriction mobilized adipose tissue, as demonstrated by the % lipid reduction by nearly 50% in rats restricted to 50% of ad libitum intake. This mobilization would increase the levels of PCBs and PBBs in the circulation resulting in higher concentrations being presented to and eliminated through the intestinal wall. In this case, it would be expected that feed restriction itself should cause a greater reduction than has been demonstrated in the literature. The use of mineral oil presumably would not allow reabsorption of the xenobiotics and speed passage out of the body via the feces. Mineral oil also stimulates the excretion of xenobiotics directly through the intestinal wall, as demonstrated with hexachlorobenzene (Rozman, K., et al., 1983). The mechanism

involved in the enhanced elimination seen with the use of feed restriction and mineral oil needs to be further studied to determine if the level of feed restriction and mineral oil used is optimal. It would be advantageous to be able to increase the elimination of xenobiotics without such a high restriction of feed intake with its associated reduction of body weight gains. Use of the combined feed restriction and mineral oil withdrawal treatment has many possible applications. Some livestock destroyed during the PBB incident in Michigan could have been salvaged, especially those animals in which a short-term reduction in body weight gains would not be a problem (i.e. valuable breeding stock). Use in humans to reduce levels of xenobiotics which accumulate in the adipose tissue may be a future application after more research has been done.

There was a 5-fold difference between total PBB residues (total of all peaks equalling 0.86 ug/g b.w.) and PCB residue (4.123 ug/g b.w.) in whole body rat samples at day 0 withdrawal. Other studies conducted in our laboratory using 10 mg/kg PCBs or PBBs in the diet have produced day 0 residue values in rats equivalent to those for PCB residues in this experiment. In double checking calculations for adding 10 mg/kg of Aroclor 1254 or fireMaster® FF-1 to the ground rodent chow, there was no error evident. Unfortunately, the diet saved for analysis was inadvertently thrown out, and therefore the actual concentrations present in the diets were not available. The cause of the low concentration of PBB residue in the rat tissues is unknown, but

the ability to measure the reduction in residues due to the different withdrawal treatments was not affected.

Rats treated with 5% mineral oil in their diets and previously fed diet containing 10 ug/kg PBBs, a significant ($p \leq 0.05$) increase in the % lipid was seen. No other references to this occurring were found in the literature. The significance of this increase would require further research to determine if it is repeatable or not.

APPENDICES

Appendix A. Data for feed consumption and body weight gains of rats fed PBBs and PCBs for 14 days, and then after withdrawal of these xenobiotics for 21 days.

Treatment	Feed consumption-g Avg. for all cages				Cage no.	Body weights at-g		
	During trt	During withdrawal Week 1	Week 2	Week 3		Start	End Contamin	End withdrwl
None	19.25	20.60	19.97	21.18	1a	323	346	372
					2a	348	366	389
					3a	351	372	391
		10.95	10.95	11.33	4b	342	360	323
					5b	349	371	329
					6b	361	380	333
	killed on day 14				7	354	378	NA
		22.19	22.41	22.48	8c	349	372	403
					9c	336	357	387
					10c	343	358	385
	killed on day 14				11	348	377	NA
		11.81	11.71	12.22	12d	337	358	317
					13d	343	360	320
					14d	326	350	307
	killed on day 14				15	341	363	NA
10 ppm PCBs	18.95	21.16	21.43	22.22	16a	344	360	393
					17a	353	368	391
					18a	364	383	413
		10.95	10.95	11.33	19b	339	368	313
					20b	342	367	323
					21b	336	360	317

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APPENDIX A (continued)

	killed on day 14				22	337	360	NA
		22.38	21.02	22.33	23c	334	353	390
					24c	353	373	406
					25c	349	371	402
	killed on day 14				26	351	373	NA
		11.81	11.71	12.22	27d	369	386	333
					28d	345	369	301
					29d	354	373	320
	killed on day 14				30	343	357	NA
10 ppm PBBs	19.06	21.94	21.87	22.31	31a	357	372	399
					32a	349	365	403
					33a	349	363	401
	killed on day 14				34	348	357	NA
		10.95	10.95	11.33	35b	349	365	316
					36b	347	363	310
					37b	328	350	311
	killed on day 14				38	356	376	NA
		22.63	21.94	23.64	39c	349	367	393
					40c	345	366	393
					41c	339	359	390
	killed on day 14				42	345	363	NA
		11.81	11.71	12.22	43d	351	375	317
					44d	353	347	310
					45d	359	373	313

NA = not alive on specified day.

a Fed ad lib.

b During withdrawal rats were restricted to 50% of ad lib intake.

c Fed a diet with 5% mineral oil.

d Fed a diet with 10% mineral oil at 50% of ad lib intake.

Appendix B. Coding of rats by cage, treatment, day killed and for residue analysis.

A. Control rats

Cage #[1]	Withdrawal Treatment	Day killed[2]	Codes
1	None[3]	21	30,87
2	None[3]	21	10,50
3	None[3]	21	17,59
4	50% feed restriction	21	4,75
5	50% feed restriction	21	38,91
6	50% feed restriction	21	20,82
7	None	0	2,74
8	5% mineral oil	21	27,64
9	5% mineral oil	21	36,90
10	5% mineral oil	21	43,83
11	None	0	26,85
12	10% MO @ 50% FR[4]	21	11,56
13	10% MO @ 50% FR[4]	21	41,71
14	10% MO @ 50% FR[4]	21	9,55
15	None	0	1,51

1 Each cage housed three rats, which were analyzed as a composite sample.

2 Represents the day of withdrawal.

3 These rats were fed ad libitum.

4 MO = mineral oil; FR = feed restriction.

Appendix B (con't)

B. PBBs rats

Cage #[1]	Withdrawal Treatment	Day killed[2]	Codes
16	None[3]	21	15,58
17	None[3]	21	31,66
18	None[3]	21	21,61
19	50% feed restriction	21	32,88
20	50% feed restriction	21	13,57
21	50% feed restriction	21	3,52
22	None	0	7,54
23	5% mineral oil	21	14,79
24	5% mineral oil	21	33,67
25	5% mineral oil	21	12,78
26	None	0	24,84
27	10% MO @ 50% FR[4]	21	16,80
28	10% MO @ 50% FR[4]	21	22,72
29	10% MO @ 50% FR[4]	21	29,65
30	None	0	35,68

1 Each cage housed three rats, which were analyzed as a composite sample.

2 Represents the day of withdrawal.

3 These rats were fed ad libitum.

4 MO = mineral oil; FR = feed restriction.

Appendix B (con't)

C. PCBs rats

Cage #[1]	Withdrawal Treatment	Day killed[2]	Codes
31	None[3]	21	8,77
32	None[3]	21	6,76
33	None[3]	21	23,62
34	None	0	42,93
35	50% feed restriction	21	39,70
36	50% feed restriction	21	34,89
37	50% feed restriction	21	25,63
38	None	0	28,86
39	5% mineral oil	21	37,69
40	5% mineral oil	21	40,92
41	5% mineral oil	21	5,53
42	None	0	19,60
43	10% MO @ 50% FR[4]	21	18,81
44	10% MO @ 50% FR[4]	21	45,73
45	10% MO @ 50% FR[4]	21	44,94

1 Each cage housed three rats, which were analyzed as a composite sample.

2 Represents the day of withdrawal.

3 These rats were fed ad libitum.

4 MO = mineral oil; FR = feed restriction.

Appendix C. Raw data for percent water and percent lipid.

I. Body water content - %

A. Control rats

Withdrawal Treatment	Cage #	% Water	Cage #	% Water	Cage #	% Water
None[1]	1	65.83	16	65.26	31	66.68
None[1]	2	62.97	17	67.00	32	66.65
None[1]	3	65.24	18	66.09	33	65.73
50% feed restriction	4	67.16	19	68.62	35	68.03
50% feed restriction	5	68.59	20	69.63	36	67.39
50% feed restriction	6	68.12	21	68.81	37	68.06
None[2]	7	68.67	22	67.90	38	68.49
5% mineral oil	8	64.29	23	65.70	39	65.69
5% mineral oil	9	66.98	24	64.37	40	65.30
5% mineral oil	10	65.03	25	64.95	41	66.42
None[2]	11	65.41	26	66.53	42	68.19
10% MO @ 50% FR[3]	12	68.90	27	70.77	43	68.74
10% MO @ 50% FR[3]	13	68.93	28	68.78	44	69.34
10% MO @ 50% FR[3]	14	68.61	29	68.78	45	69.23
None[2]	15	69.21	30	69.64	34	69.82

1 Rats were fed ad libitum ground rodent chow.

2 Rats were euthanized on day 0 of the withdrawal phase and analyzed for PCBs and PBBs residue.

3 MO = mineral oil; FR = feed restriction.

Appendix C (con't)

II. Body lipid content - %.

A. Control rats

Cage #	Withdrawal Treatment	% lipid (DM)[1]	% lipid (as is)[2]	Actual lipid (g/rat)[3]
1	None[4]	13.80	4.71	17.52
2	None[4]	16.00	5.92	23.03
3	None[4]	16.38	5.69	22.25
4	50% feed restriction	7.21	2.37	7.65
5	50% feed restriction	8.11	2.55	8.39
6	50% feed restriction	7.57	2.41	8.02
7	None[5]	14.80	4.64	17.54
8	5% mineral oil	18.71	6.68	26.92
9	5% mineral oil	14.87	4.91	19.00
10	5% mineral oil	16.75	5.86	22.56
11	None[5]	16.93	5.86	22.09
12	10% MO @ 50% FR[6]	8.02	2.49	7.89
13	10% MO @ 50% FR[6]	6.82	2.12	6.78
14	10% MO @ 50% FR[6]	7.25	2.28	7.00
15	None[5]	16.32	5.02	18.22

1 Percent lipid is based on dry matter (DM) - this value is the average of duplicate samples.

2 Percent lipid is based on an as is basis - this value was calculated using the formula (100 - percent water) x (DM percent lipid/100) = percent lipid as is.

3 Actual lipid was calculated using the formula (percent lipid as is/100%) x body weight in grams = grams of lipid.

4 Rats were fed ad libitum.

5 Rats were euthanized on day 0 of the withdrawal phase and analyzed for PCBs and PBBs residue.

6 MO = mineral oil; FR = feed restriction.

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This is to certify that the

thesis entitled

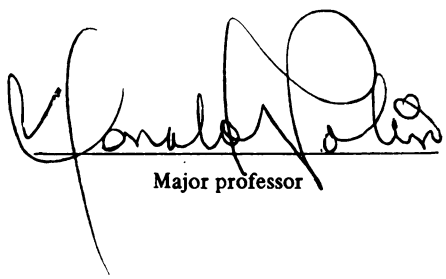
WITHDRAWAL OF POLYCHLORINATED BIPHENYL (PCB) AND POLYBROMINATED BIPHENYL (PBB) RESIDUES FROM RATS USING FEED RESTRICTION AND/OR MINERAL OIL IN THE DIET

presented by

Patricia A. Wiggers

has been accepted towards fulfillment
of the requirements for

MASTER OF SCIENCE degree in Dept. of Animal Sci.



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