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RESPONSES OF [redacted] DAIRY [redacted] POLYCHLORINATED [redacted]

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MONS 020847

RESPONSES OF LACTATING DAIRY COWS TO POLYCHLORINATED BIPHENYLS

FINAL REPORT

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By
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This study required the integration of many disciplines to answer the complex questions on the distribution and clearance, clinical effects, pathology, and target organ modifications that are associated with exposure of cattle to polychlorinated biphenyls. The investigators wish to acknowledge the professional and technical personnel who contributed to the extensive understanding of this halogenated hydrocarbon. The persons that conducted this project are listed on the following pages. We also thank Dr. John H. Craddock for his excellent assistance as Project Officer for Monsanto, as well as Mr. Paul R. Michael for quality control liaison and Mr. Art F. Uelner, Mr. Steven M. Haag, and Ms. Charm K. Russell, the members of the Quality Control Assurance Unit.

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1.	Willett, L. B., H. I. Durst, and T-T. Y. Liu. 1985. Responses of lactating dairy cows to polychlorinated biphenyls. The Toxicologist 5(1):64.	77
2.	Willett, L. B., T-T. Y. Liu, H. I. Durst, K. L. Smith, D. R. Redman, and P. D. Moorhead. 1985. Productivity and health of dairy cows fed polychlorinated biphenyls. J. Anim. Sci. 61 (Suppl. 1):360	78
3.	Willett, L. B., H. I. Durst, and T-T. Y. Liu. 1986. Concentration of polychlorinated biphenyls in lactating cows and their calves. The Toxicologist 6(1):318	79

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INTRODUCTION

Public awareness of potential harmful effects from halogenated hydrocarbons or industrial chemicals in general, together with increasing awareness of the use or occurrence of chemicals in the environment, has created an atmosphere of public concern for the safety of these agents in such proximity to man or his food supply. There is no question that in some instances clear harm has been inadvertently caused by the uses of chemicals. However, a new realm of concern has risen. Because our knowledge of chemical harm is incomplete, it sometimes appears to be nonspecific or ill-defined. Particularly in situations of relatively low or chronic exposure, the specter of nonspecific harm, caused by chemicals, is now claimed. In some cases, this claim is made when it is difficult to distinguish harm caused by chemicals from that resulting from procedure, environment, or circumstance. In food animal agriculture, proper animal housing, nutrition, and management are paramount to good animal health and production. These interactive and subjective factors confound safety assessment.

Polychlorinated biphenyls (PCB) have entered the food supplies of some dairy cattle for at lease 40 years. Farm silos, coated with a material that contained PCB, have been the msjor source. Thousands of these structures were constructed and coated between 1941 and 1970 across the eastern

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United States. Four hundred and ninety (490) have been identified in Michigan alone. Other sources of livestock contamination with PCB have been identified, but generally these are isolated incidents affecting relatively few animals.

Typically, concentrations of PCB detected in livestock diets have been low and have not been considered a health hazard to animals. However, claims of harm to livestock, resulting from exposure to PCB, have been made in recent years. Some of the conditions allegedly caused by PCB in lactating dairy cows are outlined below:

1. Impaired milk production:
   a. decreased milk production
   b. no milk when they calve
   c. only lactate for a short time after parturition
   d. never reach expected peak lactation.

2. Abnormal health problems:
   a. rough hair coat
   b. thin and gaunt
   c. don't eat right
   d. abscesses and wounds that won't heal
   e. mastitis
   f. metritis
   g. swollen joints
   h. lameness

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i. cannot withstand stress

j. won't respond to veterinary treatment

k. immunosuppressed, susceptible to disease.

3. High death loss.

4. Poor reproductive performance:

   a. irregular estrous cycles

   b. no estrus or "silent heat"

   c. will not conceive

   d. abortions.

5. Problems with calves:

   a. born dead

   b. deformed

   c. stunted or small

   d. too weak to stand

   e. susceptible to scours, pneumonia, and infections

   f. poor growth and performance

   g. die at a young age.

6. Residue concentrations do not reflect exposure:

   a. exposure may be much higher than reflected in residues

   b. fat biopsies, blood or milk don't reflect amounts of PCB in deep body stores

   c. any detectable amount of PCB will have an adverse effect.

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These claimed dysfunctions are not specific and do not suggest involvement of particular physiological systems. In fact, many of the claimed syndromes can result from natural, environmental, and/or management practices.

Studies had not been previously conducted to specifically determine the response of cattle to polychlorinated biphenyls. This was due in part to the necessary complexity and expense of this research. Studies that had been conducted in our laboratories had indicated that the amounts of PCB typically consumed by cattle receiving feed from a coated silo were not acutely toxic. There was need for carefully controlled and integrated studies that could identify if changes in productivity, health, or major physiological systems of dairy cows were affected by daily intake of polychlorinated biphenyls.

OBJECTIVES

The study represented in this report was the first detailed study to investigate the responses of dairy cattle to prolonged intake of polychlorinated biphenyls. The specific objectives were to:

1. Determine if polychlorinated biphenyls (Aroclor 1254) affects performance and production efficiency, clinical health, biochemical responses and alterations in target organs, hematology, fertility,

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and their interactions of dairy cows, when fed up to 1,000 mg/day.

2. Relate the responses (if any) attributed to intake of polychlorinated biphenyls to exposure and body burdens of PCB residue.

3. Monitor the long-term health and productivity of PCB-dosed animals through an entire reproductive cycle.

4. Determine the viability and health of offspring from PCB-dosed animals.

5. Examine tissue and organs of PCB-dosed cows and offspring for signs of damage and determine distribution of PCB residues.

METHODS AND MATERIALS

Experimental Design. The experimental design for the study entitled "Responses of Lactating Dairy Cows to Polychlorinated Biphenyls" is diagramatically represented in Figure 1. The overall study period for cows was approximately 480 days with individual variation the result of differing parturition dates of cows. This experimental design allowed for the study of the cows through one entire lactation and 42 days of a second. Although this was a continuing experiment, data were collected and identified as three distinct phases. The first, designated as PCB-R, included

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EXPERIMENTAL DESIGN

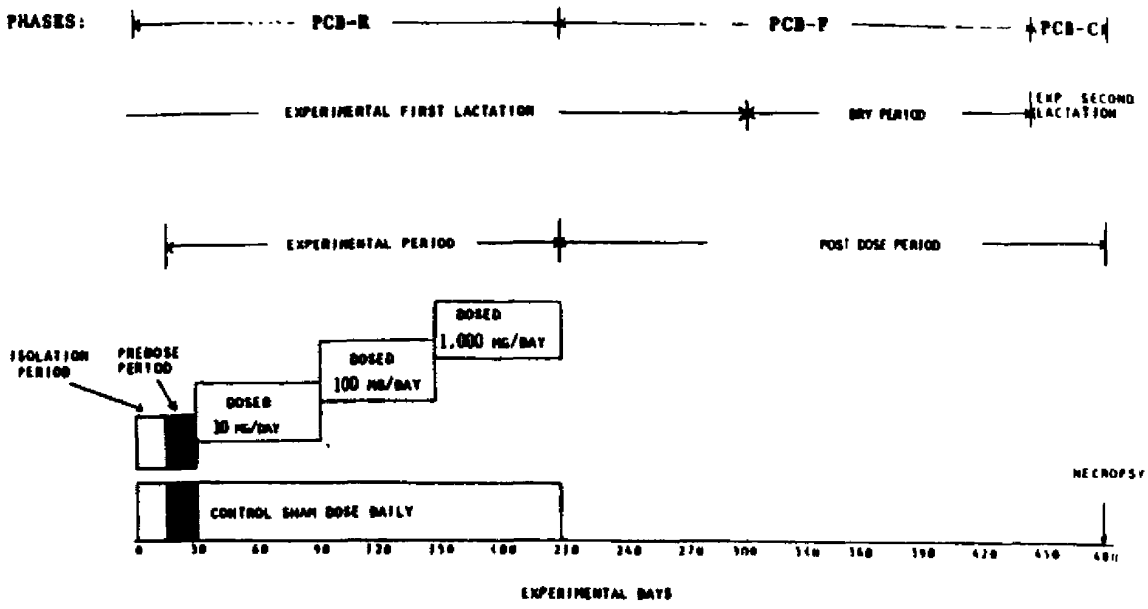

Figure 1. Diagramatic representation of the experimental design for the study "Responses of lactating dairy cows to polychlorinated biphenyls".

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all observations made during the experimental period. This represents the isolation, predose, and dosing periods for all PCB-exposed and control animals. The 15 day isolation period started after animals were shipped to OARDC. At the end of this quarantine period, animals were selected and allotted to the control or dosed group. The 15 day predose period allowed for the establishment of the basal status of each animal in the experimental environment prior to administration of PCB or sham doses. All physical and chemical measurements were made during this period so that each animal could serve as her own control, as well as be compared to animals in the corresponding (dosed or control) groups. Three consecutive 60-day dosing periods followed when PCB dosed animals were given 10, 100, or 1000 mg of Aroclor 1254 daily and control animals received daily sham doses. All animals were intensively studied during this 180 day period (see measurements section).

The second phase, designated PCB-F, covered observations made from the end of dosing through the end of the 305 day lactation and extended through the nonlactating (dry) period. While lactating, the cows were managed as they had been during the dosing period. Rigorous health and productivity recording was maintained throughout this period, whereas the frequency of chemical measurements was reduced.

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A large number of samples were collected from the dosed animals to determine the clearance rate of PCB following exposure. At the end of lactation, each animal was moved to pasture and observed daily during the nonlactating period.

The final phase, designated PCB-C, commenced with the parturitions of the second experimental lactation. Cows and their calves were intensively studied for approximately 42 days which allowed cows to reach peak lactation and calves to be weaned from milk feeding. After three days, the cows and calves were separated, with cows returning to individual stalls and calves to individual pens in a "warm-type" calf nursery. The termination of this phase of the study was marked by the necropsy of all dosed cows and their calves.

This study was conducted following Federal Good Laboratory Practices (GLP) procedures. The Quality Assurance Unit (QAU) was provided by the sponsor and frequent site visits were conducted to verify progress and the integrity of the research data.

Animals. Ten pregnant dairy cows completing their first or second lactation were purchased from the State of Ohio institutional herds. These animals were moved to Wooster in shipments on Auguet 8 and August 11, 1983. The animals were placed in querantine pastures and observed for any health disorders. The cows were allowed to calve in the

quarantine pastures, then were moved to the OARDC intensive care unit. Two cows calved within the intensive care unit as they required assistance at parturition. These animals were 2971 and 2979.

One calf was delivered posteriorly and suffocated during the assisted delivery. All other calves survived and were normal. Calves were returned to the institutional herds.

Animal identification numbers were assigned as the animals calved. One cow was too mean to be incorporated into an intense experimentation protocol. After this animal inflicted injuries on several of our personnel, she was exchanged for another cow (2971) on September 26, 1983.

Prior to calving, cows were paired based on their origin, size, lactation number, and productivity during previous lactations. Then individual animals of the pairs were randomly allotted to either the control group or those that would be dosed with PCBs.

After parturition, when the animals were placed in the intensive care unit, it was discovered that the purchased cows were too large for the comfort stalls. Several of these animals received leg injuries on the metal grates which cover the manure gutter. This problem was corrected by installing new grates and larger rubber bedding mats. The injuries sustained from the grates by cow 2972 became

infected and inhibited her mobility. It was evident that she could not hold up to the rigors of the experimental protocol and had to be replaced with cow 2722 from the OARDC herd. Table 1 summarizes the cows, identifies experimental groups, lists the dates of parturition, and when they entered the protocol.

The experimental cows were inseminated during the experimental period commencing with the first estrus (heat) after the 60th day of lactation. Accurate detection of estrus was difficult due to the restraints imposed by the experimental protocol. All PCB-dosed and control animals required multiple inseminations before pregnancy was established. One cow, 2973, failed to conceive, while cow 2975 conceived twins.

All animals were continued past the 180 consecutive days of dosing and were milked for 305 days when lactations were terminated irrespective of quantity of milk produced. Cows were observed during the dry period and intensive study resumed with subsequent parturitions. Table 2 lists the dates of these important experimental transitions for each cow. Table 3 identifies the calves born to the experimental cows which entered the study protocol.

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TABLE 1. SUMMARY OF COWS FOR THE STUDY ENTITLED "RESPONSES OF LACTATING DAIRY COWS TO POLYCHLORINATED BIPHENYLS"

Cow number	Experimental group	First experimental parturition	Date* entered protocol
2970	Control	8/26/83	10/13/83
2971	Control	11/03/83	12/07/83
2722	Control	10/05/83	11/09/83
2973	Dosed	9/02/83	10/26/83
2974	Dosed	9/08/83	10/13/83
2975	Control	10/07/83	10/19/83
2976	Dosed	10/11/83	10/19/83
2977	Control	10/14/83	12/07/83
2978	Control	10/20/83	11/09/83
2979	Dosed	10/30/83	11/09/83

*Date equals experimental day 15.

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TABLE 2. DATES THAT PCB-DOSED AND CONTROL COWS COMPLETED DOSINGS AND LACTATIONS AND DATES OF PARTURITION

Cow number	Last dosing	Last milking	Second experimental parturition
2970	4/25/84	6/25/84	12/11/84
2971	6/19/84	9/03/84	12/21/84
2722	5/22/84	8/03/84	01/29/85
2973	5/08/84	7/02/84	N Px*
2974	4/25/84	7/09/84	12/28/84
2975	5/01/84	8/06/84	04/03/85
2976	5/01/84	8/10/84	11/14/84
2977	6/19/84	8/13/84	10/31/84
2978	5/22/84	8/20/84	03/25/85
2979	5/22/84	8/29/84	01/31/85

*N Px = Not pregnant.

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TABLE 3. LISTING OF CALVES BORN TO PCB-DOSED AND CONTROL COWS AT THE SECOND EXPERIMENTAL PARTURITION

Cow no.	Experimental group	Parturition date	Calf no.	Calf sex	Parturition remarks
2970	Control	12/11/84	M2898	M	Unassisted
2971	Control	12/21/84	M2902	M	Unassisted
2722	Control	01/29/85	M2916	M	Unassisted
2974	Dosed	12/28/84	3101	F	Manual Assistance
2975	Control	04/03/85	3143	F	Manual Assistance
			3144	F	Manual Assistance
2976	Dosed	11/14/84	M2888	M	Manual Assistance
2977	Control	10/31/84	3081	F	Unassisted
2978	Control	03/25/85	3134	F	Unassisted
2979	Dosed	01/31/85	M2918	M	Unassisted

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Doses and Dosing. Dosing began at the end of the 15-day acclimation period. A staggered start was utilized to facilitate scheduling of the various parts of the study. Daily doses of Aroclor 1254 were administered orally in gelatin capsules, a procedure that had been proven effective in other xenobiotic studies in our laboratory. The PCB was mixed in lots with finely ground corn and weighed into individual dose capsules. Control animals received gelatin capsules that contained ground corn as a sham treatment.

The lot of Aroclor 1254 used for these studies was an unnumbered lot for investigational use. This lot has been maintained in this laboratory since 1972, and frequently utilized for studies in which the composition has been verified. Before the onset of this study, a portion of this Aroclor 1254 lot was examined by capillary GC and capillary CG/MS to verify that the composition was consistent with the accepted distribution of congeners. The percent chlorobiphenyl composition was di 0.2, tri 0.8, tetra 20.0, penta 56.0, and hexa 23.0. After doses were prepared, capsules were drawn at random from each batch for verification of the PCB content.

Oral capsule dosing insured that the total dose was consumed, provided an accurate method of dose accounting, and helped control environmental and cross-contamination. Doses were individually numbered and color coded to minimize the chance of improper administration. However, on December

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8, 1983, a technician erroneously dosed control cows 2970, 2975, 2978, and 2722 with 10 milligram doses of Aroclor 1254 each. Cows 2971 and 2977 were allocated to control and treated groups, respectively, and neither had yet received dose capsules (intentionally or accidentally). Although it seemed highly unlikely that 10 milligrams of PCB would influence the experiment, incorporation of additional controls was deemed to be appropriate to verify the no-effect contention. Thus, supplementary samples were collected for clinical chemistry and PCB residue analysis, and both 2971 and 2977 were allotted to the control group.

Housing. Following the first experimental parturition, cows were moved to the Intensive Care Barn of the Ohio Agricultural Research and Development Center. This concrete block building was designed for studies where animals can be managed in a routine manner, yet many types of measurements and samples can be safely collected. Control and PCB dosed cows were maintained in individual stalls in adjacent rooms. Stalls were bedded with rubber mats and pelleted corn cobs. All daily management of animals could be conducted at the stall including milking, feeding, and health observations. Animals were moved to adjacent rooms for weighing, surgical procedures, and intensive sample collections. Separate, out-of-doors, concrete lots were provided for daily exercise of dosed and control cows.

At the end of lactation, cows were maintained on pasture with no housing during summer and autumn months. Cows not beginning the second experimental lactation until winter were moved to comfort stalls for the latter portion of the dry period.

Following the second experimental parturition, each cow and her calf were housed for three days in a maternity box stall, after which cows were returned to individual stalls and calves to a nursery. The calf nursery was a "warm-type", mechanically ventilated, with individual tie stalls that prevented direct contact between animals.

Feeding. Rations were formulated to meet the nutrient requirements of dairy cows as recommended by the National Research Council. The composition of the rations changed through the experiment due to seasonal availability of some ingredients. However, the major components were corn silage, grass-legume hay and grain concentrate. Trace mineralized salt was available free choice. Feeding was on an individual basis with feed intake recorded daily.

Feed intake was not determined when cows were on pasture. Pasture feeding was supplemented with legume hay and/or grain when needed to meet the nutrient requirements of pregnant cows.

Calves remained with their dams to receive an adequate supply of colostrum by nursing, then were transferred to the

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nursery for controlled feeding. Calves from dosed cows received milk containing PCB from their dams, whereas control calves received milk free of PCB at approximately 10% of body weight daily. The calf feeding program was designed to have them weaned from milk and entirely on solid feed by 42 days of age. Thus, the ration was principally milk during the first few weeks with the gradual addition of a high-protein grain supplement and small amounts of hay roughage.

Health and Productivity Observations. Since accurate determination of health and productivity were a critical objective of this study, routine measurements and observations, plus specific tests were conducted. The schedule for collection of health and productivity information is shown in table 4. In order to assure that productivity and health information were recorded daily, computer generated data sheets were available daily for each animal. Measures of productivity recorded daily included A.M. and P.M. milk production plus the quantities of feed fed and refused. Body weight of cows was recorded weekly. Specific health items required particular attention of the technical assistant on a daily basis. These included recording whether the animal was normal, abnormal, or had a change from the previous day. Any abnormal observations required a written explanation. Specific items requiring a daily scoring were conditions of the head and neck, feed and legs, mammary gland, the torso,

TABLE 4. TIMETABLE OF MEASUREMENTS TO EVALUATE THE HEALTH AND PRODUCTIVITY OF ALL ANIMALS

Measurements	Study phases		
	PCB-R	PCB-F[a]	PCB-C
Feed intake	Daily	Daily	Daily
Milk production	Daily	Daily	Daily[b]
Milk appearance	Daily	Daily	--
Health observations	Daily	Daily	Daily
Estrus observations	Daily	--	--
Clinical Evaluations	Weekly	Monthly	Weekly[c]
Reproductive tract palpation	Weekly	--	Weekly
Body weight	Weekly	Weekly	Weekly
Photographed	Bi-weekly	Monthly	Bi-weekly
Skeletal growth	--	--	Weekly[d]

[a]On and before lactation day 305

[b]Cows only

[c]Vital signs only; i.e. heart rate, respiration rate, and body temperature

[d]Calves only

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the external urogenital tract, and the secretions from each quarter of the mammary gland. Signs of estrus were also recorded on a daily basis as either being present, not present, or suspicious. In addition, clinical evaluations were performed weekly which included determinations of heart rate, respiratory rate, and body temperature, along with rating the following on a scale of 1 - 10 (10 being excellent): hair coat, dermatitis, brightness of eyes, lacrimation, eye discharge, salivation, presence of abscesses, general condition, and wound healing. Particular attention was paid to wound healing processes as each animal had four liver biopsies and monthly fat biopsies.

Reproductive function was assessed by observation of estrus activity during exercised periods, records of discharges from the external urogenital tract, and by rectal palpation of the reproductive tract. Palpations occurred at approximately weekly intervals until animals were diagnosed pregnant. Breeding was conducted through artificial insemination utilizing semen from the same bull for both PCB-treated and control animals. Semen from a second bull was required late in the experiment.

Occurrences of intramammary infections were monitored by daily observations of foremilk for abnormalcy and routine microbiological culturing of milk plus determination of the

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somatic cell count. The following procedures for microbiological culturing and sampling were instituted on the day of parturition, then on days 3, 7, 14, 30, then monthly until the final 14 days of lactation. The sampling was also instituted whenever clinical mastitis was detected by the technical assistant. The clinical condition was defined as the presence of clots, flakes, or abnormal color or consistency of the milk. When sampled, individual samples for each of the four quarters of the mammary gland were obtained. Samples were collected aseptically. Following the described sanitation procedure for the teats, stripped foremilk was examined by use of a strip cup for evidence of clinical mastitis. Foremilk samples were collected aseptically and examined microbiologically using Esculin Blood Agar (.01 ml of milk) and MacConkey's Agar (.1 ml of milk). All plates were incubated for a total of 48 hours and microbiological growth identified following secondary culturing and biochemical screening. An intramammary infection was diagnosed when: 1) the same pathogen was recovered from 2 of 3 consecutive samples, 2) a single isolation was from a clinical quarter, and 3) occurrence of a clinical quarter from which no pathogen could be isolated. Somatic cell counts were determined in composite milk samples. A composite milk sample was the total excretion from all four quarters at a single milking. Cell counts were conducted by Coulter Counter and recorded as $\log_{10}$.

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Clinical Chemistry of Blood and Urine. Blood and urine samples were collected for clinical chemistry determinations from all animals in the morning prior to feeding and dosing. Samples were collected on the days indicated in table 5. Special samples were collected when an important change in the health status of an animal was evident.

Blood was collected by venous puncture in untreated (serum) and sodium heparin treated (whole blood) vacuum tubes. Urine was collected by induced micturition, filtered through glass wool, and stored in tubes with benzoate preservative.

Smears of whole blood were used for complete blood counts. The following components of serum were determined: glutamic-oxaloacetic transaminase (sGOT), gamma-glutamyl transpeptidase (sGGT), alkaline phosphatase (sAP), lactate dehydrogenase (sLDH), glutamic-pyruvate transaminase (sGPT), total protein, albumin, bilirubin, urea nitrogen (BUN), uric acid, creatinine, cholesterol, triglycerides, calcium, inorganic phosphorus, potassium, sodium, chloride and iron. Globulin was calculated from total protein and albumin concentrations. The albumin:globulin ratio also was calculated. Glucose was also determined in plasma. Chemstrip 9 test strips were used to determine pH and screen for glucose, protein, ketones, and blood in the urine. Color

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TABLE 5. DAYS WHEN SAMPLES WERE COLLECTED FOR CLINICAL CHEMISTRY, LIVER BIOPSY, AND LIVER FUNCTION

	Experimental day no.	Clinical chemistry	Liver biopsy	Liver function
Isolation	15	x		
Predose	25	x	x	
	30	x		x
10 mg/day	60	x		
or	85	x	x	
Sham	90	x		x
100 mg/day	120	x		
or	145	x	x	
Sham	150	x		x
1000 mg/day	180	x		
or	205	x	x	
Sham	210	x		x
Postdosing	240	x		
	270[a]	x		
	300[a]	x		x
	Second experimental lactation day no.			
Post-parturition	1	x		
	42	xb		xb

[a]Sampled this date or on the last day of lactation

[b]Calves and cows.

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and appearance of the urine were evaluated by observation and compared to controls. Specific gravity was measured by refractometer or hydrometer. Microscopic examination of the urine sediment was used to detect epithelial cells, erythrocytes, leukocytes, crystals, casts, mucous, and bacteria.

Liver Cytochrome P_{450}. Measurement of liver cytochrome P_{450} in samples of biopsied tissue allowed comparison of the inductive response with physiological and production measurements made at various levels of exposure to PCB. Liver samples (1.0-3.0 g) were obtained by biopsy on the days indicated in table 5. Each biopsy was made by an intercostal incision immediately behind the diaphragm to expose the liver. With the aid of manual palpation and guidance, a biopsy specimen was removed with a special instrument, care being taken to avoid previous biopsy sites. After removal, the tissue was quickly rinsed in iced saline to remove blood and clots. One portion of the tissue was placed in 10% buffered formalin for histological evaluation while the remainder was quick frozen at -70°C and stored at -20°C until homogenized. Unfortunately, we ran out of our normally used storage bags during the experiment and new supplies were unavailable. Replacement bags used for part of the experimental day 85, 145 and 205 biopsies did not adequately protect the tissue, so we did not feel the cytochrome P_{450} could be assayed with confidence in these samples.

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For the P_{450} assay, tissues were thawed in a petri dish containing physiological saline. The sample was blotted dry to remove excess moisture, weighed, and the weight in g was multiplied by nine to equal the number of mls of a sucrose-Tris homogenization medium (250 mM sucrose, 20 mM Tris-HCl, pH 7.4) to be added to the tissue. The sample was minced with a single edge razor blade and was transferred to a Kontes conical glass tissue homogenizer immersed in ice. Ten passes at medium motor speed were made with a motor driven Teflon pestle. A 50 µl aliquot of the homogenate was saved for protein determination. Preparation of microsomes was done by the calcium precipitation method. Approximately .6 g of liver tissue and 10 mM calcium cnloride were used. A 50 µl aliquot of the microsomal preparation was saved for protein determination. Quantification of cytochrome P-450 was done by the dithionite difference spectrum of carbon monoxide treated microsomes.

Bromosulfophthalein Dye Clearance. The dye clearance tests were conducted on the experimental day shown in table 5. Forty-five ml of blood for preparing a standard curve was drawn by venipuncture before beginning the dye clearance test. The BSP dye was obtained from Sigma and 1 g was dissolved in 20 ml distilled deionized water. The solution was filtered through a .45 µm millipore filter to eliminate bacteria and other particulate matter. The dye solution was

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rapidly injected into the jugular vein with a 16 gauge needle and timed blood samples were taken at 3, 6, 9, 12, and 15 minutes postinjection by venipuncture using a 20 gauge needle and 15 ml heparinized evacuated tubes. All blood samples were protected from light until assayed. Plasma was obtained by centrifuging the blood samples for 20 minutes at 3000 rpm. Standard curves were prepared from presampling plasma which had added 0 to 20 µg BSP/ml taken from a stock solution of .025 mg BSP dissolved in 10 ml of distilled deionized water. Reagents of .1 N NaOH and .1 N HCl were added to the serum samples containing the dye and blanks, respectively. All samples were analyzed in triplicate and read at 650 nm. The standard curve was calculated using a linear regression program.

Analysis of PCB Residues. In order to better understand the distribution, clearance, and kinetics of PCBs consumed by cattle, samples of subcutaneous fat, blood, feces, and milk were collected at predetermined intervals (table 6). The PCB content of major organs and tissues were also determined for all PCB exposed animals that were necropsied. Polychlorinated biphenyls were selectively separated from biological materials by extraction with organic solvents; separated from interfering compounds by column chromatography procedures; with final separation, detection, and quantification by gas-liquid chromatography with packed columns and electron capture detection (GC-EC).

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TABLE 6. DAYS WHEN SAMPLES WERE COLLECTED FOR POLYCHLORINATED BIPHENYL RESIDUE ANALYSIS

	Experimental day no.	Fat	Blood	Feces	Milk
Isolation	15	x	x	x	x
Predose	25		x	x	x
	30	x	x	x	x
10 mg/day	45		x	x	x
or	60	x	x	x	x
sham	85	x			
	90		x	x	x
100 mg/day	105		x	x	x
or	120	x	x	x	x
sham	135		x	x	x
	145	x			
	150		x	x	x
1000 mg/day	165		x	x	x
or	180	x	x	x	x
sham	195		x	x	x
	205	x			
	210		x	x	x
Postdosing	211		x	x	x
period[a]	212		x	x	x
	214		x	x	x
	216		x	x	x
	220		x	x	x
	224	x	x	x	x
	230		x	x	x
	240	x	x	x	x
	250		x	x	x
	260		x	x	x
	270[b]	x	x	x	x
	290[b]		x	x	x
	310[b]	x	x	x	x
	Second experimental lactation day				
Post-parturition	1[c]	x	x	x	x
	2[d]		x	x	x
	3[d]		x	x	x
	6[d]		x	x	x
	10[c]		x	x	x
	20[d]	x	x	x	x
	42[c]	x	x	x	x

[a]For dosed cows only; except on day 270 and dry date; fat, blood, feces, and milk samples were taken for control cows.
[b]Sampled this date or on date dry.
[c]Blood, feces samples also taken for calves.
[d]For dosed cows only.

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Modifications of the polybrominated biphenyl procedures, utilizing disposable glassware, which were developed in our laboratories, were used for milk, blood plasma, and feces. Samples of body fat and other organs were hydrolyzed with H_2SO_4:H_2O (1:1) prior to solvent extraction. The extraction solvent was 2% benzene in petroleum ether. Extracts were cleaned up on miniature columns of florisil and silica gel capped with sodium sulfate to remove fats, pigments, and selected interfering hydrocarbons.

Gas chromatography was on a Hewlett Packard 5736A with ^{63}Ni electron capture detectors. Columns were 3 mm x 2 m packed with 5% OV-101 on chromosorb WHP. The polychlorinated biphenyls were quantified by comparison of GC-EC peak areas of Aroclor 1254 with peak areas determined by electronic integrations of corresponding peaks in sample materials. Chromatograms were quantified by the methods of Webb and McCall. Method losses were determined in appropriate samples by fortification with known quantities of Aroclor 1254. Quality control was monitored within each material, with approximately 10% fortified samples (spikes), 10% blanks, 10% duplicates, and 20% split samples with the sponsor's analytical laboratory.

Necropsies of Cows and Calves. All of the dosed cows and their calves were necropsied, plus control cow 2975.

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The latter was done to see if we could determine the cause of periods of fever and anorexia for this cow.

Animals were euthanized with succinyl choline followed by electrocution. The gross condition of 28 organs or tissues were recorded and histological sections examined by light microscopy on 24 materials. Nine organs were weighed. Interpretation of normalcy of organs and tissues was based on the large data base of normal, diseased, and intoxicated bovines routinely necropsied by our experienced pathologists.

The bodies of necropsied animals that contained PCB were shipped to Rollins Environmental Services for incineration. This procedure prevented any possibility of meat or animal products containing PCB from entering the animal or human food chain.

RESULTS

Productivity. For dairy cows, the most frequently used measurement of productivity is milk production. Whereas this is an important measurement, the ability of cows to effectively utilize energy for the production of milk is of even greater importance. The high-producing dairy cow simply cannot consume sufficient energy to meet the energy requirements of lactation immediately following parturition. Body fat stores are utilized during peak lactation and replenished during the later phases. This study has shown

that daily dosing with polychlorinated biphenyls did not adversely affect the productivity of dairy cows.

Figure 2 illustrates the average daily milk production of the PCB dosed and control cows during the first experimental lactation (305 day) and figure 3 is for the second experimental lactation (42 day). Figures 4 and 5 are the average daily dry matter intake of cows for the same experimental lactation periods. Figure 6 shows the mean weekly changes in body weight throughout the entire study period.

Table 7 shows the average daily milk production, dry matter intake, and net energy of lactation for the PCB dosed and control cows. None of these parameters was significantly different for the dosed or control cows. It is particularly interesting to note that the net energy figures were nearly identical for all cows.

Table 8 compares the average daily production of the cows with their last previous lactation. Cows from both groups clearly produced milk comparable to their previously demonstrated potential.

MONS 020885

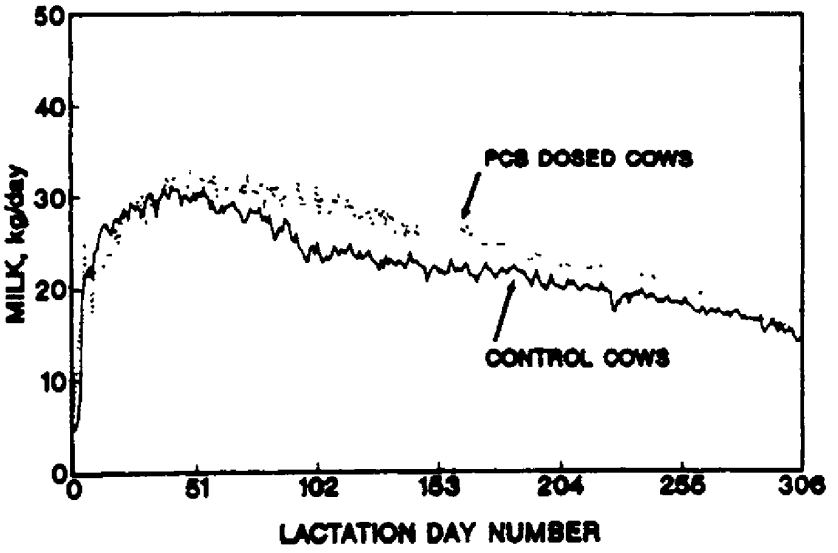

Figure 2. Mean daily milk production of control and PCB dosed cows through the first experimental lactation.

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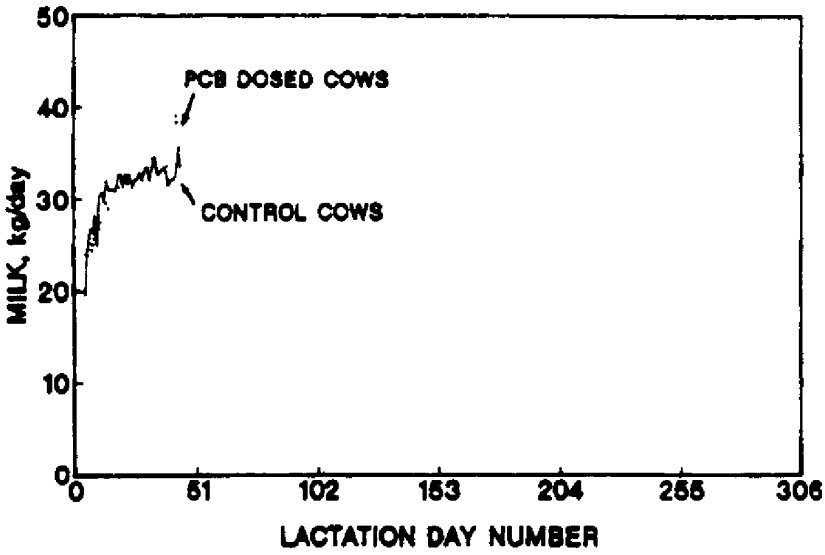

Figure 3. Mean daily milk production of control and PCB dosed cows through the second experimental lactation.

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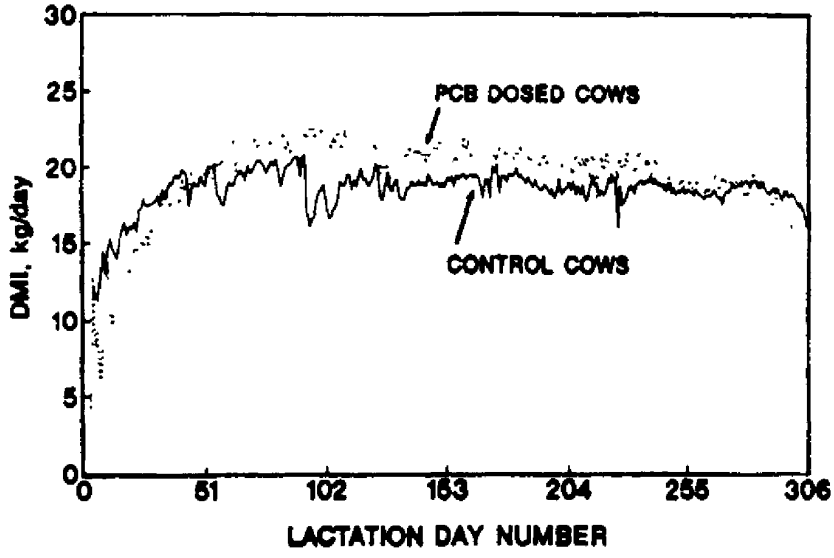

Figure 4. Mean daily dry matter intake of control and PCB dosed cows through the first experimental lactation.

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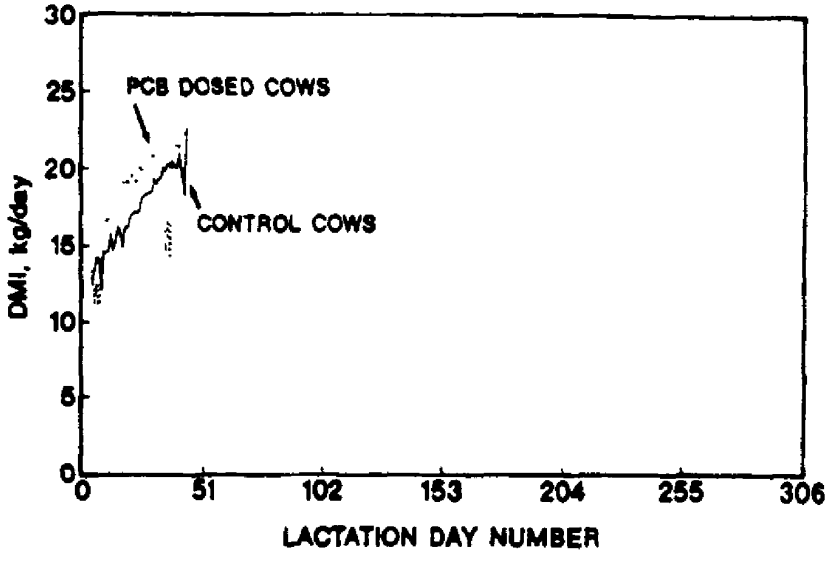

Figure 5. Mean daily dry matter intake of control and PCB dosed cows through the second experimental lactation.

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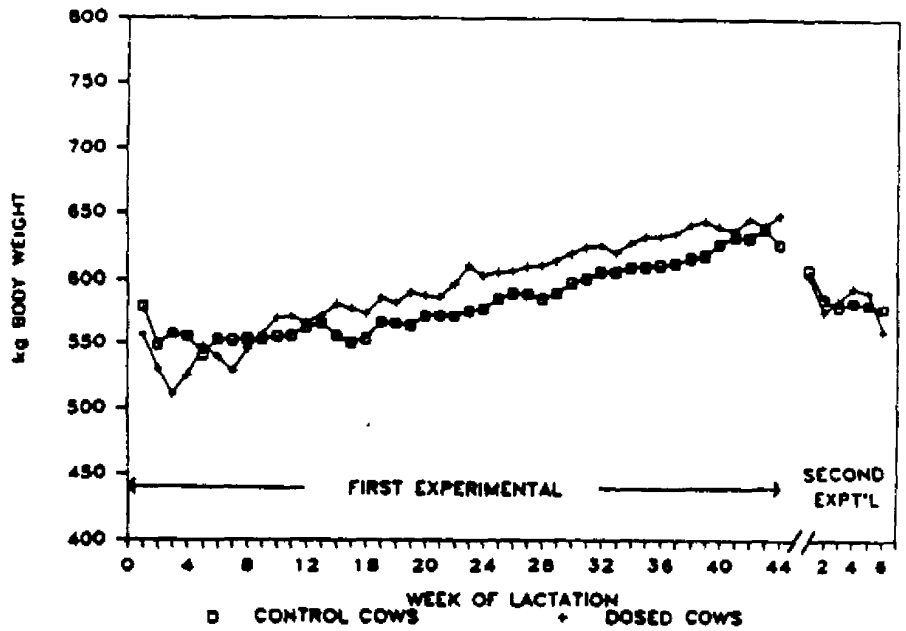

Figure 6. Mean weekly changes in body weight of control and PCB dosed cows throughout the entire study.

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OF CONTROL AND PCB DOSED
RIMENTAL LACTATIONS

	Average daily dry matter intake	Average neta energy of lactation
	(kg)	(Mcal/kg DMI)
First	(305 days)	
Contr	18.6 ± 0.7	1.46 ± 0.05
PCB d	19.5 ± 0.2	1.45 ± 0.03
Secon	(42 days)	
Contr	17.2 ± 0.7	1.36 ± 0.06
PCB d	18.1 ± 0.8	1.35 ± 0.06

[a]NE$_L$ = W_{DMI}

y of lactation
ht
ected milk
r intake

[b]Stand

MONS 020891

TABLE 8. PRODUCTIVITY OF CONTROL AND PCB DOSED COWS AS COMPARED TO PREVIOUS LACTATIONS

	Average daily milk production (Kg)		
	Previous lactation	Experimental lactation	Difference
Control	21.5	22.4	+0.9
PCB Dosed	22.2	24.8	+2.6

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Health. The procedures of daily reporting of all injuries or dysfunctions of each cow insured that minor occurrences could not progress to a serious condition without record of date of onset or original cause. In this regard, the procedures worked well, but resulted in an accumulated record of minor cuts, scrapes, bruises, lumps, and discharges from various body orifices. The majority of the conditions recorded were so minor that they would have gone unnoticed and certainly unrecorded in even the best managed dairy herd or normal research project. The research protocol for this study also induced some of the observations through surgery or special testing procedures.

Tables 9 and 10 summarize the occurrences of injuries and dysfunctions observed among all experimental cows. The numbers in these tables do not reflect the number days that a condition existed, only the occurrences. When the same condition occurred repeatedly for a cow, it was only counted as a new occurrence when the condition had not been observed for a reasonable period to preclude continuation of the same problem.

None of the conditions reported in tables 9 and 10 could be related to the onset, quantity or duration of PCB exposure. Many of the conditions observed were functions of management or season of the year. For example, sunburns of

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TABLE 9. THE OCCURRENCES OF INJURIES OR DYSFUNCTIONS OBSERVED AMONG CONTROL AND PCB DOSED COWS DURING THE FIRST EXPERIMENTAL LACTATION (305 DAY).

Condition	Classification	Description	Number of Occurrences: Control n=6	Number of Occurrences: PCB dosed n=4
Injury:	Abrasion:	Limb	7	11
		Neck	11	0
		Teat	1	0
		Torso	3	4
	Contusion:	Head	1	1
		Torso	0	4
	Decubitus ulcer:	Limb	4	6
		Udder	0	3
	Dermatitis:	Chapped	5	8
		Sunburn	4	3
		Unclass.-fied	5	2
	Hematoma	Neck	4	0
	Laceration:	Head	4	2
		Limb	3	5
		Teat	0	5
	Trauma:	Limb	2	2
		Tooth	0	1
Surgery:	Complications:	Collapsed lung	0	1
		Cut bile duct	1	0
	Hoofs trimmed		2	3
	Laparotomy:	Liver biopsy	24	16
		L.D. Abomasum	1	0
	Cutaneous	Fat biopsy	34	28
	Synovial tap		1	0
Infections:	Mastitis:	Clinical	6	7
		Hemorrhagic	2	0
	Secondary/injury	Hock	1	0
		Teat	0	1
Dysfunctions:	Diarrhea		5	1
	Fever		1	0
	Nasal discharge		6	2
	Inappetence		7	2
	Polyurea		1	0
Reproduction:	Dystocia		0	1
	Discharge/vulve:	Normal	76	94
		Abnormal	19	39
	Estrus		30	16
	Insemination		24	18
	Metestrus bleeding		32	16
	Ovarian cyst		3	8
	Retained placenta		1	0

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TABLE 10. THE OCCURRENCES OF INJURIES OR DYSFUNCTIONS OBSERVED AMONG CONTROL AND PCB DOSED COWS DURING THE SECOND EXPERIMENTAL LACTATION (42 DAYS)

Condition	Classification	Description	Number of Occurrences: Control n=6	Number of Occurrences: PCB Dosed n=3
Injury:	Abrasion:	Limb	0	1
		Teat	0	1
		Torso	2	1
	Dermatitis	Chapped	2	1
Surgery:	Laparotomy	L.D.Abomasum	0	1
	Cutaneous	Fat biopsy	6	6
Infections:	Mastitis	Clinical	7	4
	Pneumonia		0	1
	Secondary/injury	Hock	1	0
Dysfunctions:	Ataxia		0	1
	Edema	Udder	3	3
	Fever		1	0
	Straining	Abdominal	0	1
Reproduction:	Discharge/vulva:	Normal	7	3
		Abnormal	12	3
	Estrus		3	1
	Metestrus bleeding		2	0
	Ovarian cyst		1	0

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areas clipped for fat or liver biopsies became more prevalent in spring and summer months. Some conditions occurred with greater frequency in either the control or PCB dosed groups simply due to a persistent problem with one cow. An example is that 25 of the 39 abnormal discharges from the vulva (table 9) were from cow 2973.

Most of the conditions reported in tables 9 and 10 were minor or routine observations. Certain animals had more serious or persistent disorders during this study. Those cows and conditions are shown in table 11. All of these conditions had an impact on the individual animals; however, they were distributed between the two experimental groups and not deemed to have adversely affected the overall study. These conditions could not be related to PCB exposure.

Considerable effort has been directed toward determining the causes of the less than optimal reproductive performance of both controls and PCB dosed animals (table 9). The services per conception were 4.0 and 4.3 for control and PCB dosed animals, respectively; thus, there was an overall problem without relationship to experimental treatments. Following a review of the reproductive data, we suspect that the reproductive problems may be attributable in part to the management scheme required for this toxicity study. Due to the isolation and separation of control and dosed animals from each other and the rest of the OARDC herd, an exercise

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TABLE 11. MAJOR OCCURRENCES OF INJURIES, ILLNESS, AND DYSFUNCTIONS TO INDIVIDUAL ANIMALS

Cow no.	Experimental day	Description of condition
2971	71	Swollen LR leg, condition remained throughout the experiment.
2971	77	Left displaced abomasum, corrected by surgery.
2971	205	Severed bile duct during liver biopsy.
2973	Many	Infertile, cystic ovaries, persistent pyometria, occluded left oviduct evident at necropsy.
2974	26	Collapsed right lung during biopsy surgery.
2974	35 & 60	Stepped on and cut off 2.5 cm of RR teat. Subsequently infected with coliforms.
2974	81	Seriously injured both front legs in stall accident.
2974	481	Coliform mastitis in previously injured quarter of mammary gland and pneumonia.
2975	85 & 147	Anorexia and fever of unknown etiology, necropsy revealed pericarditis.
2979	470	Left displaced abomasum, corrected by surgery.

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period could only be provided once daily. The remainder of the day the animals were maintained in their assigned stalls. Once daily exercise and observation for signs of estrus apparently was inadequate to provide for optimal detection of animals at estrus. The scientific literature confirms a loss of reproductive performance in animals observed for estrus once daily. Our records suggest that heats were missed (observation of postestrus bleeding) and that the time of insemination may not have been optimal (12 hours after standing heat) for optimal conception. It appears that timing between the technician observing and reporting estrus and the technician doing the insemination was not coordinated. Some of the cows appear to have been bred less than 12 hours after standing heat.

The semen used on these PCB dosed and control cows was also used successfully in the OARDC herd by the same insemination technicians. Therefore, semen quality and insemination technique are not suspect.

Cow 2973 did not conceive during the study and a review of her records indicates several reproductive problems. Twelve days postpartum, the technical assistant reported a purulent discharge from the vulva of 2973 to the attending veterinarian (in the absence of both the principal investigator and project supervisor). He infused the uterus with Furacin and injected ECP (an estrogen preparation) to induce

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uterine contraction. Unfortunately, the latter drug has the side effect of inducing ovarian cysts. Subsequently, ovarian cysts were palpated on eight occasions and gonadotropin releasing hormone (GNRH) or prostaglandins (Lutylase) were used to treat the condition. The purulent discharge from the reproductive tract was intermittent early postpartum, then became persistent throughout the first half of lactation. The uterus was infused on eight occasions. Late in the experiment, estrus cycles occurred but were detected irregularly and pregnancy could not be established. Necropsy revealed that she was ovulating; however, a small cyst completely blocked the left oviduct 2 cm from the infundibulum. Fluids forced into the oviduct could not pass the blockage in either direction.

The weekly clinical evaluation scores further confirmed that PCBs were not adversely affecting the health of the cows. Ratings on the condition of hair coat, dermatitis, brightness of eyes, lacrimation, eye discharge, salivation, abscesses, and wound healing were related to individual animals or not evident. General condition scores were related to stage of lactation.

Average heart and respiratory rates and body temperatures are shown in figures 7, 8, and 9, respectively. Although these means changed with season of the year and stage of lactation (confounded), there were no significant differences between the control and PCB dosed groups.

MONS 020899

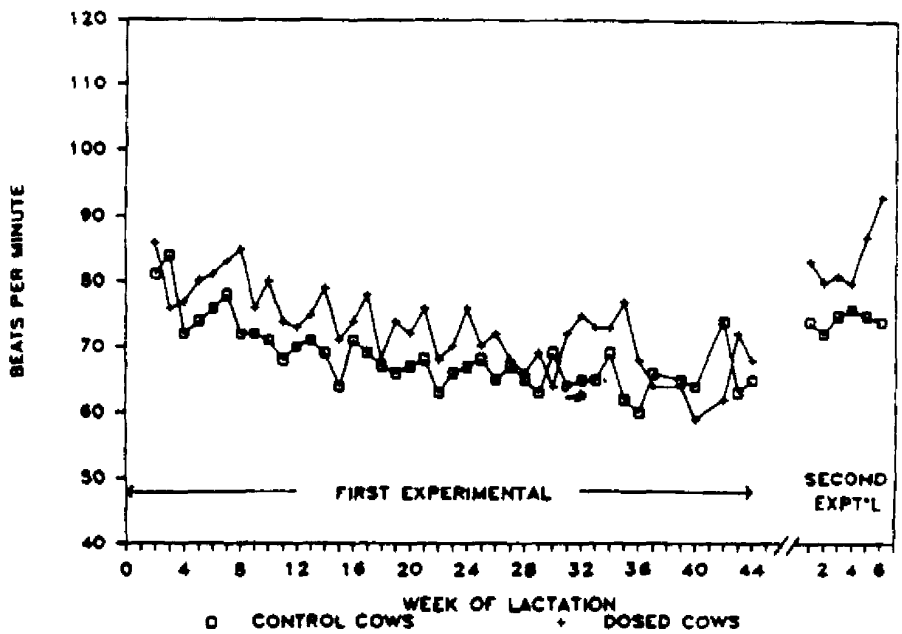

Figure 7. Mean weekly changes in heart rate of control and PCB dosed cows throughout the entire study.

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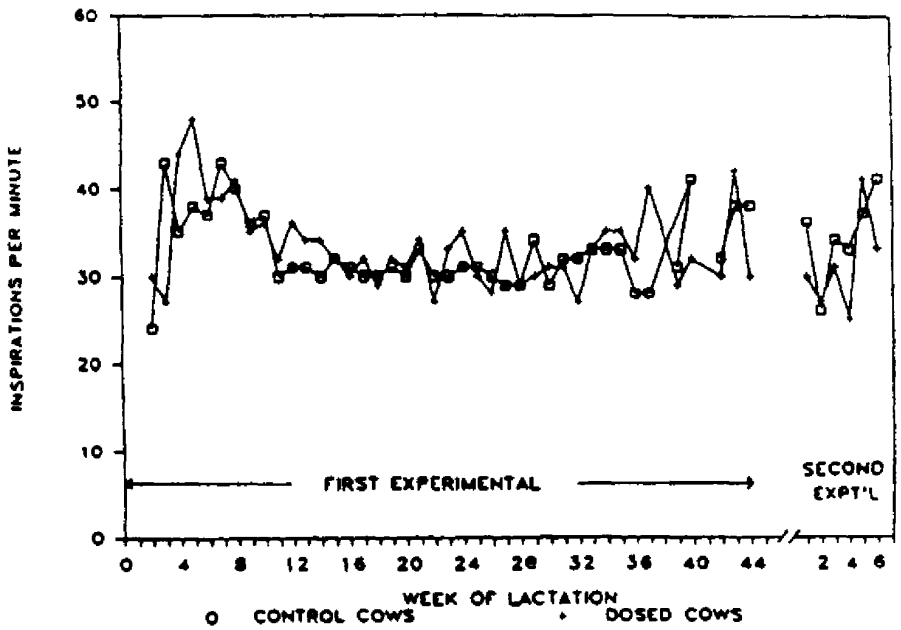

Figure 8. Mean weekly changes in respiratory rate of control and PCB dosed cows throughout the entire study.

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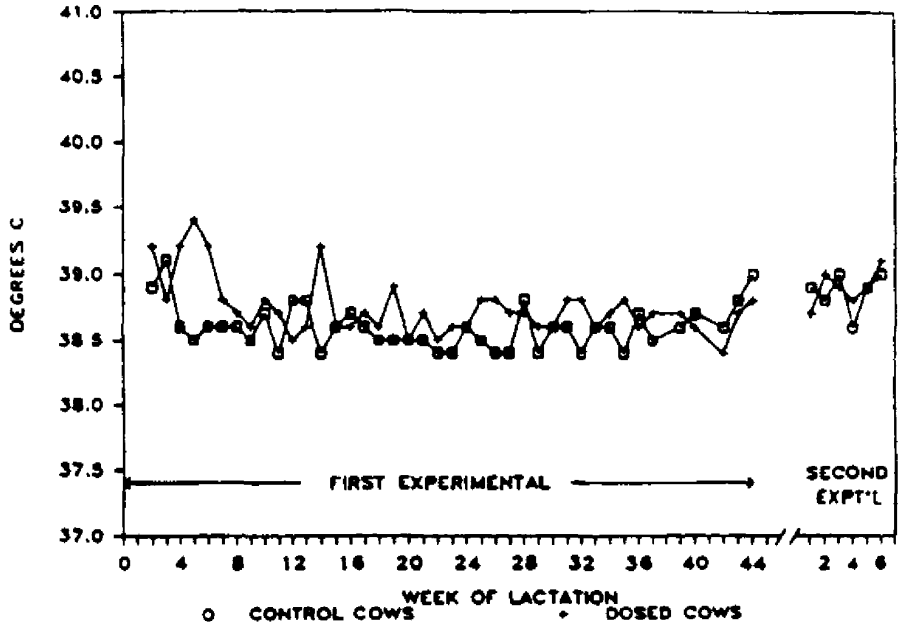

Figure 9. Mean weekly changes in body temperature of control and PCB dosed cows throughout the entire study.

MONS 020902

The intensive study of the health of the mammary gland provided the basis to determine if the PCB dosed cows were more susceptible to infection by pathogenic organisms present in the livestock environment. It must be remembered that the daily sampling of foremilk for abnormalcy and routine microbiological culturing of milk, plus determination of somatic cell counts, detect infections that would go undetected in normally managed dairy herds. Table 12 shows the occurrences of mastitis and the specific pathogens isolated from cows. Three points of explanation are necessary. First, the clinical condition was defined as the presence of clots, flakes, or an abnormal consistency of the milk. Secondly, cases or infections were based on individual quarters of the mammary gland and not on the basis of cows. Thirdly, an intramammary infection was diagnosed when: 1) the same pathogen was recovered from two of three consecutive samples, 2) a single isolation was from a clinical quarter, and 3) occurrence of a clinical quarter from which no pathogen could be isolated.

Clearly the majority of intramammary infections were related to Corynebacterium bovis, an infective organism that does not produce clinical signs, and thus would not be detected in other than extensive culturing conditions. Mastitis was not a problem in this experiment as one cow from each group required antibiotic treatment. Infections in the quarter of Cow 2974 with the excised teat represented the

MONS 020903

TABLE 12. OCCURRENCES OF MASTITIS AND SPECIFIC PATHOGENS ISOLATED FROM CONTROL AND PCB DOSED COWS

	Number of Occurrences			
	Control n=6	n=6	PCB dosed n=4	n=3
Mastitis	305 day Lactation	42 day Lactation	305 day Lactation	42 day Lactation
Clinical infections	6	7	7	4
Cultured infections	30	21	22	10
Hemorrhagic	2	0	0	0
Quarters involved	18	19	14	8
Clinical infections antibiotic treated	1	0	2	2
Classification				
Coliform	1	0	1	1
Streptococci	3	1	2	0
Staphylococci	4	4	1	5
Corynebacterium bovis	20	13	16	0
Clinical no isolation	2	3	2	4

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most severe problem with two acute occurrences of Coliform mastitis.

Monthly mean somatic cell counts for control and PCB dosed cows are shown in figure 10. The cell counts were not significantly different between groups of cows.

Clinical Chemistry. Our laboratories have extensive experience utilizing blood and urine clinical chemistry profiles in conjunction with the responses of dairy cattle to toxicants. The close interval profiling conducted during this study revealed no indications of dysfunction resulting from exposure to polychlorinated biphenyls. As was expected, specific elements of the profiles were altered in response to specific disease processes in individual animals. Tables 13, 14, 15, and 16 list mean concentrations of chemical constituents of blood for control and dosed cows on the scheduled experiments. Tables 17 and 18 similarly represent the hematology and table 19 the specific gravity and pH of urine.

Cytochrome P_{450}. The mean cytochrome P_{450} content in liver microsomes are shown in figure 11. Daily dosing for 55 days with 10 mg apparently had no stimulatory effect on this element of the mixed function oxidase system. Unfortunately, all samples from dosed animals near the end of 100 mg daily dosing had deteriorated during storage (see Methods and Materials). The P_{450} content was clearly elevated by

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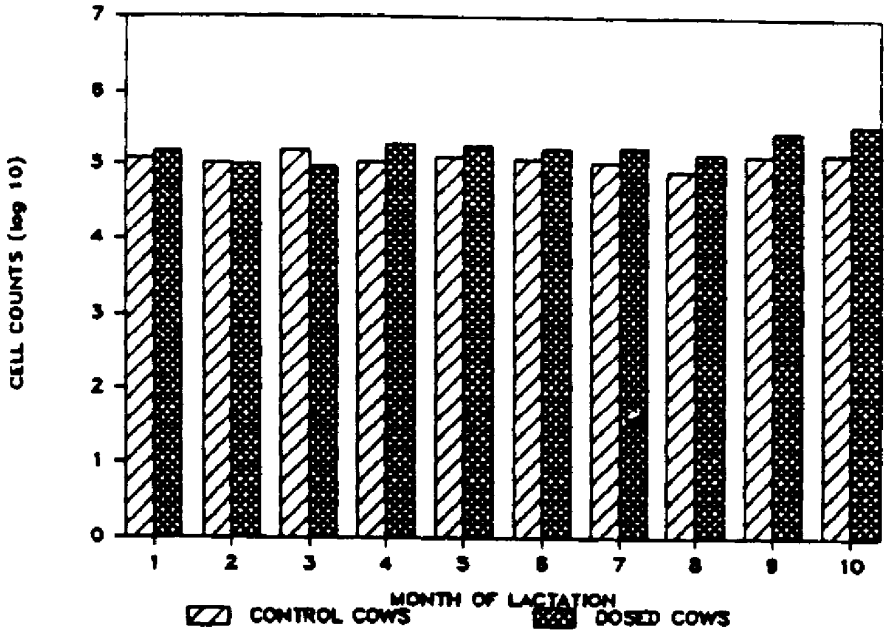

Figure 10. Monthly mean somatic cell counts for control and PCB dosed cows during the first experimental lactation.

MONS 020906

TABLE 13. MEAN CHEMICAL CONSTITUENTS OF BLOOD OF CONTROL (C) AND PCB DOSED (D) COWS: ENZYME ACTIVITIES

DAY NO.	TRT	ALKALINE PHOSPHATASE (IU/l)	GGT,a (IU/l)	aGOT (IU/l)	aGPT (IU/l)	LDH (mU/dl)
E- 15[a]	C	64.5	31.8	64.8	29.7	1120.8
	D	67.8	21.8	85.8	23.3	1119.8
E- 25	C	59.8	32.8	56.2	28.3	1041.7
	D	57.8	26.8	66.5	24.5	1115.8
E- 30	C	64.7	37.8	76.2	29.8	1465.8
	D	54.8	28.3	78.5	21.8	1366.3
E- 60	C	65.5	33.2	55.8	26.8	1285.8
	D	56.5	29.5	61.5	27.8	1278.8
E- 85	C	68.8	32.3	56.7	31.5	1089.2
	D	74.5	32.3	62.7	33.8	1253.3
E- 90	C	63.2	38.8	75.7	26.5	1469.2
	D	68.3	32.5	67.5	31.5	1395.8
E-120	C	62.7	34.8	58.8	22.8	1318.3
	D	62.3	27.8	62.3	28.8	1328.8
E-145	C	65.8	37.5	55.2	38.8	1288.3
	D	66.8	26.8	56.8	34.8	1238.8
E-158	C	62.3	42.8	61.5	35.5	1262.5
	D	68.8	34.5	62.8	42.5	1352.8
E-188	C	68.3	33.7	58.7	29.3	1196.7
	D	64.8	34.8	65.3	40.3	1266.3
E-205	C	61.3	33.7	53.7	29.5	988.3
	D	57.3	39.3	57.8	35.8	1288.8
E-218	C	68.3	34.8	57.8	31.3	1268.3
	D	57.8	39.3	69.5	34.3	1292.5
E-278	C	58.5	24.8	51.8	28.5	1856.3
E-248	D	55.5	37.8	58.3	48.3	1233.8
L1-385[b]	C	57.8	34.5	51.2	29.7	952.5
	D	51.8	38.5	56.8	34.8	1168.8
L2- 1[c]	C	188.8	31.8	72.2	15.2	947.5
	D	94.8	26.3	75.8	13.8	978.3
L2-42	C	55.5	38.3	78.2	28.7	1151.7
	D	95.3	36.7	76.3	22.3	1435.8
REF.RANGE[d]		98-488		23-165	28-77	853-1255

a E- Experimental day no.

b L1- Experimental first lactation day no.

c L2- Experimental second lactation day no.

d Taken from "Herd Health Memo". The Pennsyvania State University, Dec. 94

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TABLE 14. MEAN CHEMICAL CONSTITUENTS OF BLOOD OF CONTROL (C) AND PCB DOSED (D) COWS: COMPOUNDS CONTAINING NITROGEN

DAY NO.	TRT	PROTEIN (g/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)	CREATININE (mg/dl)	BUN (mg/dl)	URIC ACID (mg/dl)
E- 15[a]	C	7.9	3.7	4.2	1.0	15.5	1.02
	D	7.7	3.6	4.1	.9	15.3	1.10
E- 25	C	7.6	3.7	3.9	1.0	10.0	.90
	D	7.8	3.7	4.2	.9	16.0	1.30
E- 30	C	7.9	3.7	4.2	1.0	15.5	1.15
	D	7.9	3.5	4.3	.9	14.0	1.10
E- 60	C	8.0	3.7	4.3	.9	20.3	1.20
	D	8.2	3.7	4.6	.0	19.3	1.35
E- 85	C	8.2	3.7	4.5	1.0	18.5	1.07
	D	8.4	3.9	4.5	.9	10.0	1.03
E- 90	C	8.1	3.6	4.5	.9	19.5	.99
	D	8.6	3.7	4.9	.0	16.3	.93
E-120	C	8.2	3.6	4.6	1.1	17.5	1.07
	D	8.0	3.9	4.9	.9	16.0	.95
E-145	C	8.2	3.7	4.5	1.0	19.0	1.17
	D	8.5	3.8	4.7	.9	18.5	1.03
E-150	C	8.4	3.7	4.7	1.0	17.0	1.10
	D	8.7	3.9	4.8	.9	15.0	.95
E-180	C	8.3	3.6	4.6	1.0	19.0	.90
	D	8.5	3.9	4.5	.9	10.3	1.35
E-205	C	8.0	3.6	4.4	1.1	10.2	1.00
	D	8.1	3.9	4.2	.9	16.3	.95
E-210	C	8.1	3.6	4.5	1.0	17.3	.97
	D	8.2	3.8	4.4	.0	15.5	1.13
E-270	C	7.0	3.6	4.2	1.1	17.3	1.05
E-240	D	8.2	3.9	4.4	.0	10.0	1.00
L1-305[b]	C	7.9	3.7	4.2	1.2	13.3	1.05
	D	8.2	3.7	4.4	1.1	15.3	1.30
L2- 1[c]	C	6.0	3.6	3.2	1.6	17.2	1.03
	D	6.7	3.0	2.9	1.5	14.7	1.09
L2-42	C	7.8	3.7	4.2	1.1	17.7	1.10
	D	7.6	3.5	4.1	1.0	19.7	1.00
REF. RANGE[d]		6.4-8.8	2.1-4.1	3.5-5.5	.5-2.2	9.7-10.7	0-2.50

[a] E- Experimental day no.

[b] L1- Experimental first lactation day no.

[c] L2- Experimental second lactation day no.

[d] Taken from "Herd Health Memo", The Pennsylvania State University, Dec. 84

MONS 020908

TABLE 15. MEAN CHEMICAL CONSTITUENTS OF BLOOD OF CONTROL (C) AND PCB DOSED (D) COWS: BILIRUBIN, FATS AND CARBOHYDRATES

DAY NO.	TRT	GLUCOSE PLASMA (mg/dl)	GLUCOSE SERUM (mg/dl)	BILIRUBIN (mg/dl)	CHOLESTEROL (mg/dl)	TRIGLYCERIDE (mg/dl)
E- 15[a]	C	63.8	73.6	.20	122.5	8.8
	D	65.3	71.8	.20	109.5	7.8
E- 25	C	66.5	73.3	.12	119.8	4.2
	D	63.3	71.3	.15	128.3	8.5
E- 30	C	67.3	73.3	.13	131.8	3.8
	D	66.8	72.8	.15	134.3	9.8
E- 60	C	61.8	72.3	.08	139.8	8.3
	D	64.3	72.5	.13	133.5	10.5
E- 85	C	70.3	77.2	.10	133.2	11.3
	D	74.8	78.8	.10	156.8	9.3
E- 90	C	66.7	76.8	.12	135.3	8.8
	D	69.3	77.3	.10	149.3	6.3
E-120	C	69.8	82.3	.08	129.3	12.3
	D	72.8	82.8	.10	151.8	9.8
E-145	C	65.7	78.8	.08	133.5	11.8
	D	78.3	88.8	.13	151.3	14.3
E-150	C	65.2	88.3	.12	139.8	8.7
	D	69.5	79.5	.10	151.3	8.8
E-180	C	63.7	73.8	.07	122.7	11.8
	D	78.3	79.8	.15	144.5	19.3
E-205	C	62.8	72.8	.12	124.2	12.3
	D	64.5	75.8	.15	137.5	14.5
E-210	C	64.8	75.3	.10	123.8	10.7
	D	65.5	74.8	.13	132.8	11.8
E-270	C	64.3	73.8	.10	106.8	8.5
E-240	D	66.3	78.3	.10	129.3	13.5
L-305[b]	C	64.7	77.5	.12	105.8	12.5
	D	62.8	73.8	.13	118.3	11.5
L2- 1[c]	C	65.7	85.3	.40	78.3	5.8
	D	78.8	97.8	.43	76.8	4.7
L2-42	C	65.7	88.2	.12	126.7	11.8
	D	62.3	78.8	.60	131.8	15.8
REF. RANGE[d]			42-86	.04-.44	184-194	17.4+1.4

[a] E- Experimental day no.

[b] L1- Experimental first lactation day no.

[c] L2- Experimental second lactation day no.

[d] Taken from "Herd Health Memo", The Pennsylvania State University, Dec. 84

MONS 020909

TABLE 16. MEAN CHEMICAL CONSTITUENTS OF BLOOD OF CONTROL (C) AND PCB DOSED (D) COWS: MINERALS AND IONS

DAY NO.	TRT	CALCIUM (mg/dl)	PHOSPHORUS (mg/dl)	POTASSIUM (meq/l)	SODIUM (meq/l)	CHLORIDE (meq/l)	IRON (ug/dl)
E- 15[a]	C	9.5	3.6	4.3	140.0	104.2	147.0
	D	9.6	3.7	3.9	140.3	103.5	110.3
E- 25	C	9.2	3.9	4.3	139.0	102.2	144.3
	D	9.6	4.9	4.1	130.0	99.5	154.0
E- 30	C	9.7	4.1	4.2	140.0	100.3	120.0
	D	9.8	4.0	4.1	139.3	99.0	111.3
E- 60	C	9.7	4.4	4.1	130.0	100.0	160.0
	D	9.7	4.4	4.5	138.5	100.0	174.0
E- 85	C	9.6	4.7	4.3	140.3	102.3	150.2
	D	9.7	3.8	3.8	139.3	99.5	95.3
E- 90	C	10.1	4.4	3.9	139.5	99.3	130.5
	D	9.7	4.0	4.2	137.3	99.5	129.8
E-120	C	9.9	4.6	4.0	130.5	100.0	156.2
	D	10.0	4.0	4.3	138.0	101.5	146.0
E-145	C	10.0	5.0	4.5	140.7	99.3	164.7
	D	10.1	4.0	4.0	143.3	100.5	162.5
E-150	C	9.0	4.0	4.1	143.3	101.0	152.2
	D	10.0	4.4	4.1	130.5	101.3	157.0
E-180	C	9.9	5.0	4.1	139.0	90.0	102.0
	D	10.3	5.1	4.3	139.0	100.5	160.3
E-205	C	9.9	4.9	4.0	141.2	99.0	165.5
	D	9.9	4.0	4.0	141.3	99.0	159.0
E-210	C	9.7	4.7	4.2	141.0	101.3	132.5
	D	10.1	4.0	4.1	139.3	100.0	156.5
E-270	C	9.0	5.3	4.1	139.0	101.0	145.0
E-240	D	10.0	5.2	4.3	140.0	101.0	140.0
L1-305[b]	C	10.1	4.6	4.2	141.5	102.5	167.7
	D	10.3	5.4	4.3	140.0	101.0	145.0
L2- 1[c]	C	8.4	4.3	4.3	144.5	106.0	104.3
	D	8.7	3.3	4.2	146.7	107.3	100.7
L2-42	C	9.6	4.2	4.1	140.0	101.0	137.3
	D	9.1	5.9	3.0	130.0	97.0	106.3
REF. RANGE[d]		8.4-10.2	4.6-7.4	4.1-6.1	133-143	97-105	119-225

[a] E- Experimental day no.

[b] L1- Experimental first lactation day no.

[c] L2- Experimental second lactation day no.

[d] Taken from "Herd Health Memo". The Pennsylvania State University, Dec. 84

MONS 020910

TABLE 17. MEAN BLOOD CELL COUNT AND COMPOSITION OF CONTROL (C) AND PCB DOSED (D) COWS

DAY NO.	TRT	WBC ($/mm^3$)	RBC ($10^6/mm^3$)	HGB (g/dl)	HCT (%)	MCV (u^3)	MCH (pg)	MCHC (%)
E- 15[a]	C	7917	6.51	11.2	31.0	49.0	17.2	35.2
	D	8700	6.17	10.9	31.0	50.3	17.6	35.0
E- 25	C	9100	6.63	11.7	33.3	50.2	17.7	35.2
	D	9025	6.21	10.8	30.9	49.8	17.4	35.0
E- 30	C	7517	6.69	11.5	32.6	48.7	17.3	35.5
	D	9325	6.07	10.4	29.6	48.5	17.2	35.4
E- 60	C	7067	6.69	11.5	32.2	48.0	17.2	35.8
	D	8775	6.20	10.8	31.1	49.8	17.6	35.8
E- 85	C	8550	7.17	12.1	34.5	48.3	16.9	35.2
	D	12150	6.64	11.6	33.8	49.8	17.5	35.2
E- 98	C	8250	6.84	11.6	33.1	48.5	17.0	35.1
	D	9175	6.34	11.0	31.6	49.8	17.4	35.0
E-120	C	8067	7.04	11.7	33.8	47.7	17.0	35.5
	D	9925	6.83	12.8	33.3	49.8	17.6	36.1
E-145	C	9433	7.29	12.1	33.8	49.2	17.6	35.9
	D	9600	6.68	11.8	32.4	48.3	17.8	36.6
E-150	C	7933	6.95	11.9	34.8	48.7	17.1	35.8
	D	8550	6.94	11.5	33.8	48.3	16.7	34.3
E-180	C	8133	7.41	12.3	35.2	50.8	17.6	35.8
	D	8900	6.90	12.1	34.1	49.5	17.5	35.4
E-205	C	9367	7.09	12.1	34.8	49.7	17.1	34.8
	D	9425	7.04	12.2	35.8	50.3	17.4	34.7
E-210	C	8167	7.13	12.8	34.3	48.5	16.8	35.8
	D	8125	7.09	12.2	35.1	49.8	17.3	34.7
E-270	C	8200	6.79	11.6	33.9	50.5	17.1	34.1
E-240	D	23325	6.43	11.3	31.7	49.8	17.5	35.7
L1-305[b]	C	8717	6.99	12.1	34.2	48.8	17.4	35.5
	D	9100	6.33	11.5	32.6	51.8	18.1	35.2
L2- 1[c]	C	15567	6.89	12.1	35.4	51.5	17.7	34.3
	D	30733	7.30	13.2	38.3	52.3	18.1	34.3
L2-42	C	9817	6.17	10.6	30.4	49.3	17.2	34.6
	D	17500	6.83	10.6	30.1	50.8	17.6	35.2
REP.RANGE[d]		4000-12000	5.0-10.0	8-15	24-46	40-60	11-17	30-60

[a] E- Experimental day no.

[b] L1- Experimental first lactation day no.

[c] L2- Experimental second lactation day no.

[d] Roche Biomedical Laboratories, Inc.

MONS 020911

TABLE 18. MEAN DIFFERENTIAL WHITE BLOOD CELL COUNTS OF CONTROL (C) AND PCB DOSED (D) COWS

DAY NO.	TRT	NEUT. BANDS (%)	NEUT. SEGS. (%)	T. NEUTRO-PHILS (%)	LYMPHO-CYTES (%)	MONO-CYTES (%)	EOSINO-PHILS (%)	BASO-PHILS (%)
E- 15[a]	C	.2	37.0	37.2	54.0	2.0	6.5	.3
	D	.3	49.0	50.0	38.5	4.0	3.3	.3
E- 25	C	.3	28.2	32.2	53.5	7.0	6.2	.3
	D	.0	49.0	49.0	42.0	3.0	4.5	.0
E- 30	C	.4	30.2	30.6	52.4	6.6	9.0	.6
	D	.7	48.3	49.0	39.7	3.0	7.7	.7
E- 60	C	.0	37.0	37.0	55.2	4.5	2.5	.0
	D	.3	46.3	46.5	43.0	6.5	3.5	.5
E- 85	C	1.3	30.2	31.5	59.3	4.5	3.0	.0
	D	1.3	49.3	50.7	39.7	4.0	5.7	.0
E- 90	C	.2	28.0	29.0	56.5	5.3	8.5	.7
	D	.0	34.3	34.3	50.5	5.0	9.3	.0
E-120	C	.0	28.0	28.0	62.3	2.0	6.0	.0
	D	.0	44.3	44.3	45.0	2.0	7.5	.3
E-145	C	.0	31.5	31.5	58.3	2.3	7.3	.0
	D	.0	47.5	47.5	40.5	1.0	9.3	.3
E-150	C	.0	32.3	32.3	58.3	2.5	6.0	1.0
	D	.0	41.5	41.5	48.0	2.5	6.5	.0
E-180	C	.0	27.0	27.0	58.3	3.0	10.2	.7
	D	.3	43.0	44.0	42.5	5.3	8.0	.3
E-205	C	.0	27.2	27.2	64.0	1.4	7.2	.4
	D	.0	40.3	40.3	44.7	4.0	10.0	1.0
E-210	C	.0	26.4	26.4	61.6	1.4	10.6	.2
	D	.0	35.0	35.0	55.5	1.0	8.0	.3
E-270	C	.0	36.7	36.7	57.3	2.0	4.0	.0
E-240	D	.3	48.0	48.3	49.0	3.0	4.5	.5
L1-385[b]	C	.0	34.4	34.4	56.6	1.0	5.0	1.4
	D	1.0	40.0	41.0	48.5	6.5	4.0	.0
L2- 1[c]	C	2.5	59.5	62.0	32.3	5.0	.7	.0
	D	.7	70.0	70.7	24.3	4.0	.7	.3
L2-42	C	.5	33.7	34.2	55.0	5.7	4.3	.0
	D	14.0	50.3	31.0	37.7	3.7	4.0	.0
REF. RANGE[d]		0-2	15-45		45-75	2-7	2-20	

[a] E- Experimentel dey no.

[b] L1- Experimentel first lectation dey no.

[c] L2- Experimental second lectation day no.

[d] Roche Biomedical Laboratories, Inc.

MONS 020912

TABLE 19. MEAN SPECIFIC GRAVITY AND pH OF URINE OF CONTROL (C) AND PCB DOSED (D) COWS

DAY NO.	TREATMENT	SPECIFIC GRAVITY	pH
E- 15[a]	C	1.036	8.2
	D	1.039	8.3
E- 25	C	1.035	8.2
	D	1.038	6.8
E- 38	C	1.035	8.2
	D	1.039	8.3
E- 60	C	1.033	8.0
	D	1.037	8.3
E- 85	C	1.033	8.0
	D	1.039	7.5
E- 98	C	1.026	8.0
	D	1.036	8.4
E-120	C	1.030	8.0
	D	1.034	7.5
E-145	C	1.030	8.2
	D	1.036	8.0
E-150	C	1.025	8.0
	D	1.036	8.5
E-180	C	1.027	8.5
	D	1.033	8.3
E-205	C	1.032	8.5
	D	1.036	8.0
E-210	C	1.030	8.2
	D	1.037	8.3
E-270	C	1.031	8.0
E-240	D	1.037	7.0
L1-305[b]	C	1.033	8.0
	D	1.037	7.0
L2- 1[c]	C	1.031	6.2
	D	1.039	6.3
L2-42	C	1.038	7.3
	D	1.036	7.3

[a] E- Experimental day no.

[b] L1- Experimental first lactation day no.

[c] L2- Experimental second lactation day no.

MONS 020913

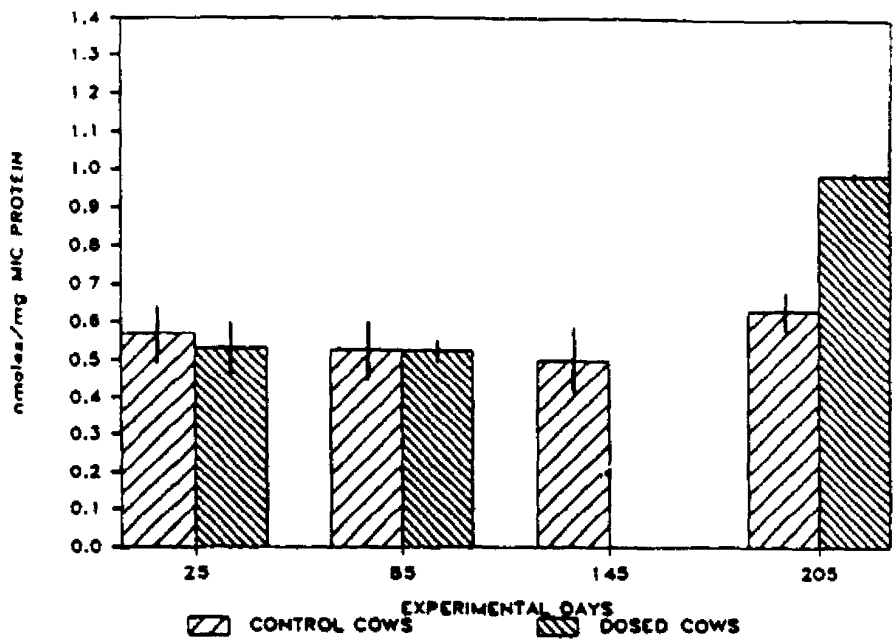

Figure 11. Mean (± standard error) cytochrome P_{450} content per mg of liver microsomal protein for control and PCB dosed cows.

MONS 020914

nearly 40% as compared to controls near the end of the 1,000 mg/day dosing period. This elevation in P_{450} could not be associated with significant changes in blood constituents, health or productivity.

Bromosulfophthalein Dye Clearance. Figure 12 shows the mean BSP clearance for control and PCB dosed cows determined predose, at the end of each dosing period and at the end of each experimental lactation. There were no significant differences between the two groups of cows. This test, along with the results of clinical chemistry determinations, suggest that PCBs at these concentrations do not harm the liver of cattle.

Calves. The calves born to the PCB dosed cows were all conceived after the onset of dosing. Therefore, the conceptus and developing fetus were continually exposed to PCBs until birth. Since these calves were fed milk from their own dams, exposure to PCB continued until termination of the experiment at the time of weaning. The PCB content of tissues of calves at birth was not determined so the importance of PCB exposure *in utero* vs exposure from the milk diet cannot be determined at this time. The PCB residue content of tissues collected at necropsy revealed that the calves had accumulated significant quantities of PCB. Table 20 shows the relationship between the average PCB content of selected

MONS 020915

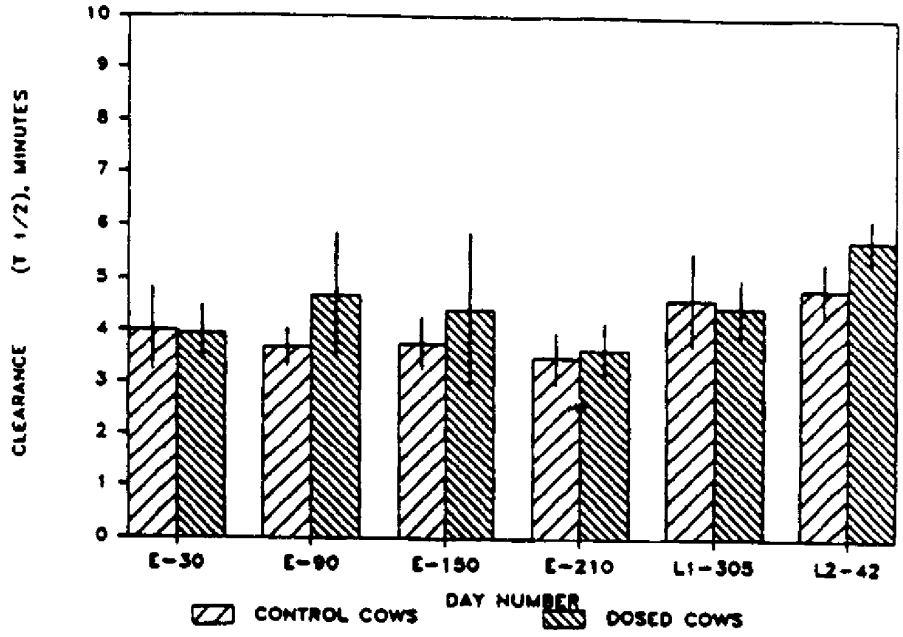

Figure 12. Mean clearance (± standard error) of bromosulfophthalein by control and PCB dosed cows. Day E-30 represents predose samples; E-90, 150 and 210 the ends of the dosing periods; and L1-305 and L2-42 the last day of the two experimental lactations.

MONS 020916

TABLE 20. MEAN PCB CONCENTRATIONS FROM AROCLOR 1254 AT NECROPSY IN SELECTED TISSUES OF LACTATING PCB DOSED COWS AND THEIR CALVES

	Concentration ppm in tissue	
Tissue	Dosed[1] cows	Calves[2]
Adipose (fat)	12.91	24.12
Liver	0.30	1.03
Kidney	0.11	0.33
Brain	0.18	0.41
Blood Plasma	0.05	0.08

[1]250-300 days after last dose.

[2]Received PCB milk from their dam.

MONS 020917

organs of cows and their calves at necropsy. When interpreting this table, it is important to remember that the cows had not been dosed for 250 to 300 days while the calves had been daily dosed with milk containing PCB to the time of necropsy.

The birth weights of calves are shown in figure 13. Whereas the PCB dosed calves tended to be larger than the control calves, the significance of this observation is unclear with the confounding factors of few observations and twins in the control group.

Three measurements of calf growth were made during the experiment at weekly intervals. Body weight was the most reliable measurement where measurement of body length and height are more variable since positioning of the calf is critical for precise measurement. Rarely do young calves cooperate by standing squarely and still during the measurement process. Figures 14, 15, and 16 represent the mean growth of calves from control and PCB dosed cows. Figure 14 particularly illustrates that, despite differences in birth weight, the growth rate of both groups of calves was nearly identical.

The health of calves was scored on a daily basis, as was the health of cows. Thus, observations were recorded that are normally overlooked in a commercial dairy operation. Conditions encountered with these calves (table 21)

MONS 020918

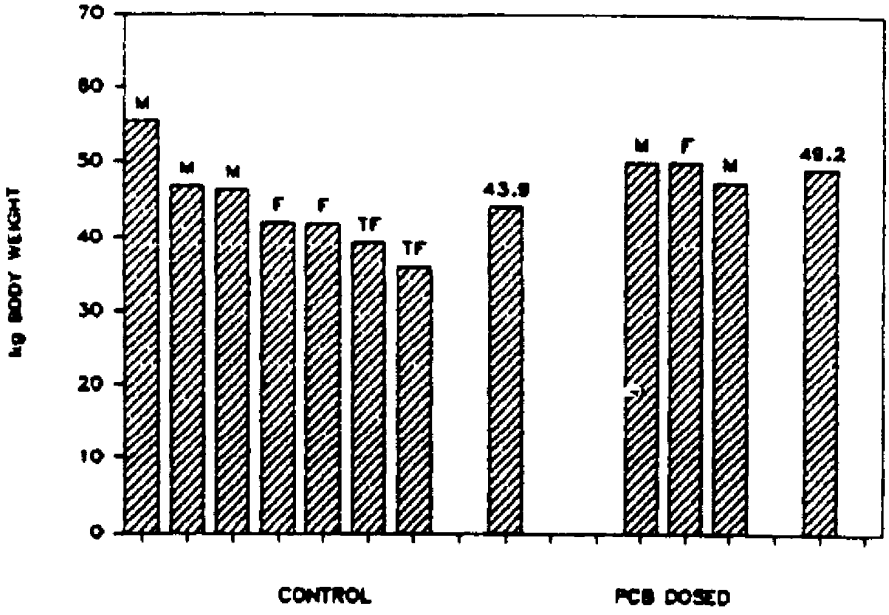

Figure 13. Individual and mean birth weights of calves from control and PCB dosed cows. Labels indicate: M=male, F=female, and TF=twin female.

MONS 020919

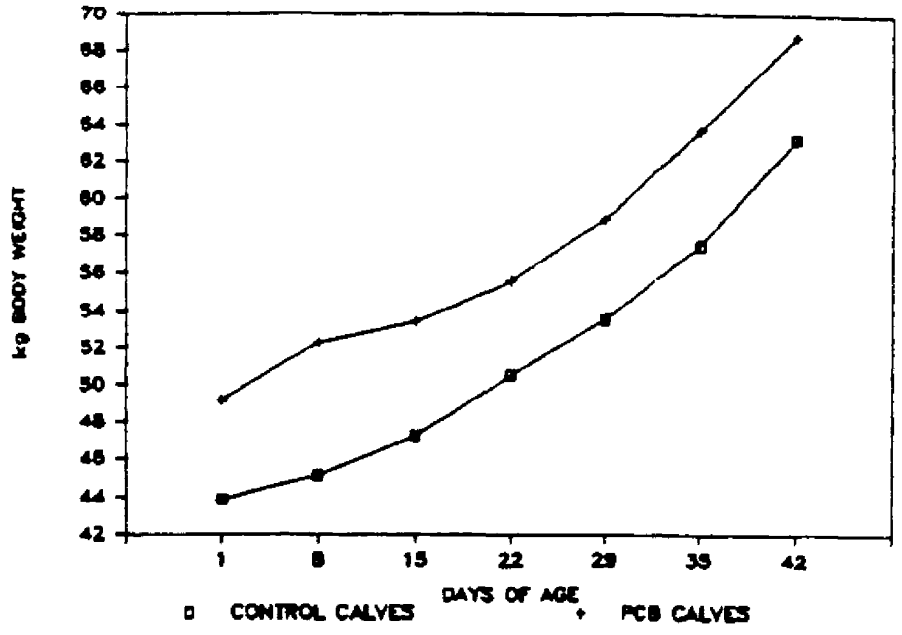

Figure 14. Mean body weight of calves from control and PCB dosed cows.

MONS 020920

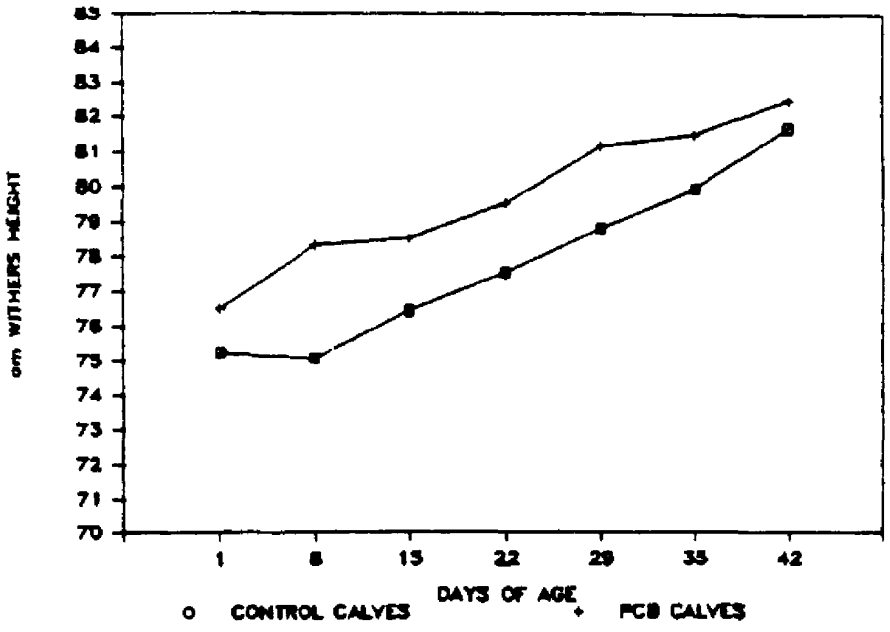

Figure 15. Mean withers height of calves from control and PCB dosed cows.

MONS 020921

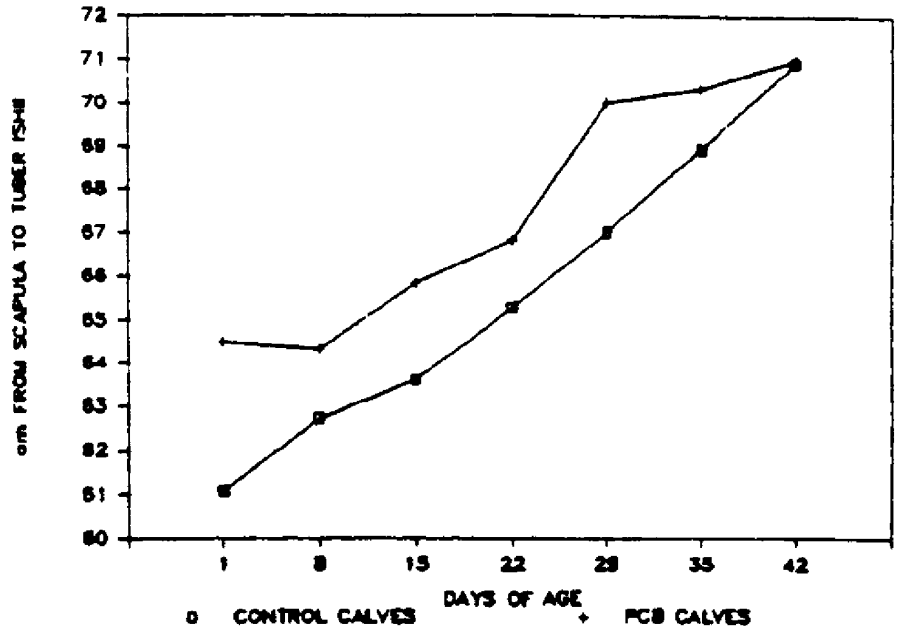

Figure 16. Mean skeletal length of calves from control and PCB dosed cows.

MONS 020922

TABLE 21. OCCURRENCES OF HEALTH DISORDERS AND DYSFUNCTIONS AMONG CALVES FROM CONTROL AND PCB DOSED COWS

Condition	Classification	Description	Number of Occurrences	
			Control n=7	PCB dosed n=3
Dermatitis	Unclassified	Scurf	0	1
Infections:	Abcess	Cutaneous	1	0
	Enteritis	Clostridial	1	0
Feces	Abnormal:	Medicated	6	3
		Not medicated	5	4
Dysfunctions:	Tendons	Contracted	2	1
	Discharge	Eye	2	0

MONS 020923

were typical and revealed no differences between the control and PCB dosed groups. Clinical chemistry profiles and complete blood counts were on all calves conducted at the end of the experiment. These results are shown in tables 22 and 23 and revealed normal values for both groups of calves of that age.

PCB Residues in Cows. Mean concentrations of PCBs from Aroclor 1254 in the subcutaneous fat (SQF), milk fat, feces, and blood plasma are shown in figure 17. This figure clearly shows the relationship of residue concentrations in the four materials sampled as daily dosing increased from 10 to 100 to 1,000 mg per day. Equilibrium of residue concentrations were quickly achieved in feces and blood plasma with slower movement of residues into milk fat and particularly into adipose tissue. Since milk fat concentrations are the most frequently sampled for regulatory purposes, these concentrations at the end of each dosing period were of particular interest. Mean concentrations at the end of the 10, 100, and 1,000 mg/day dosing were 1.9, 10.0 and 91.0 µg/g (ppm), respectively. The current federal tolerance for PCB in milk fat is 1.5 ppm. Residues in this study exceeded tolerance by over 60 times, yet we saw no indications of impaired health or performance.

MONS 020924

TABLE 22. MEAN CHEMICAL CONSTITUENTS OF BLOOD AND PROPERTIES OF URINE OF SIX WEEK OLD CALVES FROM CONTROL AND PCB DOSED COWS

COMPONENT	CONTROL	DOSED
BLOOD CHEMISTRY		
GLUCOSE, PLASMA (mg/dl)	84.4	83.7
GLUCOSE, SERUM (mg/dl)	184.9	183.8
BILIRUBIN (mg/dl)	.17	.13
CHOLESTEROL (mg/dl)	145.4	116.7
TRIGLYCERIDE (mg/dl)	34.6	39.3
PROTEIN (g/dl)	6.8	6.8
ALBUMIN (g/dl)	3.4	3.5
GLOBULIN (g/dl)	2.6	2.4
CREATININE (mg/dl)	1.8	.9
BUN (mg/dl)	10.8	12.7
URIC ACID (mg/dl)	1.14	.63
ALKALINE PHOSPHATASE (IU/l)	295.8	293.8
GGT,s (IU/l)	37.9	41.7
sGOT (IU/l)	45.4	47.3
sGPT (IU/l)	7.6	18.8
LDH (mU/dl)	852.9	818.3
CALCIUM (mg/dl)	18.6	18.7
PHOSPHORUS (mg/dl)	8.4	7.2
POTASSIUM (meq/dl)	5.8	5.8
SODIUM (meq/dl)	141.6	139.3
CHLORIDE (meq/dl)	108.1	99.8
IRON (ug/dl)	271.9	146.7
URINE CHEMISTRY		
SPECIFIC GRAVITY	1.021	1.026
pH	5.9	5.3

MONS 020925

TABLE 23. MEAN COMPLETE BLOOD COUNTS OF SIX WEEK OLD CALVES FROM CONTROL AND PCB DOSED COWS

COMPONENT	CONTROL	DOSED
BLOOD CELL COUNTS		
WBC (/mm^3)	10514	10250
HGB (g/dl)	10.8	10.7
HCT (%)	32.7	32.0
DIFFERENTIAL		
NEUT. BANDS (%)	.7	.0
NEUT. SEGS (%)	35.9	23.0
T. NEUTROPHILS (%)	36.6	33.7
LYMPHOCYTES (%)	55.9	53.7
MONOCYTES (%)	8.0	2.0
EOSINOPHILS (%)	.4	.0
BASOPHILS (%)	.6	.3

MONS 020926

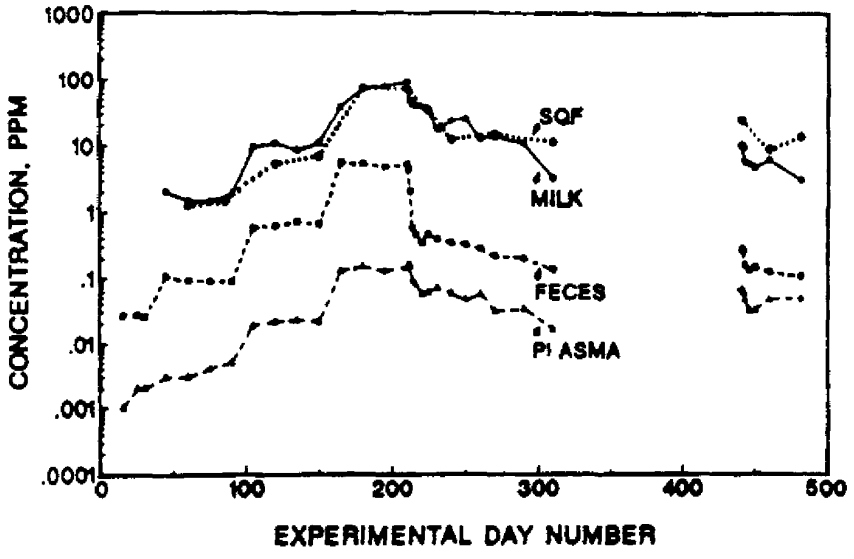

Figure 17. Mean concentrations of PCBs from Aroclor 1254 in subcutaneous fat, milk fat, feces, and blood plasma of cows orally dosed with 10, 100, and 1,000 mg/day in sequential 60 day periods.

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The behavior of the residue concentrations following the end of dosing has confirmed many of our previously conducted short-term studies. With passage of daily doses of PCB from the gut, concentrations in feces, blood, and milk decline rapidly. Feces becomes a minor excretory pathway when compared to milk, and PCB concentrations in milk fat approximate those in adipose tissue. At the onset of subsequent lactations, residue concentrations appeared elevated as compared to those at the end of the previous lactation. This is known to be a very dynamic metabolic period for cows; however, the mechanisms by which PCB kinetics were altered is yet unknown.

Polychlorinated biphenyls were distributed through the tissues and organs of the body and had a strong predilection for lipids. Table 24 gives some examples of quantities of PCB present in organs 250 to 300 days after dosing for cows and approximately day 42 for calves.

Necropsies of Cows and Calves. The necropsies of the PCB dosed cows and their calves revealed no gross or microscopic lesions suggestive of harm from PCB. The weights of major organs are listed in table 24. The majority of all organs and tissues were normal with some conditions observed that could be related to experimental procedures or other disease process. Some examples include scars and adhesions associated with liver biopsy and left displaced abomasum

MONS 020928

TABLE 24. AVERAGE ORGAN WEIGHTS AND CONCENTRATIONS OF POLYCHLORINATED BIPHENYLS IN TISSUES OF PCB DOSED COWS AND CALVES

	Cows		Calves	
Tissue	Organ weight (g)	PCB content Tissue basis (ug/g)	Organ weight(g)	PCB content Tissue basis (ug/g)
Liver	10731.88	.483	1485.46	1.031
Kidney	1846.78	.150	344.18	.330
Perirenal fat		15.790		24.118
Adrenal	43.93	.850	6.75	.883
Spleen	1102.10	.125	175.56	.113
Small intestine		.121		.155
Myocardium	2641.34	.328	454.23	.416
Lung	5391.57	.417	832.52	.301
Thymus		4.599		.148
Thyroid	35.89	3.636	10.75	.702
Skeletal muscle		.470		.106
Femur marrow		14.775		16.060
Sub-Q-Fat		5.230		.227
Ovary/Testis	25.38	.083	24.28	.141
Bile		.082		.115
CSF		.012		.008
Mammary Gland		1.601		5.892
Brain	459.03	.206	317.28	.408

MONS 020929

surgery, pneumonia and acute mastitis (2974), and blocked oviduct (2973). The necropsy of control cow 2975 revealed pericarditis possibly resulting from an old traumatic gastritis.

CONCLUSIONS

Research on polychlorinated biphenyl contamination of dairy cattle began at the Ohio Agricultural Research and Development Center in 1971 and has been ongoing to this date. Through this period of time, studies have been conducted with commercial and research herds that were exposed to PCBs via their food supplies. Other studies were conducted where cows were intentionally dosed with known quantities of PCBs for varying durations to determine the distribution and clearance of residues. Through all of these studies, health and productivity records and/or observations were made.

The sum total of observations from the aforementioned studies with cows exposed to PCBs indicated that these halogenated hydrocarbons were not toxic nor did they impair productivity. The highest exposure, based on concentrations of PCB in milk fat, was approximately 16 ppm for environmentally exposed cows to 100 ppm in short-term exposure animals. However, since none of these studies was designed to specifically obtain health and productivity information, they have not been considered conclusive.

MONS 020930

The study represented in this report had a limited number of animals, but intensively investigated many aspects of health and productivity. The design was such that animals were dosed for 60 days, with amounts of PCBs that produced residue concentrations near 1.5 ppm, the Federal tolerance. Dosing was increased so that animals received amounts of PCB similar to those often encountered by animals fed from a silo that had been coated with a PCB-containing coating. Then the dosing was increased another 10-fold so that concentrations in milk fat approached 100 ppm. The total duration of dosing was 180 days and spanned many of the important productive and reproductive stages of the normal lactation cycle. The studies were continued to nearly 500 days to determine if there were either adverse effects on offspring or delayed toxicity.

This study has clearly pointed out that polychlorinated biphenyls from Aroclor 1254 do not impair the production of milk either during exposure, following withdrawal, or in a subsequent lactation. Cows fed PCBs did not have unusual health problems, were not more susceptible to normal infections, nor did they have altered chemical composition of blood. Cows fed doses as high as one gram of PCB per day conceived and carried to term normal calves which were healthy and grew properly until the experiment was terminated.

MONS 020931

The residue concentrations found in adipose tissues, milk fat, and blood plasma were all reliable estimates of daily PCB intake. Many of the changes of residue concentrations confirm our previously published reports on the declines postdosing, changes with stage of lactation, the effects of weight gain and loss, plus dramatic rises in concentrations associated with parturition.

This report does not contain completed data from all aspects of the study. Analyses of portions of the data from this study are still in progress and will for many months in the future. Three abstracts of portions of the study have been published or submitted for publication (Attachments 1, 2, and 3). Journal articles reporting the various aspects of the study will be forthcoming.

MONS 020932

ATTACHMENT 1

Willett, L. B., H. I. Durst, and T-T. Y. Liu. 1985. Responses of lactating dairy cows to polychlorinated biphenyls. The Toxicologist 5(1):64.

255 RESPONSES OF LACTATING DAIRY COWS TO POLYCHLORINATED BIPHENYLS. L.B. Willett, H.I. Durst, and T.-T. Y. Liu. Ohio State Univ./Ohio Agricultural Research & Development Center, Wooster, OH

Polychlorinated biphenyls (PCB) have entered the feed of some dairy cattle over the last 40 years. Farm silos, coated with a material that contained PCB, have been a major source. Four lactating Holstein cows (mean body weight = 576 kg) were orally dosed with 10, 100 and 1,000 mg/day of Aroclor 1254 in sequential 60-day periods. Six control cows received daily sham doses. The following were recorded: (daily) milk production, feed intake, health observations; (weekly) body weight, heart and respiretory rates; plus 12 determinations of milk PCB residue and blood clinical chemistry (22 components) from predose through the end of dosing. Each cow had a liver biopsy and sulfobromophthalein (BSP) clearance predose and at the end of each dosing period. Average 305-day lactations were not significantly different between control (6,822 kg) and PCB-dosed (7,545 kg) animals. Clinical signs indicating toxicosis were not evident, mean clinical chemistries were within normal range, and liver histologies and BSP clearance were not different for dosed and control cows. Average PCB residue concentrations in milk fat at the end of the three dosing periods were 2, 13, and 91 µg/g, respectively. PCB exposure, resulting in milk fat residues 10 times those typically encountered, had no apparent effect on health and productivity. (Supported in part by Monsanto Contract No. RF 715618.)

ATTACHMENT 2

Willett, L. B., T-T. Y. Liu, H. I. Durst, K. L. Smith, D. R. Redman, and P. D. Moorhead. 1985. Productivity and health of dairy cows fed polychlorinated biphenyls. J. Anim. Sci. 61 (Suppl. 1):360.

375 Productivity and health of dairy cows fed polychlorinated biphenyls. L. B. Willett*, T.-T. Y. Liu, H. I. Durst, K. L. Smith, D. R. Redman, and P. D. Moorhead. Ohio State University/Ohio Agricultural Research and Development Center, Wooster

Lactating Holstein cows (n=4) were studied through a complete lactation for overt and subtle responses to consecutive 60-day periods of daily dosing with 10, 100, and 1,000 mg of Aroclor 1254, a commercial mixture of polychlorinated biphenyls (PCB). Control cows (n=4) received daily sham doses. The following were recorded: (daily) milk production, feed intake, health observations; (weekly) body weight and temperature, heart and respiratory rate, rectal palpation; (monthly) milk fat, microbiological culture of quarter (ore-milk samples and composite milk somatic cell counts; plus 3 determinations of blood cell counts and clinical chemistry. Liver biopsies and sulfobromophthalein (BSP) clearance tests were conducted predose and at the end of each dosing period. Mean daily milk production (22.4 ± 1.1 vs 24.8 ± 1.0 kg) and net energy of lactation (1.459 ± .042 vs 1.446 ± .052 Mcal/kg DM1) were not different (P>.35) for control vs PCB-dosed cows. Means of clinical chemistry were within the normal range. Liver histology and BSP clearance were not different among control and dosed cows. Occurrences of injuries, dysfunctions and general infections were not related to PCB exposure. Six cases of clinical mastitis were detected in each group with 30 and 20 cultured infections, respectively, for control and dosed groups. Environmental pathogens were most frequently isolated from cases of clinical mastitis. The majority of quarter infections detected were due to Corynebacterium bovis. Only one animal (dosed, necropsy revealed left oviduct obstructed) failed to conceive with services per conception of 4.0 and 4.7 for control and dosed groups. PCB exposure, resulting in residues in milk fat near 100 µg/g had no apparent effect on health and productivity.

KEY WORDS: Polychlorinated Biphenyls, Toxicity, Dairy Cattle.

MONS 020934

ATTACHMENT 3

Willett, L. B., H. I. Durst, and T-T. Y. Liu. 1986. Concentration of polychlorinated biphenyls in lactating cows and their calves. The Toxicologist 6(1):318.

CONCENTRATIONS OF POLYCHLORINATED BIPHENYLS IN LACTATING COWS AND THEIR CALVES. L. B. Willett, H. I. Durst, and T-T. Y. Liu. Ohio State University/Ohio Agricultural Research and Development Center, Wooster, Ohio 44691

Polychlorinated biphenyls (PCB) occasionally enter the feed supplies of lactating dairy cattle. It is important to understand the distribution of residues in animals that produce and are used for human food. Four Holstein cows were orally dosed with 10, 100, and 1,000 mg/day of Aroclor 1254 in sequential 60-day periods. Average concentrations of PCB (µg/g) in blood plasma (BP), milk fat (MF), and adipose tissue (A), at the end of the dosing periods were: 10 mg/day BP=0.005, MF=1.9, and A=1.4; 100 mg/day BP=0.02, MF=10, and A=6.9; and 1,000 mg/day BP=0.14, MF=91, and A=70. Cows were maintained through 40 days of the subsequent lactation which was between 250 and 300 days after the last PCB dose. The cows, plus their calves which were conceived during dosing and fed milk from their dams, were necropsied after 40 days post-partum. Concentrations of PCB (µg/g) in cows averaged BP=0.05, A=17.7, liver (L)=0.30, kidney (K)=0.11, and brain (B)=0.18; and in calves BP=0.08, A=26.3, L=1.03, K=0.33, and B=0.41. High concentrations of PCB had no apparent adverse effect on health or productivity.
(Supported in part by Monsanto Contract No. RF7156681.)