RESPONSES OF LACTATING DAIRY COWS

TO POLYCHLORINATED BIPHENYLS

PHASE II
(EXPANDED)

Submitted to:

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by

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INTRODUCTION

In September of 1983 a study to determine the specific responses of dairy cows to polychlorinated biphenyls was initiated. The specific objectives of this research were to: 1) determine if polychlorinated biphenyls (Aroclor 1254) affected performance and production efficiency, clinical health, biochemical responses and alterations in target organs, hematology, fertility and their interactions of dairy cows when fed up to 1,000 mg per day; and 2) relate the responses (if any) attributed to intake of polychlorinated biphenyls to exposure and body burdens of PCB residues.

After the cows had completed the initial 210-day study period, the program was expanded into Phase II which allowed continuation of study of the PCB and control cows through the end of normal lactation and subsequent parturition. The specific objectives of Phase II were: 1) determine the effects of 180-day exposure of cows to PCB (up to 1,000 mg per day) on complete lactation milk production; 2) determine the pharmacokinetics of PCB following cessation of long-term exposure; 3) monitor responses of PCB-dosed animals through an entire reproductive cycle; and 4) determine the viability and health of offspring from PCB-dosed animals and maintain for possible future study.

By mid-October of 1984 the original objectives had been addressed as well as the first two objectives of Phase II. The protocol established for Phase II would supply some limited information to accomplish objectives 3 and 4; however, expansion of this protocol will allow us to study dairy cows which have substantial body burdens of PCB during the postpartum and early

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lactation periods. Further, survival and growth of calves can be established through the most critical phase of the development period, from birth until weaning.

Necropsies of animals, at the end of the 42-day study period, will provide new information on the distribution of PCB residues in cows and calves. Further, gross and histological evaluations will verify the presence or absence of lesions that may be related to PCB consumption.

OBJECTIVES OF PHASE II (EXPANDED)

As previously stated, the objectives of the expansion of Phase II are identical to your objectives of Phase II Protocol. This expansion will enhance the information to accomplish objectives 2, 3 and 4. To reiterate, the objectives are:

1) determine the effects of 180-day exposure of cows to PCB (up to 1,000 mg/day) on complete lactation production of milk;
2) determine the pharmacokinetics of PCB following cessation of long-term exposure;
3) monitor health responses of PCB-dosed animals through an entire reproductive cycle;
4) determine the viability and health of offspring from PCB-dosed animals and maintain for possible future study.

EXPERIMENTAL DESIGN

Specific protocol have been submitted and accepted to study the responses of lactating dairy cows to polychlorinated biphenyls for study periods through 180 days of dosing (PCB-R) and from last dosing through the sixth day of the subsequent parturition (PCB-F). This protocol for the expanded portion of the protocol will

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commence at the time of parturition through the 42 day postpartum. This protocol replaces that portion of the previously submitted protocol between parturition and day 6.

Table 1 lists the dates that experimental animals will have completed their dosings and lactations, plus indicates the dates of expected parturition. From the termination date of each lactation, animals have been reared on pasture with nutritional supplementation when necessary. It is anticipated that the animals will continue to be pasture-reared until the first part of November, weather permitting. During this pasture period animals have been checked daily but body weight, feed intake and specific scored health records have not been maintained. All observed injuries or abnormal conditions have been recorded. This procedure will continue until parturition. Parturitions will occur in the maternity or physiology section of the Dairy Research Unit.

Beginning at parturition, these studies will be referred to as PCB-C and intensive measurements of health and performance will resume. Table 2 is a time table of measurements to determine the responses of cows and the calves to PCBs. Table 3 lists the days when samples are scheduled for clinical chemistry, liver function and residue analyses for dosed and control cows plus the calves.

Cows will be closely monitored near the time of expected parturition. Attempts will be made to have all parturitions attended; however, this is not always possible or practical. Collection of day 1 samples will be made as close to the time of parturition as practical.

Calves will remain with their dams to receive an adequate supply of colostrum by nursing, then will be transferred to a

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Table 1

DATES THAT PCB-DOSED AND CONTROL COWS COMPLETE DOSINGS AND LACTATIONS AND EXPECTED DATES OF PARTURITION

Cow Number	Last Dosing	Last Milking	Parturition
2970	4-25-84	6-25-84	12-07-84
2973	5-08-84	7-02-84	N Px
2974	4-25-84	7-09-84	12-24-84
2722	5-22-84	8-03-84	01-26-85
2975	5-01-84	8-06-84	04-09-85
2976	5-01-84	8-10-84	11-12-84
2977	6-19-84	8-13-84	11-02-84
2978	5-22-84	8-20-84	03-25-85
2979	5-22-84	8-29-84	01-21-85
2971	6-19-84	9-03-84	12-20-84

N Px = Not pregnant.

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Table 2

TIMETABLE OF MEASUREMENTS TO DETERMINE THE RESPONSES OF COWS AND CALVES TO PCB (PCB-C)

Measurement	Dosed Cows	Control Cows	Calves
Feed intake	Daily	Daily	Daily
Milk production	Daily	Daily	
Health observations	Daily	Daily	Daily
Body weight[a]	Weekly	Weekly	Weekly
Vital signs[b]	Weekly	Weekly	Weekly
Palpation	Weekly	Weekly	
Skeletal growth[a]			Weekly
Photograph[a]	Semi-monthly[c]	Semi-monthly[c]	Semi-monthly[d]
Mastitis check[e]	Scheduled	Scheduled	

[a] These observations are also made on experimental day 42.
[b] Body temp., heart rate, and respiration rate.
[c] Conducted during the weeks with Mondays nearest the 1st and 15th of the month.
[d] Conducted during the weeks with Wednesdays nearest the 1st and 15th of the month.
[e] Conducted at parturition and days 3, 7, 14, 30, and 40 thereafter (± 1 day).

Table 3

DAYS WHEN SAMPLES ARE SCHEDULED FOR CHEMICAL CHEMISTRY, LIVER FUNCTION AND RESIDUE ANALYSIS (PCB-C)

Day	Clinical Chemistry	Liver Function	Fat	Blood	Feces	Milk
			Dosed Cows			
1[a]	X		X	X	X	X
2				X	X	X
3				X	X	X
6				X	X	X
10[b]				X	X	X
20[b]			X	X	X	X
42[b]	X	X	X	X	X	X
			Control Cows			
1[a]	X		X	X	X	X
10[b]				X	X	X
42[b]	X	X	X	X	X	X
			Calves			
1[a]				X	X	
10[b]				X	X	
42[b]	X	X		X	X	

[a] Day one equals parturition of cows and day of birth for calves. Since parturition sometimes occurs during nighttime hours, parturition and birth samples will be collected within 16 hours.

[b] Collected ± 2 days to avoid weekends and holidays.

nursery area. Calves from dosed cows will receive milk containing PCB from their dam, whereas control calves will receive milk free of PCB. The calf feeding program will be designed to have them weaned from milk and entirely on solid feed by 42 days of age. Thus, the ration will be principally milk during the first few weeks with the gradual addition of a high-protein grain supplement and small amounts of hay roughage.

Postparturition cows will be transferred to comfort tie stalls. Daily feeding and management of the lactating cows will be similar to that outlined in the original proposal. Daily evaluation sheets will be utilized as they have in previous phases of this study.

At the end of 42 days postparturition, cows and calves that were exposed to PCB will be necropsied by Drs. P. D. Moorhead and/or R. F. Cross, OARDC Pathologists. Removal and disposal of animal carcasses will be responsibility of the sponsor. Table 4 is a modification of the OARDC necropsy check list indicating tissues to be weighed and samples to be collected for residue analyses and histology. The pathologist will make a gross examination of all organ systems and those selected for histological evaluation will be fixed in neutral buffered 10% formalin, processed for parafin embedding, sectioned at 6 μm and stained with azure and eosin. Organs and tissues will be evaluated as normal or abnormal based on the pathologists' extensive experience with bovines. Abnormal conditions (if present) will be described, and if necessary, compared to organs and tissues from necropsied control animals. Sections for residue analysis will be stored frozen until extraction and gas chromatography analysis utilizing ongoing

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Table 4

AN OARDC NECROPSY CHECK LIST INDICATING ORGANS TO BE WEIGHED AND SAMPLES TO BE COLLECTED FOR RESIDUE ANALYSIS AND HISTOLOGY

NECROPSY √-LIST COW NO.

DATE CAUSE OF DEATH EXP. DAY

ORGAN OR TISSUE	NUMBER	ORGAN WEIGHT	RESIDUE ANALYSIS	Check List HISTOLOGY	EM
Liver	101	X	X	X	
Kidney	102	X	X	X	
Peri-renal fat	103		X		
Adrenals	104	X	X	X	
Spleen	105	X	X	X	
Pancreas	106			X	
Small intestine	107		X	X	
Rumen	108			X	
Tongue	109				
Myocardium	110	X	X	X	
Lung	111	X	X	X	
Thymus	112		X	X	
Lymph nodes	113			X	
Thyroid	114	X	X	X	
Sk. muscle	115		X	X	
Spinal cord	116				
Sternum marrow	117				
Femur marrow	118		X	X	
Pericardial fat	119				
Omental fat	120				
Sub-Q-Fat	121		X		
Bladder	122			X	
Ovaries	123	X	X	X	
Uterus	124			X	
Fetus	125				
Eyelid	126			X	
Bile	127		X		
Rumen contents	128				
CSF	129		X		
Synovial	130				
Amniotic/Allantoic	131/132				
Trachea/Esophagus	133/141				
Blood	134				
Mammary gland	135		X	X	
Parotid gland	136			X	
Pituitary	137			X	
Brain	138	X	X	X	
Gall Bladder	140			X	
Colon	145			X	

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procedures. In the event of death of any animal prior to the scheduled 42 days postpartum, complete necropsies will be performed as described.

BUDGET

The proposed research will require technical assistance for the care of experimental animals, health observations, sample collection, analysis of PCB residue, assistance with necropsies, and statistical summarization of data.

PHASE II - EXPANDED BUDGET

Personnel		
Research Assistant 2 B/H, (50%) x 4 mo. T.-T. Liu	$ 2,880	
Research Assistant 1 B/H, 4 mo. R. Wachtel	4,800	
Total Salaries	$ 7,680	
Fringe Benefits:		
Retirement @ 13.71%	$ 1,053	
Insurance @ 175/man/mo	1,400	
Total Fringe Benefits	$ 2,453	
Total Research Assistants	10,133	
Hourly Assistants	1,200	
Total Personnel	$11,333	
Supplies and Reagents	$15,300	
Travel	800	
Total Personnel and Research Materials*		$27,433
Research Services @ 45% of Personnel and Research Materials		12,345
Total Budget		$39,778

*The sponsor will dispose of dosed animals and their offspring.

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We intend to utilize personnel already trained for various aspects of this project to continue the research. We propose to continue the appointment of R. Wachtel (special animal handling and residue analyses) and reestablish the appointment of C. Jordan to handle the daily management of the animals. Dr. T.-T. Liu will continue with data analyses and assistance with cytochrome analyses. Some hourly personnel will be required during and following the parturition of cows. Travel allocation is for presentation of results at the Society of Toxicology Annual Meeting.

TIME TABLE

The research in this expansion of Phase II begins effectively with the parturition of each cow (see Table 1). Since considerably more information and samples requiring analysis will be collected, the termination of the date of this project must be revised from July 1, 1985, to November 1, 1985. The latter is the proposed termination date for Phase II (Expanded).