AN ABSTRACT OF THE THESIS OF

Jill Annette Meyer Youngberg for the degree of Master of Science in Veterinary Science presented on August 20, 1991.

Title: The Effect of 3,4,5,3',4',5'-Hexachlorobiphenyl on Plasma Corticosterone and Prolactin Concentrations in the Mouse

Abstract Approved: Redacted for Privacy

(Bradford B. Smith)

It was hypothesized that alterations in plasma concentrations of corticosterone (CS) and prolactin (PRL) may be at least partially responsible for polychlorinated biphenyl (PCB)-induced immunosuppression.

A 2 by 2 factorial experiment examined the interactions of PCB and P815, an allogeneic tumor, on plasma concentrations of CS and PRL, and on body, spleen, and thymus weights. The PCB dosage used (10 mg/kg) was previously shown to suppress immune response to the tumor. The four study groups were: Group A (vehicle control), Group B (tumor only), Group C (PCB only), and Group D (tumor plus PCB). Mice received one dose of PCB (Groups C and D) or carrier (Groups A and B) on day -1; tumor (Groups B and D) or carrier (Groups A and C) was injected intraperitoneally on day 0. In Experiment 1, animals were killed on days -1, -0.6, 0 through 10, 21, 42, and 84. Body, spleen, and thymus weights were measured. Plasma samples were obtained for CS and PRL measurements. In Experiment 2, the study was

repeated with samples obtained only on days 3 and 10.

Group A body weights increased steadily throughout Experiment 1. Relative to Group A, the weight gain in Group B was significantly ($p < 0.05$) higher. Group C lost weight on days 0 through 6, and gained significantly ($p < 0.05$) less weight than Group A. Group D gained significantly ($p < 0.05$) less weight than Groups A, B, and C.

As a percent of body weight, spleen weight remained constant over 21 days in Experiment 1 in both Groups A and D. Compared to Group A, Group B showed significantly ($p < 0.05$) increased spleen percent body weight while Group C showed significantly ($p < 0.05$) decreased spleen percent body weight.

As a percent of body weight, thymus weight remained constant for 21 days in Experiment 1 in Group A. Groups B and C were similar ($p > 0.05$) and showed a decreased thymus percent body weight compared to Group A. Group D showed significantly ($p < 0.05$) decreased thymus percent body weight relative to the other three groups.

Mean CS concentrations in Experiment 1 in Groups A and B were similar ($p < 0.05$). Relative to Groups A and B, Group C CS concentrations were elevated, with a peak of 126.1 ng/ml on day 4. Group D CS concentrations were higher than the other three groups, peaking at 294.1 ng/ml on day 10.

There was no significant difference in PRL concentrations in Groups A, B, and C in Experiment 1 ($p > 0.05$). Mean PRL concentration in Group D was significantly ($p < 0.05$) lower than in the other three groups.

The results of Experiment 2 validated those of Experiment 1. Although absolute values differed, the pattern of changes seen in body and organ

weights and in CS and PRL concentrations was similar.

An acute exposure to PCB and tumor resulted in an increase in circulating CS concentration and a decrease in circulating PRL concentrations. These changes may contribute to PCB-induced immunosuppressioin.

The Effect of 3,4,5,3',4',5'-Hexachlorobiphenyl on Plasma Corticosterone and Prolactin Concentration in the Mouse

by

Jill Annette Meyer Youngberg

A THESIS

Submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed August 20, 1991

Commencement June 1992

APPROVED:

Redacted for Privacy

Associate Professor of Veterinary Medicine in charge of major

Redacted for Privacy

Associate Dean, College of Veterinary Medicine

Redacted for Privacy

Dean of Graduate School

Date thesis is presented August 20, 1991

Typed by Suzi Schamp for Jill Annette Meyer Youngberg

ACKNOWLEDGEMENTS

The assistance of my committee is greatly appreciated. Dr. Peter Burke, graduate representative, was very supportive. Drs. Brad Smith, Nancy Kerkvliet, and Donald Mattson began as faculty and became, over time, colleagues and friends.

Several faculty, staff, and other graduate students gave assistance, without which this project would have taken much longer. They are Nancy Hollingshead, Carol Forsyth, Gil LeCren, Abbie Moos, Pamela Reed, Linda Steppan, Julie Oughten, and Greg DeKrey.

Several good friends and relatives were a great source of moral support: Dan Youngberg, Penny Snyder, Nancy Hollingshead, Si and Elsie Meyer, Shirley Spidle, Mary Kay Schuette, and, especially, Brad Smith.

My heartfelt gratitude goes to all of them.

TABLE OF CONTENTS

INTRODUCTION	1
LITERATURE REVIEW	3
MATERIALS AND METHODS	11
RESULTS	16
DISCUSSION	34
BIBLIOGRAPHY	50
APPENDIX A: PROCEDURES	54
APPENDIX B: DATA TABLES	94

LIST OF FIGURES

Figure		Page
1.	Experimental Design	12
2.	Weight Change as a Function of Time	17
3.	Spleen Weight as a Percent of Body Weight as a Function of Time	21
4.	Thymus Weight as a Percent of Body Weight as a Function of Time	23
5.	Corticosterone Concentration as a Function of Time for Group A and Group B	25
6.	Corticosterone Concentration as a Function of Time for Group A and Group C	26
7.	Corticosterone Concentration as a Function of Time for Group A and Group D	27
8.	Corticosterone Concentration as a Function of Time for Group B and Group D	28
9.	Corticosterone Concentration as a Function of Time for Group C and Group D	29
10.	Prolactin Concentration as a Function of Time for Group A, Group B, Group C, and Group D	32
11.	Prolactin Concentration (Logarithmic scale) as a Function of Time for Group A, Group B, Group C, and Group D	33

LIST OF TABLES

Table		Page
1.	Mortality Table	18
2.	Weight Data Comparison, Experiments 1 and 2	19
3.	Hormone Data Comparison, Experiments 1 and 2	30
4.	Weight Data, Experiment 1: Summary	96
5.	Weight Data, Experiment 1: Edited Raw Data	100
6.	Weight Data, Experiment 2: Edited Raw Data	113
7.	Corticosterone Data, Experiment 1: Summary	116
8.	Prolactin Data, Experiment 1: Summary	118
9.	Hormone Data, Experiment 1: Edited Raw Data	121
10.	Hormone Data, Experiment 2: Edited Raw Data	134

THE EFFECT OF 3,4,5,3',4',5'-HEXACHLOROBIPHENYL ON PLASMA CORTICOSTERONE AND PROLACTIN CONCENTRATIONS IN THE MOUSE

INTRODUCTION

In C57Bl/6J mice challenged with P815, an allogeneic tumor, the major immune response is generation of alloantigen specific cytotoxic T lymphocytes (CTL), leading to total clearance of the tumor. Previous studies (Kerkvliet 1988a) have shown that this response was suppressed by an acute exposure to PCB. When the PCB was added directly to **in vitro** mixed lymphocyte cultures, however, CTL response was not suppressed (Kerkvliet 1988b). This suggested an indirect effect of PCB on the CTL response. The dosage of PCB needed to suppress CTL in these mice also resulted in thymic involution (Kerkvliet 1988a), a hallmark of both PCB toxicity and of elevated concentrations of corticosterone (CS). When CS concentrations were measured, they were found to be elevated (Kerkvliet 1990). Elevated concentrations of glucocorticoids (GC) have been shown to inhibit CTL response (Gillis 1979, Schleimer 1984). In some species, an elevation of GC concentration has been correlated with a decrease in the concentration of prolactin (PRL). Elevated concentrations of GC and decreased concentrations of PRL have both been associated with immunosuppression (Griffin and Ojeda 1988, Jones 1987).

It was hypothesized that altered concentrations of CS and/or PRL may be at least partially responsible for PCB-induced immunosuppression. The objective

of this study was to clarify the temporal changes in plasma concentrations of CS and PRL following acute exposure to a known immunosuppressive dose of PCB and challenge with P815 tumor. This data may provide the basis for future experiments examining the relationship between these hormonal changes and CTL suppression.

LITERATURE REVIEW

Halogenated aromatic hydrocarbons (HAHs) have been widely used in industry as organic solvents, flame retardants, dielectric fluids, hydraulic fluids, lubricants, insulators, and plasticizers. Chemicals which comprise this group include the polychlorinated biphenyls (PCBs), (industrial heat transfer agents in electrical transformers and capacitors), polychlorinated naphthylenes (PCNs), polychlorinated terphenyls (PCTs), hexachlorobenzene (HxCB), and the polybrominated biphenyls (PBBs) (chemical fire retardants) (Safe 1982, Raloff 1989).

Additional members of this class are not primary industrial chemicals, but rather are formed as by-products in the commercial preparation of PCBs, PCNs, and chlorinated phenol-derived products and in the synthesis and biodegradation of chlorinated anilines and their derived pesticides. Included in this group are the polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), and polychlorinated azo- and azoxy-benzenes. PCDDs, PCDFs, PCBs, and HCB are also formed during the combustion of some organic chlorine-containing wastes (Safe 1982). Likewise, PCDDs and PCDFs are produced during the manufacture of bleached paper products (Raloff 1989).

PCBs, PCTs, PBBs, and HCB have been widely identified as contaminants of air and water, and of domestic animals, fish, wildlife, and humans. PCDFs and PCDDs have been identified in both environmental

samples and in occupationally exposed individuals (Safe 1982). The concentrations of PCDDs and PCDFs in the environment have increased in recent decades, as evidenced by analysis of dated sediments from Lake Huron (Byard 1987).

From 1870 to 1930, low levels of PCDDs and PCDFs were present in the environment, presumably due to combustion of wood and coal. An increase began in 1930 that parallels the production of synthetic chlorinated hydrocarbons (Byard 1987). Today, incineration of waste material is thought to be the major source of these chemicals in the environment, with high temperature combustion of hydrocarbons and chlorinated additives in automobile engines suggested as other sources (Byard 1987). The release of PCDDs and PCDFs into the air and soil is primarily by combustion processes and by the manufacture, utilization, and disposal of chlorinated phenols. These processes provide the background dermal and inhalation exposure for the general population (Byard 1987).

However, the major source of human exposure to HAHs appears to be the food chain (Byard 1987). As a class, these chemicals are highly lipophilic and resistant to chemical, thermal, and photochemical degradation. Consequently, they are persistent in the environment, accumulating in the fatty tissues of animals which may then become part of food chains (Safe 1982). They are biomagnified up the aquatic food chain to fish and then to predatory birds and humans. They may also enter the human diet by way of residues in animal feed that results in contaminated meat, milk, and egg (Byard 1987).

The toxic effects of HAHs are a result of the chemicals' ability to act as

a ligand for the cytosolic aromatic hydrocarbon (Ah) receptor (Silkworth 1982, Poland 1977, Silkworth 1984). Specific isomers have different binding affinities for the receptor, depending on the degree and nature of halogen substitution (Safe 1982, Poland 1977). The most potent HAH, 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD), also has the greatest affinity for the receptor. In general, the closer a compound resembles TCDD in shape (flat or coplanar aromatic rings), size (3A x 10A), halogen substitution (bromine or chlorine), and spatial orientation (3 lateral bromines or 4 lateral chlorines, one or two on each phenyl ring), the higher the affinity for the Ah receptor (Safe 1982). Among the PCBs, the most toxic isomers (3,4,3',4'-tetra, 3,4,5,3',4'-penta, and 3,4,5,3',4',5'-hexachlorobiphenyl) have chlorine substitutions in at least one meta and one para position of each phenyl ring and have no ortho-chloro substitutions. They are all approximate stereoisomers of 2,3,7,8-TCDD (Safe 1982). Many studies of HAH toxicity have been complicated by the use of mixtures of isomers, each with different receptor affinity and hence different potential to induce toxic effects.

A number of toxic effects of HAHs, both as individual compounds and as mixtures, have been reported in the literature. These include a wasting syndrome (characterized by weight loss and decreased food intake), skin disorders (chloracne, alopecia, edema, hyperkeratosis, hypertrophy of Meibomian glands), thymic involution and atrophy, porphyria, endocrine disorders, modulation of chemical carcinogenesis, immune suppression, and the induction of several enzyme systems, including the cytochrome P-448 (or 450)-dependent monooxygenases (Safe 1982). For any specific HAH, the

observed toxicity depends on the age, sex, and species of experimental animal used.

The only effect consistently observed in humans with known exposure to TCDD is chloracne (Muzi 1987). Congenital poisoning of transplacentally exposed fetuses has been reported following an outbreak of PCB and PCDF-contaminated in cooking oil in Taiwan (Rogan 1988). The exposed children demonstrated small size, gum hypertrophy, hyperpigmentation, nail deformities, eyebrow flare, clinodactyly, a history of bronchitis, and developmental delay, when compared to age- and sex-matched neighborhood controls (Rogan 1988). Humans appear to be less susceptible to HAH toxicity than do other species, possibly due to the ingestion of less food per unit body mass, sequestration of these compounds in adipose tissue, away from target organs, and/or a lower tissue susceptibility (Byard 1987).

It has been shown that exposure to such HAHs as PCDD, PCB, PBB, and HCB can alter immune response in laboratory animals. Several investigators have shown evidence of suppression of both the humoral and cellular immune responses following treatment with PCBs, although the reports on the nature of the suppression have been conflicting (reviewed by Vos, 1980).

PCB-induced immunosuppression could result from either cytotoxicity to lymphocytes or other lymphoid tissues or from action through some other pathway. Despite much research, there is no firm evidence supporting cytotoxic effects of PCBs on the thymus or lymphocytes. One proposed alternative mechanism is immunosuppression due to PCB-induced alterations

of hormone concentrations.

A variety of hormones have been shown to be modulated by acute PCB exposure, including GCs, PRL, thyroxine, and the sex steroids (Sanders 1977, Jones 1987, Umbreit 1988). These hormones have all been shown to have significant immunomodulatory functions.

Elevated concentrations of GCs are known to suppress immune function in many species through a variety of mechanisms, including, but not limited to, stabilizing lysosomal membranes, decreasing capillary permeability, depressing phagocytosis, and suppressing T-lymphocytes (Griffinand Ojeda 1988). Gene regulation by direct GC receptor-GC receptor enhancer interaction with DNA is the primary mechanism of action of GCs and accounts for a number of immunomodulatory effects of this hormone. A second messenger system, however, also mediates a number of GC functions. The most important of these second messenger systems for the GCs is the family of phospholipase inhibiting proteins represented by the lipocortins. Lipocortin appears to play an important role in such immune system activities as glycosylation of the Fc receptors, natural killer cell function, and suppressor cell function (Gustaffson 1987).

A variety of species have shown a dramatic increase in circulating levels of GCs upon exposure to PCBs. This phenomenon may be in response to the hypoglycemia of the wasting syndrome, following acute PCB exposure in many species (Gorski 1988a). This increase in GCs, an important factor in the regulation of blood glucose concentrations during starvation, is probably protective, by increasing gluconeogenesis and decreasing peripheral utilization

of glucose. Rats adrenalectomized before PCB exposure die rapidly of hypoglycemic shock, but are protected by administration of exogenous CS, the main GC of rodents (Gorski 1988b). This protective response, however, may also be an indirect mediator of PCB-induced immunosuppression.

Several lines of evidence suggest that PRL should also be considered as a potential mediator of PCB-induced immunosuppression. This evidence falls into two main categories, specifically the interactions between GCs and PRL and the known immunomodulatory characteristics of PRL itself.

PRL and GCs appear to interact considerably. There seems to be a parallel **in vivo** modulation of liver PRL and GC receptors in the rat (Dave 1985), and there is some evidence that ACTH and PRL may share a common releasing factor in the anterior pituitary (Harms 1973). In rats, large dosages of dexamethasone suppressed the PRL response to handling (Euker 1975). Adrenalectomy increased the PRL response to ether stress, while dexamethasone given 4 hours before ether reduced the PRL response in a dose dependent manner (Harms 1975). Witorsch and Kitay have demonstrated that PRL can decrease the adrenal enzyme 5-a-reductase, thereby leading to increased concentrations of circulating GC in the rat (Witorsch 1972).

It has also been proposed that a major function of PRL is the regulation of adrenal cortical function. Adrenal glands of rats have large numbers of PRL receptors, and it has been suggested that PRL is important in the regulation of CS secretion in response to ACTH. Hypophysectomized rats have a decreased ability to secrete CS in response to ACTH. This effect can be

reversed following PRL administration (Jones 1987). GCs also have an interactive effect on PRL by their ability to regulate PRL binding to its receptor (Jones 1987) and to regulate release of PRL from the anterior pituitary (Harms 1973).

Low concentrations of PRL have been associated with immuno-suppression in mice and rats (Jones 1987), while high concentrations have been demonstrated to be immunostimulatory in humans (Rovensky 1991). Hypophysectomized rats show a humoral suppression that can be reversed by administration of PRL (Berczi 1981). Mice made hypoprolactinemic by administration of bromocryptine, a semisynthetic ergot alkaloid dopamine agonist, show T-cell immunosuppression and thymic atrophy which can be prevented by administration of exogenous PRL (Bernton 1988). Finally, cyclosporine, a fungal peptide which inhibits T-cell function, inhibits PRL binding to lymphocyte receptors by competitive inhibition (Hiestand 1986, Russell 1984). Humans made hyperprolactinemic within physiological limits by breast feeding or injection of dopamine blockers, dompiridone and chlorpromazine, demonstrate stimulation of both cell-mediated and nonspecific immunity (Rovensky 1991).

The relationship between one particular Ah receptor binding isomer, TCDD, and PRL concentrations has been studied in rats. Administration of TCDD produced a fall in PRL concentrations within 4 hours, followed by a hyperprolactinemic state in 7 days and abolition of normal PRL circadian rhythm (Jones 1987). Russell et al (1988) evaluated the effects of TCDD administration on the activity of the tuberoinfundibular nucleus in the

hypothalamus of rats and reported that this resulted in a two-fold elevation of dopamine turnover in the median eminence. Dopamine is generally considered to be the prolactin inhibiting substance in the hypothalamus, and the authors suggest a hypothalamic site of TCDD action. Also, PRL receptors have been demonstrated in lymphocyte-containing tissues in the rat and on human lymphocytes, and stimulation of these receptors has been shown to induce the expression of ornithine decarboxylase, the rate-limiting enzyme in polyamine biosynthesis and a marker of hormone mediated transmembrane signalling (Russell 1985). Clevenger (1991) demonstrated that nuclear PRL was necessary for the proliferation of cloned T lymphocytes in response to interleukin-2. His studies suggested that PRL can function in the nucleus without binding to its cell surface receptor.

Based on the above information, it was hypothesized that exposure to PCB would cause an increase in CS concentration and a decrease in PRL concentration. To study these changes over time, mice were given PCB, challenged with a tumor, and followed for 84 days. C57Bl/6J mice were chosen because they have high affinity Ah receptors (Poland 1977). Purified 3,4,5,3',4',5'-hexachlorobiphenyl was selected as the PCB isomer because it binds strongly to the Ah receptor (Safe 1982).

MATERIALS AND METHODS

Animals: Six-week-old C57Bl/6J (H-2b) intact male mice were purchased from Jackson Laboratories, Bar Harbor, Maine. Upon arrival, the animals were eartagged for identification, weighed, and randomly assigned, three to a polycarbonate shoebox cage (Experiment 1) or individually (Experiment 2), and cages were assigned to groups. Random number generation was used to determine which cages were used on which days. The animals were allowed to adjust to their new environment for three days before the experiment began. The animals were maintained at Laboratory Animal Resources in one room on a 12 hour light (fluorescent):dark cycle (0730 lights on). Room temperature was 72±2°F. Humidity was 40-60%. Food (Wayne Rodent Blox) and water were available *ad libitum*. One day prior to termination, the animals were moved to the dissection laboratory and left undisturbed overnight.

The animals were killed between 0930 and 1200 hours by cervical dislocation followed by immediate decapitation in Experiment 1 and by carbon dioxide overdose followed by cardiac puncture in Experiment 2. Spleen and thymus were dissected out, trimmed of fat, and weighed. Body weight was obtained after blood collection. Blood was collected into 10% ethylenediaminetetraacetate (EDTA), thoroughly mixed, and placed on ice until all samples were collected. The blood was centrifuged, and plasma was transferred into two labelled plastic microvials. The plasma was frozen at -20°C until analysis (see Appendix I, Procedure 1). In experiment 1, the CS assays were completed within two months of collection; the PRL assays were

completed within 15 months of collection. In Experiment 2, PRL and CC assays were run within three months of sample collection.

Experimental Design: A 2 x 2 factorial experimental design examined the effects and interactions of PCB and an allogeneic tumor. Animals were randomly assigned, 96 (Experiment 1) or 16 (Experiment 2) per group, to one of the following treatments:

Group A: vehicle controls

Group B: P815 tumor cells and PCB vehicle

Group C: P815 vehicle and PCB

Group D: P815 tumor cells and PCB

This is shown in a 2 x 2 matrix form as:

Figure 1: Experimental Design

		tumor (-)	tumor (+)
	(-)	A	B
PCB	(+)	C	D

On day -1 all animals received either PCB or PCB carrier (peanut oil) by gavage. On day 0 all animals received either P815 tumor cells or vehicle solution by intraperitoneal injection. Six (Experiment 1) animals from each group were killed on days -1, -.6, 0 to 10, 21, 42, and 84, except on days 42 and 84 when no animals from Group D remained alive. In Experiment 2, eight animals from each group were killed on days 3 and 10. Body weights, and

spleen and thymus weights were measured, and blood was collected for determination of CC and PRL concentrations.

Tumor: The P815 mastocytoma was maintained in ascites form by weekly intraperitoneal passage in a syngeneic host, female DBA/2J (H-2d) mice. See Procedures 2 and 3, Appendix 1 for preparation of cells for injection. Group B and D animals received 3×10^5 cells in suspension by intraperitoneal injection. Carrier solution was injected into Group A and C animals in the same manner. A different passage of the tumor was used for Experiments 1 and 2.

PCB: 3,4,5,3',4',5'-hexachlorobiphenyl (HxCB) (Ultra Scientific, Hope, RI) was prepared as outlined in Appendix I, Procedure 4. Group C and D animals received 0.1 ml HxCB (at a concentration of 1 mg/ml peanut oil) per 10 g body weight (10 mg/kg) by gavage, while Group A and B animals received 0.2 ml peanut oil by gavage.

Assays: Corticosterone: CS concentrations were measured using a commercial double antibody radioimmunoassay (RIA) kit (ICN Biomedicals, Inc., Carson, California), previously validated for use with rodent plasma by the manufacturer (see Appendix I, Procedure 5). High and low controls were included in the kit. An internal pool of mouse plasma was prepared and an aliquot analyzed with each assay. The interassay coefficients of variation (CVs) for Experiment 1 were 16.1% (low control), 7.3% (high control) and 7.1% (mouse pool). The intraassay CVs for Experiment 1 were 10.0% (low control),

5.5% (high control), and 5.4% (mouse pool). Only one assay was run for Experiment 2; the interassay CVs were 2.5% (low control) and 4.3% (high control).

Assays: Prolactin: PRL concentrations were measured using a double antibody RIA procedure. Mouse prolactin and rabbit antimouse prolactin were obtained from Dr. A. F. Parlow of the Pituitary Hormones and Antisera Center at Harbor-UCLA Medical Center in Torrence, California (see Appendix I, Procedures 6a and 6b). Two internal pools of mouse plasma were prepared and an aliquot was run with each assay. A male (low) and a female (high) pool were used. The interassay CVs for Experiment 1 were 17.6% (male) and 11.3% (female). The intraassay CVs for Experiment 1 were 11.2 % (male) and 6.5% (female). The interassay CV for Experiment 2 was 7.9% (male). The intraassay CV for Experiment 2 was 5.9% (male).

Statistical Analysis: Statistical analyses was performed by Jim Pratt, Department of Statistics, Oregon State University, under the direction of Dr. Lyle Calvin, using SAS software for the general linear models procedure. An analysis of variance (ANOVA, type III sums of squares option, due to imbalance) was performed on each data set (body weight, organ weights, CC concentration, and PRL concentration) to test the effects of group. There was no indication that the imbalance in sample size was caused by the treatment. An analysis of residual plots confirmed the assumption of constant variance in the data sets. The two sample t-test method with unequal sample sizes was

used to compare specific values. A p value of < 0.05 was considered significant for all determinations.

RESULTS

Body Weight (See Appendix II, Tables 4, 5, and 6 for raw data):
In Experiment 1, the change in body weight from time of initial weighing to time of death was significantly different in each of the four treatment groups (Figure 2). Untreated control animals gained weight throughout the experiment. From day 5 to day 10, animals that received tumor alone showed a greater weight gain than animals in the other three groups. Animals exposed to PCB alone lost weight on days 0 to 6, and gained less weight than the untreated control animals throughout the experiment. Animals that received both PCB and tumor were similar to untreated control animals on days 0 to 7 and then lost weight from day 9 until day 21. No animals in this group survived until day 42. Information on the animals' deaths is summarized in Mortality Table, Table 1. Similar patterns of weight change were observed among the four groups in Experiment 2 (see Table 2).

Spleen Weight (See Appendix II, Tables 4, 5, and 6 for raw data):
Splenic weight (SW) was evaluated as a percentage of body weight to offset the effect of decreased body weight seen in Group C and D animals and provided a rough indicator of immune stimulation. In Experiment 1, SW remained constant over 21 days in the untreated control animals (see Figure 3). Animals that received tumor alone showed a increased SW on days 6 to 10, then returned to a value similar to untreated control animals on day 21.

Figure 2: Weight change as a function of time for Group A (control o), Group B (tumor ●), Group C (PCB▽), and Group D (tumor plus PCB▼) from day 0 to day 84. Plotted as mean plus standard error.

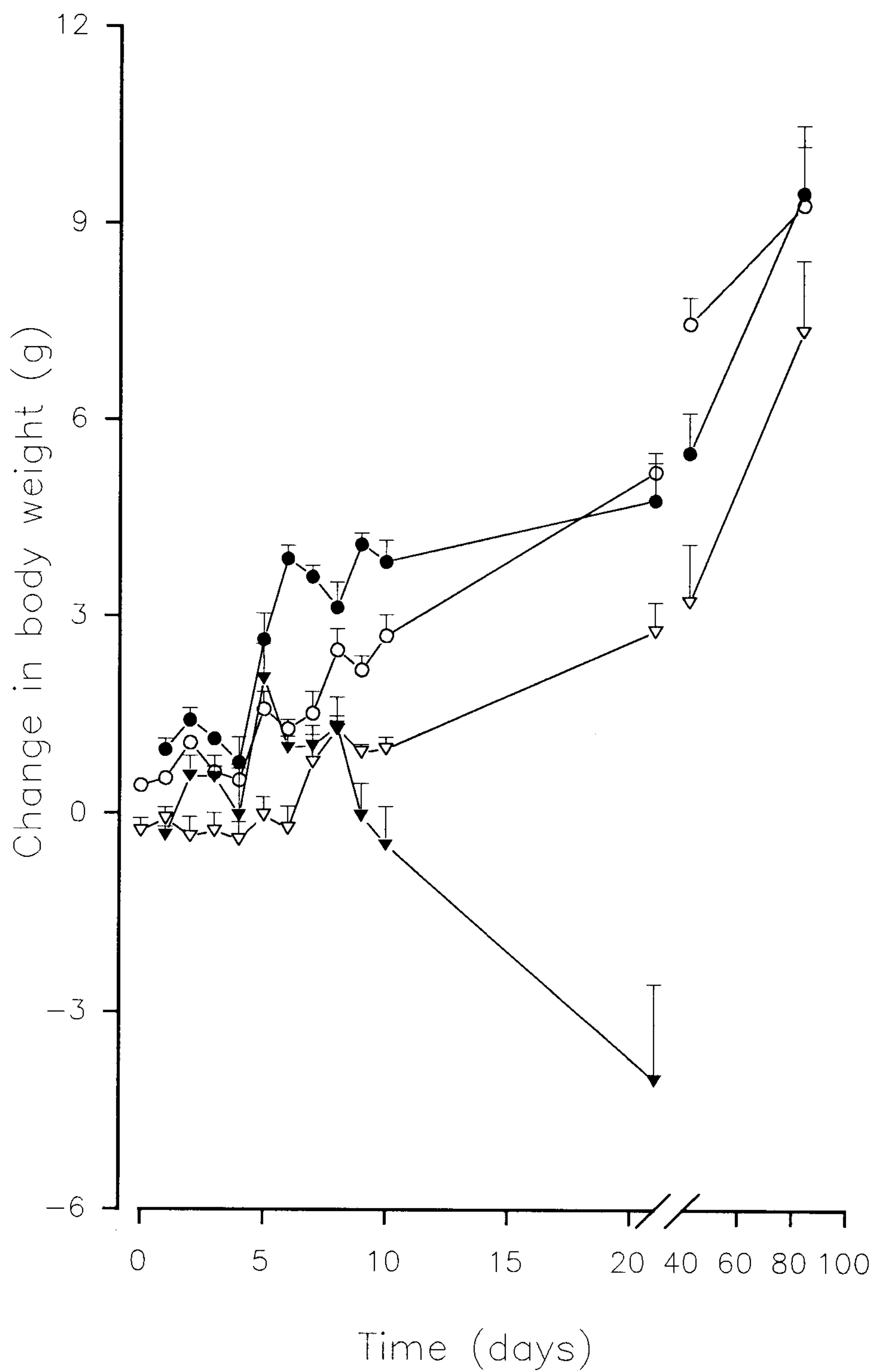


TABLE 1

MORTALITY TABLE

Date	Day	Animal	Cage	Necropsy
5/08/89	13	255	D-21	
5/08/89	13	280	D-23	Cannibalized, some tumor
5/08/89	13	257	D-21 (cull)	Massive tumor
5/08/90	13	339	D-28	Massive tumor
5/09/89	14		cull	Wasted, no teeth
5/09/89	14		cull	Bites
5/09/89	14	219	D-18	Ascites and tumor
5/09/89	14	221	D-18	Ascites and massive tumor
5/09/89	14	220	D-18	Cannibalized, severe autolysis
5/10/89	15	329	D-27	Massive tumor
5/11/89	16	073	D-6	Massive tumor
5/11/89	16	340	D-28	Massive tumor
5/12/89	17	074	D-12	Massive tumor

TABLE 2

WEIGHT DATA COMPARISON, EXPERIMENTS 1 AND 2

file name: compwt12
created by: A. Youngberg printer: printer: 12 pt
day 0 = 25 April 1989, exp't 1
day 0 = 11 Dec 1990, exp't 2
animals administered pcb or vehicle on day -1
animals administered tumor or vehicle in day 0
Group A = controls
Group B = tumor only
Group C = pcb only
Group D = tumor plus pcb
contains: comparison of body and organ weight data,
from exp'ts 1 and 2
no day 3 spleen wt information from exp't 1; day 2 used
no day 10 thymus wt data from exp't 1; day 9 1; day 9 used

CHANGE IN BODY WEIGHT IN GRAMS

	Experiment 1 mean (se)	Experiment 2 mean (se)
Day 3:		
Group A	0.62 (.64)	-0.07 (.24)
Group B	1.13 (.10)	0.59 (.20)
Group C	-0.27 (.68)	-0.34 (.31)
Group D	0.55 (.40)	0.21 (.24)
Day 10:		
Group A	2.70 (.81)	1.06 (.50)
Group B	3.83 (.80)	1.94 (.32)
Group C	0.98 (.44)	1.23 (.21)
Group D	-0.47 (1.43)	-2.42 (.68)

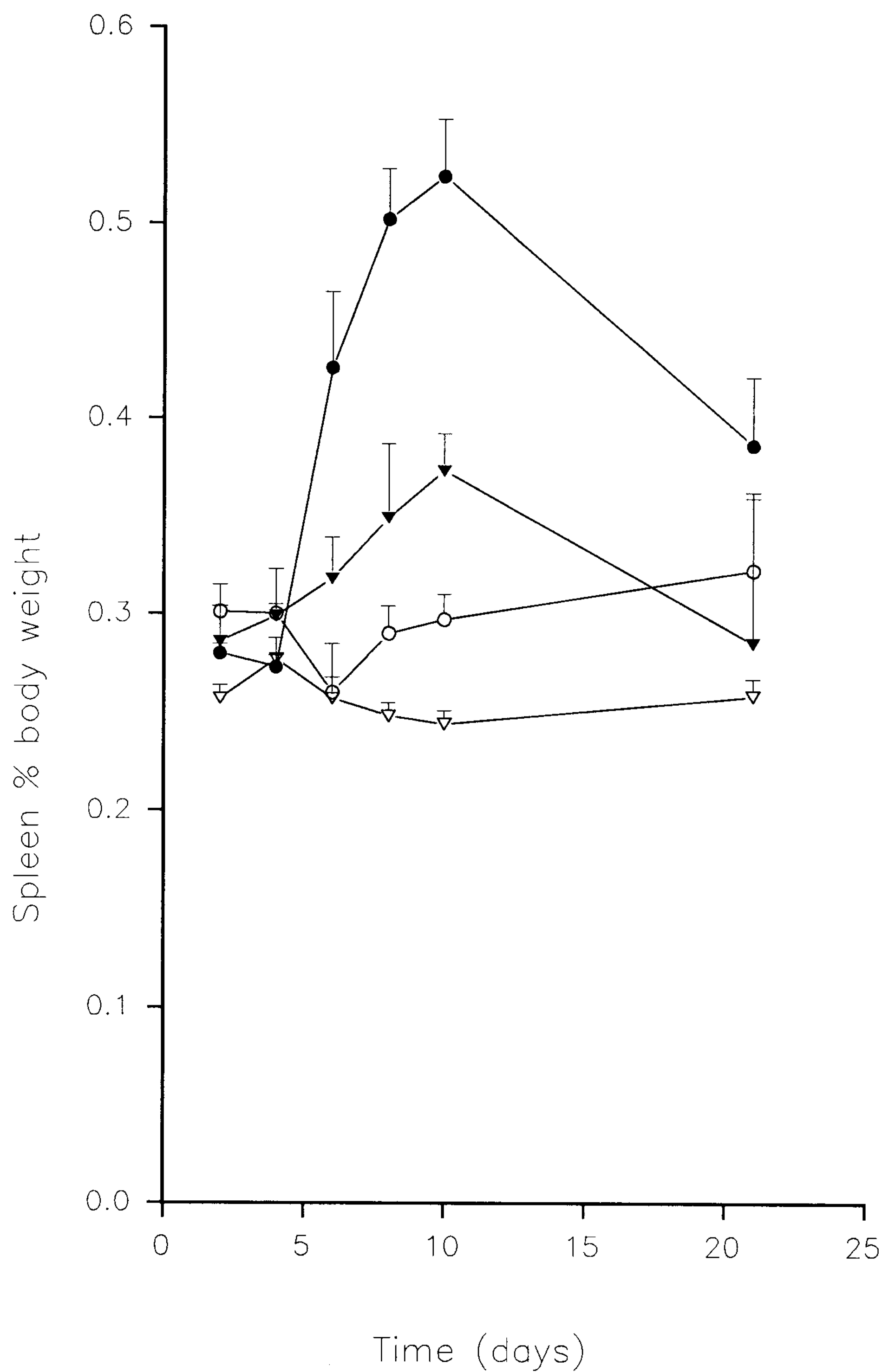
SPLEEN PERCENT BODY WEIGHT

	Experiment 1 mean (se)	Experiment 2 mean (se)
Day 3:		
Group A	0.300 (.057)	0.268 (.012)
Group B	0.280 (.012)	0.276 (.005)
Group C	0.257 (.017)	0.249 (.003)
Group D	0.286 (.045)	0.239 (.007)
Day 10:		
Group A	0.297 (.032)	0.252 (.011)
Group B	0.524 (.070)	0.508 (.020)
Group C	0.244 (.017)	0.215 (.004)
Group D	0.373 (.047)	0.308 (.026)

THYMUS PERCENT BODY WEIGHT

	Experiment 1 mean (se)	Experiment 2 mean (se)
Day 3:		
Group A	0.126 (.042)	0.105 (.012)
Group B	0.101 (.029)	0.112 (.008)
Group C	0.089 (.032)	0.111 (.014)
Group D	0.085 (016)	0.078 (.007)
Day 10:		
Group A	0.143 (.050)	0.146 (.011)
Group B	0.082 (.008)	0.071 (.005)
Group C	0.108 (.023)	0.105 (.008)
Group D	0.017 (.004)	0.031 (.003)

Figure 3: Spleen weight as a percent of body weight as a function of time for Group A (control o), Group B (tumor o), Group C (PCB), and Group D (tumor plus PCB) from day 2 to day 21. Plotted as mean plus standard error.

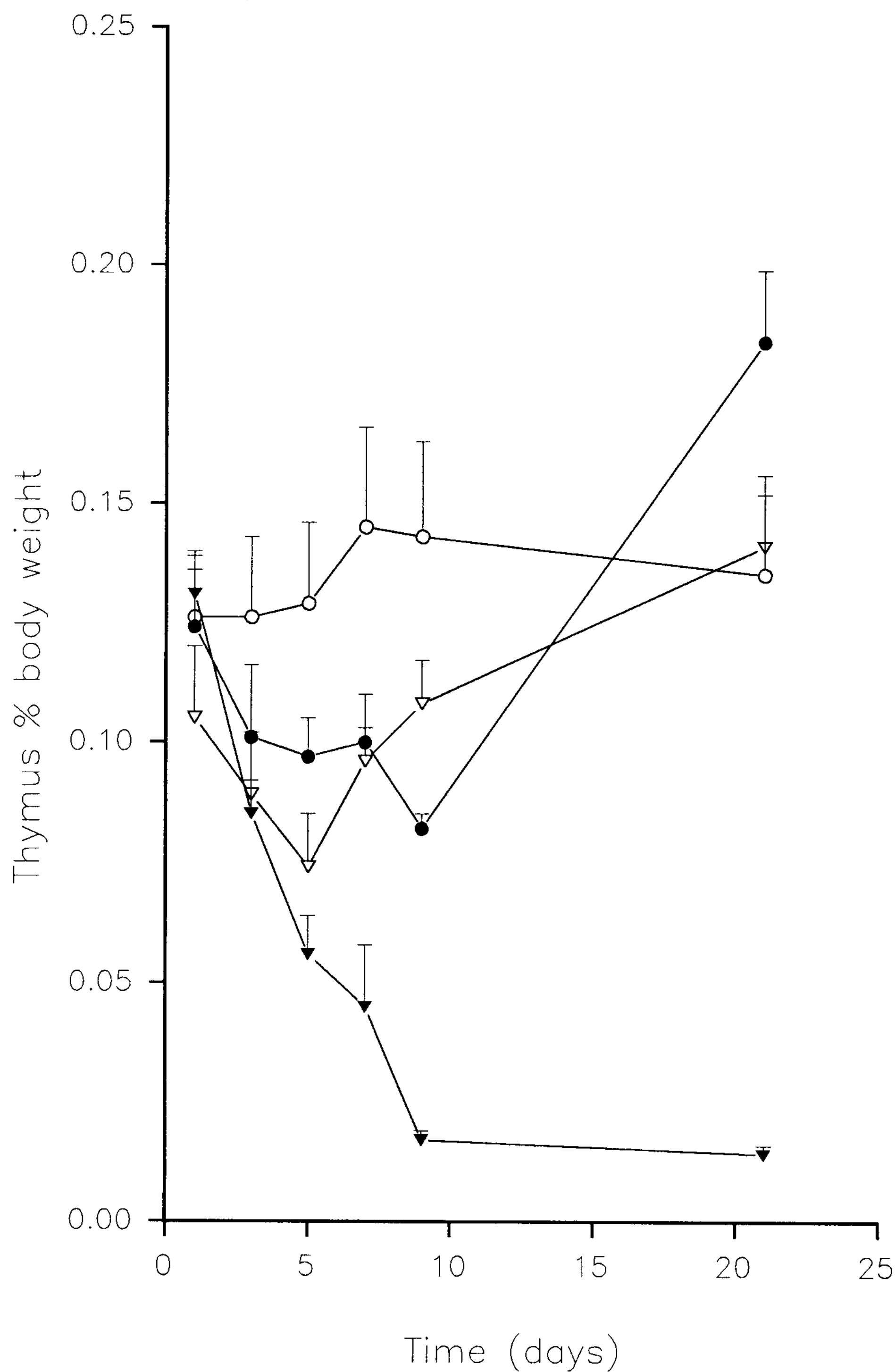


Animals that received PCB alone showed a decrease in SW relative to untreated controls. The animals treated with both tumor and PCB did not differ significantly from untreated control animals in SW except on day 10, when SW was higher in the dual treatment group. Compared to animals injected with tumor only, animals who received tumor plus PCB showed a significantly smaller increase in SW. Similar results were observed in Experiment 2 (see Table 2).

Thymus Weight (See Appendix II, Tables 4, 5, and 6 for raw data): Thymic weight (TW) was evaluated as a percentage of body weight to offset the effects of weight loss in Groups C and D. Over the course of Experiment 1, TW remained constant for the untreated control animals (see Figure 4). Animals that received either tumor alone or PCB alone showed an initial decrease in TW which was no longer observed by day 21. Animals that received both tumor and PCB showed a decrease in TW that began on day 3 and continued throughout the study. These results were validated in Experiment 2 (see Table 2).

Corticosterone Concentration (See Appendix II, Tables 7, 9, and 10 for raw data): CS concentrations on day -1 for Group A were unusually high and were significantly higher than CS concentrations for the same group on any other day. The animals may have been unduly stressed by nearby elevator construction on this day which caused excessive noise. Mice have been shown to develop high concentrations of CS following exposure to acute, but

Figure 4: Thymus weight as a percent of body weight as a function of time for Group A (control o), Group B (tumor o), Group C (PCB) and Group D (tumor plus PCB) from day 1 to day 21. Plotted as mean plus standard error.



not chronic, noise (Kugler 1990). For these reasons, the data for CS concentration for day -1 were not included in the statistical analysis.

Control animals and animals that received tumor alone had similarly low concentrations of CS throughout Experiment 1 (less than 25 ng/ml to 52.2 ng/ml) (see Figure 5). CS concentrations in animals that received PCB alone and PCB plus tumor were higher than those of the control animals. In comparison to control animals, animals that receiveid PCB alone had higher CS concentrations on all days, although the difference was significant only on day 4 in Experiment 1, when a peak CS concentration of 126.1 ng/ml was observed (Figure 6). Animals that received tumor plus PCB also showed CS concentrations higher than control animals on all days of the study, rising to a peak in Experiment 1 of 294.1 ng/ml on day 10 (Figure 7). Although both Groups B and D received tumor cells, the CS concentrations were significantly ($p < 0.05$) higher in Group D (tumor plus PCB) than in Group B (tumor alone) on days 4 through 21 (Figure 8). Both Groups C and D received PCB. The graph of CS concentrations versus time for these two groups (Figure 9) was similar on days 1 through 4. From day 5 through day 10 the concentration of CS in Group C (PCB only) was significantly lower than that of Group D (tumor plus PCB). The two were similar again on day 21.

In Experiment 2, the same patterns of CS concentration changes were observed (see Table 3), although the absolute concentrations of CS were higher in all groups in Experiment 2.

Figure 5: Corticosterone concentration as a function of time for Group A (control o) and Group B (tumor ●) from day -0.6 to day 84. Plotted as mean plus standard error.

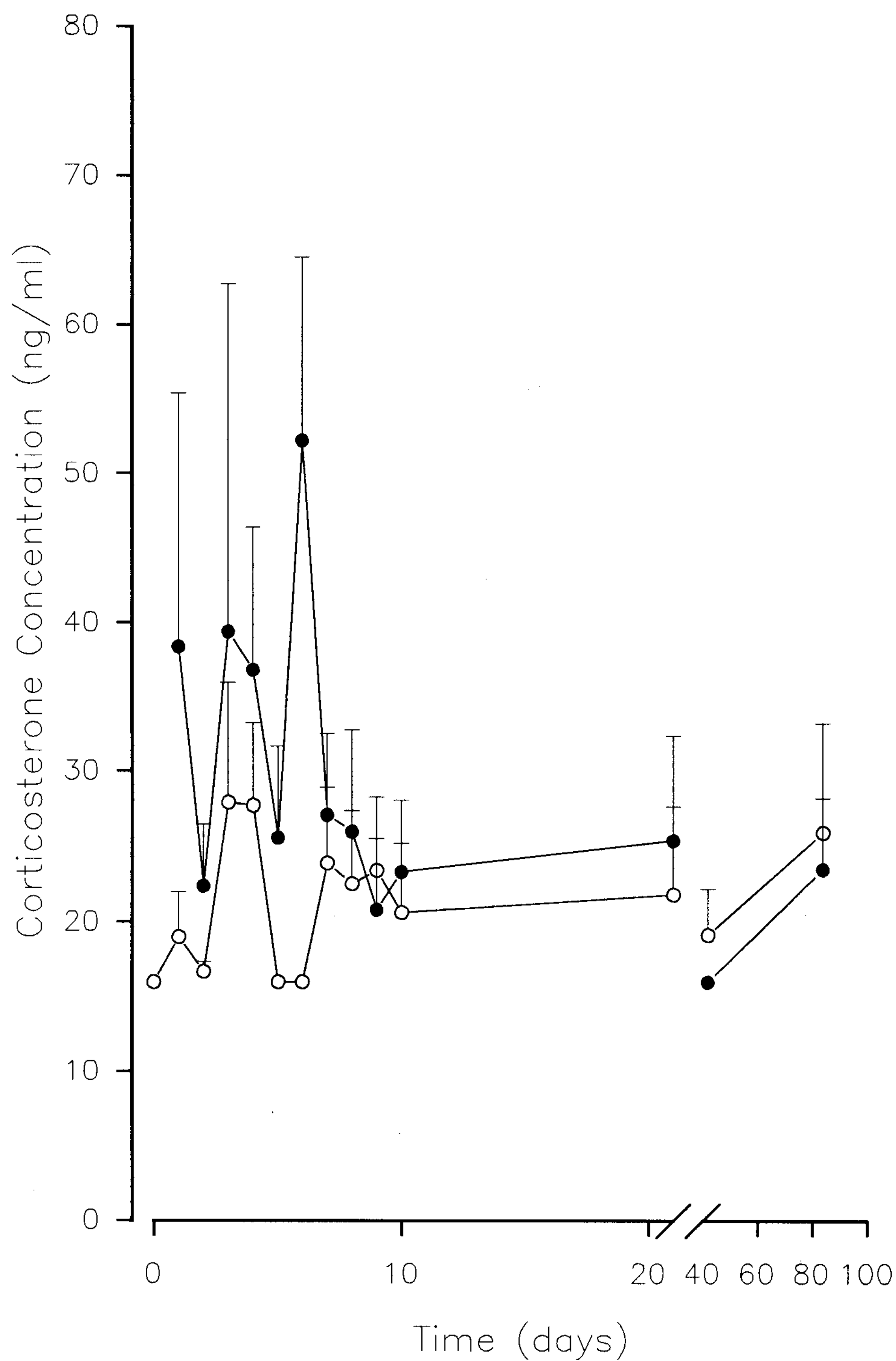


Figure 6: Corticosterone concentration as a function of time for Group A (control o) and Group C (PCB ●) from day -0.6 to day 84. Plotted as mean plus standard error.

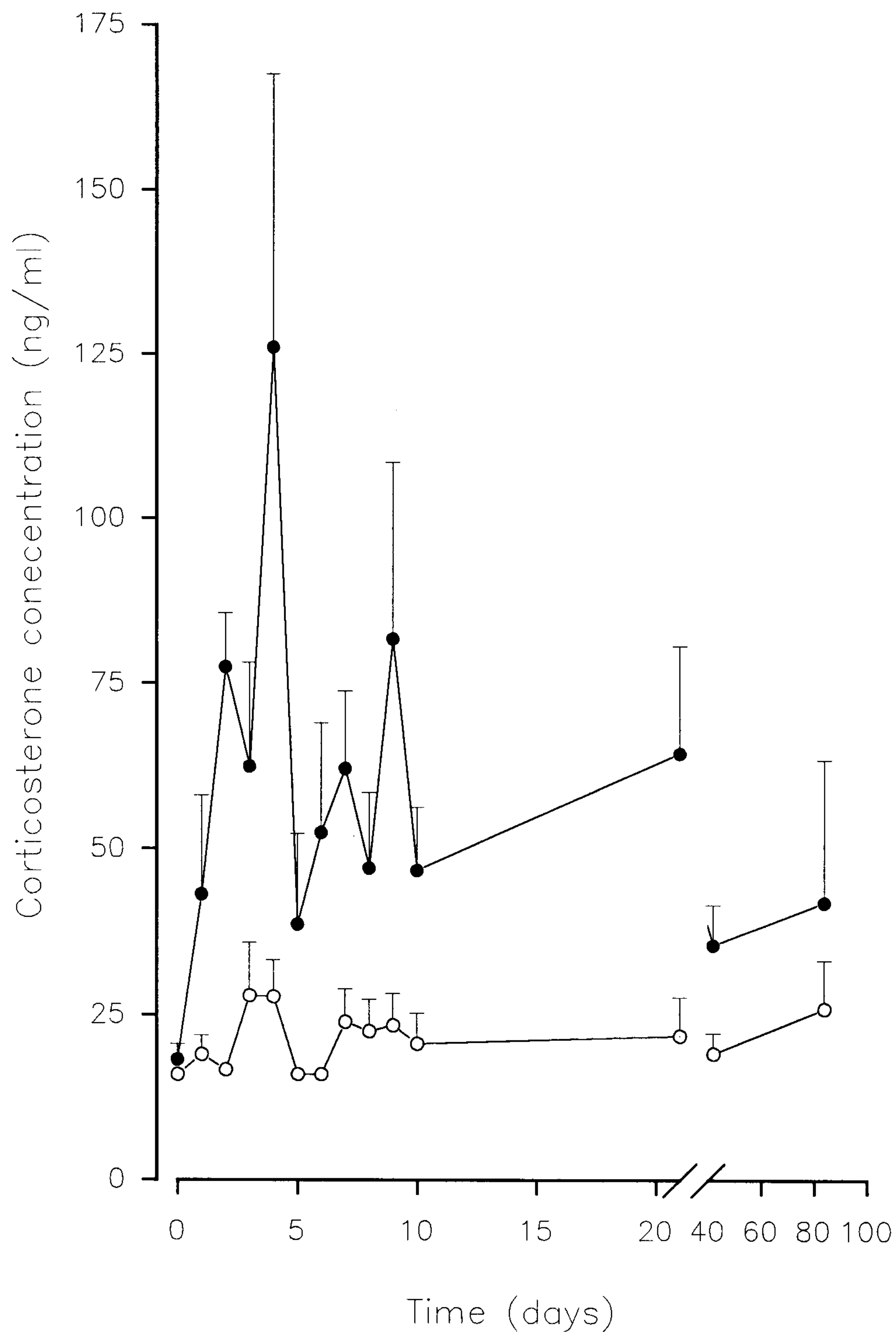


Figure 7: Corticosterone concentration as a function of time for Group A (control o) and Group D (tumor plus PCB ●) from day -0.6 to day 84. Plotted as mean plus standard error.

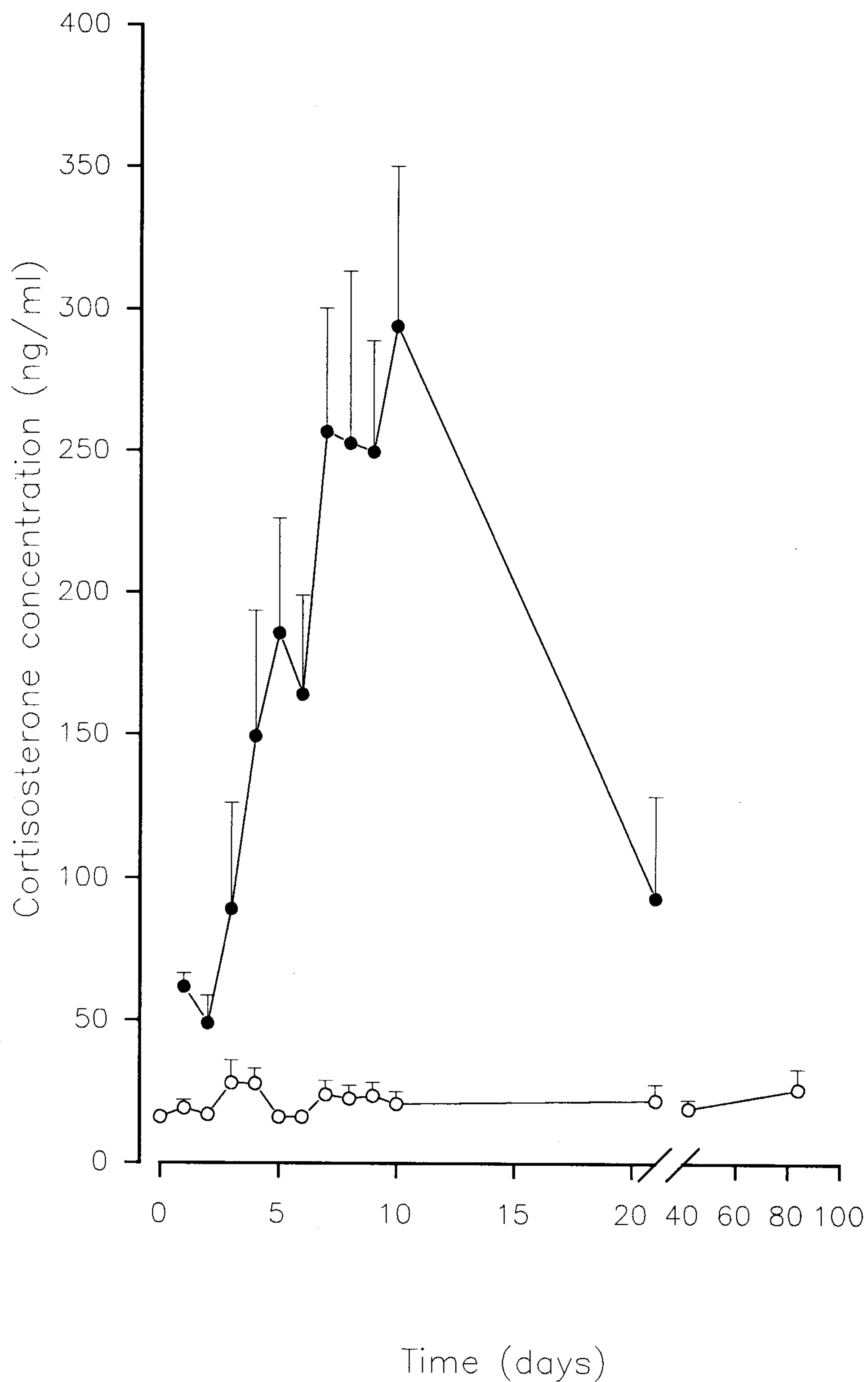


Figure 8: Corticosterone concentratioin as a function of time for Group B (tumor o) and Group D (tumor plus PCB ●) from day 1 to day 84. Plotted as mean plus standard error.

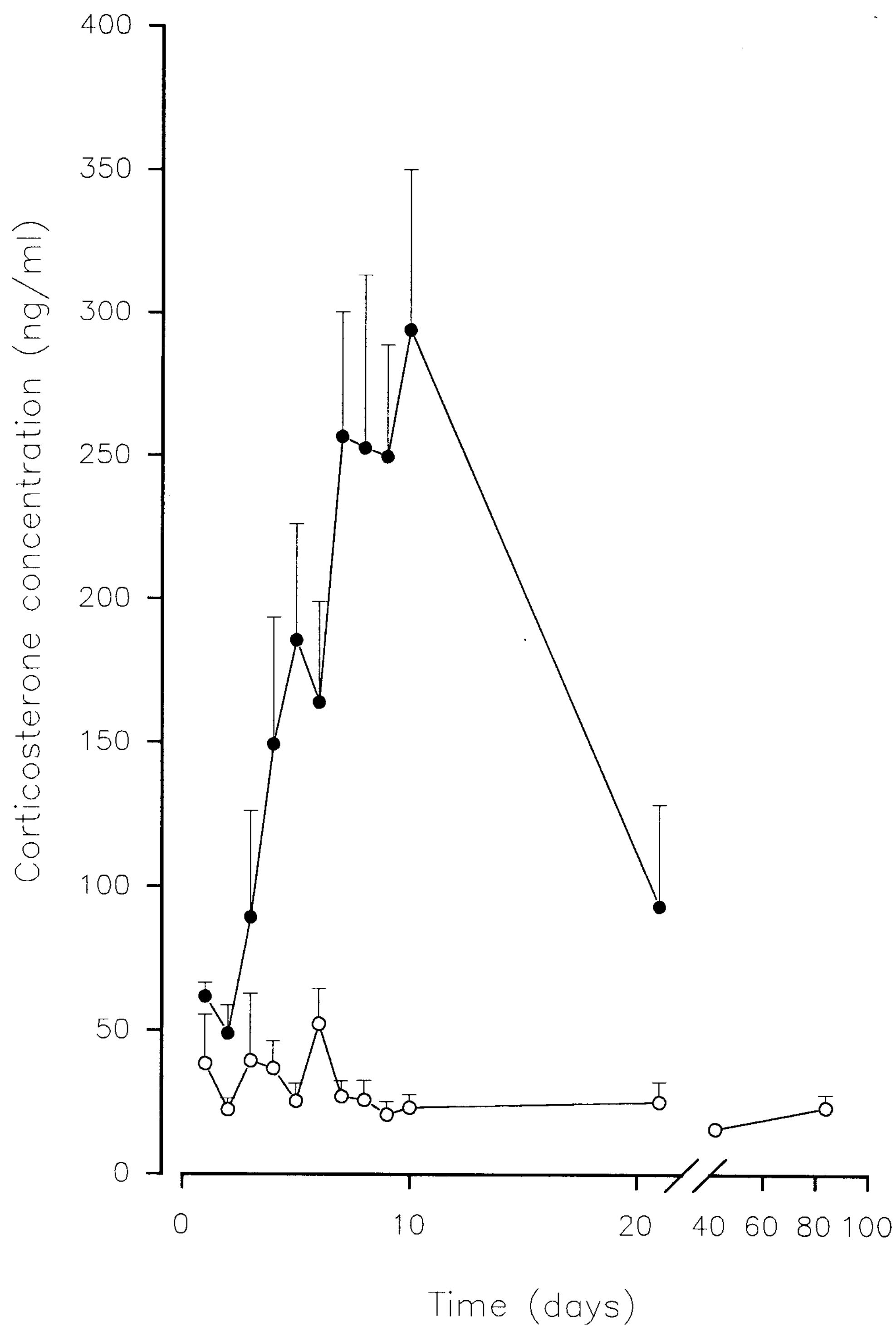


Figure 9: Corticosterone concentration as a function of time for Group C (PCB o) and Group D (tumor plus PCB •) from day -0.6 to day 84. Plotted as mean plus standard error.

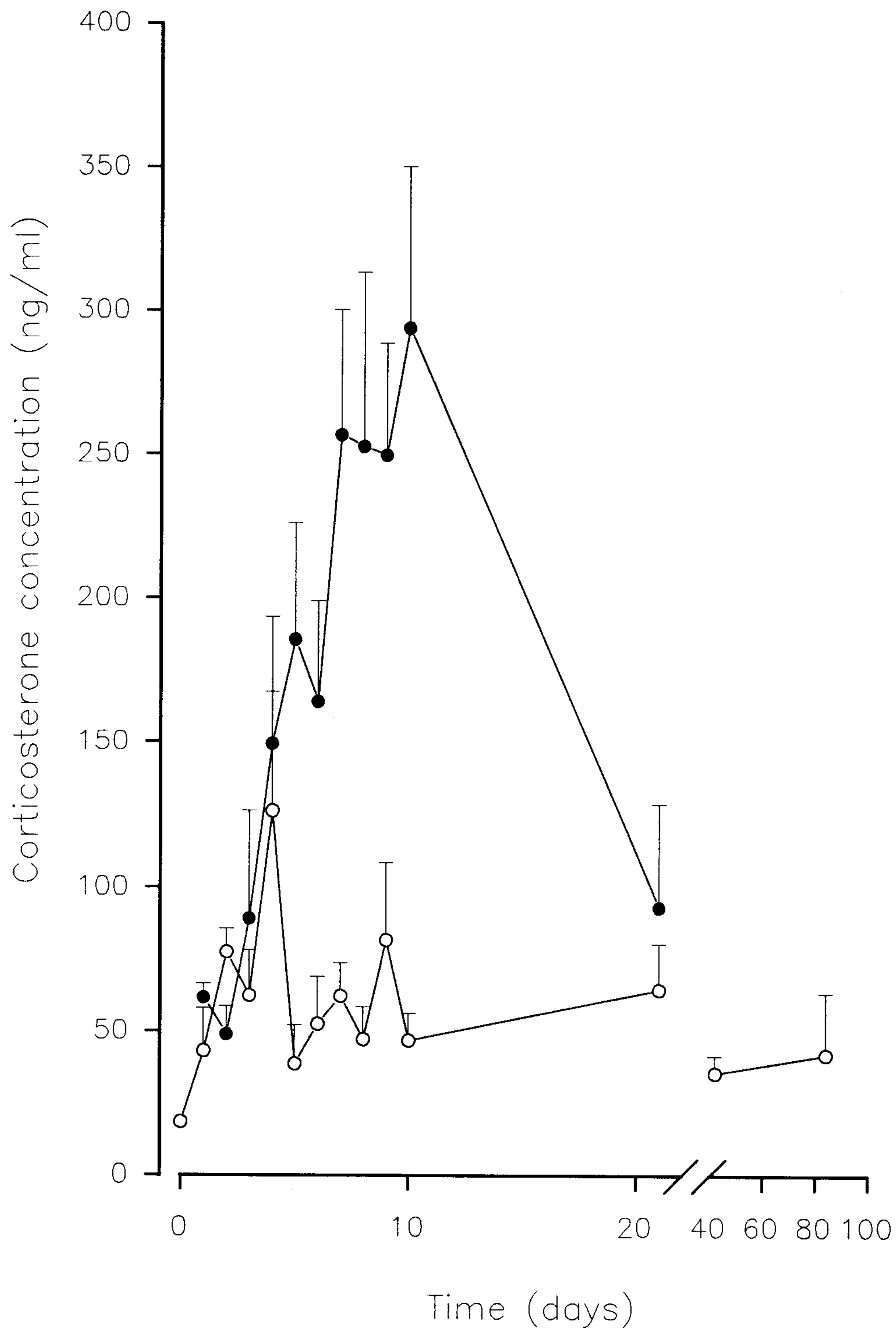


TABLE 3

HORMONE DATA COMPARISON, EXPERIMENTS 1 AND 2

file name: comp12 (scf)
created by: A. Youngberg printer: 12 pt
day 0 = 25 April 1989, exp't 1
day 0 = 11 Dec 1990, exp't 2
animals administered pcb or vehicle on day -1
animals administered tumor or vehicle in day 0
Group A = controls
Group B = tumor only
Group C = pcb only
Group D = tumor plus pcb
contains: summary of prl and cc data from expts 1 and 2
for prl assays: 1 is used for results less than 1.25
for cc assays: 16 is used for results less than 25
units are ng/ml

PROLACTIN CONCENTRATIONS IN NG/ML

	Experiment 1 mean (se)	Experiment 2 mean (se)
Day 3:		
Group A	1.54 (0.54)	27.84 (10.41)
Group B	2.30 (1.30)	28.53 (6.01)
Group C	1.51 (0.27)	33.0 (10.12)
Group D	2.09 (1.09)	18.76 (3.54)
Day 10:		
Group A	6.31 (5.13)	20.13 (3.85)
Group B	1.77 (0.25)	34.27 (8.05)
Group C	2.64 (0.96)	17.51 (2.14)
Group D	1.13 (0.13)	3.74 (1.13)

CORTICOSTERONE CONCENTRATION IN NG/ML

	Experiment 1 mean (se)	Experiment 2 mean (se)
Day 3:		
Group A	27.9 (8.0)	78.0 (23.1)
Group B	39.4 (23.4)	55.9 (37.4)
Group C	62.4 (15.8)	154.9 (29.6)
Group D	89.2 (37.0)	169.0 (36.4)
Day 10:		
Group A	20.6 (4.6)	66.4 (17.1)
Group B	23.3 (4.7)	40.5 (14.2)
Group C	46.8 (9.4)	171.8 (49.4)
Group D	294.1 (56.1)	799.2 (93.2)

Prolactin Concentration (See Appendix II, Tables 8, 9, and 10 for raw data): Analysis of variance of Experiment 1 PRL showed no significant effect of tumor or PCB or group assignment (Figure 10). Analysis of variance of the data after logarithmic transformation (Figure 11), showed no difference in PRL concentrations among Groups A, B, and C, although the average PRL concentrations were 3.7 ng/ml (Groups A (control) and B (tumor alone)) and 2.5 ng/ml Group C (PCB alone). Group D (tumor plus PCB), however, had an average PRL concentration of 1.5 ng/ml and was significantly lower than the other three groups. Because many PRL concentrations were close to the lower limit of detection of the assay, the data was also examined as the percentage of values in each group that fell below the assay's limit of detection. This percentage was 44.2% for Group A, 40.7% for Group B, 46.7% for Group C, and 76.9% for Group D.

When this experiment was repeated on days 3 and 10 only with modifications in the PRL assay procedure (Experiment 2), the PRL concentrations obtained were higher, all falling above the lower limit of assay detection. On day 3, animals treated with both tumor and PCB had a lower concentration of PRL than the other groups. The PRL concentration fell even lower in this group on day 10 -- 3.7 ng/ml versus 17.5, 20.1, and 34.3 ng/ml for the other three groups (see Table 3).

Figure 10: Prolactin concentration as a function of time for Group A (control o), Group B (tumor o), Group C (PCB), and Group D (tumor plus PCB) from day -0.6 to day 94. Plotted as mean.

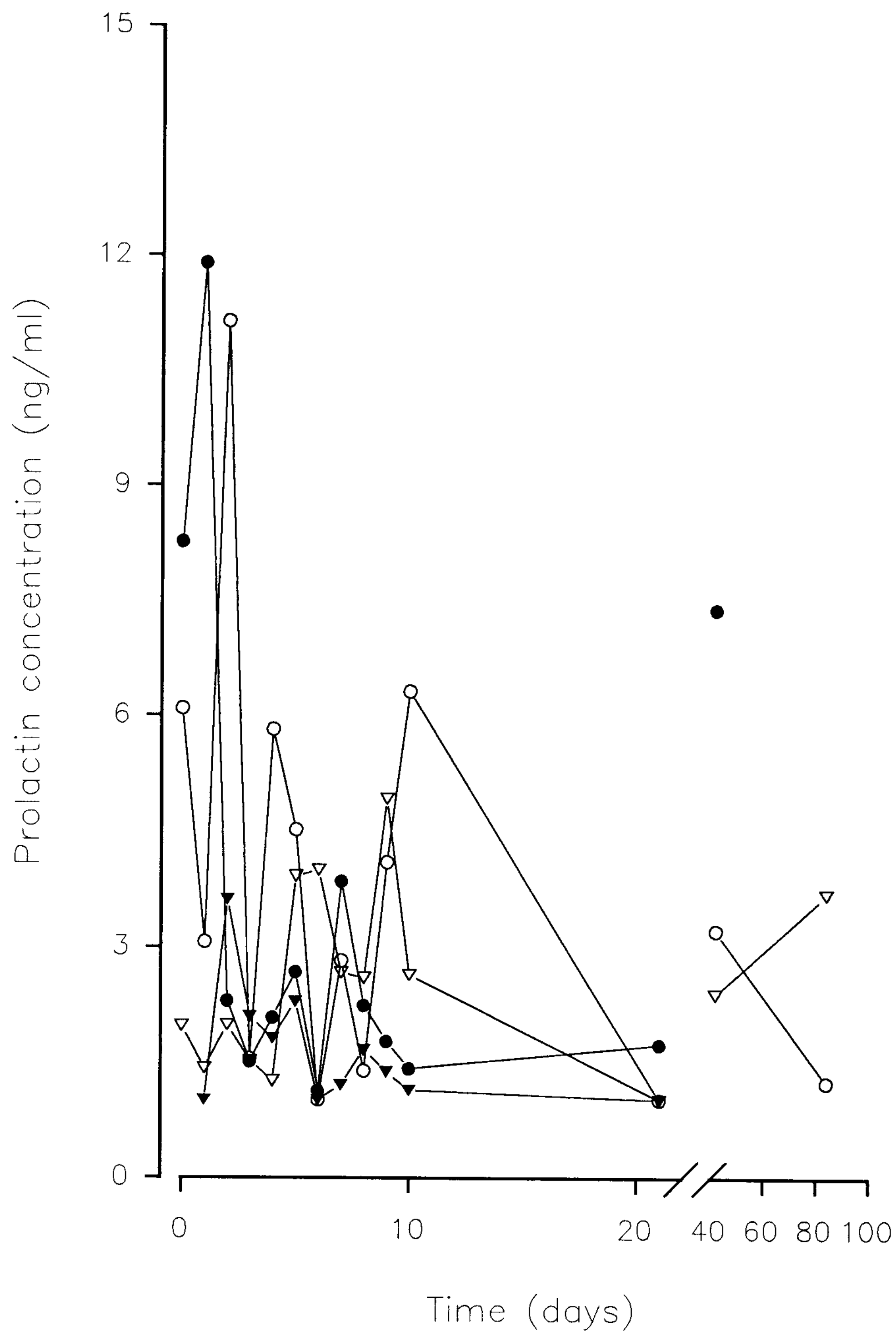
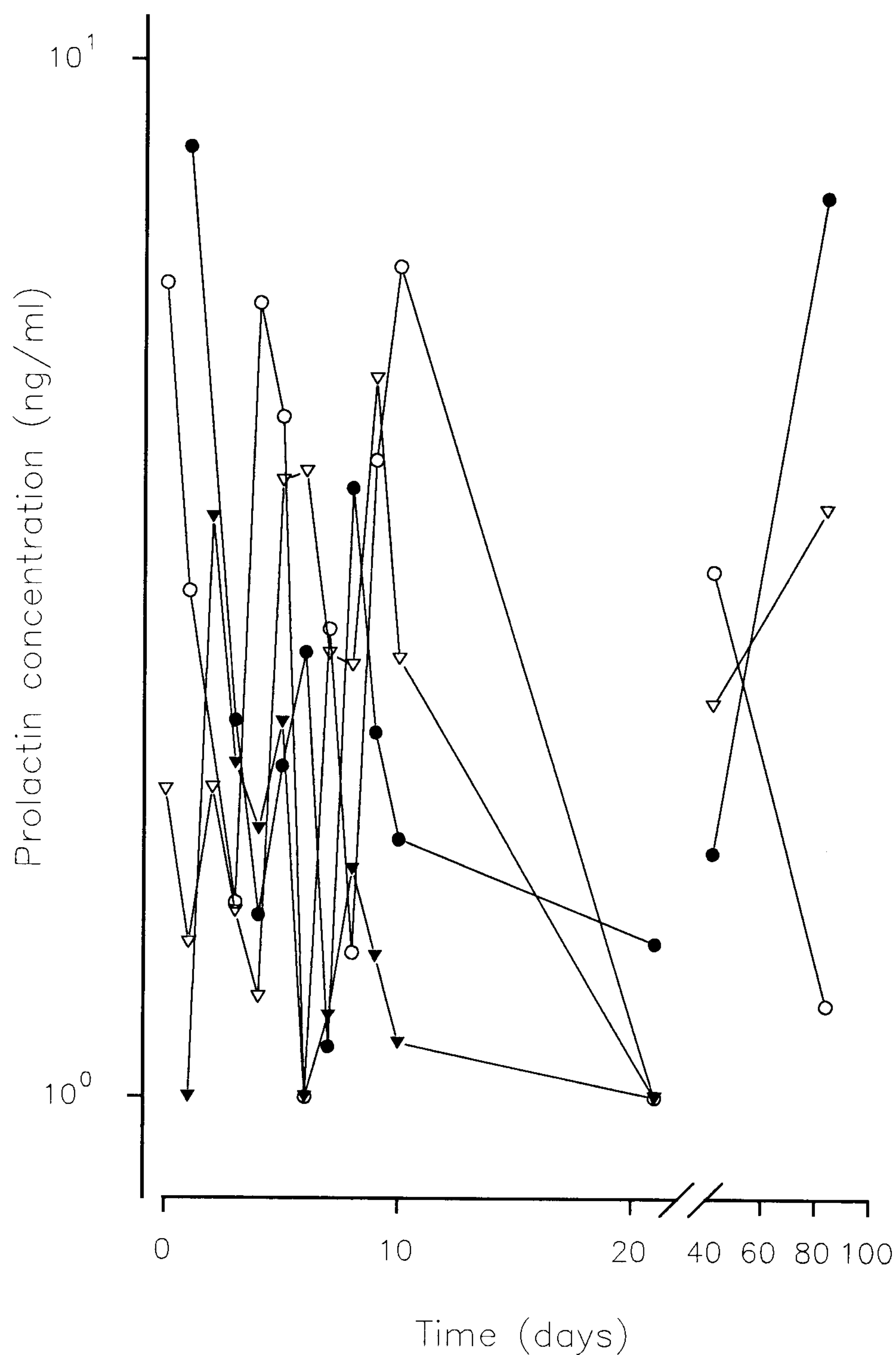


Figure 11: Prolactin concentration (logarithmic scale) as a function of time for Group A (control o), Group B (tumor o), Group C (PCB), and Group D (tumor plus PCB) from day -0.6 to day 84. Plotted as mean.



DISCUSSION

Literature reports suggest that acute exposure of laboratory animals to PCBs results in weight loss, thymic involution, and a rise in the concentration of circulating glucocorticoids. It was hypothesized that a decrease in the concentration of circulating PRL would also occur, due to the elevation of CS. These changes were observed in this project.

Body Weight (Figure 2): The changes in body weight following PCB and tumor exposure were similar to those observed in earlier studies (Kerkvliet 1988a, Kerkvliet 1990). In Experiment 1, the body weight of the untreated control animals increased throughout the experiment. On days 5 to 10, animals that received tumor showed a greater weight gain, presumably due to tumor growth during this period. Once the tumor was cleared, weight gains returned to those seen in control animals. Compared to untreated control animals, animals that received PCB gained less weight gain on all days. This "wasting syndrome," a decrease in weight gain in growing animals or an actual weight loss in grown animals, is a hallmark of PCB toxicity in many species. This wasting appears to be dose-dependent and has been observed after both acute and chronic exposure to PCBs (Seefeld 1984). At lethal dosages, the weight loss continues until death, while at sublethal dosages the wasting is temporary, and the animals eventually resume a normal rate of growth (Seefeld 1984).

The cause of this syndrome has not been determined, although malabsorption has been suggested (Seefeld 1984). It has also been suggested that the wasting may be due to a disturbance in glucose and/or fat metabolism (Muzi 1987, Gorski 1987). This hypothesis is supported by the observation that diet can modify the toxicity of TCDD in cold-adapted rats (Muzi 1987) and by the changes observed in concentrations of thyroid hormones and insulin in TCDD-treated rats (Gorski 1987). Seefeld (Seefeld 1984), also working with TCDD in rats, hypothesized a lowering of a body weight regulatory "set point" and his data suggested that changes in food intake were the result of this altered set point and were sufficient alone to account for the resulting weight loss.

PCB-treated tumor injected animals showed a weight gain similar to that seen in the control animals on days 1 through 7 and a significant decrease in weight gain on days 8, 9, 10, and 21. When compared to the animals that received PCB alone, they showed similar weight changes except for an increased weight gain on days 5 and 6 and a decreased weight gain on days 10 and 21. Growth of the tumor on days 2 through 7 was probably sufficient to offset the PCB-induced weight loss during that time period. On day 8, when the animals who received PCB alone were beginning to gain weight, the animals who were treated with both tumor and PCB were starting to lag behind the control animals. By day 9 the doubly treated animals were losing weight. This weight loss was presumably due to the combined effects of the wasting syndrome and impending death from the tumor. Most of the animals treated with tumor plus PCB died between days 13 and 17. Those that were examined after

death showed generally massive tumor and/or ascites. (See Mortality Table, Table 1). Presumably the PCB treatment suppressed the animals' ability to reject the tumor, allowing unrestrained tumor spread. Previous studies using this model system and measuring cytotoxic T-lymphocyte (CTL) function showed a decrease in CTL function in animals that received both PCB and tumor (Kerkvliet 1988a). The CTL response is a major component of the mouse's natural ability to reject this tumor.

Similar patterns of weight changes were observed among the groups in Experiment 2. The magnitude of weight loss in Group D on day 10 was higher in Experiment 2 than in Experiment 1 (-2.42 g vs. -0.47 g), possibly related to the higher CS concentration on this day in Experiment 2 (799.2 ng/ml vs. 294.1 ng/ml).

Spleen Weight (Figure 3): In Experiment 1, control animals showed a relatively constant SW over time, and all groups had SWs that were not significantly different on days 2 or 4. Animals given the tumor alone showed a significant increase in SW on days 6, 8, and 10, which was greater than that seen in the other three groups. This increase in SW is presumably due to the animals' immune response to the tumor. Compared to the control animals, animals exposed to PCB alone showed a decrease in SW on days 6, 8, and 10, which was not significant on any given day. This decrease has been previously reported to be associated with a decrease in the number of viable nucleated spleen cells (Kerkvliet 1988a). In other studies using lethal dosages

of PCB, both the weight of the spleen and the size of the gut associated lymphoid tissue were often reduced. Microscopically, the reduction in spleen size was due to a reduction in the size of the germinal centers, primarily due to a reduction in the periarterial lymphoid sheath portion (McConnell, 1980). Spleen weight and cellularity also have been shown to correlate negatively with CS concentration in C57Bl/6J female mice (del Rey, 1984). Animals that received both tumor and PCB (Group D) showed an increase in SW on days 6, 8, and 10 that was greater than that seen in the nonimmunized control animals; but less than that seen in the animals that received tumor alone. This suggests that PCB decreased, but did not obliterate, the animals' ability to respond to the tumor. The data obtained from Experiment 2 was similar to that from Experiment 1.

Thymus Weight (Figure 4): The results of this study are similar to those previously reported (Kerkvliet 1988a, Kerkvliet 1990) for the control, PCB alone and tumor plus PCB groups. A tumor alone group was not included in those studies. Weights were obtained only on day 10 or day 12 of those studies.

In Experiment 1, the control animals show a fairly constant TW. Animals that received tumor alone showed a lower TW than the controls on days 5 to 9. It has been suggested that this may represent an accelerated export of CTL precursor cells from the thymus to meet the demand for CTLs to reject the rapidly growing tumor (Kerkvliet 1990). In similarly treated animals in another study (Kerkvliet 1988a), CTL activity was detectable on day 7 and peaked on

day 10.

Animals exposed to PCB alone had a TW was less than the controls. Thymic atrophy is a classic symptom of PCB toxicity (Gorski 1987). Microscopically, this has been reported to be due almost entirely to loss in the cortical lymphocytes (McConnell, 1980). These animals were given a sublethal dose of PCB, and their thymic atrophy showed signs of reversal at day 9, which was complete by day 21. Animals exposed to both tumor and PCB (Group D) showed thymic atrophy similar to that seen in both the PCB alone and the tumor alone groups on days 1, 3, and 5, but then continued declining throughout the study. This suggests that tumor and PCB had an additive effect on the animals. Either treatement alone caused a depression in TW which was overcome by day 10 (PCB alone) or 21 (tumor alone). Together they depressed TW further and with no recovery. It has been suggested that the thymic atrophy caused by the PCB may be sufficient to preclude effective recruitment of CTL precursor cells, leading to a depressed peripheral CTL response, followed by death from the tumor (Kerkvliet 1990). The results of Experiment 2 were similar to those of Experiment 1.

Thymic atrophy in response to PCB may also be due, in some part, to the effects of starvation. To address that issue, control animals pair-fed to TCDD-treated rats were examined by Gorski (Gorski 1988a). He noted that, following lethal dosages of TCDD, treated animals showed a significant decrease in thymic percent body weight, which was paralleled by pair-fed controls, though

at a later date and to a slightly lesser extent. In animals given a nonlethal dosage, thymic atrophy occurred in treated animals, but only transiently and to a small extent in pair-fed controls. The authors, therefore, suggested that TCDD either exerts a direct effect on the thymus or it causes an additional stress (e.g. a metabolic stress) beyond that of starvation stress.

Corticosterone Concentration (Figures 5 through 9): In Experiment 1, the control animals had very low concentrations of CS on all days of the experiment except day -1, indicating that nothing in the study design caused undue stress to the animals. The results of the CS determinations on day -1 were deleted from the study, due to noise from construction work in the hall outside the laboratory where the animals were being handled. Acute exposure to noise has been shown to increase CS concentrations in the mouse (Kugler 1990), and these mice showed higher concentrations of CS than did the control mice on any other day of the study. CS concentrations in animals that received tumor only did not differ significantly from those in the control group, indicating that neither the presence of the tumor nor its rejection alone was sufficient to elevate CS concentrations.

Animals exposed to PCB alone showed elevated CS concentrations compared to control animals on all days of the study. However, this elevation was statistically significant only on day 4, when the CS concentration peaked at 126.1 ng/ml. A PCB-induced rise in circulating CS has been observed by other investigators (Sanders 1977, Gorski 1988a, 1988b). Increased

concentrations of circulating CS may be one effect of the binding of the PCB to the Ah-receptor. Increased concentrations of CS may also be produced secondarily in response to the hypoglycemia of the wasting syndrome. Gorski (1988a) has addressed the effects of weight loss on CS concentrations by using control animals that were fed the same amount of food as was eaten by the treated animals, with one days' delay. He reported that when sublethal dosages of TCDD were given to rats, CS concentrations rose in the treated animals but not in the pair-fed controls. When lethal dosages were used, however, the CS concentrations showed similar increases in both treated animals and pair-fed controls. These results suggested that, while lethal dosages of TCDD can cause hypoglycemia sufficient to increase CS concentrations, nonlethal dosages of TCDD can by themselves cause an increase in circulating CS concentrations, even when the weight loss that occured was not sufficient to do so. The authors concluded, therefore, that starvation stress alone was unlikely to account for all of the increased CS concentrations observed. They supported this conclusion by histological studies of adrenal glands, which secrete CS, and of thymus glands, which suffer atrophy in the face of elevated concentrations of CS. The glands show more pronounced changes in the TCDD-treated group than in the pair-fed controls, again suggesting that the decreased caloric intake was not sufficient alone to account for the effects seen (Gorski 1988a).

In addition to the effect of starvation, another mechanism by which PCBs could affect CS concentrations is interference with glucose metabolism (Gorski 1988a, Messner 1976, Gorski 1987). One feeding study demonstrated that oral dosages of a PCB (Clophen T64) that were not enough to decrease food intake caused a decrease of 50% in the activity of phosphoenolpyruvate carboxykinase (PEPCK), a key regulatory enzyme of gluconeogenesis. Blood glucose concentrations, however, fell only slightly following this treatment. At higher PCB doses, PEPCK activity increased, due to the effect of decreased food intake, but to only about half the level seen in control animals (Messner 1976). Normal rats fed PCB (Aroclor 1254) for 2 weeks showed decreased food intake, weight gain, and blood glucose concentration. The activity of PEPCK was also decreased by approximately 40% (Mehlman 1975). If PCBs suppress blood glucose concentrations by decreasing gluconeogenesis, then CS concentrations would be expected to increase in response to the hypoglycemia. The elevated CS concentrations would increase gluconeogenesis and decrease peripheral utilization of glucose. At low dosages of PCBs, glucose and CS concentrations eventually returned to normal or near normal, and the animals survived. Nonsurvivors at higher doses were not able to return concentrations of either CS or glucose to normal (Gorski 1987). In these animals, gluconeogenesis may be blocked or exhausted, as might be indicated by the fact the TCDD-treated rats have a different respiratory quotient than pair-fed controls, suggesting gluconeogenic conversion of proteins in control animals, but not in experimental ones (Muzi 1989).

Also supporting the theory that increased concentrations of CS are a protective response of the animal to hypoglycemia is the observation that adrenalectomy greatly enhances the mortality of TCDD-treated rats and PCB-treated mice (Gorski 1988b, DeKrey, unpublished data). The rats treated with TCDD suffered severe hypoglycemia (15 mg/ml) immediately preceding death. These effects were reversed by administration of CS replacement therapy.

In rats, TCDD produced an elevation in plasma CS concentrations without altering adrenocorticotropic hormone (ACTH) concentrations (DiBartolomeis, 1987). The authors suggested that exposure to TCDD altered the responsiveness of the adrenal gland to ACTH, possibly by effects on adrenal mitochondrial cholesterol side chain cleavage activity. This increase in plasma CS concentrations with unaltered ACTH concentrations has also been observed in C57Bl/6J mice exposed to PCB (DeKrey, unpublished data).

Animals exposed to tumor plus PCB had higher CS concentrations than those seen in the other three groups. The CS concentrations were similar to those seen in the PCB only animals on days 1 through 4. On day 5 the CS concentrations in the PCB only animals began to fall, but they continued to rise in the tumor plus PCB group until at least day 10. By day 21 the CS concentrations in these two groups were not significantly different. Statistical

analysis showed that the interaction of PCB and tumor was greater than the additive effects of the two alone. One possible explanation is that the hypoglycemia that occurred due to PCB exposure was further exacerbated by the diversion of glucose to the rapidly growing tumor cells, requiring an even greater secretion of CS in an attempt to maintain a normal blood glucose concentration.

All animals were bled during the morning. No attention was given to the possible effects of PCB on the diurnal rhythm of CS secretion. Previous studies using rats exposed to Aroclor 1254 and to TCDD showed no changes in the diurnal rhythm (Dunn 1983, DiBartolomeis 1987), although one study showed an increase in the morning and a decrease in the evening concentrations of CS in rats exposed to TCDD (DiBartolomeis 1987).

The CS concentrations measured in all groups in Experiment 2 were higher than those seen in Experiment 1, although the relationships between the treatment groups was the same (see Table 2). The method of killing may have been partially responsible for the elevation of CS concentrations in Experiment 2. If the method used in Experiment 2 (carbon dioxide overdone) was more stressful to the animals than the cervical dislocation used in Experiment 1, elevated CS concentrations would have been expected. It may also be that this stress caused a disproportionately greater effect on PCB-exposed animals (Groups C and D) due to their preexisting stress from toxic exposure.

The tumor used in both Experiments 1 and 2 was a P815 mastocytoma of the same cell line, but different passages were in use for these two experiments. It has been noticed that different CS concentrations have been measured in animals receiving different passages of the tumor (DeKrey, unpublished data).

Prolactin Concentration (Figures 9 and 10): Analysis of PRL concentrations from Experiment 1 showed that the tumor injected PCB-treated animals had significantly lower concentrations of PRL than animals of any of the other groups. This data was confusing due to the large number of samples that yielded undetectable results in the assay. This caused a reevaluation of the experimental protocol. Three issues seemed worth pursuing. First, a larger sample volume would permit larger sample size during the assay, which would allow use of the main portion of the standard curve, rather than the less sensitive low end. A different method of killing, such as carbon dioxide overdose, would be equally humane and could allow the collection of more blood.

Second, plasma samples should have been analyzed sooner after collection. The samples in Experiment 1 were frozen for up to 15 months before analysis, due to difficulties in perfecting the assay for use. If the concentration of PRL in the samples decreased over time, any but the most dramatic differences between groups may have been unrecognizable.

Third, no consideration was given to a circadian rhythm for prolactin secretion in these mice. Their blood may have been drawn at a time of day when the levels were not at their peak, thus possibly blunting observation of effects. Determining the peak time for blood drawing may not completely solve the problem, however, as Jones et al (1987) reported that TCDD may abolish the native circadian rhythm of prolactin secretion in rats.

Experiment 2 was performed to address the first two issues. Animals were killed by carbon dioxide overdose, allowing greater blood sample volume, and samples were analyzed within one month of collection. The results of this study are shown on Table 2. All animals had PRL concentrations within the detectable range of the assay, and the mean concentrations were higher than those seen in the original study. The concentration of PRL may have been elevated by the blood collection procedure. Mock and Frankel (Mock 1978) compared PRL concentrations from rats killed by decapitation to those obtained from rats killed by ether, followed by cardiac puncture. Samples obtained by cardiac puncture had higher PRL concentrations than samples obtained by decapitation. Although reference PRL concentrations for male C57Bl/6J mice have not been published, a concentration of 19.3 ± 3.9 ng/ml has been reported for male C57Bl/St mice at 80 days of age (Sinha 1972). This compares favorably with the means of 27.8 and 20.1 ng/ml measured in

control animals analyzed shortly after collection. In Experiment 1, when only days 3 and 10 are considered, no significant difference was shown between the four groups. In Experiment 2, however, Group D (tumor plus PCB) had significantly lower PRL concentrations than the other groups on day 10. The final conclusion, that Group D had lower PRL concentrations than the other groups, was the same for both experiments, although in Experiment 2 this was shown with less data. Perhaps more or more subtle changes could have been detected had this protocol been used for the entire study.

Jones et al (1987) have studied the effects of TCDD administration of PRL concentrations on rats. They observed an initial decrease in the concentration of PRL at four hours post exposure. At 3 days post exposure, PRL concentrations were similar in both treated and pair-fed control animals. At seven days post exposure, the concentration of PRL was increased in the TCDD-treated group versus the pair-fed control animals. In our study we saw no difference between the control animals and the animals that received PCB alone in PRL concentrations. This may be due to use of a different species of animal, a different chemical, a different effective dosage, or a change too small to detect with our assay protocol.

Animals that received both tumor and PCB showed a decrease in PRL

concentrations. This may be due to a lack of sensitivity in the assay protocol. It may also be that PCB alone at the dosage given is not enough to depress circulating PRL, but that the tumor acts to somehow potentiate the effects of the PCB. Severe hypoglycemia in rats has been shown to decrease serum PRL (Sinha 1972). If mice that received tumor plus PCB were hypoglycemic due to the combined effects of PCB and tumor, this may be a factor contributing to the decrease in the concentration of PRL.

Another possible explanation may be found in the concentrations of CS, which were most elevated in animals that received both tumor and PCB. If CS has a role in the regulation of PRL concentrations, the decrease in PRL seen in the animals that received both tumor and PCB may be due to the fact that this group also had the highest concentration of CS.

Russell (1988) confirmed Jones' work and demonstrated not only the rapid (four hours post TCDD exposure) decrease in PRL concentration, but also a reversal of this effect when pimozide, a dopamine receptor antagonist, was given with TCDD. The authors interpreted this to mean that TCDD had an action on the tuboinfundibular dopaminergic system of the hypothalamus. They then measured the dopamine concentrations in the median eminence of vehicle- and TCDD-treated rats. The rate of dopamine synthesis and turnover

was nearly doubled in the TCDD-treated animals. Since dopamine exerts inhibitory control over PRL release from the pituitary gland, this could explain the decrease in circulating prolactin following TCDD exposure. They also suggested that a hypothalamic body weight set point could be altered in a similar fashion and that altered PRL release could cause an alteration in proliferation and differentiation of thymocytes. Thus both weight loss and thymic atrophy, two hallmarks of PCB toxicity, as well as suppression of PRL concentrations, could be explained by hypothesizing a primary hypothalamic site of action for PCB.

In summary, in response to acute exposure to tumor, PCB, and PCB plus tumor, mice responded in a manner that suggested that:

1. Immunosuppression occurred at the dosage of PCB used.
2. The dose of PCB alone was sublethal, but sufficient to cause transient weight loss.
3. Tumor alone caused no change in either CC or PRL concentrations.
4. PCB alone was sufficient to elicit a small rise in CC concentrations, but caused no change in PRL concentrations.

5. PCB plus tumor were sufficient to cause a large rise in CS concentrations and a decrease in PRL concentrations.

BIBLIOGRAPHY

Berczi, I., E. Nagy, K. Kovacs and E. Horvath. 1981. Regulation of humoral immunity in rats by pituitary hormones. Acta Endocrinol. 98:506.

Bernton, E.W., M.S. Meltzer and J.W. Holaday. 1988. Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice. Science 239:401.

Byard, J.L. 1987. The toxicological significance of 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds in human adipose tissue. J. Toxicol. Environ. Health 22:381.

Clevenger, C.V., S.W. Altmann and M.B. Prystowsky. 1991. Requirement of nuclear prolactin for interleukin-2-stimulated proliferation of T lymphocytes. Science 253:182.

Dave, J.R., R.J. Witorsch, M.Y. Kalimi. 1985. Endocrine-mediated parallel changes in hepatic glucocorticoid and prolactin receptors. Biochim. Biophys. Acta 845:276.

DeKrey, Greg. Unpublished data.

del Rey, A., H. Besedousky and E. Sorkim. 1984. Endogenous blood levels of corticosterone control the immunologic cell mass and B cell activity in mice. J. Immunol. 133;2:572.

DiBartolomies, M.J., R.W. Moore, R.E. Peterson, B.J. Christian and C.R. Jefcoate. 1987. Altered regulation of adrenal steroidogenesis in 2,3,7,8-tetrachlorodibenzo-p-dioxin-treated rats. Biochem. Pharmacol. 36:59.

Dunn, J.O., J.W. Carter and D.A. Henderson. 1983. Effect of polychlorinated biphenyls (Aroclor 1254) on rhythmic pituitary-adrenal function. Bull. Environ. Contam. Toxicol. 31:322.

Euker, J., J. Meites and G.D. Riegle. 1975. Effects of acute stress on serum LH and prolactin in intact, castrate, and dexamethasone-treated male rats. Endocrinology 96;1:85.

Gillis, S., G.R. Crabtree and K.A. Smith. 1979. Glucocorticoid-induced inhibition of T cell growth factor production. II. The effect on the **in vitro** generation of cytolytic T cells. J. Immunol. 123;4:1632.

Gorski, J.R., G. Muzi, L.W. Weber, D.W. Pereira, M.J. Iatropoulos and K. Rozman. 1988a. Elevated plasma corticosterone levels and histopathology of the adrenals and thymuses in 2,3,7,8-tetrachlorodibenzo-p-dioxin treated rats. Toxicology 53:19.

Gorski, J.R. and K. Rozman. 1987. Dose-response and time course of hypothyroxinemia and hypoinsulinemia and characterization of insulin hypersensitivity in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treated rats. Toxicology 44:297.

Gorski, J.R., T. Rozman, H. Greim and K. Rozman. 1988. Corticosterone modulates acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in male Sprague-Dawley rats. Fundam. Appl. Toxicol. 11:494.

Griffin,J.E. and S.R. Ojeda. 1988. Textbook of Endocrine Physiology. oxford University Press, New York.

Gustafsson, J.A., J. Carlstedt-Duke, L. Poellinger, S. Okret, A. Wilkstrom, M. Bronnegard, M. Gillner, Y. Dong, K. Fuxe, A. Cintra, A. Harfstrand and L. Agnati. 1987. Biochemistry, molecular biology, and physiology of the glucocorticoid receptor. Endocr. Rev. 8:185.

Harms, P.G., P. Langlier, and S.M. McCann. 1973. Modification of stress-induced prolactin release by dexamethasone or adrenalectomy. Endocrinology 96:475.

Harms, P.G., P. Langlier and S.M. McCann. 1975. Modification of stress-induced prolactin release by dexamethasone or adrenalectomy. Endocrinology 96;2:475.

Hiestand, P.C., P. Mekler, R. Nordmann, A. Grieder and C. Permmongkol. 1986. Prolactin as a modulator of lymphocyte responsiveness provides a possible mechanism of action for cyclosporine. Proc. Natl. Acad. Sci., USA 83:2599.

Jones, M.K.,W. Weisenburger, I.G. Sipes, D.H. Russell. 1987. Circadian alterations in prolactin, corticosterone, and thyroid hormone levels and down-regulation of prolactin receptor activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 87:337.

Kerkvliet, N.I. and Baecher-Steppan, L. 1988a. Suppression of allograft immunity by 3,4,5,3',4',5'-hexachlorobiphenyl. I. Effects of exposure on tumor rejection and cytotoxic T cell activity **in vivo**. Immunopharmacology 16:1.

Kerkvliet, N.I. and L. Baecher-Steppan, L. 1988b. Suppression of allograft immunity by 3,4,5,3',4',5'-hexachlorobiphenyl. II. Effects of exposure on mixed lymphocyte reactivity and induction of suppressor cell activity **in vitro.** Immunopharmacology 16:13.

Kerkvliet, N.I.,L. Baecher-Steppan, B.B. Smith, J.A. Youngberg, M.C. Henderson and D.R. Buhler. 1990. Role of the Ah locus in suppression of cytotoxic T lymphocyte activity by halogenated aromatic hydrocarbons (PCBs and TCDD): Structure-activity relationships and effects in C57BL/6 mice congenic at the Ah locus. Fundam. Appl. Toxicol. 14:532.

Kugler, J., K.T. Kalveram and K.W. Lange. 1990. Acute, not chronic exposure to unpredictable noise periods affects splenic lymphocytes. Intern. J. Neuroscience 51:233.

McConnell, E.E. 1980. In Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. R.D. Kimbrough, ed. Elsevier/North-Holland BioMedical Press. Amsterdam, p. 109-150.

Mehlman, M.A.,R.B. Tobin, B. Friend and C.R. Mackerer. 1975. The effects of a polychlorinated biphenyl mixture (Aroclor 1254) on liver gluconeogenic enzymes of normal and alloxan-diabetic rats. Toxicology 5:89.

Messner, B., J. Berndt and J. Still. 1976. Inhibition of PEP-carboxykinase in rat liver by polychlorinated biphenyl. Nature (London) 263:599.

Mock, E.J. and A.I.Frankel. 1978. Blood-collecting methodology does not affect the maturational pattern of serum hormone concentrations (testosterone, LH, FSH, and prolactin) in the male rat. Neuroendocrinology 26:202.

Muzi, G., J.R. Gorski, and K. Rozman. 1987. Composition of diet modifies toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in cold-adapted rats. Arch. Toxicol. 61:34.

Muzi, G., J.R. Gorski and K. Rozman. 1989. Mode of metabolism is altered in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treated rats. Toxicol. Lett. 47:77.

Poland, A. and E. Glover. 1977. Chlorinated biphenyl induction of aryl hydrocarbon hydroxylase activity: a study of the structure-activity relationship. Mol. Pharmacol. 13:924.

Raloff, J. 1989. Dioxin: Paper's trace. Sci. News 135:104.

Rogan, W.J., B.C. Gladen, K. Hung, S. Koong, L. Shih, J.S. Taylor, Y. Wu, D. Yang, N.B. Ragan, and C. Hsu. 1988. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 241:334.

Rovensky, J., M. Vigas, J. Marek, S. Blazickova, L. Korcakova, L. Vyletelkova and A. Takac. 1991. Evidence for immunomodulatory properties of prolactin in selected **in vitro** and **in vivo** situatioins. Int. J. Immunopharmac. 13;213:267.

Russell, D.H. and A.R. Buckley. 1988. Hypothalamic site of action of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol. Appl. Pharmacol. 94:496.

Russell, D.H., R. Kibler, L. Matrisian, D.F. Larson, B. Poulos and B. Magun. 1985. Prolactin receptors on human T and B lymphocytes: binding site in human erythrocytes and lymphocytes. Life Sci. 41:951.

Russell, D.H., L. Matrisian, R. Kibler, D.F. Larson, B. Poulos and B. Magun. 1984. Prolactin receptors on human lymphocytes and their modulation by cyclosporine. Biochem. Biophys. Res. Commun. 121:899.

Safe, S., L.W. Robertson, L. Safe, A. Parkinson, S. Bandiera, T. Sawyer, and M.A. Campbell. 1982. Halogenated biphenyls: molecular toxicity. Canad. J. Physiol. Pharmacol. 60:1057.

Sanders, O.T., R.L. Kirkpatrick, P.E. Scanlon. Polychlorinated biphenyls and nutritional restriction: their effects and interactions on endocrine and reproductive characteristics of white male mice. 1977. Toxicol. Appl. Pharmacol. 40:91.

Schleimer, R.P., A. Jacques, H. Shin, L.M. Lichtenstein and M. Plant. 1984. Inhibition of T cell-mediated cytotoxicity by antiinflamatory steroids. J. Immunol. 132;1:266.

Seefeld, M.D., S.W. Corbett, R.E. Keesey and R.E. Peterson. 1984. Characterization of the wasting syndrome in rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 73:311.

Silkworth, J.B., L. Antrim, L. Kaminsky. 1984. Correlations between polychlorinated biphenyl immunotoxicity, the aromatic hydrocarbon locus, and liver microsomal enzyme induction in C57BL/6 and DBA/2 mice. Toxicol. Appl. Pharmacol. 75:156.

Silkworth, J.B. and E.M. Grabstein. 1982. Polychlorinated biphenyl immunotoxicity: dependence on isomer planarity and the Ah gene complex. Toxicol. Appl. Pharmacol. 65:109.

Sinha, N., F.W. Selby, U.J. Lewis and P. Vanderlaan. 1972. Studies of prolactin secretion in mice by a homologous radioimmunoassay. Endocrinology 91:1045.

Umbriet, T.H. and M.A. Gallo. 1988. Physiological implications of estrogen receptor modulation by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Lett. 42:5.

Vos, J.G., R.E. Faith and M.I. Luster. 1980. In Halogenated biphenyls, naphthalenes, dibenzodioxins and related products. R.D. Kimbrough, ed. Elsevier/North-Holland Biochemical Press. Amsterdam, p. 241-266.

Witorsch, R.J. and J.I. Kitay. 1972. Pituitary hormones affecting adrenal 5-a-reductase activity: ACTH, growth hormone and prolactin. Endocrinology 91:764.

APPENDICES

APPENDIX A

PROCEDURES

Procedure			Page
1.	Mouse Bleeding Procedure		55
2.	Mouse Laminar Flow Room		58
3.	Maintenance of P815 Tumor		60
4.	Preparation of 3,3'4,4'5,5'-Hexachlorobiphenyl		65
5.	Corticosterone Assay		72
6.	a.	Prolactin Assay: Iodination	79
6.	b.	Prolactin Assay: Radioimmunoassay	86

Procedure 1

MOUSE BLEEDING PROCEDURE

Prepared by: Annette Youngberg

I. Reagents and Supplies

A. balance (Kerkvliet's lab), model # Mettler PN163
B. paper towels for carcass
C. 1 cc tuberculin syringe (1 per mouse + extras)
D. metal scissors
E. 15% EDTA (K_2EDTA) (w/v)
F. sample vials - labelled with mouse number (plastic microcentrifuge tubes with cap, VWR #20170-331)
G. bucket with ice for samples
H. gloves for handling mice
I. weigh boats (large) for balance - carcass
J. bags for carcass disposal (1 qt. Ziploc)
K. 70% ethanol
L. black pens
M. Sharpies
N. Kim Wipes
O. data book

II. Procedure

A. The afternoon before mice are to be bled, move the mice to Dr. Kerkvliet's laboratory, Room 306, Dryden Hall. Randomize the order of the cages, and do not move them after that.

B. Prepare supplies before bleeding time (see supply list).

C. Mouse bleeding begins at 0930 each morning. All supplies are kept in VRL, Room 114. 15 minutes should be allowed for setting up.

D. Calibrate the balance (Mettler PN163) with a 10 g calibration weight. Log this in data book.

E. Set out two paper towels on which to bleed the mouse.

F. With a 1 cc tuberculin syringe draw up 0.05 ml of 15% EDTA. Place the EDTA in the syringe case cap. Draw the plunger back to 0.2 ml.

G. Take a mouse from the first cage. Quickly kill the mouse by cervical dislocation. Pull mainly on the head. Pulling on the tail may cause rupture of the aorta, leading to blood in the abdominal cavity rather than in the heart.

H. Mat down the fur around the neck with 70% alcohol. Dry with a Kim Wipe.

I. Cut the head off the mouse.

J. Collect the blood from the neck area into the syringe case cap with EDTA. Mix well.

K. After checking the ear tag number of the mouse, draw the blood up into the syringe and place into a plastic microcentrifuge tube with cap, prelabelled. Mix well. Place in ice.

L. Weigh mouse carcass and head in balance tared with large weigh boat.

M. Record in data book: Study day, date, initials of people working that day, animal number, cage number, spleen weight, and thymus weight.

N. Label paper towel with animal number and cage number of mouse. Place carcass and head on paper towel and pass to immunology person.

O. Repeat steps 7-14 on all mice. Take one mouse from each cage from left to right (facing cages) then repeat. Keep doing this until all mice are done.

P. All cage cards should be placed in data book.

Q. Return empty cages to LAR, separating out and labelling with biohazard sticker all cages, lids, and water bottles from PCB exposed mice.

R. Take samples to VRL 112 for processing. Centrifuge all samples in the Beckman Microfuge 12 in VRL, Room 114 for 10 minutes at speed setting "11".

S. Remove plasma from cells into two plastic microcentrifuge tubes with lids - 25 ul into one tube for corticosterone and the rest into a second tube for prolactin. Label tubes with animal number.

T. Store sample in -20°C freezer in VRL Room 112 until analysis.

Procedure 2

MOUSE LAMINAR FLOW ROOM: GUIDELINES

Prepared by: Linda Steppan

I. Reagents and Supplies

A. Reagents

Disinfectant: 70% Ethanol + 4% Nolvasan
70% EtOH: 2000 ml EtOH + 714 RO water
Nolvasan, #0626-1E, Fort Dodge Labs
Mix 960 ml 70% EtOH with 40 ml Nolvasan. Transfer to spray bottle for use.

B. Supplies

Shoe covers, #69551, Kimberly Clark
Reagent bottle, 1000 ml
Spray bottle, 250 ml
Gloves, latex type; small, med, large, #312, Perry

II. Procedure

A. Do not enter the mouse laminar flow room if you have been in other animal rooms that day--**NO EXCEPTIONS!!!**

B. Do not walk into the area where dirty animal cages are sitting before going into the animal room.

C. Leave "street" lab coat in the ante room.

D. Use **new** shoe covers each time you enter the animal room.

E. Foreign materials (test tubes, racks, vials, etc.) must be sprayed with disinfectant (Appendix A) in the ante room before entry into the animal room.

F. Wear animal room lab coat whenever you are in the room.

G. Animal room lab coats willbe cleaned biweekly.

H. Gloves must be worn while handling mice.

I. Wash hands before putting on gloves.

Procedure 3

MAINTENANCE OF P815 TUMOR CELLS

Prepared by: Linda Steppan

I. Reagents and Supplies

A. Reagents

a. HBSS (see Appendix for formula)
b. 70% alcohol
   200 ml 95% EtOH plus 714 ml deionized water
c. Trypan Blue, 0.2% (see Appendix for formula)

B. Supplies

a. Centrifuge
b. Bioflow hood
c. CO_2 tank and killing jar
d. Gilson 'Pipetman' pipettor, P200
e. Microscope
f. Hemacytometer
g. Small sharp scissors
h. Dressing forceps
i. 10 cc syringe
j. 20 or 22 g 1 inch needle
k. 50 ml sterile conical centrifuge tube, Corning 25330
l. 15 ml sterile conical centrifuge tubes, Corning 25319
m. Plastic disposable tips for pipettor
n. Pipets 2, 5, and 10 ml, sterile
0. 12 x 75 disposable glass tubes
p. Small autoclave bag

II. Procedure

A. Harvesting of P815 cells in ascites

1. Under the Bioflow hood, fill 10 ml syringe with cold HBSS and attach needle.

2. Kill mouse in carbon dioxide jar.

3. Lay mouse on its back and wet abdominal hair with 70% alcohol.

4. Cut skin and pull back to reveal peritoneum. Don't touch or otherwise contaminate the surface.

5. Lift peritoneum with forceps and insert needle with bevel down about 1 cm, taking care not to puncture organs.

6. Inject the 10 ml HBSS quickly to flush cavity. Withdraw as much fluid as possible (usually 8-10 ml).

7. Remove needle from syringe and transfer cell suspension to 50 ml centrifuge tube. Add an additional 20 ml cold HBSS.

8. Centrifuge at 800-1000 rpm for 10 minutes.

9. Resuspend cell pellet in 10-20 ml cold HBSS. Aspirate the suspension in and out of a 10 ml pipet several times to dissociate the cells.

NOTE: All steps involving manipulation of cells with the container open should be performed in the Bioflow hood.

NOTE: All items contained with tumor cells should be autoclaved or otherwise decontaminated.

B. Counting Cells

1. Dilute cells 1:50 - 1:100 (depending on pellet size) by diluting 100 ul in 5 ml or 10 ml HBSS. Mix well and

transfer 100 ul to a 12 x 75 mm tube containing 100 ul of 0.2% trypan blue. The final dilution willbe 1:100 or 1:200.

2. Fill both chambers of hemacytometer. Count all viable cells in four corner squares of each chamber. Calculate cells per ml:

$$\frac{\text{viable cell count}}{\text{\# of chambers}} \times \text{dilution} = \text{viable cells} \times 10^4/\text{ml}$$

3. Keep cells on ice until needed for injection.

4. Record requested information in culture book.

C. Injection of Cells

1. Normal maintenance of P815 cells in ascites is done on a weekly basis. Two mice are usually injected. A seven day harvest of 6×10^5 cells injected will yield $3\text{-}5 \times 10^8$ cells. The general condition of the animals should be noted, along with the viability of the cells (when counting) and the numbers of red blood cells present in the ascites.

2. Calculate the amount of cell suspension needed to make 8×10^5 cells per ml. Each mouse willbe given an ip injection of 0.5 ml or 4×10^5 P815 cells.

   Injections should be done as soon as possible after harvesting.

3. Select two DBA mice and inject the cells intraperitoneally. If the mice are in a cage with others, earpunch them to differentiate. Make a label for the front of the cage and record the date and the number of cells transferred.

III. Appendix: Reagent Sheet

A. Hank's Balanced Salt Solution (HBSS), 1X

HBSS, 10x Gibco #310-4060, lot number 24P7384

Gentamicin, dilute to 5 mg/ml in RO water, Gibco #600-5750, lot number 14N3384

HEPES, 2M, Sigma #H3375, lot number 4-12-89

NaOH, 1N, prepared 10-19-88

Final Volume (ml)	100	200	400	600
Distilled water	88	176.5	347	520
HBSS	10	20	40	60
Gentamicin	1	2	4	6
HEPES	1.0	2.0	4.0	6.0
NaOH (pH to 7.2)	0.25	0.5	1	1.5

B. 0.2% Trypan Blue

45 ml RO water

5 ml HBSS (10x)

0.1 g Trypan blue

0.05 g Sodium Azide (toxic chemical, wear gloves
and mask)

Combine, stir until stain is dissolved and filter through filter paper. Store at 4℃.

Procedure 4

PREPARATION OF 3,3',4,4',5,5'-HEXACHLOROBIPHENYL

Prepared by: Linda Steppan

I. Reagents and Supplies

A. 3,3',4,4',5,5'-HCB(Ultra Scientific; #RPC-090; 5 mg/vial), lot number A-0123, received 11-88
B. Acetone (HPLC grade), lot number 09020PP
C. Peanut Oil, Planters, lot number 0066A, received 4-89
D. Evaporator with manifold system and N_2 source (Craig's lab)
E. 18 G x 1 1/2" needles

II. Procedure

A. Wear gloves at all times when handling the HCB. Cover the work surface with a lab mat.

B. Add 3 ml acetone to each vial of HCB using a 3 or 5 cc syringe and 26 G needle. Insert the needle through the rubber plug in the vial cap. Place a small piece of time tape over the hole left by the needle to seal the cap. vortex each vial.

C. Incubate the vials in a 37 °C incubator for 4-6 hours and vortex periodically.

D. Add 1 ml peanut oil to each vial. The caps can be removed and the oil added with a 1 ml disposable pipet or using a pipetting device. Vortex.

E. Incubate the vials in a 37 °C incubator for 1-2 hours.

F. Evaporate the acetone using an evaporator and N_2 stream. Add distilled water to the evaporator and heat to 45 °C. Attach 18 G

needles to the manifold and position over the HCB vials. Adjust the gas stream so that the liquid bubbles slightly but does not splash. Evaporation of the acetone takes 45-60 min.

G. Dilute HCB to 1 mg/ml (i.e., dilute each vial to a total of 5 ml) and dose by gavage with 0.2 ml per 20 g BW to give **10 mg/kg.**

III. Appendix

A. Reagent log

DATE PREPARED	CONC	LOT	AMOUNT	ANALYSIS
11-15-88	1 mg/ml	2-88	30 mg	
4-19-89	1 mg/ml	A-0123	60 mg	1.1 mg/ml (by EPA)
6-23-89	1 mg/ml	A-0123	35 mg	

DATE	STOCKS RECEIVED	LOT	AMOUNT
11-88	$(3,4,5)^2$-HCB	A-0123	20 x 5 mg/vial
4-89		A-0123	1 x 5 mg vial to Paul Wickster, EPA for use as standard

B. Guidelines for the Safe Handling of Chlorinated Dioxins and Related Compounds

(Prepared by: Julie A. Brauner)

Dioxins and related compounds are extremely hazardous and classified as Poison B reagents (Radian Corporation). TCDD and

p-dioxane (solvent for TCDD for **in vivo** use) have been defined by the National Toxicology Program as chemicals that may reasonably be anticipated to be carcinogenic. However, OSU's Committee on Hazardous Chemicals did not include TCDD on the list of carcinogens. On the other hand, PCBs are defined by OSU's Committee on Hazardous Chemicals as a Class B chemical carcinogen. Therefore, certain precautions are in order so that these chemicals can be handled, stored, and maintained with proper care to minimize the possibility of unforeseen accidents. In general, techniques used in handling radioactive and infectious materials are applicable to dioxins.

Protective Clothing

1. A fully fastened lab coat must be worn over street clothes. A lab coat or coveralls impervious to these chemicals would offer the most protection.
2. Two dissimilar types of impervious gloves must be worn and discarded after each use.
3. Safety glasses in accordance with ANSI standards willbe worn at all times.
4. Open-toe sandals, clogs, and bare feet are prohibited.
5. Respirators must be used whenever handling dry chemicals that may produce airborne contamination or in the case of an accident or spill.

Work Areas

1. Any procedure that may produce airborne contamination must be conducted in Dr. Craig's chemical fume hood (room 311). This chemical fume hood has been certified by OSU's Office of Environmental Health and Safety at 107 fpm (7-87) which exceeds the minimum average face velocity of 100 feet per min.
2. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazard. Therefore, chemicals in dilute solutions can be utilized on the open bench.
3. All work surfaces which could be potentially exposed to these chemicals need to be protected by disposable plastic-backed absorbent paper. If a surface becomes contaminated or if a spill occurs, the work surface must be decontaminated or disposed of immediately (as indicated below).

Personal Hygiene

1. Hands and forearms must be thoroughly washed after each manipulation and before coffee and lunch breaks.
2. Eating, drinking, smoking, chewing gum, applying cosmetics, or storing food is prohibited in the laboratory where these chemicals are being used or stored.

3. Oral pipetting is absolutely prohibited. Pipetting must be performed by mechanical means.

4. Hands must be kept away from areas of the face, especially the mouth, nose, and eyes, when working with these chemicals.

Disposal of Wastes

1. According to KOR Isotopes, low-level waste such as absorbent paper, tissues, animal remains, and plastic gloves may be burned in a "good" incinerator because dioxins decompose at high temperatures. VMAIL's incinerator has been certified for burning biohazard waste.

2. Low-level waste must be disposed in the duly marked "bio-hazard" covered waste can that is double lined with plastic bags. **Do not dispose waste in the general waste cans.** The double-packaged low level waste then will be taken to Laboratory Animal Resource (LAR) to be delivered to VMAIL for incineration.

3. Gross quantities (milligrams) must be packaged securely and disposed of through OSU's Office of Environmental Health and Safety.

4. Liquids are allowed to evaporate in Craig's certified hood and in a disposable container. Residues may then be handled as above.

5. Good technique includes minimizing contaminated waste.

Decontamination

1. Personal--any mild soap with plenty of scrubbing action.
2. Glassware, tools, and surfaces--According to KOR Isotopes, chlorothene NU is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with chlorothene, then washing with any detergent and water. Dish water may be disposed of in the sewer. It is prudent to minimize solvent wastes because they may require special disposal through OSU's Office of Environmental Health and Safety which is expensive.
3. Accidents: Remove contaminated clothing immediately, taking precautions not to contaminate skin or other articles. Carefully place such clothing in a plastic bag for burning. Wash exposed skin vigorously and repeatedly.

Wipe Tests

1. According to KOR Isotopes, a useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper which is then extracted for 30 min with agitation in 5 ml chloroform or benzene at room temperature. For prompt analysis, gas chromatography may be used. Less than 1 lg on a wipe sample indicates an acute hazard and requires prompt cleaning before further use of the equipment of work

space. It indicates further that unacceptably sloppy work habits have been employed in the past.

Storage

1. Stock quantities of these chemicals must be stored in a tightly sealed primary container and placed inside an unbreakable, sealed outer container that is impervious to these chemicals.

2. All biohazardous material are stored in the small refrigerator outside room 305 that is locked at all times. See Julie or Linda, room 307-309, for key.

IV. References

Dornhogger, Mary K. 1986. **Handling Chemical Carcinogens: A Safety Guide for the Laboratory Researcher.** Chemsyn Science Laboratories, Lenexa, Kansas.

KOR Isotopes. Summary of safe handling of dioxins and related compounds in the research laboratory. **Letter**, Cambridge, MA.

Procedure 5

CORTICOSTERONE ASSAY

Prepared by: Annette Youngberg

I. Reagents and Supplies

A. Kit: ordered from ICN Biochemicals, Inc., Carlson, California 90746, catalogue numbers 07-120102 (100 tubes) and 70-012013 (200 tubes).

Store kit at 2-8 °C. All reagents are stable until expiration date on label. Included in kit (all are ready to use):

1. steroid diluent--phosphosaline gelatin buffer (pH 7.00 $\pm$ 0.1) containing rabbit gamma globulins.
2. anticorticosterone antibody--first antibody--corticosterone-3-carboxymethyloxime--BSA was used as the antigen to generate the antibody in rabbits. The antiserum is titered to bind 50-60% of the corticosterone- ^{125}I derivative in the absence of nonradioactive corticosterone. Yellow color.
3. corticosterone standards--for preparation of standard curve, prepared in steroid diluent. Six standards are provided: 25, 50, 100, 250, 500, and 1000 ng/ml. The concentrations of the standards are expressed in terms of serum equivalence. To obtain actual concentrations of corticosterone in ng/ml, divide value by 200.

4. precipitin solution--a mixture of PEG and goat antirabbit gamma globulins (second antibody) in a phosphosaline buffer. 500 ul of this precipitin will immediately precipitate all the antibody-bound antigen. Red color.

5. corticosterone- ^{125}I derivative--contains less than 3.5 uCi per vial (100 tube kit) or less than 7 uCi per vial (200 tube kit) on the date of shipment. 0.2 ml of this radioactive derivative will provide approximately 50,000 cpm at 75% counter-efficiency on the date of shipment. Blue color.

6. high and low level controls--ready to use after reconstitution with 2.0 ml deionized water and being allowed to sit at room temperature for at least 30 min.

B. Not included in kit:

1. DPC repeating pipetter
2. syringes for DPC pipetter
3. COBRA gamma counter (in Dryden Hall)
4. vortex mixer
5. gloves
6. test tube racks
7. 12 x 75 mm glass test tubes
8. centrifuge
9. lab mat
10. corks
11. pools -- rat (dilute 1:200)

mallard (dilute 1:200)

mouse (dilute 1:200)

12. 1000 1m Pipetman, 200 11 Pipetman, and 20 11 Pipetman.
13. yellow and blue Pipetman tips

II. Procedure

A. Before the assay:

1. Reserve gamma counter.
2. Prepare worksheet.
3. Label tubes.
4. Remove specimens from ultra-low freezer and set in refrigerator to thaw on the day before running the assay. Samples may be diluted the afternoon before a morning assay.
5. Dilute mice samples 1:200 (1990 ul diluent and 10 ul sample. A COBRA factor of 1.0 is necessary.
6. Dilute pools:

rat: 1:200

mallard 1:200

mouse 1:200

Do <u>not</u> dilute controls or standards.

B. Day of the assay:

1. Be sure all tubes are labeled properly and in duplicate as per the sample worksheet.

2. Bring all reagents to room temperature.

3. Set water bath to 25 /C and be sure there is enough water.

4. Reconstitute controls with 2.0 ml deionized water and let sit for 30 minutes.

5. Using Pipetman, add steroid diluent to assay tubes as follows:

   300 ul to tubes 3 and 4 (TC) and TC tubes at end of assay, if used.

   100 ul to tubes 5 and 6 (0 standard) and 0 standard tubes at end of assay, if used.

6. Using Pipetman, add 100 ul of appropriate standard to each standard tube (7 to 18).

7. Using Pipetman, add 100 ul of low level control (ready to use as is after reconstitution) to tubes 19 and 20 and low control tubes at end of assay.

8. Using Pipetman, add 100 ul of high level control (ready to use as is after reconstitution) to tubes 21 and 22 and high level control tubes at end of assay.

9. Using Pipetman, add 100 ul of rat, mallard, and mouse pools (diluted 1:200) to tubes 23-28 and rat, mallard, and mouse pool tubes at end of assay.

10. Using Pipetman, add 100 ul of 1:200 samples to appropriate tubes.

11. Using DPC pipetter, add 200 ul corticosterone- ^{125}I (blue reagent) to all tubes. Wear gloves from this point in the assay until it is finished. Also, wear radiation safety badge and rings.

12. Cork total count tubes.

13. Using DPC pipetter, add 200 ul anticorticosterone to all tubes, EXCEPT 1 and 2 and 3 and 4 and TC and NSB tubes at end of assay, if used. Use the DPC pipetter syringe that is labelled for ^{125}I corticosterone.

    WARNING: ^{125}I tracer <u>must</u> be added before antiserum.

14. Vortex mix all tubes.

15. Incubate for two hours in 25 °C water bath. Cover rack with aluminum foil. Do not shake.

SUMMARY SHEET				
Diluent	STD CNT PT	Trace	AB	
0	0	200	0	TC (1,2)
300	0	↓	0	NSB (3 4)
100	0	↓	200	0 (5,6
0	100	↓	↓	25-1000 stds (7-18)
↓	↓	↓	↓	controls, pool, patients

16. Add 500 ul precipitin solution (red reagent) to all tubes EXCEPT first two and last two tubes (TC tubes). Micromedic may be used (see "Use of Micromedic" for instructions).

17. Vortex THOROUGHLY.

18. Centrifuge for 15 minutes at 1000 g (about 2500 rpm in lab Beckman TJ-6) at setting "10".

19. Pour off supernatant. Blot tubes, allow to drain for 30 minutes. Blot again.

20. Count in gamma counter for one minute.

21. Prepare a front sheet for the print-out from the COBRA. Place all paperwork in corticosterone assay book.

22. Dispose of all liquid and solid actually and potentially radioactive waste in liquid and solid waste barrels, respectively.

23. Log the amount of isotope used, assay number, and assay date on the isotope order sheet for corticosterone.

III. References

Product Insert, ICN Biomedicals, Inc., Carson, California. June, 1986.

Procedure 6a

MURINE PROLACTIN ASSAY: IODINATION

Prepared by: Annette Youngberg

I. Reagents and Supplies

A. Purified mouse prolactin for iodination -- ordered from Dr. A. F. Parlow, Pituitary Hormones and Antisera Center, Harbor-UCLA Medical Center, 1000 W. Carson Street, Torrence, CA 90509: Phone (213) 533-3537; FAX (213) 533-3432; Lot #AFP-6476-C.

Approximately 50 ug is supplied (the exact weight is noted on the vial label). The antigen is lyophilized and should be stored frozen until just before use. At the time of the first iodination, the prolactin should be solubilized in 0.01 M $NaHCO_3$ at a concentration of 100 ug prolactin to 1 ml $NaHCO_3$. Multiple aliquots of 2.5 ug prolactin in 25 ul $NaHCO_3$ should be made in Sarsted 1.5 ml vials and frozen at -70°C until use. Label the vials with the amount and concentration of prolactin, date aliquoted, and lot number of prolactin. One vial is used per iodination.

B. Analytical grade anion exchange resin, AG 2 x 8, 50-100 mesh, chloride form, BioRad.

C. Chloramine T solution - 5 mg in 25 ml 0.05 M phosphate (pH 7.5) (see reagent sheet at end for preparation), make just prior to use.

D. Sodium metabisulfite - 6 mg in 25 ml 0.05 M phosphate (pH 7.5) (see reagent sheet at end for preparation), make just prior to use.

E. 0.05 M phosphate (see reagent sheet at end for preparation), prepare just prior to use.

F. 0.01 M $NaHCO_3$ (see reagent sheet at end for preparation).

G. 0.5 M phosphate (see reagent sheet at end for preparation).

H. 5% BSA in 0.05 M PBS (see reagent sheet at end for preparation).

I. 0.01 M PBS - 0.1% Knox gelatin (see reagent sheet at end for preparation).

J. ^{125}I in NaOH, product number IMS.30, carrier free iodine, ordered from Amersham.

K. Barrel of 3 ml syringe.

L. Glass wool.

M. Tygon tubing, size .05 x .09. VWR 63013-029.

N. Ring stand and clamp.

O. Tubing clamp.

P. 250 ml beaker.

Q. Various pipets and pipet bulb and test tube racks.

R. Parafilm.

S. 12 mm x 75 mm test tube, glass, not plastic.

T. Balance and associated supplies.

U. 2 10 ul Hamilton syringes (the "hot" syringe is stored in the hood).

V. One 20 ul Pipetteman.

W. Radioactive waste disposal.

X. TB syringe.

II. Procedure

A. Preparation of Column

1. Remove plunger from 3 ml syringe. The barrel will be used as the column.

2. Add a small amount of glass wool to the bottom of the column.

3. Add about 1 ml of BioRad AG 2 x 8 (chloride form, 50-100 mesh) packing to the column. Tap gently to avoid air pockets.

4. Place a small piece of Tygon tubing on the end of the column.

5. Set up a ring stand and clamp, and set the column in the clamp. Set a beaker under the column to collect the effluent.

6. Prerinse the column with 2 ml of 0.5 M phosphate, pH 7.5.

7. Rinse next with 2 ml 5% BSA - .05 M phosphate, pH 7.5.

8. Rinse last with 2 ml 0.05 M phosphate, pH 7.5.

9. Clamp tubing shut.

10. Place 0.5 ml 0.05 M phosphate on top of column.
11. Column may be used immediately or covered with parafilm, stored in the refrigerator, and used within 2-3 days. If the column is stored cold, it should be warmed to room temperature (preferably overnight) before use.
12. Buffers for the iodination should be made up fresh for each iodination.

B. Iodination

1. Warm column to room temperature if necessary.
2. In a 12 x 75 glass test tube place 1.0 ml of 0.01 M PBS-0.1% Knox gelatin. Place this tube under the column on the ring stand and move the entire assembly into the hood. Coat the sides of the tube with the gelatin mixture by inverting the tube several times.
3. Drain the reserve buffer (0.5 ml 0.05 M PBS) from column into the 12 x 75 test tube.
4. Rinse the 10 ul Hamilton syringes to be used (Chloramine T, sodium metabisulfite, and iodine) in LiftAway,, water, and then 70% ethanol, ten times each. Discard waste with radioactive waste.
5. Prepare the chloramine T and sodium metabisulfite solutions and store them in foil.
6. Turn on the hood.

7. Thaw the prolactin aliquot.
8. The vial containing the prolactin is used as the reaction vial.
9. Fill the chloramine T and sodium metabisulfite syringes; label them and set them aside in easy reach. Also prepare a TB syringe with 100 ul of 0.05 M phosphate.
10. Double gloves, lab coat, radiation badge and finger rings are to be worm for the rest of the procedure.
11. Just prior to use, coat the sides of the 12 x 75 glass tube with the gelatin mixture by inverting the tube several times.
12. To the reaction vial, add a 0.5 mCi ^{125}I (5 ul, using the "hot" Hamilton syringe). Add just above the prolactin. Be sure to observe that a "drop" of ^{125}I is seen. Mix by gently tapping side of reaction vial.
13. Using the chloramine T Hamilton syringe, add 5 ul of chloramine T to the reaction vial. Start a stopwatch as this is done.
14. Turn the reaction vial and agitate it gently for 60 seconds by tapping the side.
15. Terminate the reaction by adding 20 ul of sodium metabisulfite. (We use the Pipetteman.)
16. Briefly agitate the vial by tapping and turning.
17. Remove the reaction mixture from the reaction vial with a Pasteur pipet, and place it on the column.

18. Rinse the reaction vial with 100 ul of 0.05 M phosphate and add this also to the column.

19. Add 1.0 ml of 0.05 M phosphate to the column and allow it to drain through.

20. Repeat step 19. The collection tube contains purified labelled prolactin. Free iodine is retained on the column.

21. After column has drained, count 10 ul of eluate to determine activity. Usually, over 200,000 cpm are obtained, but not always.

22. Allow to equilibrate overnight in the refrigerator. Gently "finger-mix"the test tube. Prepare a dilution in another vial that gives an approximate count of 50,000 - 80,000 cpm per 100 ul. Prepare the dilution in 1% BSA-PBS. This is the storage form of the iodinated prolactin. Use this for your dilutions of trace to 10,000 cpm for your assay.

C. Clean Up

1. Cap the collection tube and the dilution tube and label them with the iodination number, date, your initials, and the cpm of 10 ul (for the collection tube) and of 100 ul (for the dilution vial). Store the tubes in the lead container in the refrigerator.

2. Rinse the Hamilton syringes with LiftAway,, water and 70% ethanol, ten times each.

3. Dispose of all contaminated wastes in the radioactive waste barrels.
4. Return all reagents and supplies to their proper locations.
5. Fillout an iodination sheet, and file it in the prolactin iodination book.
6. Do a room survey, and file the form in the Radiation Safety Book.
7. Log use of the isotope on the isotope sheet in the Radiation Safety Book.

IV. Reference

Adapted from Doug J. Bolt, USDA, Beltsville, MD 1986.

MURINE PROLACTIN ASSAY: RADIOIMMUNOASSAY

Prepared by: Nancy Hollingshead

I. Reagents and Supplies

A. Trace - use radiolabelled prolactin, diluted in 1% BSA-PBS to obtain approximately 10,000 to 11,000 cpm in 100 ul.

B. First antibody - rabbit antimouse prolactin, lot # AFP-13078, obtained from Dr. A. F. Parlow (see Procedure 6a for address). About 0.8 ml of a 1:50 dilution of first antibody in 2% normal rabbit serum in PBS is obtained. When received, this is further diluted 1:10 (for a final dilution of 1:500) in PBS. Multiple aliquots of 0.5 ml are prepared, placed in labelled Sarsted 1.5 ml vials, and stored at -70°C until use. The aliquot is diluted for use to a final dilution of 1:40,000 in 0.3% NRS-EDTA-PBS.

C. Second antibody - sheep antirabbit immunoglobulin, sheep #404 and #405 bled 7-9-87, pooled 10-13-89, mixed 1:1, stored in -70°C freezer. Thaw and dilute 1:30 in PBS. Mix 1:1 with PEG before use.

D. 6% polyethylene glycol (PEG), prepare in 0.01 M PBS, mix with second antibody 1:1 before use.

E. Prolactin lot # AFP-6476-C for standard curve - mouse prolactin reference preparation for RIA, obtained from Dr. A. F. Parlow

(see IA for address). 5 ug are received, lyophilized in 1 ml 1% BSA-PBS. Store in desiccant in refrigerator until use. To use, reconstitute with 1.0 ml distilled water. Dilute this 5 ug/ml solution to a 50 ng/ml solution in 1% BSA-PBS. Aliquot into labelled vials and store at -70°C until use.

F. Controls - normal mouse pool, NCH 5/23/90, 200 ul aliquots, stored at -70°C (male) and female, NCH 7/23/90, 200 ul aliquots.

G. "Buffer"- 1% BSA-PBS (see reagent sheet at end for preparation).

H. .3% NRS-EDTA-PBS - (see reagent sheet at end for preparation).

I. 12 mm x 75 mm glass test tubes. (NB - plastic tubes have been tried and do not work).

J. Caps for total count tubes.

K. 25°C water bath.

L. Radioactive waste disposal.

M. Various pipets, pipet bulb, test tube racks.

N. Micromedic - for pipetting buffer.

O. Internal pools of mouse plasma - stored in -70°C freezer.

II. Procedure

A. Dilute trace to approximately 13,000 cpm/100 ul (see IIA).

B. Dilute first antibody to 1:40,000 (see IIA).

C. Thaw samples and controls and reference prolactin.

D. Label sufficient 12 x 75 glass test tubes for assay.

(Run samples in triplicate. Run internal pools every 8-10 samples.)

E. Refer to Table I for tube designations and volumes.

F. Use Micromedic to pipet buffer. Check calibration prior to each use.

G. Prepare standard curve and NSB and NSB-P tubes (see Table I).

H. Pipet 20 ul of sample and controls to tubes with 480 ul buffer (see Table I).

I. In the hood, add 100 ul labelled prolactin trace to all tubes. (Gloves, lab coat, and radiation badges should be worn from this step on.)

J. Cap the total count tubes.

K. Add 200 ul of first antibody to all tubes **except** 1-7. Cover with parafilm.

L. Shake the rack gently (avoid foaming).

M. Incubates tubes in 25 °C water bath, with shaking, for 24 hours.

N. Remove tubes from water bath, and add 400 ul of second antibody with PEG to all tubes **except** total count tubes.

O. Shake all tubes gently (avoid foaming).

P. Incubate tubes in 25 °C water bath, with shaking, for 2 hours.

Q. Remove tubes from water bath. Centrifuge at 2-8 °C for 30-45 minutes at full speed (Beckman TJ-6R tabletop centrifuge with TH-4 rotor and buckets). Be sure the centrifuge is at 2-8 °C for the centrifugation.

R. Decant tubes gently into liquid waste bottle in hood. Rotate tubes while pouring off to prevent pellet from coming off bottom of tube. (Decant two buckets at a time, centrifuging the remaining tubes while decanting the first set.)

S. Let tubes sit inverted in hood for several hours or until dry (overnight works best).

T. Count on COBRA gamma counter for 2 minutes, using protocol #29.

U. Assay sheets are filed in the Prolactin Assay Book.

V. Clean Micromedic after each use. Disassemble to allow the tubing to dry completely.

TABLE I
(All volumes in ul)

Tube #	Name	Buffer	EDTA-NRS-Buffer	25 ng/ml Std	Sample or Control	PRL	1° Ab
1,2,3	total count	-	-	-	-	200	-
4,5	NSB	500	200	-	-	-	
6,7	NSB-P	480	200	-	(Control) 20		-
8,9,10 0	500	-	-	-	200		
11,12,13	0.025	499	-	1	-		
14,15,16	0.05	498	-	2	-		
17,18,19	0.10	496	-	4	-		
20,21,22	0.20	492	-	8	-		
23,24,25	0.50	480	-	20	-		
26,27,28	1.0	460	-	40	-		
29,30,31	2.0	420	-	80	-		
32,33,34	4.0	340	-	160	-		
35,36,37	10.0	100	-	400	-		
38,39,40	Samples + Controls	480	-	-	20		

IV. Appendix: Reagents

A. <u>10% EDTA</u> (anticoagulant for blood)

10.0 g of Na_2EDTA
100 ml (Q.S.) double distilled H_2O

Use 1-2 mg/ml of whole blood (.01 ml per sample)

(J.T. Baker, lot #314338)
(Sigma, lot #496-0506)

B. <u>.3 % Normal Rabbit Serum-EDTA-PBS</u> (diluent for first antibody)

1 l:
0.05 M disodium EDTA (18.42 g)
3.0 mls Rabbit serum
1 l (Q.S.) PBS

pH to 7.4 with NaOH

100 ml:
(1.8424 g) 0.05 M disodium EDTA
.3 mls Rabbit serum
100 mls (Q.S.) PBS

pH to 7.4 with NaOH

50 mls:
(.921 g) 0.05 M disodium EDTA
.15 mls Rabbit serum
50 mls (Q.S.) PBS

pH to 7.4 with NaOH

C. <u>0.01 M Phosphate Buffered Saline - PBS pH 7.4</u> (Basic PBS, used as diluent for second antibody)

NaCl (VWR Scientific, lot #918409, J.T. Baker, lot #407706)	16.364 g
Monobasic sodium phosphate (EM Science, lot #5226)	0.203 g
Dibasic sodium phosphate (Mallinkrodt, lot #KTXG)	2.6306 g
Thimersol (Sigma, lot #84F-0342)	0.2 g
Q.S. to	2 liters

- Make sure Na_2HPO_4 is anhydrous (141.96 F.W.)
- pH to 7.4 with NaOH
- Refrigerate buffer

D. 1% BSA - PBS (pH 7.4) (diluent for trace, "buffer" in assay)

1 l: 10 g Bovine serum albumin, fraction V
1000 mls PBS (Q.S.)

pH to 7.4 with NaOH

500 mls: 5 g Bovine serum albumin, fraction V
500 mls PBS (Q.S.)

pH to 7.4 with NaOH

(Research Products International Corp., lot #8372)
(Sigma, lot #99F00101)

E. 0.5 M Phosphate Buffer pH 7.4 (used for iodination)

100 mls: 1.159 g Monobasic sodium phosphate
5.906 g Dibasic sodium phosphate

Q.S. to 100 mls

- pH to 7.4 with NaOH
- may aliquot and freeze
- store at room temperature

0.05 M Phosphate Buffer pH 7.5 (used for iodination)

1 l: 1.095 Monobasic sodium phosphate
5.960 Dibasic sodium phosphate

Q.S. to 1 liter

- pH to 7.5 with NaOH

100 mls: .1095 g Monobasic sodium phosphate
.5960 g Dibasic sodium phosphate

Q.S. to 100 mls

- pH to 7.5 with NaOH

200 mls: .2190 g Monobasic sodium phosphate
1.192 g Dibasic sodium phosphate

F. 0.1 M Phosphate Buffer pH 7.5 (used for iodination)

100 mls: 1.380 g Monobasic sodium phosphate
Q.S. to 100 mls with distilled H_2O

1.420 g Dibasic sodium phosphate
Q.S. to 100 mls with distilled H_2O

USE MONOBASIC TO CORRECT pH to 7.5

G. 5% BSA (Bovine Serum Albumin) in .05 Phosphate Buffer (used for iodination)

25 mls: 1.25 g BSA
Q.S. to 25 mls with .05 M Phosphate buffer

H. 0.01 M PBS + .1% Knox Gelatin (used for iodination)

50 mls: .05 g Knox gelatin
Q.S. to 50 mls with .01 M PBS
- heat to disolve

I. Chloramine T (used for iodination)

5 mg Chloramine T (Sigma)
Q.S. to 25 ml in volumetric flask with 0.05 M phosphate buffer
make fresh just prior to use
store chloramine T protected from light in the -20°C dessicator; it is easily oxidized

J. Sodium Metabisulfite (used for iodination)

6 mg Sodium metabisulfite
Q.S. to 25 ml in volumetric flask with 0.05 M phosphate buffer
make fresh just prior to use

(J.T. Baker, lot #005396)

K. 0.01 M $NaHCO_3$ (used for iodination)

.042 g: $NaHCO_3$
Q.S. to 50 ml with distilled water

L. 6% PEG (Polyethylene Glycol) (MW ~ 8000) (used with second antibody)

100 mls: 6 g PEG
Q.S. to 100 mls with .01 M PBS (ph 7.4)

(Sigma, lot #18F-0033)

M. Purified Mouse Prolactin for Iodination (used for iodination)
lot #AFP6476C

N. Rabbit Antimurine Prolactin (used for first antibody)
lot #AFP-13078

O. Reference Prolactin (used for standard curve)
lot # AFP-6476-C

P. Sheep Anti-Rabbit Immunoglobulin (used as second antibody)
Sheep #404 and 405
Bled 7-9-87
Pooled 10-13-89

APPENDIX B

DATA

Table		Page
1.	Table 4	96
2.	Table 5	100
3.	Table 6	113
4.	Table 7	116
5.	Table 8	118
6.	Table 9	121
7.	Table 10	134

TABLE 4

WEIGHT DATA, EXPERIMENT 1: SUMMARY

file: pcbsum2 (scf)
created by A. Youngberg printer: 16.67
day 0 = 25 April 1989
animals administered pcb or vehicle on day -1
animals administerby gavage on day -1
This spreadsheet in edited data. The following mice were deleted:
Day 3: 189, 191.....scabby
Day 6: 077, 017.....chewed, 016.....+/- ascites
Day 7: 316.....nonresponder
Day 8: 285.....nonresponder
Day 21: 246, 248.....scabby, 048, 123.....nonresponder
Day 42: 278.....scabby
Day 84: 217, 118.....scabby
all weight in grams
change in weight = final - initial weights
Group A = control
Group B = tumor only
Group C = pcb only
Group D = tumor plus pcb

		Change in body weight				Spleen % body weight			
Day	Group	Mean	SD	N	SE	Mean	SD	N	SE
no body weights on day -1									
no body weight change on day -.6									
-1	A	N/A	N/A		N/A	N/A	N/A	N/A	N/A
-.6	A	N/A	N/A		N/A	N/A	N/A	N/A	N/A
0	A	.42	.19	6	.08	N/A	N/A	N/A	N/A
1	A	.53	.20	6	.08	N/A	N/A	N/A	N/A
2	A	1.07	.20	6	.08	.301	.034	6	.014
3	A	.62	.64	6	.26	N/A	N/A	N/A	N/A
4	A	.50	.46	6	.19	.300	.057	6	.023
5	A	1.58	.67	6	.27	N/A	N/A	N/A	N/A
6	A	1.28	.36	6	.15	.260	.019	5	.008
7	A	1.52	.82	6	.33	N/A	N/A	N/A	N/A
8	A	2.48	.82	6	.33	.290	.034	6	.014
9	A	2.18	.53	6	.22	N/A	N/A	N/A	N/A
10	A	2.70	.81	6	.33	.297	.032	6	.013
21	A	5.20	.76	6	.31	.322	.074	4	.037
42	A	7.48	1.00	6	.41	N/A	N/A	N/A	N/A
84	A	9.28	2.98	6	1.22	N/A	N/A	N/A	N/A
no Group B on days -1, -.6, 0									
-1	B	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
-.6	B	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0	B	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1	B	.97	.40	6	.16	N/A	N/A	N/A	N/A

Thymus % body weight			
Mean	SD	N	SE
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
.126	.032	6	.013
N/A	N/A	N/A	N/A
.126	.042	6	.017
N/A	N/A	N/A	N/A
.129	.042	6	.017
N/A	N/A	N/A	N/A
.145	.051	6	.021
N/A	N/A	N/A	N/A
.143	.050	6	.020
N/A	N/A	N/A	N/A
.135	.042	4	.021
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
.124	.029	6	.012

Day	Group	Change in body weight Mean	SD	N	SE	Spleen % body weight Mean	SD	N	SE
2	B	1.42	.43	6	.18	.280	.012	6	.005
3	B	1.13	.10	4	.05	N/A	N/A	N/A	N/A
4	B	.78	.92	6	.38	.273	.014	6	.006
5	B	2.65	.97	6	.40	N/A	N/A	N/A	N/A
6	B	3.88	.40	4	.20	.426	.077	4	.039
7	B	3.60	.44	6	.18	N/A	N/A	N/A	N/A
8	B	3.14	.93	6	.38	.502	.064	6	.026
9	B	4.10	.41	6	.17	N/A	N/A	N/A	N/A
10	B	3.83	.80	6	.33	.524	.070	6	.029
21	B	4.77	1.42	6	.58	.386	.085	6	.035
42	B	5.50	1.50	6	.61	N/A	N/A	N/A	N/A
84	B	9.46	1.60	5	.72	N/A	N/A	N/A	N/A

no Group C on day -1
no body weight change on day -.6

Day	Group	Change in body weight Mean	SD	N	SE	Spleen % body weight Mean	SD	N	SE
-1	C	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
-.6	C	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0	C	-.27	.50	6	.20	N/A	N/A	N/A	N/A
1	C	-.08	.42	6	.17	N/A	N/A	N/A	N/A
2	C	-.35	.73	6	.30	.257	.017	6	.007
3	C	-.27	.68	6	.28	N/A	N/A	N/A	N/A
4	C	-.40	.69	6	.28	.277	.028	6	.011
5	C	-.03	.68	6	.28	N/A	N/A	N/A	N/A
6	C	-.23	.83	6	.34	.257	.069	6	.028
7	C	.78	1.03	6	.42	N/A	N/A	N/A	N/A
8	C	1.28	.49	6	.20	.248	.017	6	.007
9	C	.93	.30	6	.12	N/A	N/A	N/A	N/A
10	C	.98	.44	6	.18	.244	.017	6	.007
21	C	2.77	1.11	6	.45	.258	.023	6	.009
42	C	3.22	1.99	5	.89	N/A	N/A	N/A	N/A
84	C	7.36	2.43	5	1.09	N/A	N/A	N/A	N/A

no Group D on days -1, -.6, 0

Day	Group	Change in body weight Mean	SD	N	SE	Spleen % body weight Mean	SD	N	SE
-1	D	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
-.6	D	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0	D	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1	D	-.33	.32	6	.13	N/A	N/A	N/A	N/A
2	D	.57	.75	6	.31	.286	.045	6	.018
3	D	.55	.40	6	.16	N/A	N/A	N/A	N/A
4	D	-.03	1.89	6	.77	.299	.015	6	.006
5	D	2.05	1.29	6	.53	N/A	N/A	N/A	N/A
6	D	1.00	.42	6	.17	.318	.052	6	.021
7	D	1.03	.76	6	.31	N/A	N/A	N/A	N/A
8	D	1.32	1.10	6	.45	.349	.093	6	.038
9	D	-.03	1.19	6	.49	N/A	N/A	N/A	N/A
10	D	-.47	1.43	6	.58	.373	.047	6	.019
21	D	-4.03	2.91	4	1.46	.285	.154	4	.077

Thymus % body weight			
Mean	SD	N	SE
N/A	N/A	N/A	N/A
.101	.029	4	.015
N/A	N/A	N/A	N/A
.097	.019	6	.008
N/A	N/A	N/A	N/A
.100	.025	6	.010
N/A	N/A	N/A	N/A
.082	.008	6	.003
N/A	N/A	N/A	N/A
.184	.036	6	.015
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
.105	.037	6	.015
N/A	N/A	N/A	N/A
.089	.032	6	.013
N/A	N/A	N/A	N/A
.074	.028	6	.011
N/A	N/A	N/A	N/A
.096	.017	6	.007
N/A	N/A	N/A	N/A
.108	.023	6	.009
N/A	N/A	N/A	N/A
.141	.028	6	.011
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A
.131	.021	6	.009
N/A	N/A	N/A	N/A
.085	.016	6	.007
N/A	N/A	N/A	N/A
.056	.020	6	.008
N/A	N/A	N/A	N/A
.045	.032	6	.013
N/A	N/A	N/A	N/A
.017	.004	6	.002
N/A	N/A	N/A	N/A
.014	.004	4	.002

TABLE 5

WEIGHT DATA, EXPERIMENT 1: EDITED RAW DATA

files: pcb2ayh and pcb2ayno (scf)
created by A. Youngberg printer: 16.67
day 0 = 25 April 1989
animals administered pcb or vehicle on day -1
animals administered tumor or vehicle on day 0
Group A = control
Group B = tumor only
Group C = pcb only
Group D = tumor plus pcb
contains: body and organ weight data
this is edited raw data; following were deleted:
Day 3: 189, 191.....scabby
Day 6: 077, 017.....chewed, 016.....+/- ascites
Day 7: 316.....nonresponder
Day 8: 285.....nonresponder
Day 21: 246, 248.....scabby, 048, 123.....nonresponder
Day 42: 278.....scabby, 075.....nonresponder
Day 84: 217, 118.....scabby
all weights in grams
change in body weight = final weight - initial weight

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in Body Wt (in g)	Spleen Wt (in g)	Spleen % BW	Thymus Wt (in g)	Thymus % BW
345		-1.0				.074		.043	
347		-1.0				.066		.024	
348		-1.0				.066		.036	
346		-1.0				.067		.024	
350		-1.0				.071		.025	
349		-1.0				.068		.026	
		mean				.069		.030	
		s d				.003		.008	
		n				6.000		6.000	
351	A	-.6	24.3			.102	.420	.035	.144
352	A	-.6	23.1			.081	.351	.029	.126
353	A	-.6	24.2			.084	.347	.029	.120
357	A	-.6	24.5			.083	.339	.038	.155

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in BW (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
358	A	-.6	19.7			.061	.310	.024	.122
359	A	-.6	22.0			.067	.305	.021	.095
		mean	23.0			.080	.345	.029	.127
		s d	1.9			.014	.041	.006	.021
		n	6.0			6.000	6.000	6.000	6.000
354	C	-.6	23.3			.079	.339	.042	.180
355	C	-.6	23.2			.064	.276	.039	.168
356	C	-.6	26.6			.089	.335	.033	.124
360	C	-.6	20.4			.056	.275	.035	.172
361	C	-.6	25.5			.069	.271	.032	.125
362	C	-.6	23.8			.068	.286	.023	.097
		mean	23.8			.071	.297	.034	.144
		s d	2.1			.012	.031	.007	.034
		n	6.0			6.000	6.000	6.000	6.000
318	A	.0	22.5	23.2	.70	.077	.332	.029	.125
321	A	.0	24.7	25.1	.40	.069	.275	.027	.108
324	A	.0	21.4	22.0	.60	.090	.409	.036	.164
367	A	.0	22.8	23.1	.30	.073	.316	.036	.156
368	A	.0	23.5	23.8	.30	.065	.273	.030	.126
369	A	.0	23.4	23.6	.20	.072	.305	.024	.102
		mean	23.1	23.5	.42	.074	.318	.030	.130
		s d	1.1	1.0	.19	.009	.050	.005	.025
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
342	C	.0	25.8	25.2	-.60	.078	.310	.032	.127
343	C	.0	23.6	22.9	-.70	.085	.371	.034	.148
344	C	.0	22.6	22.6	.00	.068	.301	.034	.150
364	C	.0	22.8	22.0	-.80	.101	.459	.037	.168
365	C	.0	22.8	22.9	.10	.099	.432	.029	.127
366	C	.0	23.0	23.4	.40	.060	.256	.032	.137
		mean	23.4	23.2	-.27	.082	.355	.033	.143
		s d	1.2	1.1	.50	.016	.080	.003	.016
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
050	A	1.0	22.0	22.8	.80	.067	.294	.042	.184
051	A	1.0	23.3	23.9	.60	.072	.301	.032	.134
053	A	1.0	23.7	23.9	.20	.066	.276	.027	.113
294	A	1.0	23.7	24.2	.50	.070	.289	.022	.091
295	A	1.0	24.3	24.8	.50	.074	.298	.028	.113
296	A	1.0	21.2	21.8	.60	.068	.312	.027	.124
		mean	23.0	23.6	.53	.070	.295	.030	.126
		s d	1.2	1.1	.20	.003	.012	.007	.032
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
055	B	1.0	22.7	23.9	1.20	.079	.331	.036	.151

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in BW (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
056	B	1.0	21.9	23.5	1.60	.085	.362	.035	.149
057	B	1.0	21.2	21.8	.60	.066	.303	.032	.147
297	B	1.0	25.4	26.4	1.00	.072	.273	.023	.087
298	B	1.0	24.6	25.5	.90	.082	.322	.024	.094
299	B	1.0	23.0	23.5	.50	.063	.268	.027	.115
		mean	23.1	24.1	.97	.075	.310	.030	.124
		s d	1.6	1.6	.40	.009	.036	.006	.029
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
058	C	1.0	20.7	20.8	.10	.052	.250	.022	.106
059	C	1.0	22.5	22.1	-.40	.063	.285	.039	.176
060	C	1.0	22.8	22.8	.00	.077	.338	.016	.070
300	C	1.0	24.4	25.0	.60	.090	.360	.023	.092
301	C	1.0	25.9	25.3	-.60	.066	.261	.022	.087
302	C	1.0	25.3	25.1	-.20	.095	.378	.025	.100
		mean	23.6	23.5	-.08	.074	.312	.025	.105
		s d	2.0	1.9	.42	.017	.054	.008	.037
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
061	D	1.0	22.1	22.1	.00	.062	.281	.033	.149
062	D	1.0	23.7	23.1	-.60	.075	.325	.036	.156
063	D	1.0	20.7	20.8	.10	.055	.264	.028	.135
303	D	1.0	24.7	24.3	-.40	.075	.309	.024	.099
304	D	1.0	24.0	23.6	-.40	.066	.280	.027	.114
305	D	1.0	22.3	21.6	-.70	.080	.370	.029	.134
		mean	22.9	22.6	-.33	.069	.305	.030	.131
		s d	1.5	1.3	.32	.009	.039	.004	.021
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
101	A	2.0	22.6	23.5	.90	.060	.255	.032	.136
102	A	2.0	20.7	21.7	1.00	.070	.323	.029	.134
103	A	2.0	21.7	23.1	1.40	.066	.286	.044	.190
258	A	2.0	20.7	21.7	1.00	.066	.304	.028	.129
259	A	2.0	21.1	22.0	.90	.063	.286	.062	.282
260	A	2.0	20.1	21.3	1.20	.075	.352	.032	.150
		mean	21.2	22.2	1.07	.067	.301	.038	.170
		s d	.9	.9	.20	.005	.034	.013	.059
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
104	B	2.0	22.3	23.1	.80	.066	.286	.039	.169
105	B	2.0	25.1	26.4	1.30	.073	.277	.031	.117
106	B	2.0	21.2	22.8	1.60	.059	.259	.037	.162
261	B	2.0	23.0	24.4	1.40	.071	.291	.033	.135
262	B	2.0	22.8	24.1	1.30	.069	.286	.042	.174
263	B	2.0	26.0	28.1	2.10	.080	.285	.028	.100
		mean	23.4	24.8	1.42	.070	.280	.035	.143
		s d	1.8	2.0	.43	.007	.012	.005	.030

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in BW (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
107	C	2.0	22.1	21.1	-1.00	.057	.270	.025	.118
108	C	2.0	21.3	20.8	-.50	.057	.274	.031	.149
109	C	2.0	22.4	21.1	-1.30	.057	.270	.056	.265
264	C	2.0	25.3	25.9	.60	.061	.236	.023	.089
265	C	2.0	24.1	23.9	-.20	.057	.238	.018	.075
266	C	2.0	21.4	21.7	.30	.055	.253	.030	.138
		mean	22.8	22.4	-.35	.057	.257	.031	.139
		s d	1.6	2.0	.73	.002	.017	.013	.068
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
110	D	2.0	21.4	20.9	-.50	.060	.287	.020	.096
111	D	2.0	23.3	24.7	1.40	.089	.360	.026	.105
112	D	2.0	20.1	21.3	1.20	.054	.254	.015	.070
267	D	2.0	21.1	21.0	-.10	.049	.233	.024	.114
268	D	2.0	23.3	23.8	.50	.074	.311	.023	.097
269	D	2.0	22.2	23.1	.90	.063	.273	.045	.195
		mean	21.9	22.5	.57	.065	.286	.026	.113
		s d	1.3	1.6	.75	.015	.045	.010	.043
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
025	A	3.0	23.9	24.8	.90	.089	.359	.043	.173
026	A	3.0	22.1	23.2	1.10	.068	.293	.042	.181
027	A	3.0	24.1	24.8	.70	.075	.302	.029	.117
186	A	3.0	24.9	26.0	1.10	.072	.277	.025	.096
187	A	3.0	21.6	22.1	.50	.056	.253	.025	.113
188	A	3.0	25.0	24.4	-.60	.074	.303	.019	.078
		mean	23.6	24.2	.62	.072	.298	.031	.126
		s d	1.4	1.4	.64	.011	.035	.010	.042
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
028	B	3.0	20.1	21.1	1.00	.061	.289	.027	.128
029	B	3.0	22.2	23.3	1.10	.067	.288	.014	.060
030	B	3.0	23.0	24.2	1.20	.076	.314	.025	.103
190	B	3.0	23.2	24.4	1.20	.077	.316	.027	.111
		mean	22.1	23.3	1.13	.070	.302	.023	.101
		s d	1.4	1.5	.10	.008	.015	.006	.029
		n	4.0	4.0	4.00	4.000	4.000	4.000	4.000
031	C	3.0	21.8	22.1	.30	.099	.448	.018	.081
032	C	3.0	21.1	21.8	.70	.081	.372	.033	.151
033	C	3.0	23.4	22.3	-1.10	.051	.229	.017	.076
192	C	3.0	22.7	22.2	-.50	.061	.275	.021	.095
193	C	3.0	24.3	23.5	-.80	.057	.243	.016	.068
194	C	3.0	24.9	24.7	-.20	.064	.259	.016	.065
		mean	23.0	22.8	-.27	.069	.304	.020	.089

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in BW (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
		s d	1.5	1.1	.68	.018	.087	.007	.032
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
034	D	3.0	21.4	21.5	.10	.061	.284	.015	.070
035	D	3.0	22.5	23.3	.80	.069	.296	.015	.064
036	D	3.0	22.2	23.1	.90	.063	.273	.023	.100
195	D	3.0	25.2	25.2	.00	.064	.254	.021	.083
196	D	3.0	24.9	25.8	.90	.087	.337	.022	.085
197	D	3.0	22.9	23.5	.60	.068	.289	.025	.106
		mean	23.2	23.7	.55	.069	.289	.020	.085
		s d	1.5	1.6	.40	.009	.028	.004	.016
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
001	A	4.0	21.4	22.0	.60	.090	.409	.039	.177
002	A	4.0	22.0	22.2	.20	.056	.252	.038	.171
003	A	4.0	23.0	22.9	-.10	.066	.288	.032	.140
137	A	4.0	25.0	25.8	.80	.080	.310	.044	.171
138	A	4.0	20.9	22.1	1.20	.063	.285	.036	.163
140	A	4.0	25.4	25.7	.30	.066	.257	.040	.156
		mean	23.0	23.5	.50	.070	.300	.038	.163
		s d	1.9	1.8	.46	.012	.057	.004	.014
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
004	B	4.0	23.4	23.7	.30	.070	.295	.031	.131
005	B	4.0	23.6	25.0	1.40	.069	.276	.023	.092
006	B	4.0	20.6	20.4	-.20	.054	.265	.020	.098
141	B	4.0	22.8	22.6	-.20	.057	.252	.033	.146
142	B	4.0	24.9	26.6	1.70	.074	.278	.043	.162
143	B	4.0	22.8	24.5	1.70	.067	.273	.034	.139
		mean	23.0	23.8	.78	.065	.273	.031	.128
		s d	1.4	2.1	.92	.008	.014	.008	.027
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
007*	C	4.0	23.2	22.4	-.80	.057	.254	.027	.121
008*	C	4.0	19.9	19.8	-.10	.046	.232	.019	.096
009*	C	4.0	24.1	22.8	-1.30	.069	.303	.034	.149
144	C	4.0	23.7	23.5	-.20	.065	.277	.029	.123
145	C	4.0	26.1	25.4	-.70	.076	.299	.030	.118
146	C	4.0	21.0	21.7	.70	.064	.295	.028	.129
		mean	23.0	22.6	-.40	.063	.277	.028	.123
		s d	2.2	1.9	.69	.010	.028	.005	.017
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
010	D	4.0	24.9	25.0	.10	.077	.308	.019	.076
011	D	4.0	26.1	22.6	-3.50	.065	.288	.022	.097
012	D	4.0	22.3	21.6	-.70	.063	.292	.026	.120
147	D	4.0	25.7	27.0	1.30	.079	.293	.020	.074
148	D	4.0	22.7	23.9	1.20	.069	.289	.026	.109

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in BW (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
149	D	4.0	21.0	22.4	1.40	.073	.326	.021	.094
		mean	23.8	23.8	-.03	.071	.299	.022	.095
		s d	2.1	2.0	1.89	.006	.015	.003	.018
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
174	A	5.0	23.2	25.1	1.90	.076	.303	.026	.104
198	A	5.0	22.4	22.7	.30	.114	.502	.022	.097
199	A	5.0	24.5	26.0	1.50	.091	.350	.022	.085
200	A	5.0	22.5	24.3	1.80	.069	.284	.043	.177
319	A	5.0	23.1	24.9	1.80	.075	.301	.033	.133
370	A	5.0	17.1	19.3	2.20	.071	.368	.035	.181
		mean	22.1	23.7	1.58	.083	.351	.030	.129
		s d	2.6	2.4	.67	.017	.081	.008	.042
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
177	B	5.0	22.6	26.3	3.70	.107	.407	.027	.103
178	B	5.0	23.4	27.2	3.80	.101	.371	.033	.121
179	B	5.0	23.5	26.3	2.80	.109	.414	.023	.087
201	B	5.0	24.5	26.8	2.30	.086	.321	.030	.112
202	B	5.0	22.8	24.7	1.90	.093	.377	.018	.073
203	B	5.0	22.7	24.1	1.40	.087	.361	.020	.083
		mean	23.3	25.9	2.65	.097	.375	.025	.097
		s d	.7	1.2	.97	.010	.034	.006	.019
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
180	C	5.0	23.7	23.8	.10	.056	.235	.008	.034
181	C	5.0	22.0	22.4	.40	.075	.335	.013	.058
204	C	5.0	24.4	23.4	-1.00	.069	.295	.019	.081
205	C	5.0	26.0	25.3	-.70	.066	.261	.018	.071
206	C	5.0	22.4	22.6	.20	.064	.283	.026	.115
338	C	5.0	25.8	26.6	.80	.064	.241	.023	.086
		mean	24.1	24.0	-.03	.066	.275	.018	.074
		s d	1.7	1.6	.68	.006	.037	.007	.028
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
183	D	5.0	22.1	24.8	2.70	.077	.310	.021	.085
184	D	5.0	24.3	23.8	-.50	.087	.366	.006	.025
185	D	5.0	24.2	26.3	2.10	.090	.342	.013	.049
207	D	5.0	24.3	27.4	3.10	.092	.336	.017	.062
208	D	5.0	23.9	26.3	2.40	.087	.331	.018	.068
209	D	5.0	24.2	26.7	2.50	.095	.356	.013	.049
		mean	23.8	25.9	2.05	.088	.340	.015	.056
		s d	.9	1.3	1.29	.006	.019	.005	.020
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
013	A	6.0	22.2	23.3	1.10	.063	.270	.039	.167
014	A	6.0	21.8	22.9	1.10	.064	.279	.030	.131

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in BW (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
015	A	6.0	22.6	23.5	.90	.054	.230	.034	.145
076	A	6.0	24.7	26.2	1.50	.070	.267	N/A	N/A
078	A	6.0	21.2	23.0	1.80	.058	.252	N/A	N/A
		mean	22.5	23.8	1.28	.062	.260	.034	.148
		s d	1.3	1.4	.36	.006	.019	.004	.018
		n	5.0	5.0	5.00	5.000	5.000	5.000	3.000
018	B	6.0	22.8	26.7	3.90	.139	.521	.020	.075
079	B	6.0	20.5	24.7	4.20	.113	.457	.029	.117
080	B	6.0	23.4	27.5	4.10	.101	.367	.031	.113
081	B	6.0	18.1	21.4	3.30	.077	.360	.027	.126
		mean	21.2	25.1	3.88	.108	.426	.027	.108
		s d	2.4	2.7	.40	.026	.077	.005	.023
		n	4.0	4.0	4.00	4.000	4.000	4.000	4.000
019	C	6.0	21.7	22.0	.30	.050	.227	.028	.127
020	C	6.0	19.5	19.6	.10	.068	.347	.016	.082
021	C	6.0	23.4	21.5	-1.90	.071	.330	.029	.135
082	C	6.0	22.0	21.8	-.20	.043	.197	.007	.032
083	C	6.0	21.6	21.8	.20	.039	.179	.022	.101
085	C	6.0	20.2	20.3	.10	.053	.261	.012	.059
		mean	21.4	21.2	-.23	.054	.257	.019	.089
		s d	1.4	1.0	.83	.013	.069	.009	.040
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
022	D	6.0	23.8	25.1	1.30	.082	.327	.022	.088
023	D	6.0	22.0	23.7	1.70	.087	.367	.020	.084
024	D	6.0	22.2	22.8	.60	.052	.228	N/A	N/A
086	D	6.0	23.3	24.0	.70	.080	.333	.020	.083
087	D	6.0	23.4	24.2	.80	.070	.289	.018	.074
088	D	6.0	19.8	20.7	.90	.075	.362	.020	.097
		mean	22.4	23.4	1.00	.074	.318	.020	.085
		s d	1.5	1.5	.42	.012	.052	.001	.008
		n	6.0	6.0	6.00	6.000	6.000	5.000	5.000
125	A	7.0	20.8	23.4	2.60	.070	.299	.035	.150
126	A	7.0	22.7	23.9	1.20	.069	.289	.032	.134
127	A	7.0	23.4	24.1	.70	.066	.274	.018	.075
306	A	7.0	20.2	22.7	2.50	.060	.264	.043	.189
307	A	7.0	21.6	22.6	1.00	.057	.252	.025	.111
308	A	7.0	21.4	22.5	1.10	.063	.280	.048	.213
		mean	21.7	23.2	1.52	.064	.276	.034	.145
		s d	1.2	.7	.82	.005	.017	.011	.051
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
130	B	7.0	22.6	27.0	4.40	.109	.404	.024	.089
309	B	7.0	23.5	26.7	3.20	.053	.199	.019	.071

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in BW (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
310	B	7.0	19.3	22.5	3.20	.103	.458	.029	.129
311	B	7.0	23.3	26.8	3.50	.126	.470	.026	.097
322	B	7.0	22.2	25.9	3.70	.116	.448	.034	.131
323	B	7.0	22.4	26.0	3.60	.135	.519	.021	.081
		mean	22.2	25.8	3.60	.107	.416	.026	.100
		s d	1.5	1.7	.44	.029	.113	.005	.025
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
131	C	7.0	22.8	23.0	.20	.053	.230	.018	.078
132	C	7.0	24.1	24.7	.60	.070	.283	.026	.105
133	C	7.0	21.7	22.2	.50	.055	.248	.022	.099
312	C	7.0	23.2	23.1	-.10	.058	.251	.019	.082
313	C	7.0	22.8	23.5	.70	.062	.264	.029	.123
314	C	7.0	23.8	26.6	2.80	.069	.259	.024	.090
		mean	23.1	23.9	.78	.061	.256	.023	.096
		s d	.9	1.6	1.03	.007	.018	.004	.017
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
134	D	7.0	23.3	24.1	.80	.092	.382	.004	.017
135	D	7.0	23.5	24.3	.80	.085	.350	.006	.025
136	D	7.0	24.7	26.8	2.10	.070	.261	.013	.049
315	D	7.0	23.0	24.8	1.80	.101	.407	.007	.028
316	D	7.0	21.2	21.8	.60	.068	.312	.023	.106
317	D	7.0	24.0	24.1	.10	.068	.282	.011	.046
		mean	23.3	24.3	1.03	.081	.332	.011	.045
		s d	1.2	1.6	.76	.014	.057	.007	.032
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
162	A	8.0	22.5	23.9	1.40	.059	.247	.024	.100
163	A	8.0	23.4	25.8	2.40	.068	.264	.022	.085
164	A	8.0	21.4	24.0	2.60	.077	.321	.033	.138
282	A	8.0	23.2	25.3	2.10	.070	.277	.036	.142
283	A	8.0	21.3	25.2	3.90	.085	.337	.046	.183
284	A	8.0	24.0	26.5	2.50	.078	.294	.041	.155
		mean	22.6	25.1	2.48	.073	.290	.034	.134
		s d	1.1	1.0	.82	.009	.034	.009	.036
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
165	B	8.0	22.7	24.5	1.80	.126	.514	.019	.078
166	B	8.0	20.7	24.7	4.00	.106	.429	.026	.105
167	B	8.0	21.4	24.1	2.70	.145	.602	.037	.154
286	B	8.0	24.6	28.6	4.00	.134	.469	.021	.073
287	B	8.0	23.5	26.7	3.20	.133	.498	.016	.060
		mean	22.6	25.7	3.14	.129	.502	.024	.094
		s d	1.6	1.9	.93	.014	.064	.008	.037
		n	5.0	5.0	5.00	5.000	5.000	5.000	5.000

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in BW (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
168	C	8.0	23.4	24.3	.90	.053	.218	.027	.111
169	C	8.0	21.6	23.3	1.70	.059	.253	.036	.155
170	C	8.0	22.2	23.9	1.70	.062	.259	.023	.096
288	C	8.0	23.6	24.9	1.30	.059	.237	.027	.108
289	C	8.0	22.9	23.4	.50	.061	.261	.020	.085
290	C	8.0	21.4	23.0	1.60	.060	.261	.023	.100
		mean	22.5	23.8	1.28	.059	.248	.026	.109
		s d	.9	.7	.49	.003	.017	.006	.024
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
171	D	8.0	22.4	23.4	1.00	.068	.291	.013	.056
172	D	8.0	23.1	25.9	2.80	.077	.297	.021	.081
173	D	8.0	23.2	25.1	1.90	.075	.299	.011	.044
291	D	8.0	23.8	23.8	.00	.079	.332	.013	.055
292	D	8.0	21.0	23.0	2.00	.078	.339	.014	.061
293	D	8.0	21.9	22.1	.20	.118	.534	.010	.045
		mean	22.6	23.9	1.32	.083	.349	.014	.057
		s d	1.0	1.4	1.10	.018	.093	.004	.014
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
089	A	9.0	22.1	24.6	2.50	.070	.285	.035	.142
090	A	9.0	25.2	27.3	2.10	.075	.275	.038	.139
091	A	9.0	22.2	24.9	2.70	.115	.462	.057	.229
150	A	9.0	26.9	29.1	2.20	.085	.292	.031	.107
151	A	9.0	20.9	22.1	1.20	.081	.367	.018	.081
152	A	9.0	21.4	23.8	2.40	.074	.311	.035	.147
		mean	23.1	25.3	2.18	.083	.332	.036	.141
		s d	2.4	2.5	.53	.016	.072	.013	.050
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
092	B	9.0	19.7	23.8	4.10	.136	.571	.019	.080
093	B	9.0	21.8	26.6	4.80	.159	.598	.020	.075
094	B	9.0	19.9	23.9	4.00	.142	.594	.021	.088
153	B	9.0	21.7	26.0	4.30	.142	.546	.022	.085
154	B	9.0	21.1	24.8	3.70	.139	.560	.018	.073
155	B	9.0	21.6	25.3	3.70	.184	.727	.024	.095
		mean	21.0	25.1	4.10	.150	.600	.021	.082
		s d	.9	1.1	.41	.018	.066	.002	.008
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
095	C	9.0	21.7	23.0	1.30	.060	.261	.032	.139
096	C	9.0	23.7	24.4	.70	.054	.221	.025	.102
097	C	9.0	22.4	23.0	.60	.054	.235	.020	.087
156	C	9.0	22.0	23.3	1.30	.067	.288	.030	.129
157	C	9.0	22.1	22.9	.80	.059	.258	.018	.079
158	C	9.0	23.6	24.5	.90	.066	.269	.027	.110
		mean	22.6	23.5	.93	.060	.255	.025	.108

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in BW (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
		s d	.9	.7	.30	.006	.024	.006	.023
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
098	D	9.0	19.2	19.6	.40	.069	.352	.003	.015
100	D	9.0	23.0	21.3	-1.70	.074	.347	.003	.014
159	D	9.0	24.5	26.4	1.90	.064	.242	.003	.011
160	D	9.0	23.1	22.8	-.30	.072	.316	.005	.022
161	D	9.0	23.8	23.9	.10	.078	.326	.004	.017
327	D	9.0	24.2	23.6	-.60	.084	.356	.005	.021
		mean	23.0	22.9	-.03	.074	.323	.004	.017
		s d	1.9	2.3	1.19	.007	.043	.001	.004
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
222	A	10.0	23.3	26.4	3.10	.088	.333	.033	.125
223	A	10.0	20.5	23.6	3.10	.064	.271	.047	.199
224	A	10.0	23.8	27.3	3.50	.086	.315	.020	.073
234	A	10.0	26.2	28.4	2.20	.070	.246	.048	.169
235	A	10.0	22.8	25.8	3.00	..081	.314	.047	.182
236	A	10.0	21.2	22.5	1.30	.068	.302	.046	.204
		mean	23.0	25.7	2.70	.076	.297	.040	.159
		s d	2.0	2.2	.81	.010	.032	.011	.051
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
225	B	10.0	21.2	25.1	3.90	.156	.622	.060	.239
226	B	10.0	23.3	26.8	3.50	.123	.459	.051	.190
227	B	10.0	25.5	28.3	2.80	.145	.512	.026	.092
237	B	10.0	21.8	25.1	3.30	.150	.598	.029	.116
238	B	10.0	23.1	27.7	4.60	.127	.458	.036	.130
239	B	10.0	24.3	29.2	4.90	.144	.493	.033	.113
		mean	23.2	27.0	3.83	.141	.524	.039	.147
		s d	1.6	1.7	.80	.013	.070	.013	.056
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
228	C	10.0	22.2	23.6	1.40	.065	.275	.029	.123
229	C	10.0	21.9	22.4	.50	.054	.241	.025	.112
230	C	10.0	21.3	22.3	1.00	.051	.229	.023	.103
242	C	10.0	26.5	26.9	.40	.066	.245	.021	.078
325	C	10.0	24.6	25.8	1.20	.063	.244	.028	.109
326	C	10.0	23.3	24.7	1.40	.057	.231	.029	.117
		mean	23.3	24.3	.98	.059	.244	.026	.107
		s d	2.0	1.9	.44	.006	.017	.003	.016
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
231	D	10.0	24.6	23.3	-1.30	.079	.339	.031	.133
232	D	10.0	20.8	21.6	.80	.072	.333	.024	.111
233	D	10.0	20.2	20.5	.30	.088	.429	.014	.068
243	D	10.0	20.6	20.6	.00	.090	.437	.016	.078
244	D	10.0	24.0	24.4	.40	.086	.352	.026	.107

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in BW (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
245	D	10.0	24.0	21.0	-3.00	.073	.348	.033	.157
		mean	22.4	21.9	-.47	.081	.373	.024	.109
		s d	2.0	1.6	1.43	.008	.047	.008	.033
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
037	A	21.0	21.2	25.8	4.60	.084	.326	.024	.093
038	A	21.0	19.7	25.4	5.70	.108	.425	.029	.114
039	A	21.0	21.9	26.4	4.50	.072	.273	.050	.189
247	A	21.0	21.5	27.5	6.00	.073	.265	.039	.142
		mean	21.1	26.3	5.20	.084	.322	.036	.135
		s d	1.0	.9	.76	.017	.074	.012	.042
		n	4.0	4.0	4.00	4.000	4.000	4.000	4.000
040	B	21.0	25.0	29.7	4.70	.087	.293	.043	.145
041	B	21.0	20.2	27.2	7.00	.143	.526	.064	.235
042	B	21.0	19.4	24.5	5.10	.098	.400	.053	.216
249	B	21.0	23.4	26.4	3.00	.084	.318	.042	.159
250	B	21.0	22.1	27.4	5.30	.118	.431	.052	.190
251	B	21.0	21.3	24.8	3.50	.087	.351	.039	.157
		mean	21.9	26.7	4.77	.103	.386	.049	.184
		s d	2.1	1.9	1.42	.023	.085	.009	.036
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
043	C	21.0	23.6	25.4	1.80	.062	.244	.039	.154
044	C	21.0	21.4	22.8	1.40	.058	.254	.037	.162
045	C	21.0	21.2	23.9	2.70	.057	.238	.040	.167
252	C	21.0	23.0	27.5	4.50	.077	.280	.041	.149
253	C	21.0	22.5	25.4	2.90	.060	.236	.024	.094
254	C	21.0	25.4	28.7	3.30	.084	.293	.034	.118
		mean	22.9	25.6	2.77	.066	.258	.036	.141
		s d	1.6	2.2	1.11	.011	.023	.006	.028
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
047	D	21.0	21.2	20.6	-.60	.046	.223	.002	.010
122	D	21.0	22.5	19.2	-3.30	.098	.510	.003	.016
124	D	21.0	22.7	15.1	-7.60	.025	.166	.002	.013
341	D	21.0	20.3	15.7	-4.60	.038	.242	.003	.019
		mean	21.7	17.7	-4.03	.052	.285	.003	.014
		s d	1.1	2.7	2.91	.032	.154	.001	.004
		n	4.0	4.0	4.00	4.000	4.000	4.000	4.000
064	A	42.0	22.1	30.7	8.60	.088	.287	.036	.117
065	A	42.0	22.7	31.4	8.70	.111	.354	.030	.096
066	A	42.0	22.4	29.1	6.70	.078	.268	.037	.127
270	A	42.0	24.2	30.7	6.50	.086	.280	.032	.104
271	A	42.0	20.7	28.4	7.70	.094	.331	.030	.106
272	A	42.0	23.1	29.8	6.70	.105	.352	.055	.185

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in BW (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
		mean	22.5	30.0	7.48	.094	.312	.037	.122
		s d	1.2	1.1	1.00	.012	.038	.009	.032
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
067	B	42.0	21.1	28.0	6.90	.074	.264	.039	.139
068	B	42.0	21.6	28.7	7.10	.089	.310	.043	.150
069	B	42.0	24.2	28.8	4.60	.082	.285	.043	.149
273	B	42.0	21.7	26.5	4.80	.076	.287	.034	.128
274	B	42.0	22.6	28.9	6.30	.081	.280	.054	.187
275	B	42.0	25.0	28.3	3.30	.061	.216	.047	.166
		mean	22.7	28.2	5.50	.077	.274	.043	.153
		s d	1.6	.9	1.50	.009	.032	.007	.021
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
070	C	42.0	23.5	26.7	3.20	.090	.337	.042	.157
071	C	42.0	24.6	28.9	4.30	.074	.256	.049	.170
072	C	42.0	20.8	23.3	2.50	.063	.270	.070	.300
276	C	42.0	23.3	29.0	5.70	.071	.245	.041	.141
277	C	42.0	25.8	26.2	.40	.084	.321	.047	.179
		mean	23.6	26.8	3.22	.076	.286	.050	.190
		s d	1.9	2.3	1.99	.011	.041	.012	.064
		n	5.0	5.0	5.00	5.000	5.000	5.000	5.000
212	A	84.0	24.0	37.1	13.10	.102	.275	.053	.143
115	A	84.0	21.6	29.6	8.00	.083	.280	.049	.166
210	A	84.0	22.6	27.3	4.70	.091	.333	.034	.125
113	A	84.0	23.1	32.5	9.40	.096	.295	.045	.138
211	A	84.0	21.2	29.8	8.60	.079	.265	.029	.097
114	A	84.0	25.7	37.6	11.90	.085	.226	.052	.138
		mean	23.0	32.3	9.28	.089	.279	.044	.135
		s d	1.7	4.2	2.98	.009	.035	.010	.023
		n	6.0	6.0	6.00	6.000	6.000	6.000	6.000
117	B	84.0	22.8	30.4	7.60	.124	.408	.050	.164
215	B	84.0	20.0	29.8	9.80	.087	.292	.042	.141
214	B	84.0	23.0	33.3	10.30	.097	.291	.077	.231
116	B	84.0	20.9	32.4	11.50	.089	.275	.052	.160
213	B	84.0	24.7	32.8	8.10	.104	.317	.047	.143
		mean	22.3	31.7	9.46	.100	.317	.054	.168
		s d	1.9	1.5	1.60	.015	.053	.014	.037
		n	5.0	5.0	5.00	5.000	5.000	5.000	5.000
372	C	84.0	22.1	29.4	7.30	.082	.279	.019	.065
120	C	84.0	22.4	30.1	7.70	.073	.243	.035	.116
218	C	84.0	21.8	32.0	10.20	.091	.284	.034	.106
121	C	84.0	22.0	30.1	8.10	.111	.369	.043	.143

Animal Number	Group	Day	Initial Body Wt (in g)	Final Body Wt (in g)	Change in BW (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
119	C	84.0	23.7	27.2	3.50	.078	.287	.041	.151
		mean	22.4	29.8	7.36	.087	.292	.034	.116
		s d	.8	1.7	2.43	.015	.046	.009	.034
		n	5.0	5.0	5.00	5.000	5.000	5.000	5.000

TABLE 6

WEIGHT DATA, EXPERIMENT 2: EDITED RAW DATA

```
file name: pcbwt2 (scf)
created by A. Youngberg  printer: 16.67
day 0 = 11 Dec 1990
animals administerd pcb or vehicle on day -1
animals administered tumor or vehicle on day 0
Group A = controls
Group B = tumor only
Group C = pcb only
Group D = tumor plus pcb
contains: body, spleen, thymus weight data, experiment 2
                this is edited raw date; animals excluded:
                day 3:A-4.......died
                day 3: D-2......nonresponder
                day 10: D-14....died
                day 10: D-15....nonresponder
animals killed by CO2 overdose
blood collected by cardiac puncture
day 3: Carol Forsyth killed; Nancy Hollingshead bled
day 10: Nancy Hollinghead bled; faster than day 3
day 10: lab found to be very cold in am, 17.7 C
```

Animal Number	Group	Day	Initial Weight (in g)	Final Weight (in g)	Weight Change (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
1	A	3.0	20.50	19.80	-.70	.0477	.241	.0193	.097
2	A	3.0	19.50	19.63	.13	.0483	.246	.0171	.087
3	A	3.0	20.50	21.15	.65	.0519	.245	.0184	.087
4	A	3.0	22.50	dead					
5	A	3.0	24.00	22.96	-1.04	.0699	.304	.0131	.057
6	A	3.0	22.00	21.68	-.32	.0612	.282	.0253	.117
7	A	3.0	22.50	23.12	.62	.0728	.315	.0304	.131
8	A	3.0	18.50	18.67	.17	.0451	.242	.0291	.156
		mean	21.25		-.07	.0567	.268	.0218	.105
		s d	1.81		.64	.0113	.032	.0065	.033
		n	8.00		7.00	7.0000	7.000	7.0000	7.000
		se	.64		.24	.0043	.012	.0025	.012
1	B	3.0	21.00	20.43	-.57	.0530	.259	.0368	.180
2	B	3.0	20.50	21.22	.72	.0540	.254	.0168	.079
3	B	3.0	21.50	21.23	-.27	.0507	.239	.0140	.066
4	B	3.0	20.00	19.04	-.96	.0465	.244	.0201	.106
5	B	3.0	21.50	21.57	.07	.0545	.253	.0290	.134
6	B	3.0	22.00	22.45	.45	.0540	.241	.0327	.146
7	B	3.0	19.00	18.89	-.11	.0459	.243	.0124	.066
8	B	3.0	23.00	20.95	-2.05	.0546	.261	.0233	.111

Animal Number	Group	Day	Initial Weight (in g)	Final Weight (in g)	Weight Change (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
		mean	21.06		-.34	.0517	.249	.0231	.111
		s d	1.24		.87	.0036	.009	.0089	.041
		n	8.00		8.00	8.0000	8.000	8.0000	8.000
		se	.44		.31	.0013	.003	.0032	.014
1	C	3.0	21.00	22.04	1.04	.0604	.274	.0289	.131
2	C	3.0	19.00	19.34	.34	.0516	.267	.0180	.093
3	C	3.0	22.00	22.48	.48	.0564	.251	.0257	.114
4	C	3.0	22.00	21.54	-.46	.0634	.294	.0200	.093
5	C	3.0	23.00	24.20	1.20	.0714	.295	.0177	.073
6	C	3.0	20.00	21.24	1.24	.0570	.268	.0269	.127
7	C	3.0	23.00	23.56	.56	.0639	.271	.0337	.143
8	C	3.0	23.50	23.82	.32	.0685	.288	.0298	.125
		mean	21.69		.59	.0616	.276	.0251	.112
		s d	1.58		.57	.0066	.015	.0059	.024
		n	8.00		8.00	8.0000	8.000	8.0000	8.000
		se	.56		.20	.0023	.005	.0021	.008
1	D	3.0	18.00	18.08	.08	.0488	.270	.0095	.053
2	D	3.0	nonresponder						
3	D	3.0	20.00	21.08	1.08	.0494	.234	.0150	.071
4	D	3.0	22.00	22.26	.26	.0481	.216	.0203	.091
5	D	3.0	21.00	20.65	-.35	.0459	.222	forgot	
6	D	3.0	19.00	19.89	.89	.0467	.235	.0173	.087
7	D	3.0	21.50	20.82	-.68	.0504	.242	.0147	.071
8	D	3.0	19.00	19.20	.20	.0487	.254	.0184	.096
		mean	20.07		.21	.0483	.239	.0159	.078
		s d	1.48		.63	.0015	.018	.0038	.016
		n	7.00		7.00	7.0000	7.000	6.0000	6.000
		se	.56		.24	.0006	.007	.0015	.007
9	A	10.0	22.50	22.20	-.30	.0585	.264	.0410	.185
10	A	10.0	19.50	20.45	.95	.0465	.227	.0280	.137
11	A	10.0	21.00	20.60	-.40	.0440	.214	.0240	.117
12	A	10.0	23.00	23.50	.50	.0591	.251	.0220	.094
13	A	10.0	21.00	24.85	3.85	.0625	.252	.0440	.177
14	A	10.0	20.50	20.80	.30	.0637	.306	.0270	.130
15	A	10.0	18.50	20.05	1.55	.0500	.249	.0340	.170
16	A	10.0	20.00	22.05	2.05	fogot		.0360	.163
		mean	20.75		1.06	.0549	.252	.0320	.146
		s d	1.49		1.41	.0079	.029	.0080	.032
		n	8.00		8.00	7.0000	7.000	8.0000	8.000
		se	.53		.50	.0030	.011	.0028	.011

Animal Number	Group	Day	Initial Weight (in g)	Final Weight (in g)	Weight Change (in g)	Spleen Weight (in g)	Spleen % BW	Thymus Weight (in g)	Thymus % BW
9	B	10.0	18.00	19.35	1.35	.0420	.217	.0260	.134
10	B	10.0	19.00	19.60	.60	.0430	.219	.0190	.097
11	B	10.0	20.50	21.30	.80	.0458	.215	.0150	.070
12	B	10.0	21.50	22.60	1.10	.0479	.212	.0190	.084
13	B	10.0	20.50	22.75	2.25	.0432	.190	.0290	.127
14	B	10.0	22.00	22.50	.50	.0499	.222	.0250	.111
15	B	10.0	21.00	22.70	1.70	.0508	.224	.0220	.097
16	B	10.0	21.00	22.50	1.50	.0506	.225	.0260	.116
		mean	20.44		1.23	.0467	.215	.0226	.105
		s d	1.32		.60	.0036	.011	.0047	.022
		n	8.00		8.00	8.0000	8.000	8.0000	8.000
		se	.47		.21	.0013	.004	.0017	.008
9	C	10.0	22.00	23.45	1.45	.1390	.593	.0160	.068
10	C	10.0	20.00	20.30	.30	.0851	.419	.0160	.079
11	C	10.0	20.00	21.20	1.20	.1103	.520	.0140	.066
12	C	10.0	22.50	25.65	3.15	.1269	.495	.0220	.086
13	C	10.0	21.00	23.00	2.00	.1216	.529	.0170	.074
14	C	10.0	22.00	24.20	2.20	.1242	.513	.0100	.041
15	C	10.0	21.00	23.50	2.50	.1028	.437	.0180	.077
16	C	10.0	20.50	23.20	2.70	.1293	.557	.0180	.078
		mean	21.13		1.94	.1174	.508	.0164	.071
		s d	.95		.92	.0172	.058	.0035	.013
		n	8.00		8.00	8.0000	8.000	8.0000	8.000
		se	.34		.32	.0061	.020	.0012	.005
9	D	10.0	21.50	22.20	.70	.0571	.257	.0050	.023
10	D	10.0	20.00	18.10	-1.90	.0662	.366	.0060	.033
11	D	10.0	23.50	19.85	-3.65	.0469	.236	.0080	.040
12	D	10.0	19.50	16.75	-2.75	.0672	.401	.0050	.030
13	D	10.0	21.00	17.20	-3.80	.0502	.292	.0070	.041
14	D	10.0	18.50	dead					
15	D	10.0	nonresponder						
16	D	10.0	21.50	18.40	-3.10	.0542	.295	.0040	.022
		mean	20.79		-2.42	.0570	.308	.0058	.031
		s d	1.63		1.67	.0083	.064	.0015	.008
		n	7.00		6.00	6.0000	6.000	6.0000	6.000
		se	.62		.68	.0034	.026	.0006	.003

TABLE 7

CORTICOSTERONE DATA, EXPERIMENT 1: SUMMARY

```
file: cortsum (scf)
created by: A. Youngberg          printer: 12 pt
day 0 = 25 April 1  9/ 5/1  9/ 5/89
animals administered pcb or vehicle on day -1
animals administered tumor or vehicle on day 0
Group A = controls
Group B = tumor only
Group C = pcb only
Group D = tumor plus pcb
contains: summary of cc data, Experiment 1
data is edited: following are deleted:
                  Day 3: 189, 191.....scabby
                  Day 6: 077, 017.....chewed
                         016..........+/=ascites
                  Day 7: 316.....nonresponder
                  Day 8: 285.....nonresponder
                  Day 21: 246, 248.....scabby
                          048, 123.....nonresponder
                  Day 42: 278.....scabby
                  Day 84: 217, 118.....scabby
results in ng/ml
value of 16 used for all values less than 25
```

CORTICOSTERONE CONCENTRATIONS (IN NG/ML)

Day	Group	Mean	SD	N	SE
(day -1 animals received no treatment at all)					
-1	A	106.3	44.4	5	19.86
-.6	A	31.1	27.3	6	11.15
0	A	16.0	.0	3	.00
1	A	19.0	6.7	5	3.00
2	A	16.7	1.5	5	.67
3	A	27.9	19.7	6	8.04
4	A	27.7	13.6	6	5.55
5	A	16.0	.0	6	.00
6	A	16.0	.0	4	.00
7	A	23.9	12.3	6	5.02
8	A	22.5	11.9	6	4.86
9	A	23.4	11.9	6	4.86
10	A	20.6	11.3	6	4.61
21	A	21.8	10.1	3	5.83
42	A	19.1	7.6	6	3.10
84	A	25.9	17.9	6	7.31
no Group B on days -1, -.6, 0					
-1	B	N/A	N/A	N/A	N/A
-.6	B	N/A	N/A	N/A	N/A

Day	Group	Mean	SD	N	SE
0	B	N/A	N/A	N/A	N/A
1	B	38.4	34.0	4	17.00
2	B	22.4	10.0	6	4.08
3	B	39.4	46.7	4	23.35
4	B	36.8	23.5	6	9.59
5	B	25.6	14.9	6	6.08
6	B	52.2	24.6	4	12.30
7	B	27.1	13.3	6	5.43
8	B	26.0	15.1	5	6.75
9	B	20.8	11.6	6	4.74
10	B	23.3	11.6	6	4.74
21	B	25.4	17.1	6	6.98
42	B	16.0	.0	6	.00
84	B	23.5	10.5	5	4.70

no Group C on day -1

Day	Group	Mean	SD	N	SE
-1	C	N/A	N/A	N/A	N/A
-.6	C	56.2	49.5	6	20.21
0	C	18.3	4.6	4	2.30
1	C	43.2	29.8	4	14.90
2	C	77.5	18.5	5	8.27
3	C	62.4	38.8	6	15.84
4	C	126.1	92.6	5	41.41
5	C	38.7	33.2	6	13.55
6	C	52.4	40.6	6	16.57
7	C	62.1	28.7	6	11.72
8	C	47.1	25.5	5	11.40
9	C	81.8	65.5	6	26.74
10	C	46.8	23.1	6	9.43
21	C	64.3	36.4	5	16.28
42	C	35.5	13.6	5	6.08
84	C	41.9	48.0	5	21.47

no Group D on days -1, -.6, 0

Day	Group	Mean	SD	N	SE
-1	D	N/A	N/A	N/A	N/A
-.6	D	N/A	N/A	N/A	N/A
0	D	N/A	N/A	N/A	N/A
1	D	61.7	11.1	5	4.96
2	D	49.0	23.6	6	9.63
3	D	89.2	90.7	6	37.03
4	D	149.4	108.0	6	44.09
5	D	185.6	99.3	6	40.54
6	D	164.0	70.1	4	35.05
7	D	256.7	106.9	6	43.64
8	D	252.8	148.3	6	60.54
9	D	249.7	95.9	6	39.15
10	D	294.1	125.5	5	56.13
21	D	93.2	70.8	4	35.40
42	D	no surviving mice			
84	D	no surviving mice			

TABLE 8

PROLACTIN DATA, EXPERIMENT 1: SUMMARY

file: prlsum2 (scf)
created by: A. Youngberg printer: 12 pt
day 0 of study was 25 April 1989
animals administered pcb or vehicle on day -1
animals administered tumor or vehicle on day 0
Group A = controls
Group B = tumor only
Group C = pcb only
Group D = tumor plus pcb
contains: summary of prl data, experiment 1
this is edited data: following are deleted:
Day 3: 18
Day 6: 077, 017..chewed, 016..+/= ascites
Day 7: 316....nonresponder
Day 8: 285....nonresponder
Day 21: 246, 248....scabby
048, 123....nonresponder
Day 42: 278..scabby, 075..nonresponder
Day 84: 217, 118....scabby
blanks indicate deletion (above) or qns sample or bad assay
results in ng/ml
value of 1 used for all values less than 1.25

PROLACTIN CONCENTRATIONS (IN NG/ML)

Day	Group	Mean	SD	N	SE
-1.00	A	4.66	2.72	4.00	1.36
-.60	A	1.77	1.21	4.00	.61
.00	A	6.09	5.67	3.00	3.27
1.00	A	3.07	.13	3.00	.08
2.00	A	11.14	7.81	2.00	5.52
3.00	A	1.54	1.20	5.00	.54
4.00	A	5.82	9.73	5.00	4.35
5.00	A	4.52		1.00	.00
6.00	A	1.00		1.00	.00
7.00	A	2.82	3.15	3.00	1.82
8.00	A	1.38	.68	6.00	.28
9.00	A	4.10	1.87	3.00	1.08
10.00	A	6.31	8.88	3.00	5.13
21.00	A	1.00	.00	2.00	.00
42.00	A	3.21	4.42	4.00	2.21
84.00	A	1.23	.40	3.00	.23

Day	Group	Mean	SD	N	SE
no Group B on days -1, -.6, 0					
-1.00	B	N/A	N/A	N/A	N/A
-.60	B	N/A	N/A	N/A	N/A
.00	B	N/A	N/A	N/A	N/A
1.00	B	8.26	4.63	3.00	2.67
2.00	B	11.90	15.14	6.00	6.18
3.00	B	2.30	2.26	3.00	1.30
4.00	B	1.50	.87	6.00	.36
5.00	B	2.08	1.26	4.00	.63
6.00	B	2.68	1.90	3.00	1.10
7.00	B	1.12	.19	6.00	.08
8.00	B	3.86	4.04	2.00	2.86
9.00	B	2.24	1.66	4.00	.83
10.00	B	1.77	.56	5.00	.25
21.00	B	1.41	.49	4.00	.25
42.00	B	1.72	.87	4.00	.44
84.00	B	7.37	11.93	4.00	5.97
no Group C on day -1					
-1.00					
-.60	C	1.74	1.28	3.00	.74
.00	C	1.97	1.67	2.00	1.18
1.00	C	1.41	.58	2.00	.41
2.00	C	1.98	1.39	2.00	.98
3.00	C	1.51	.47	3.00	.27
4.00	C	1.25	.50	4.00	.25
5.00	C	3.92	2.61	3.00	1.51
6.00	C	4.00	4.64	4.00	2.32
7.00	C	2.67	2.49	3.00	1.44
8.00	C	2.60	2.42	3.00	1.40
9.00	C	4.92	4.79	4.00	2.40
10.00	C	2.64	1.66	3.00	.96
21.00	C	1.00	.00	2.00	.00
42.00	C	2.38	2.10	5.00	.94
84.00	C	3.68		1.00	.00
no Group D on days -1, -.6, 0, 42, 84					
-1.00	D	N/A	N/A	N/A	N/A
-.60	D	N/A	N/A	N/A	N/A
.00	D	N/A	N/A	N/A	N/A
1.00	D	1.00	.00	3.00	.00
2.00	D	3.62	4.54	3.00	2.62
3.00	D	2.09	2.18	4.00	1.09
4.00	D	1.81	1.51	6.00	.62
5.00	D	2.29		1.00	.00
6.00	D	1.00	.00	2.00	.00
7.00	D	1.20	.34	3.00	.20
8.00	D	1.66	1.11	6.00	.45

Day	Group	Mean	SD	N	SE
9.00	D	1.37	.74	4.00	.37
10.00	D	1.13	.26	4.00	.13
21.00	D	1.00	.00	5.00	.00
42.00	D	no surviving mice			
84.00	D	no surviving mice			

TABLE 9

HORMONE DATA, EXPERIMENT 1: EDITED RAW DATA

files: hormsh and hormsnoh
created by: A. Youngberg printer: 12 pt
day 0 = 25 April 1989
animals administerd pcb or vehicle on day -1
animals administered tumor or vehicle on day 0
Group A = controls
Group B = tumor only
Group C = pcb only
Group D = tumor plus pcb
contains: prl and cc data for Experiment 1
edited data: following are deleted:
Day 3: 189, 191....scabby
Day 6: 077, 017..chewed, 016..+/=ascites
Day 7: 316....nonresponder
Day 8: 285....nonresponder
Day 21: 246, 248....scabby
048, 123....nonresponder
Day 42: 278..scabby, 075..nonresponder
Day 84: 217, 118....scabby
blanks indicate deletion (above), qns sample, bad assay
results in ng/ml
in cc assays: 16 used for all values less than 25
in prl assays: 1 used for all values less than 1.25

Animal Number	Group	Day	Corticosterone, in ng/ml		Prolactin, in ng/ml
345		-1.0	79.6		7.38
347		-1.0	119.8		6.62
348		-1.0			
346		-1.0	165.4		2.38
350		-1.0	48.4		2.25
349		-1.0	118.2		
		mean	106.3	mean	4.66
		s d	44.4	s d	2.72
		n	5.0	n	4.00
351	A	-.6	16.0		1.53
352	A	-.6	16.0		
353	A	-.6	83.7		
357	A	-.6	16.0		1.00
358	A	-.6	16.0		1.00
359	A	-.6	38.9		3.54

Animal Number	Group	Day	Corticosterone in ng/ml		Prolactin in ng/ml
		mean	31.1	mean	1.77
		s d	27.3	s d	1.21
		n	6.0	n	4.00
354	C	-.6	16.0		3.22
355	C	-.6	83.7		
356	C	-.6	136.9		
360	C	-.6	68.7		
361	C	-.6	16.0		1.00
362	C	-.6	16.0		1.00
		mean	56.2	mean	1.74
		s d	49.5	s d	1.28
		n	6.0	n	3.00
318	A	.0	16.0		12.42
321	A	.0			
324	A	.0			
367	A	.0	16.0		1.49
368	A	.0	16.0		4.36
369	A	.0			
		mean	16.0	mean	6.09
		s d	.0	s d	5.67
		n	3.0	n	3.00
342	C	.0	16.0		1.00
343	C	.0	16.0		
344	C	.0	16.0		3.90
364	C	.0			
365	C	.0			
366	C	.0	25.1		1.00
		mean	18.3	mean	1.97
		s d	4.6	s d	1.67
		n	4.0	n	3.00
050	A	1.0	30.9		
051	A	1.0	16.0		3.16
053	A	1.0	16.0		3.13
294	A	1.0	16.0		2.92
295	A	1.0	16.0		
296	A	1.0			
		mean	19.0	mean	3.07
		s d	6.7	s d	.13
		n	5.0	n	3.00
055	B	1.0			
056	B	1.0	33.9		2.91
057	B	1.0	87.8		10.97

Animal Number	Group	Day	Corticosterone in ng/ml		Prolactin in ng/ml
297	B	1.0			
298	B	1.0	16.0		10.89
299	B	1.0	16.0		
		mean	38.4	mean	8.26
		s d	34.0	s d	4.63
		n	4.0	n	3.00
058	C	1.0	16.0		
059	C	1.0	85.6		
060	C	1.0			
300	C	1.0			
301	C	1.0	38.2		1.82
302	C	1.0	33.0		1.00
		mean	43.2	mean	1.41
		s d	29.8	s d	.58
		n	4.0	n	2.00
061	D	1.0			
062	D	1.0	70.3		
063	D	1.0	67.3		1.00
303	D	1.0	42.6		
304	D	1.0	61.4		1.00
305	D	1.0	66.9		1.00
		mean	61.7	mean	1.00
		s d	11.1	s d	.00
		n	5.0	n	3.00
101	A	2.0	16.0		
102	A	2.0	16.0		
103	A	2.0			
258	A	2.0	16.0		16.66
259	A	2.0	19.3		
260	A	2.0	16.0		5.62
		mean	16.7	mean	11.14
		s d	1.5	s d	7.81
		n	5.0	n	2.00
104	B	2.0	33.4		6.81
105	B	2.0	16.0		5.45
106	B	2.0	16.0		2.48
261	B	2.0	16.0		13.15
262	B	2.0	37.0		1.81
263	B	2.0	16.0		41.68
		mean	22.4	mean	11.90
		s d	10.0	s d	15.14
		n	6.0	n	6.00

Animal Number	Group	Day	Corticosterone in ng/ml		Prolactin in ng/ml
107	C	2.0	59.0		
108	C	2.0	58.7		
109	C	2.0	97.5		
264	C	2.0			
265	C	2.0	78.4		
266	C	2.0	93.8		2.96
		mean	77.5	mean	2.96
		s d	18.5	s d	ERROR
		n	5.0	n	1.00
110	D	2.0	68.4		1.00
111	D	2.0	25.9		
112	D	2.0	49.7		8.86
267	D	2.0	58.5		1.00
268	D	2.0	16.0		
269	D	2.0	75.5		
		mean	49.0	mean	3.62
		s d	23.6	s d	4.54
		n	6.0	n	3.00
025	A	3.0	16.0		3.69
026	A	3.0	16.0		1.00
027	A	3.0	26.7		1.00
186	A	3.0	25.7		
187	A	3.0	16.0		1.00
188	A	3.0	66.7		1.00
		mean	27.9	mean	1.54
		s d	19.7	s d	1.20
		n	6.0	n	5.00
028	B	3.0	16.0		1.00
029	B	3.0	109.4		4.91
030	B	3.0	16.0		
189	B	3.0			
190	B	3.0	16.0		1.00
191	B	3.0			
		mean	39.4	mean	2.30
		s d	46.7	s d	2.26
		n	4.0	n	3.00
031	C	3.0	74.4		
032	C	3.0	16.0		
033	C	3.0	127.7		1.00
192	C	3.0	40.5		1.93
193	C	3.0	73.0		1.59
194	C	3.0	43.0		
		mean	62.4	mean	1.51

Animal Number	Group	Day	Corticosterone in ng/ml		Prolactin in ng/ml
		s d	38.8	s d	.47
		n	6.0	n	3.00
034	D	3.0	259.5		5.35
035	D	3.0	113.4		1.00
036	D	3.0	16.0		
195	D	3.0	76.4		
196	D	3.0	25.4		1.00
197	D	3.0	44.7		1.00
		mean	89.2	mean	2.09
		s d	90.7	s d	2.18
		n	6.0	n	4.00
001	A	4.0	16.0		1.00
002	A	4.0	46.8		2.14
003	A	4.0	31.8		1.00
137	A	4.0	16.0		1.74
138	A	4.0	16.0		
140	A	4.0	39.4		23.20
		mean	27.7	mean	5.82
		s d	13.6	s d	9.73
		n	6.0	n	5.00
004	B	4.0	59.0		1.86
005	B	4.0	27.2		1.00
006	B	4.0	30.2		3.13
141	B	4.0	72.3		1.00
142	B	4.0	16.0		1.00
143	B	4.0	16.0		1.00
		mean	36.8	mean	1.50
		s d	23.5	s d	.87
		n	6.0	n	6.00
007*	C	4.0	95.2		1.00
008*	C	4.0	16.0		1.00
009*	C	4.0	75.3		1.00
144	C	4.0	210.8		1.99
145	C	4.0	233.1		
146	C	4.0			
		mean	126.1	mean	1.25
		s d	92.6	s d	.50
		n	5.0	n	4.00
010	D	4.0	115.3		1.00
011	D	4.0	318.6		4.75
012	D	4.0	204.0		1.00
147	D	4.0	176.4		1.00
148	D	4.0	65.8		2.09

Animal Number	Group	Day	Corticosterone in ng/ml		Prolactin in ng/ml
149	D	4.0	16.0		1.00
		mean	149.4	mean	1.81
		s d	108.0	s d	1.51
		n	6.0	n	6.00
174	A	5.0	16.0		
198	A	5.0	16.0		
199	A	5.0	16.0		4.52
200	A	5.0	16.0		
319	A	5.0	16.0		
370	A	5.0	16.0		
		mean	16.0	mean	4.52
		s d	.0	s d	ERROR
		n	6.0	n	1.00
177	B	5.0	43.4		
178	B	5.0	16.0		1.00
179	B	5.0	16.0		3.36
201	B	5.0	16.0		1.00
202	B	5.0	46.3		2.96
203	B	5.0	16.0		
		mean	25.6	mean	2.08
		s d	14.9	s d	1.26
		n	6.0	n	4.00
180	C	5.0	16.0		6.03
181	C	5.0	49.3		
204	C	5.0	100.5		
205	C	5.0	16.0		
206	C	5.0	34.4		4.73
338	C	5.0	16.0		1.00
		mean	38.7	mean	3.92
		s d	33.2	s d	2.61
		n	6.0	n	3.00
183*	D	5.0	289.4		
184	D	5.0	133.5		2.29
185	D	5.0	143.5		
207	D	5.0	180.3		
208	D	5.0	52.9		
209	D	5.0	313.8		
		mean	185.6	mean	2.29
		s d	99.3	s d	ERROR
		n	6.0	n	1.00
013	A	6.0	16.0		
014	A	6.0	16.0		

Animal Number	Group	Day	Corticosterone in ng/ml		Prolactin in ng/ml
015	A	6.0	16.0		
076	A	6.0			
077	A	6.0			
078	A	6.0	16.0		1.00
		mean	16.0	mean	1.00
		s d	.0	s d	ERROR
		n	4.0	n	1.00
016	B	6.0			
017	B	6.0			
018	B	6.0	45.3		2.30
079	B	6.0	40.7		1.00
080	B	6.0	34.3		
081	B	6.0	88.4		4.74
		mean	52.2	mean	2.68
		s d	24.6	s d	1.90
		n	4.0	n	3.00
019	C	6.0	36.8		1.00
020	C	6.0	16.0		
021	C	6.0	66.4		
082	C	6.0	127.9		10.77
083*	C	6.0	28.4		1.00
085	C	6.0	38.9		3.21
		mean	52.4	mean	4.00
		s d	40.6	s d	4.64
		n	6.0	n	4.00
022	D	6.0	213.2		1.00
023	D	6.0	128.0		
024	D	6.0	83.5		
086	D	6.0	231.4		1.00
087	D	6.0			
088	D	6.0			
		mean	164.0	mean	1.00
		s d	70.1	s d	.00
		n	4.0	n	2.00
125	A	7.0	16.0		
126	A	7.0	41.4		
127	A	7.0	38.0		1.00
306	A	7.0	16.0		
307	A	7.0	16.0		1.00
308	A	7.0	16.0		6.45
		mean	23.9	mean	2.82
		s d	12.3	s d	3.15
		n	6.0	n	3.00

Animal Number	Group	Day	Corticosterone in ng/ml		Prolactin in ng/ml
130	B	7.0	26.8		1.00
309	B	7.0	26.7		1.00
310	B	7.0	16.0		1.35
311	B	7.0	52.2		1.00
322	B	7.0	16.0		1.00
323	B	7.0	25.0		1.38
		mean	27.1	mean	1.12
		s d	13.3	s d	.19
		n	6.0	n	6.00
131	C	7.0	100.4		
132	C	7.0	49.5		1.48
133	C	7.0	79.2		
312	C	7.0	70.0		5.54
313	C	7.0	57.2		
314	C	7.0	16.0		1.00
		mean	62.1	mean	2.67
		s d	28.7	s d	2.49
		n	6.0	n	3.00
134	D	7.0	305.2		1.00
135	D	7.0	189.2		
136	D	7.0	379.5		1.00
315	D	7.0	109.1		1.59
316	D	7.0			
317	D	7.0	300.4		
		mean	256.7	mean	1.20
		s d	106.9	s d	.34
		n	5.0	n	3.00
162	A	8.0	45.5		1.00
163	A	8.0	16.0		1.00
164	A	8.0	25.5		1.62
282	A	8.0	16.0		2.68
283	A	8.0	16.0		1.00
284	A	8.0	16.0		1.00
		mean	22.5	mean	1.38
		s d	11.9	s d	.68
		n	6.0	n	6.00
165	B	8.0	16.0		
166	B	8.0	49.9		1.00
167	B	8.0	16.0		
285	B	8.0			
286	B	8.0	32.2		
287	B	8.0	16.0		6.72

Animal Number	Group	Day	Corticosterone in ng/ml		Prolactin in ng/ml
		mean	26.0	mean	3.86
		s d	15.1	s d	4.04
		n	5.0	n	2.00
168	C	8.0	16.0		1.00
169	C	8.0			
170	C	8.0	80.7		1.42
288	C	8.0	33.6		5.39
289	C	8.0	64.0		
290	C	8.0	41.0		
		mean	47.1	mean	2.60
		s d	25.5	s d	2.42
		n	5.0	n	3.00
171	D	8.0	504.5		1.00
172	D	8.0	61.8		2.34
173	D	8.0	283.2		1.00
291	D	8.0	237.1		1.00
292	D	8.0	270.3		1.00
293	D	8.0	159.6		
		mean	252.8	mean	1.27
		s d	148.3	s d	.60
		n	6.0	n	5.00
089	A	9.0	33.4		
090	A	9.0	43.1		2.54
091	A	9.0	16.0		3.58
150	A	9.0	16.0		6.17
151	A	9.0	16.0		
152	A	9.0	16.0		
		mean	23.4	mean	4.10
		s d	11.9	s d	1.87
		n	6.0	n	3.00
092	B	9.0	16.0		
093	B	9.0	16.0		1.00
094	B	9.0	16.0		
153	B	9.0	44.5		1.00
154	B	9.0	16.0		2.44
155	B	9.0	16.0		4.52
		mean	20.8	mean	2.24
		s d	11.6	s d	1.66
		n	6.0	n	4.00
095	C	9.0	50.0		3.88
096	C	9.0	55.5		
097	C	9.0	208.8		1.00
156	C	9.0	92.9		2.92

Animal Number	Group	Day	Corticosterone in ng/ml		Prolactin in ng/ml
157	C	9.0	54.1		11.88
158	C	9.0	29.3		
		mean	81.8	mean	4.92
		s d	65.5	s d	4.79
		n	6.0	n	4.00
098	D	9.0	166.4		
100	D	9.0	312.2		1.00
159	D	9.0	97.0		1.00
160	D	9.0	302.3		1.00
161	D	9.0	280.5		2.47
327	D	9.0	339.7		
		mean	249.7	mean	1.37
		s d	95.9	s d	.74
		n	6.0	n	4.00
222	A	10.0	16.0		1.00
223	A	10.0	16.0		16.56
224	A	10.0	16.0		
234	A	10.0	43.8		
235	A	10.0	16.0		1.37
236	A	10.0	16.0		
		mean	20.6	mean	6.31
		s d	11.3	s d	8.88
		n	6.0	n	3.00
225	B	10.0	34.0		2.35
226	B	10.0	16.0		2.22
227	B	10.0	16.0		1.00
237	B	10.0	16.0		1.84
238	B	10.0	16.0		1.45
239	B	10.0	41.8		
		mean	23.3	mean	1.77
		s d	11.6	s d	.56
		n	6.0	n	5.00
228	C	10.0	16.0		
229	C	10.0	58.1		4.32
230	C	10.0	62.5		1.00
242	C	10.0	25.8		2.59
325	C	10.0	41.7		
326	C	10.0	76.4		
		mean	46.8	mean	2.64
		s d	23.1	s d	1.66
		n	6.0	n	3.00
231	D	10.0	450.5		1.00

Animal Number	Group	Day	Corticosterone in ng/ml		Prolactin in ng/ml
232	D	10.0			
233	D	10.0	316.3		1.52
243	D	10.0	349.1		1.00
244	D	10.0	115.2		1.00
245	D	10.0	239.6		
		mean	294.1	mean	1.13
		s d	125.5	s d	.26
		n	5.0	n	4.00
037	A	21.0	bad dups-25 to 50		
038	A	21.0	16.0		1.00
039	A	21.0	33.5		
246	A	21.0			
247	A	21.0	16.0		1.00
248	A	21.0			
		mean	21.8	mean	1.00
		s d	10.1	s d	.00
		n	3.0	n	2.00
040	B	21.0	30.4		1.00
041	B	21.0	58.2		
042	B	21.0	16.0		2.00
249	B	21.0	16.0		1.63
250	B	21.0	16.0		
251	B	21.0	16.0		1.00
		mean	25.4	mean	1.41
		s d	17.1	s d	.49
		n	6.0	n	4.00
043	C	21.0	47.7		
044	C	21.0			
045	C	21.0	42.4		
252	C	21.0	63.4		
253	C	21.0	127.5		1.00
254	C	21.0	40.6		1.00
		mean	64.3	mean	1.00
		s d	36.4	s d	.00
		n	5.0	n	2.00
047	D	21.0	187.3		1.00
048	D	21.0			
122	D	21.0	16.0		1.00
123	D	21.0			
124	D	21.0	90.4		1.00
341	D	21.0	79.1		1.00
		mean	93.2	mean	1.00
		s d	70.8	s d	.00

Animal Number	Group	Day	Corticosterone in ng/ml		Prolactin in ng/ml
		n	4.0	n	4.00
064	A	42.0	16.0		
065	A	42.0	34.3		1.00
066	A	42.0	16.0		9.84
270	A	42.0	16.0		
271	A	42.0	16.0		1.00
272	A	42.0	16.0		1.00
		mean	19.1	mean	3.21
		s d	7.5	s d	4.42
		n	6.0	n	4.00
067	B	42.0	16.0		
068	B	42.0	16.0		2.11
069	B	42.0	16.0		1.00
273	B	42.0	16.0		2.76
274	B	42.0	16.0		1.00
275	B	42.0	16.0		
		mean	16.0	mean	1.72
		s d	.0	s d	.87
		n	6.0	n	4.00
070	C	42.0	45.2		3.19
071	C	42.0	35.5		1.00
072	C	42.0	50.7		5.73
276	C	42.0	30.1		1.00
277	C	42.0	16.0		1.00
278	C	42.0			
		mean	35.5	mean	2.38
		s d	13.6	s d	2.10
		n	5.0	n	5.00
212	A	84.0	16.0		
115	A	84.0	16.0		
210	A	84.0	60.3		1.00
113	A	84.0	16.0		1.69
211	A	84.0	31.1		
114	A	84.0	16.0		1.00
		mean	25.9	mean	1.23
		s d	17.9	s d	.40
		n	6.0	n	3.00
117	B	84.0	16.0		25.24
215	B	84.0	16.0		2.23
118	B	84.0			
214	B	84.0	31.3		1.00
116	B	84.0	38.0		1.00

Animal Number	Group	Day	Corticosterone in ng/ml		Prolactin in ng/ml
213	B	84.0	16.0		
		mean	23.5	mean	7.37
		s d	10.5	s d	11.93
		n	5.0	n	4.00
217	C	84.0			
372	C	84.0	16.0		
120	C	84.0	16.0		
218	C	84.0	16.0		
121	C	84.0	35.1		
119	C	84.0	126.4		3.68
		mean	41.9	mean	3.68
		s d	48.0	s d	ERROR
		n	5.0	n	1.00

TABLE 10

HORMONE DATA, EXPERIMENT 2: EDITED RAW DATA

file name: prlstudy (scf)
created by : A. Youngberg printer: 12 pt
day 0 = 25 April 1989, exp't 1
day 0 = 11 Dec 1990, exp't 2
animals administered pcb or vehicle on day -1
animals administered tumor or vehicle on day 0
Group A = controls
Group B = tumor only
Group C = pcb only
Group D = tumor plus pcb
contains: prl and cc data from Experiments 1 and 2
this is edited raw data; animals excluded:
exp't 1: day 3: 189, 191....scabby
exp't 2: day 10: D2, D15....nonresponder
blanks: qns sample, animal died, invalid assay
experiment 2: animals killed by CO2 overdose, blood
collected by cardiac puncture; assays run quickly
day 10: lab found to be very cold in am, 17.7 C
day 3: Carol Forsyth killed; Nancy Hollinghead bled
day 10: Nancy H. killed and bled; faster than day 3
value of 1.00 used for prl less than 1.25
value of 16 used for cc less than 25

Day 3

Animal Number	Group	Prolactin in ng/ml	Corticosterone in ng/ml
1	A	87.90	196.1
2	A	8.16	122.0
3	A	13.57	56.5
4	A	dead	dead
5	A	29.94	57.6
6	A	24.48	16.0
7	A	19.41	57.1
8	A	11.45	41.0
	mean	27.84	78.0
	sd	27.55	61.1
	n	7.00	7.0
	se	10.41	23.1
1	B	15.26	28.2
2	B	43.47	101.0
3	B	15.02	46.9
4	B	34.77	43.5
5	B	8.59	79.9
6	B	49.61	16.0
7	B	47.34	84.8
8	B	14.14	46.0

Animal Number	Group	Prolactin in ng/ml	Corticosterone in ng/ml
	mean	28.53	55.8
	sd	17.00	29.6
	n	8.00	8.0
	se	6.01	10.5
1	C	26.79	76.2
2	C	12.80	196.6
3	C	55.94	257.2
4	C	21.76	55.6
5	C	83.58	119.9
6	C	13.51	139.8
7	C	16.65	239.1
	mean	33.00	154.9
	sd	26.79	78.3
	n	7.00	7.0
	se	10.12	29.6
1	D	17.11	133.1
2	D	nonresponder	
3	D	11.82	
4	D	16.06	128.2
5	D	8.69	150.6
6	D	16.29	106.2
7	D	36.88	147.9
8	D	24.46	348.1
	mean	18.76	169.0
	sd	9.37	89.2
	n	7.00	6.0
	se	3.54	36.4
Day 10			
9	A	6.67	
10	A	10.53	
11	A	14.74	110.2
12	A	32.04	67.2
13	A	28.02	117.4
14	A	36.72	25.2
15	A	13.44	16.0
16	A	18.89	62.1
	mean	20.13	66.4
	sd	10.88	41.9
	n	8.00	6.0
	se	3.85	17.1
9	B	30.58	16.0

Animal Number	Group	Prolactin in ng/ml	Corticosterone in ng/ml
10	B	77.56	128.7
11	B	42.16	26.1
12	B	51.64	70.0
13	B	8.33	16.0
14	B	15.99	16.0
15	B	15.20	16.0
16	B	32.72	35.5
	mean	34.27	40.5
	sd	22.76	40.1
	n	8.00	8.0
	se	8.05	14.2
9	C	19.41	344.5
10	C	20.66	193.9
11	C	25.10	56.9
12	C	11.64	39.4
13	C	16.88	93.5
14	C	5.92	352.7
15	C	19.20	
16	C	21.27	121.8
	mean	17.51	171.8
	sd	6.06	130.6
	n	8.00	7.0
	se	2.14	49.4
9	D	1.08	720.4
10	D	8.49	
11	D	1.24	
12	D	5.11	579.8
13	D	3.60	896.5
14	D	dead	dead
15	D	non	nonresponder
16	D	2.91	1,000.0
	mean	3.74	799.2
	sd	2.77	186.3
	n	6.00	4.0
	se	1.13	93.2