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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

DATE: January 9, 2008

TXR#: 0054664

SUBJECT: Propanil: Immunotoxicity Study in Rats
PC Code: 028201, DP Barcode: D343051

FROM: Yung G. Yang, Ph.D.
Toxicology Branch
Health Effects Division (7509P)

THRU: Jess Rowland, Acting Chief
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Health Effects Division (7509P)

TO: Jack Arthur, Acting Chief
Reregistration Branch 2
Health Effects Division (7509P)
and
Cathryn O'Connell/Tom Myers, RM52
Rregistration Branch 2
Special Review and Reregistration Division (7508P)

CONCLUSION: The HED has reviewed an immunotoxicity study (MRID 47162901) which was submitted in response to the propanil Data-Call-In (DCI). The study is considered acceptable/guideline and fulfills the data requirement for conducting a guideline immunotoxicity study (OPPTS 870.7800). An executive summary is as follows.

Rec'd in RCC 1/15/2008

EXECUTIVE SUMMARY

In an immunotoxicity study (MRID 47162901), Propanil (99.7% a.i.; lot #SJ0588R301) was administered to Sprague-Dawley (Crl:CD[SD]) rats (10/sex/dose) in the diet at dose levels of 0, 50, 200, or 600 ppm (0, 4/5, 16/19, and 48/56 mg/kg bw/day in males/females, respectively) for 29 days. An additional group of 10 rats/sex was dosed with the known immunosuppressant cyclophosphamide by intraperitoneal injection at a dose level of 50 mg/kg/day (10 mL/kg dose volume) on Study Days 24-27 to serve as positive controls. All rats were inoculated intravenously with sheep red blood cells (SRBC) on Day 24, and the splenic anti-SRBC (IgM) response was measured with a splenic antibody-forming cell (AFC) assay.

There were no treatment-related effects on mortality, clinical observations, food consumption, or macroscopic pathology.

At 600 ppm, body weights were slightly decreased in both sexes throughout treatment. Cumulative body weight gains were decreased in the males on Days 0-7, 0-10, and 0-14, and overall (Days 0-28) body weight gains were decreased. In the females, cumulative body weight gains were decreased during all intervals, resulting in decreased overall body weight gains. Additionally at this dose, decreases were noted in mean red blood cell number (females), hemoglobin (both sexes), hematocrit (both sexes), and mean corpuscular hemoglobin concentration (females), and increases were observed in both mean absolute and percent reticulocytes (both sexes). Increases in absolute, relative to body, and relative to brain spleen weights were observed. Collectively, these findings were considered to be indicative of a mild anemia.

No significant treatment-related effects were observed in the 50 and 200 ppm groups.

The systemic LOAEL is 600 ppm (equivalent to 48/56 mg/kg/day in males/females), based on decreased body weights and body weight gains in both sexes, and mild anemia in the females. The NOAEL is 200 ppm (equivalent to 16/19 mg/kg/day in males/females).

Positive control rats (50 mg/kg/day cyclophosphamide on Days 24-27) displayed decreased body weights, body weight losses, and decreased overall body weight gains. In the females, decreases were observed in red blood cells, hemoglobin, and hematocrit. Additionally, decreases were noted in the following mean absolute parameters in both sexes: reticulocytes, platelets, and white blood cells counts. Small spleen and small thymus were noted at necropsy. Absolute and relative to body and brain spleen and thymus weights were decreased. These findings were considered to be consistent with cyclophosphamide treatment.

For immunotoxicity study, there were no significant treatment-related effects on splenic anti-SRBC (IgM) response. Spleen cell number and the splenic anti-SRBC response as indicated by specific activity (AFC/10^6 spleen cells) and total activity (AFC/spleen) were similar to controls in all treatment groups.

The NOAEL for splenic anti-SRBC response is 600 ppm (equivalent to 48/56 mg/kg/day in males/females; the highest dose tested).

In the positive controls, spleen cell numbers were decreased ($p<=0.01$) by 78-85%, and splenic anti-SRBC response (AFC/10^6 spleen cells) and total activity (AFC/spleen) were decreased ($p<=0.01$) by 99-100%. These findings were considered to be consistent with cyclophosphamide treatment.

This immunotoxicity study is classified **acceptable/guideline** and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in rats.

DATA EVALUATION RECORD

PROPANIL

Study Type: OPPTS 870.7800; Immunotoxicity Study in Rats

Work Assignment No. 5-01-155 (MRID 47162901)

Prepared for
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Disclaimer

This Data Evaluation Record my have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel

EPA Reviewer: Yung G. Yang, Ph.D. **Signature:** Yung G. Yang
Toxicology Branch, Health Effects Division (7509P) **Date:** 1/9/2008
EPA Work Assignment Manager: Myron Ottley, Ph.D. **Signature:** Myron Ottley
Registration Action Branch 3, Health Effects Division (7509P) **Date:** 1/9/08

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Immunotoxicity [diet] - rats; OPPTS 870.7800

PC CODE: 028201 **DP BARCODE:** D343051
TXR #: 0054664

TEST MATERIAL (PURITY): Propanil (99.7% a.i.)

SYNONYMS: *N*-(3,4-dichlorophenyl)propanamide

CITATION: Padgett, E. L. (2007) A splenic antibody study in rats following dietary exposure to propanil for 28 days. WIL Research Laboratories, LLC, Ashland, OH. Laboratory study number: WIL-141014, May 4, 2007. MRID 47162901. Unpublished

SPONSOR: Propanil Task Force II, c/o Edward M. Ruckert, McDermott, Will, and Emery, 600 13th Street, NW, Washington, DC

EXECUTIVE SUMMARY: In an immunotoxicity study (MRID 47162901), Propanil (99.7% a.i.; lot #SJ0588R301) was administered to Sprague-Dawley (Crl:CD[SD]) rats (10/sex/dose) in the diet at dose levels of 0, 50, 200, or 600 ppm (0, 4/5, 16/19, and 48/56 mg/kg bw/day in males/females, respectively) for 29 days. An additional group of 10 rats/sex was dosed with the known immunosuppressant cyclophosphamide by intraperitoneal injection at a dose level of 50 mg/kg/day (10 mL/kg dose volume) on Study Days 24-27 to serve as positive controls. All rats were inoculated intravenously with sheep red blood cells (SRBC) on Day 24, and the splenic anti-SRBC (IgM) response was measured with a splenic antibody-forming cell (AFC) assay.

There were no treatment-related effects on mortality, clinical observations, food consumption, or macroscopic pathology.

At 600 ppm, body weights were slightly decreased in both sexes throughout treatment. Cumulative body weight gains were decreased in the males on Days 0-7, 0-10, and 0-14, and overall (Days 0-28) body weight gains were decreased. In the females, cumulative body weight gains were decreased during all intervals, resulting in decreased overall body weight gains. Additionally at this dose, decreases were noted in mean red blood cell number (females), hemoglobin (both sexes), hematocrit (both sexes), and mean corpuscular hemoglobin concentration (females), and increases were observed in both mean absolute and percent reticulocytes (both sexes). Increases in absolute, relative to body, and relative to brain spleen weights were observed. Collectively, these findings were considered to be indicative of a mild anemia.

No significant treatment-related effects were observed in the 50 and 200 ppm groups.

The systemic LOAEL is 600 ppm (equivalent to 48/56 mg/kg/day in males/females), based on decreased body weights and body weight gains in both sexes, and mild anemia in the females. The NOAEL is 200 ppm (equivalent to 16/19 mg/kg/day in males/females).

Positive control rats (50 mg/kg/day cyclophosphamide on Days 24-27) displayed decreased body weights, body weight losses, and decreased overall body weight gains. In the females, decreases were observed in red blood cells, hemoglobin, and hematocrit. Additionally, decreases were noted in the following mean absolute parameters in both sexes: reticulocytes, platelets, and white blood cells counts. Small spleen and small thymus were noted at necropsy. Absolute and relative to body and brain spleen and thymus weights were decreased. These findings were considered to be consistent with cyclophosphamide treatment.

For immunotoxicity study, there were no significant treatment-related effects on splenic anti-SRBC (IgM) response. Spleen cell number and the splenic anti-SRBC response as indicated by specific activity (AFC/10^6 spleen cells) and total activity (AFC/spleen) were similar to controls in all treatment groups.

The NOAEL for splenic anti-SRBC response is 600 ppm (equivalent to 48/56 mg/kg/day in males/females; the highest dose tested).

In the positive controls, spleen cell numbers were decreased ($p<=0.01$) by 78-85%, and splenic anti-SRBC response (AFC/10^6 spleen cells) and total activity (AFC/spleen) were decreased ($p<=0.01$) by 99-100%. These findings were considered to be consistent with cyclophosphamide treatment.

This immunotoxicity study is classified **acceptable/guideline** and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in rats.

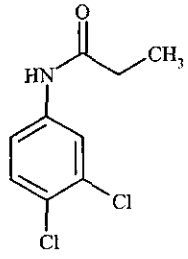
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Propanil

Description:	Irregular, off-white flakes
Lot #:	SJ0588R301
Purity:	99.7% a.i.
Compound Stability:	Stable in room temperature
CAS # of TGAI:	000709-98-8



2. **Vehicle:** Diet

Positive control: Cyclophosphamide monohydrate (Lot # 084K1328; purity 99.7%) in phosphate-buffered saline, pH 7.4 (made using sterile water for injection, USP)

3 **Test animals**

Species:	Rat	
Strain:	Crl:CD(SD)	
Age/weight at study initiation:	Approximately 7 weeks / 217-254 g males; 155-199 g females	
Source:	Charles River Laboratories, Inc. (Raleigh, NC)	
Housing:	Individually in stainless steel, wire-mesh cages suspended over cage board	
Diet:	Certified Rodent LabDiet® 5002 meal (PMI Nutrition International, LLC, St. Louis, MO), *ad libitum*	
Water:	Reverse osmosis-treated tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	21.3-21.6EC
	Humidity:	33.6-47.2%
	Air changes:	≥10/hr
	Photoperiod:	12h light/12 h dark
Acclimation period:	13-14 days	

B. STUDY DESIGN:

1. **In life dates:** Start: 12/18/06 End: 01/16/07

2. **Animal assignment:** Animals were randomly assigned, stratified by body weight in a block design, to the test groups noted in Table 1. Individual body weights at randomization were within ±20% of the mean for each sex.

TABLE 1: Study design[a]				
Test group	**Conc. in diet (ppm)**	**Dose to animal (mg/kg/day; M/F)**	**# Male**	**# Female**
Control	0	0/0	10	10
Low	50	4/5	10	10
Mid	200	16/19	10	10
High	600	48/56	10	10
Positive control[b]	0	N/A	10	10

a Data were obtained from page 25 of the study report.
b Positive control rats received basal diet, and were administered cyclophosphamide by intraperitoneal (i.p.) injection at a dose level of 50 mg/kg/day (10 mL/kg dose volume) on Days 24-27.
N/A Not applicable

3. **Dose selection:** A dose-selection rationale was not provided.

4. **Diet preparation, administration, and analysis:** For each dose level, the appropriate amount of the test substance was mixed with a small amount of basal feed to form a premix. The premix was then diluted with additional diet to achieve the desired concentration. The diets were prepared weekly. Basal diet for the control and positive control groups was weighed and placed into plastic storage bags approximately weekly. Homogeneity (top, middle, and bottom) and stability at ambient temperature for 10 days were assessed prior to the initiation of treatment on duplicate samples of the 50 and 600 ppm formulations. Concentrations of all test formulations from all preparations were analyzed.

Results

Homogeneity (% RSD): 1.7-4.1%

Stability (% of initial concentration on Day 10): 99.6-100%

Concentration (mean % nominal): 87.2-116%

Concentration analyses of the formulations prepared on 01/04/07 displayed excessive variation, particularly the 50 ppm formulation. This preparation was reanalyzed, and the results were deemed unacceptable. Therefore, all of the formulations prepared on this day were not administered to the animals, and new formulations were prepared on 01/06/07. These formulations were found to be acceptable. Otherwise, the analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the animals was acceptable.

5. **Positive controls:** Positive control rats received basal diet, and were administered the known immunosuppressant cyclophosphamide by intraperitoneal (i.p.) injection at a dose level of 50 mg/kg/day (10 mL/kg dose volume) on Days 24-27.

6. **Statistics**: The following statistical procedures (two-tailed) were used:

Parameter	Statistical procedure
Body weight, body weight gain, food consumption, hematology parameters, and organ weights	Parametric one-way analysis of variance (ANOVA; Snedecor and Cochran, 1980) followed by Dunnett's test where appropriate.
Antibody-forming cell (AFC) assay data (absolute and relative to body spleen weights, mean number of spleen cells IgM AFC/10^6 spleen cells, and IgM AFC/spleen)	Bartlett's Chi Square test for homogeneity of variances, followed by parametric one-way ANOVA and Dunnett's test where appropriate. If variances were not homogeneous, then a non-parametric ANOVA followed by the Gehan-Wilcoxon test where appropriate. Jonckheere's Test was also used to test for dose-related trend across the vehicle and treatment groups.
Positive control data	Student's t-test for comparison with vehicle controls

Statistical significance was denoted at $p \leq 0.05$ and 0.01. It was stated that statistical significance was not considered automatically to imply immunotoxicological significance. The statistical methods were considered appropriate.

C. METHODS

1. **Observations**: All animals were observed twice daily for mortality and morbidity. Clinical examinations were performed daily; detailed examinations were performed weekly.

2. **Body weight**: Body weights were recorded twice weekly, beginning approximately one week prior to study initiation (Days -10 and -11 for males and females, respectively), and at termination. Both absolute and cumulative body weight gains were calculated and presented for each weighing interval.

3. **Food consumption and compound intake**: Individual food consumption was recorded weekly, beginning approximately one week prior to study initiation (Days -10 and -11 for males and females, respectively) and presented as g/animal/day for each weekly interval. Compound intake (mg/kg/day) values were calculated by dividing the concentration of the test substance in the diet (mg/kg) by the g/animal/day food consumption for each interval. A grand mean for the entire exposure period was also calculated.

4. **Hematology**: Blood samples were collected from the inferior vena cava of all (non-fasted) rats at necropsy. The following CHECKED (X) parameters were examined.

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpuscular volume (MCV)
X	Platelet count	X	Reticulocyte count
	Blood clotting measurements	X	Red blood cell morphology
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

An additional blood sample was collected, and serum was prepared for an anti-sheep red blood cell IgM antibody ELISA; however, this assay was determined not to be warranted. These serum samples were discarded unused.

5. **Sacrifice and pathology:** At study termination on Day 28, all animals (non-fasted) were killed by carbon dioxide inhalation followed by exsanguination and subjected to a complete necropsy. The CHECKED (X) tissues were collected for microscopic examination. Additionally, the (XX) organs were weighed from each animal; paired organs were weighed together.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta, thoracic	XX	Brain
X	Salivary glands	XX	Heart	X	Peripheral nerve (sciatic)
X	Esophagus	X	Bone marrow	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	XX	Spleen	X	Eyes (optic nerve)
X	Jejunum	XX	Thymus		GLANDULAR
X	Ileum			XX	Adrenal gland
X	Cecum		UROGENITAL	X	Lacrimal gland
X	Colon	XX	Kidneys	X	Harderian gland
X	Rectum	X	Urinary bladder	XX	Parathyroid[a]
XX	Liver	XX	Testes	XX	Thyroid[a]
	Gall bladder	XX	Epididymides		OTHER
X	Pancreas	X	Prostate	X	Bone (sternum and femur)
	RESPIRATORY	XX	Ovaries (with oviducts)	X	Skeletal muscle
X	Trachea	XX	Uterus	X	Skin
X	Lungs	X	Mammary gland	X	All gross lesions and masses
	Nose	X	Cervix		
	Pharynx	X	Seminal vesicles		
	Larynx	X	Vagina		

a Weighed after fixation

Tissues were fixed in neutral-buffered 10% formalin, with the exception of the epididymides and testes (Bouin's solution) and the eyes and optic nerves (Davidson's solution). However, further processing and examination of the tissues was not performed.

6. **Splenic antibody-forming cell (AFC) assay:** On Day 24, all animals were immunized with an intravenous injection of 2 x 10^8 sheep red blood cells in 0.5 mL of Earle's Balanced Salt Solution with HEPES via a tail vein. At termination, the spleen of each animal was removed immediately following blood collection, weighed, and placed into coded individual tubes containing Earle's Balanced Salt Solution with 15 mM HEPES supplemented with gentamicin. Spleens were shipped overnight on wet ice to a testing facility (ImmunoTox Inc., Richmond, VA), and single cell suspensions were prepared from each spleen. Splenocyte viability was determined using propidium iodide and a flow cytometer. The primary response to sheep erythrocytes was measured using a modified hemolytic plaque assay[1]. Spleen cells from each suspension were mixed with guinea pig complement, sheep red blood cells, and warm agar, plated on separate Petri dishes, and incubated at 36-38° for three hours. Cell counts were performed and the number of cells/spleen, AFC/spleen and AFC/10^6 spleen cells were determined.

[1]Jerne, N.K., *et al.*, Plaque forming cells: methodology and theory. *Transpl. Rev.* **18**: 130-191, 1974

II. RESULTS

A. OBSERVATIONS

1. **Mortality:** All rats survived to scheduled termination.

2. **Clinical signs of toxicity:** There were no treatment-related clinical observations. Clinical findings in the positive control (cyclophosphamide) group were observed after beginning of injection at Day 24 and consisted of wet and/or dried yellow material on various body surfaces (urogenital area, ventral trunk, anogenital area, hindlimbs, and forelimbs). Males were affected to a greater extent than were females. It was stated that these findings were consistent with the expected effects of cyclophosphamide.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Body weight and body weight gain data are presented in Table 2. At 600 ppm, body weights were slightly decreased in both sexes throughout treatment (↓2-9%; not significant [NS] except on Day 28 in the females). Cumulative body weight gains were decreased ($p \leq 0.05$) by 18-19% in the 600 ppm males on Days 0-7, 0-10, and 0-14, and overall (Days 0-28) body weight gains were decreased (NS) by 16%. In the 600 ppm females, cumulative body weight gains were decreased ($p \leq 0.05$) during all intervals by 29-42%; overall (Days 0-28) body weight gains were decreased ($p \leq 0.01$) by 30%.

No treatment-related effects were observed on body weights or body weight gains in the 50 or 200 ppm groups.

For positive controls, both male and female rats (50 mg/kg/day cyclophosphamide) displayed decreased ($p \leq 0.05$) body weights on Day 28 (↓9-12%), body weight losses ($p \leq 0.01$) during treatment with cyclophosphamide (Days 24-28; -29 g for males; -12 g for females), and decreased ($p \leq 0.01$) overall body weight gains (↓28-30%).

TABLE 2: Mean (±SD) body weights and body weight gains (g) in rats treated with propanil for up to 28 days[a]

Parameter/interval		Dose (ppm)				
		0	50	200	600	Positive controls[b]
Males						
Body weight	Day 0	236±10.9	236±10.2	237±10.4	236±11.4	234±9.2
	Day 3	250±10.5	248±11.2	252±11.8	246±13.5 (↓2)	249±10.6
	Day 28	402±28.2	395±28.4	403±35.4	376±23.2 (↓7)	354±31.0** (↓12)
Body weight gain	Day 24-28	16±2.5	16±3.0	16±3.5	15±3.3	-29±8.0**
	Day 0-7	55±5.4	50±5.8	56±9.9	45±9.0** (↓18)	55±5.6
	Day 0-10	73±8.9	69±7.8	74±14.1	59±10.3* (↓19)	71±8.2
	Day 0-14	91±13.2	87±10.0	93±16.5	75±11.2* (↓18)	89±10.6
	Day 0-28	166±24.0	159±21.4	166±30.5	140±15.8 (↓16)	120±25.4** (↓28)
Females						
Body weight	Day 0	180±10.7	179±10.2	180±9.9	179±9.9	178±12.5
	Day 3	191±10.8	191±10.8	190±11.2	186±8.9 (↓3)	189±11.7
Body weight	Day 28	246±18.6	240±20.1	246±14.6	225±14.7* (↓9)	224±18.6* (↓9)
Body weight gain	Day 24-28	1±5.6	1±5.0	6±4.2	1±5.5	-12±8.3**
	Day 0-3	12±2.8	12±3.7	10±2.9	7±2.1* (↓42)	11±4.6
	Day 0-7	28±7.2	26±3.9	25±5.1	20±4.6** (↓29)	22±8.2
	Day 0-28	66±11.9	61±16.5	66±8.4	46±12.0** (↓30)	46±11.7** (↓30)

a Data were obtained from Tables 7-12 on pages 65-80 of the study report. Percent differences from controls are included in parentheses.
b Positive control rats were administered 50 mg/kg day cyclophosphamide by i.p. injection on Days 24-27.
* Significantly different from controls; p≤0.05
** Significantly different from controls; p≤0.01

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. **Food consumption:** There was no test-substance related effect on food consumption. Food consumption was decreased (p≤0.01) by 16-23% in the positive control rats during Days 21-28.

2. **Compound consumption:** Test substance intake is presented in Table 1.

D. HEMATOLOGY: Selected hematology parameters are presented in Table 3. At 600 ppm, decreases (p≤0.05) were noted in mean red blood cell number (↓16% in females), hemoglobin (↓6% in males, ↓16% in females), hematocrit (↓6% in males, 14% in females), and mean corpuscular hemoglobin concentration (↓3% in females). Additionally at this dose, increases (p≤0.01) were observed in both mean absolute and percent reticulocytes (↑51-60% in males, ↑159-209% in females). These findings were considered to be indicative of a mild anemia. However, the Sponsor stated that all of these findings, with the exception of the mean reticulocyte count in the females, were within the range of performing laboratory's historical controls (data not provided). Also at 600 ppm, decreases (p≤0.01) were noted in activated partial thromboplastin time (↓13%) in the males and prothrombin time (↓3%) in the females; however, decreases in these parameters are not normally considered adverse. Monocytes were also decreased (p≤0.05) in the 50 ppm females (↓33%), but this finding was considered incidental.

In the 200 ppm females, decreases (p≤0.05) were observed in mean red blood cell number (↓5%), hemoglobin (↓6%), and hematocrit (↓6%). In the absence of corroborating pathological findings, these decreases were not considered adverse.

In the positive control females, decreases ($p \leq 0.05$) were observed in red blood cells (↓6%), hemoglobin (↓6%), and hematocrit (↓7%). Additionally, decreases ($p \leq 0.01$) were noted in the following mean absolute parameters in both sexes (except where noted): reticulocytes (↓100%; both absolute and percent); platelets (↓53%); white blood cells (↓94-95%); neutrophils (89-91%); lymphocytes (↓94-95%); monocytes (↓95-96%); eosinophils (↓85-88%); basophils (90-95%); and large unstained cells (91-96%). These findings were considered to be consistent with cyclophosphamide treatment. Changes ($p \leq 0.05$) in the relative percentages of these cell types were not considered toxicologically relevant.

TABLE 3: Selected mean (±SD) hematology parameters in rats treated with propanil for up to 28 days[a]

Parameter/interval	Dose (ppm)				
	0	50	200	600	Positive controls[b]
Males					
Hemoglobin (g/dL)	16.1±0.61	16.1±0.64	16.0±0.63	15.1±0.72** (↓6)	15.4±0.94
Hematocrit (%)	47.1±1.88	47.1±1.77	47.3±1.45	44.4±2.11* (↓6)	44.8±2.81
Reticulocytes (%)	2.5±0.28	2.7±0.24	2.7±0.39	4.0±0.67** (↑60)	0.0±0.0** (↓100)
Reticulocytes ($10^3/\mu L$)	204.8±21.3	218.1±19.6	221.2±33.2	308.4±50.6** (↑51)	0.2±0.7**(↓100)
APTT (s)[c]	20.6±1.54	19.6±1.49	19.0±1.88	18.0±1.86** (↓13)	19.1±2.12
Platelets ($10^3/\mu L$)	1066±97	1157±157	1100±138	1089±167	502±92** (↓53)
White blood cells ($10^3/\mu L$)	15.3±2.25	17.1±4.05	17.9±4.81	17.8±3.72	0.75±0.13** (↓95)
Neutrophils ($10^3/\mu L$)	1.52±0.41	1.58±0.22	2.15±1.26	1.95±0.62	0.13±0.08** (↓91)
Lymphocytes ($10^3/\mu L$)	12.9±2.28	14.6±3.70	14.8±3.38	15.0±3.16	0.59±0.08** (↓95)
Monocytes ($10^3/\mu L$)	0.25±0.09	0.29±0.07	0.35±0.25	0.30±0.14	0.01±0.01** (↓96)
Eosinophils ($10^3/\mu L$)	0.16±0.61	0.16±0.04	0.13±0.06	0.15±0.06	0.02±0.01** (↓88)
Basophils ($10^3/\mu L$)	0.18±0.04	0.21±0.11	0.20±0.03	0.17±0.03	0.01±0.01** (↓95)
Large unstained cells ($10^3/\mu L$)	0.26±0.16	0.27±0.19	0.26±0.13	0.27±0.16	0.01±0.01** (↓96)
Females					
Red blood cells ($10^6/\mu L$)	8.13±0.27	7.95±0.37	7.72±0.48* (↓5)	6.87±0.25** (↓16)	7.65±0.31* (↓6)
Hemoglobin (g/dL)	15.9±0.51	15.6±0.65	14.9±0.82** (↓6)	13.3±0.35** (↓16)	14.9±0.73** (↓6)
Hematocrit (%)	45.8±1.81	44.7±1.48	42.9±2.71** (↓6)	39.4±1.68** (↓14)	42.5±2.21** (↓7)
MCHC[d] (g/dL)	34.8±0.55	34.8±0.73	34.8±0.62	33.8±0.82** (↓3)	35.0±0.38
Reticulocytes (%)	2.2±0.65	2.7±0.42	2.9±0.88	6.8±1.01** (↑209)	0.0±0.0** (↓100)
Reticulocytes ($10^3/\mu L$)	178.9±51.2	215.4±27.2	226.4±64.2	463.7±69.2** (↑159)	0.0±0.0** (↓100)
Prothrombin time (s)	16.1±0.31	16.0±0.28	15.9±0.42	15.6±0.47** (↓3)	16.4±0.34
Platelets ($10^3/\mu L$)	1026±122	1003±196	1009±68	1077±243	484±54** (↓53)
White blood cells ($10^3/\mu L$)	14.0±2.78	14.5±3.40	14.5±3.44	14.6±4.31	0.91±0.13** (↓94)
Neutrophils ($10^3/\mu L$)	1.47±0.97	1.30±0.56	1.36±0.49	1.50±0.69	0.16±0.05** (↓89)
Lymphocytes ($10^3/\mu L$)	11.8±2.38	12.6±2.89	12.5±3.11	12.5±3.70	0.68±0.10** (↓94)
Monocytes ($10^3/\mu L$)	0.21±0.07	0.14±0.04* (↓33)	0.18±0.08	0.17±0.06	0.01±0.01** (↓95)
Eosinophils ($10^3/\mu L$)	0.13±0.023	0.11±0.04	0.13±0.04	0.12±0.05	0.02±0.01** (↓85)
Basophils ($10^3/\mu L$)	0.19±0.06	0.16±0.04	0.18±0.08	0.14±0.03	0.02±0.01** (↓90)
Large unstained cells ($10^3/\mu L$)	0.22±0.120	0.21±0.08	0.21±0.08	0.18±0.06	0.02±0.01** (↓91)

a Data were obtained from Tables 17-18 on pages 85-96 of the study report. Percent differences from controls are included in parentheses.

b Positive control rats were administered 50 mg/kg day cyclophosphamide by i.p. injection on Days 24-27.

c APTT = activated partial thromboplastin time

d MCHC = mean corpuscular hemoglobin concentration

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

E. PATHOLOGY

1. **Organ weight:** At 600 ppm, increases in absolute (↑13-19%; NS), relative to body (↑22-30%; p≤0.01), and relative to brain (↑16-21%; p≤0.05 in males only) spleen weights were observed. There were no other treatment-related changes in organ weights.

   In the positive controls, absolute and relative to body and brain spleen weights were decreased (p≤0.01) by 53-63%, and absolute and relative to body and brain thymus weights were decreased (p≤0.01) by 76-79%. These findings were considered to be consistent with cyclophosphamide treatment. Other changes (p≤0.05) in organ weights were considered to be incidental or a reflection of the decreased final body weights in these animals.

TABLE 4: Mean (±SD) spleen and thymus weights in rats treated with propanil for up to 28 days[a]

Parameter/interval	Dose (ppm)				
	0	50	200	600	Positive controls[b]
Males					
Final body weight (g)	402±28.2	395±28.4	403±35.4	376±23.2 (↓7)	354±31.0** (↓12)
Absolute spleen (mg)	862±124	838±111	860±110	977±157 (↑13)	321±47** (↓63)
Relative (to body) spleen (%)	0.214±0.025	0.213±0.025	0.213±0.018	0.260±0.041** (↑22)	0.091±0.010** (↓58)
Relative (to brain) spleen (%)	42.82±6.54	42.21±6.68	43.16±4.61	49.80±8.09* (↑16)	16.53±2.49** (↓61)
Absolute thymus (mg)	644±86	614±109	642±164	599±104	140±40** (↓78)
Relative (to body) thymus (%)	0.161±0.023	0.157±0.033	0.158±0.033	0.160±0.030	0.039±0.009** (↓76)
Relative (to brain) thymus (%)	31.93±3.82	30.93±6.06	32.24±7.78	30.65±5.89	7.22±2.09** (↓77)
Females					
Final body weight (g)	246±18.6	240±20.1	246±14.6	225±14.7* (↓9)	224±18.6* (↓9)
Absolute spleen (mg)	606±106	653±73	692±164	722±155 (↑19)	260±29** (↓57)
Relative (to body) spleen (%)	0.246±0.034	0.273±0.029	0.281±0.063	0.321±0.069** (↑30)	0.116±0.012** (↓53)
Relative (to brain) spleen (%)	33.18±5.76	36.09±3.90	37.32±8.72	40.08±10.16 (↑21)	14.21±1.97** (↓57)
Absolute thymus (mg)	531±91	509±110	532±75	507±78	110±24** (↓79)
Relative (to body) thymus (%)	0.216±0.036	0.212±0.037	0.217±0.033	0.226±0.034	0.049±0.010** (↓77)
Relative (to brain) thymus (%)	28.93±4.09	28.17±6.17	28.73±4.28	28.05±5.24	6.08±1.56** (↓79)

a Data were obtained from Tables 21-22 on pages 100-115 of the study report. Percent differences from controls are included in parentheses.
b Positive control rats were administered 50 mg/kg day cyclophosphamide by i.p. injection on Days 24-27.
* Significantly different from controls; p≤0.05
** Significantly different from controls; p≤0.01

2. **Macroscopic pathology:** There were no treatment-related findings at necropsy. In the positive control animals, small spleen was observed in 3/10 males and 4/10 females, and small thymus was noted in 9/10 males and 7/10 females, all compared to 0 controls. These findings were considered to be consistent with cyclophosphamide treatment.

3. **Microscopic pathology:** Microscopic pathology was not performed.

F. SPLENIC ANTIBODY-FORMING CELL (AFC) ASSAY

Immunotoxicity findings for the AFC assay are summarized in Table 5. There were no effects of treatment on the splenic anti-SRBC (IgM) response. Spleen cell number and the anti-SRBC responses as indicated by antibody-forming cell (AFC/10^6 spleen cells) and total activity (AFC/spleen) were similar to controls in all treatment groups.

In the positive controls, spleen cell numbers were decreased ($p \leq 0.01$) by 78-85%, and suppression of anti-SRBC responses was observed as indicated by decreased specific activity (AFC/10^6 spleen cells) and total activity (AFC/spleen) by 99-100% ($p \leq 0.01$). These findings were considered to be consistent with cyclophosphamide treatment.

TABLE 5: Splenic AFC assay parameters in rats treated with propanil for up to 28 days[a]

Parameter/interval	Dose (ppm)				
	0	50	200	600	Positive controls[b]
Males					
Final body weight (g)[c]	402±28.2	395±28.4	403±35.4	376±23.2 (↓7)	354±31.0** (↓12)
Absolute spleen (mg)[c]	862±124	838±111	860±110	977±157 (↑13)	321±47** (↓63)
Spleen cells (x 10^7)[d]	103.6±4.7	95.7±4.7	106.3±6.9	104.1±6.5	22.5±9.2** (↓78)
IgM AFC/10^6 spleen cells[d]	486±65	501±81	352±65	867±214	6±6** (↓99)
IgM AFC/Spleen (x 10^3)[d]	494±65	453±65	371±70	937±245	1±1** (↓100)
Females					
Final body weight (g)[c]	246±18.6	240±20.1	246±14.6	225±14.7* (↓9)	224±18.6* (↓9)
Absolute spleen (mg)[c]	606±106	653±73	692±164	722±155 (↑19)	260±29** (↓57)
Spleen cells (x 10^7)[d]	89.2±7.0	91.1±3.7	100.9±8.3	86.2±8.1	13.0±1.0** (↓85)
IgM AFC/10^6 spleen cells[d]	399±69	704±155	595±99	649±172	4±4** (↓99)
IgM AFC/Spleen (x 10^3)[d]	373±78	643±141	624±139	557±146	1±1** (↓100)

a Data were obtained from Tables 21-22 on pages 100-115 and Tables 3-4 on pages 548-549 of the study report. Percent differences from controls are included in parentheses.
b Positive control rats were administered 50 mg/kg day cyclophosphamide by i.p. injection on Days 24-27.
c Mean ± SD
d Mean ± SE
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Exposure of rats to propanil resulted in slightly lower body weight gains in the 600 ppm males and females, lower food consumption in the 600 ppm males, and changes in red blood cell indices (lower red blood cell counts, hemoglobin, hematocrit, and corpuscular hemoglobin concentration levels) and higher reticulocyte counts in the 200 ppm females and 600 ppm males and females. There were no treatment-related effects on mortality, clinical observations, macroscopic findings, or organ weights. Additionally, there were no effects on spleen cell number, or on the humoral immune response as evaluated in the IgM antibody-forming cell response to the T-dependent antigen, sheep erythrocytes, while the positive control (cyclophosphamide) induced the expected changes in these parameters. The NOAEL for immunotoxicity is 600 ppm.

B. REVIEWERS COMMENTS: There were no effects of treatment on mortality, clinical observations, food consumption, or macroscopic pathology.

The reviewers disagree with the Sponsor concerning the effects of treatment at 600 ppm on food consumption and the lack of effect on spleen weight. The decreases in food consumption observed in the 600 ppm males were small (1-3 g/animal/day) and did not achieve statistical significance.

At 600 ppm, body weights were slightly decreased in both sexes throughout treatment (↓2-9%; not significant [NS] except on Day 28 in the females). Cumulative body weight gains were decreased ($p<0.05$) by 18-19% in the males on Days 0-7, 0-10, and 0-14, and overall (Days 0-28) body weight gains were decreased (NS) by 16%. In the females, cumulative body weight gains were decreased ($p\leq0.05$) during all intervals by 29-42%; overall (Days 0-28) body weight gains were decreased ($p\leq0.01$) by 30%.

Additionally at this dose, decreases ($p\leq0.05$) were noted in mean red blood cell number (↓16% in females), hemoglobin (↓6% in males, ↓16% in females), hematocrit (↓6% in males, 14% in females), and mean corpuscular hemoglobin concentration (↓3% in females), and increases ($p\leq0.01$) were observed in both mean absolute and percent reticulocytes (↑51-60% in males, ↑159-209% in females). These findings were considered to be indicative of a mild anemia. Also, increases in absolute (↑13-19%; NS), relative to body (↑22-30%; $p\leq0.01$), and relative to brain (↑16-21%; $p\leq0.05$ in males only) spleen weights were observed.

The systemic LOAEL is 600 ppm (equivalent to 48/56 mg/kg/day in males/females), based on decreased body weights and body weight gains in both sexes, and mild anemia, characterized by decreased hemoglobin and hematocrit and increased reticulocytes and spleen weights in both sexes, and decreased mean red blood cell number and mean corpuscular hemoglobin concentration in the females. The NOAEL is 200 ppm (equivalent to 16/19 mg/kg/day in males/females).

Positive control rats (50 mg/kg/day cyclophosphamide on Days 24-27) displayed decreased body weights on Day 28, body weight losses during treatment with cyclophosphamide, and decreased overall body weight gains. In the females, decreases were observed in red blood cells, hemoglobin, and hematocrit. Additionally, decreases were noted in the following mean absolute parameters in both sexes (except where noted): reticulocytes (both absolute and percent); platelets; white blood cells; neutrophils; lymphocytes; monocytes; eosinophils; basophils; and large unstained cells. Small spleen was observed in 3/10 males and 4/10 females, and small thymus was noted in 9/10 males and 7/10 females, all compared to 0 controls, and absolute and relative to body and brain spleen and thymus weights were decreased. These findings were considered to be consistent with cyclophosphamide treatment.

For immunotoxicity study, treatment of propanil had no effects on spleen cell numbers and splenic anti-SRBC (IgM) response as evaluation of there were no effects of treatment on splenic anti-SRBC (IgM) response. Spleen cell number and the anti-SRBC response as indicated by specific activity (AFC/10^6 spleen cells) and total activity (AFC/spleen) were similar to controls in all treatment groups.

The NOAEL for splenic anti-SRBC response is 600 ppm (equivalent to 48/56 mg/kg/day in males/females; the highest dose tested).

In the positive controls, spleen cell numbers were decreased ($p\leq0.01$) by 78-85%, and specific activity (AFC/10^6 spleen cells) and total activity (AFC/spleen) were decreased

($p \leq 0.01$) by 99-100%. These findings were considered to be consistent with cyclophosphamide treatment.

This immunotoxicity study is classified **acceptable/guideline** and satisfies the guideline requirement for an immunotoxicity study (OPPTS 870.7800) in rats.

C. **STUDY DEFICIENCIES:** No study deficiencies were observed.

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R156335

Chemical: **Propanil**

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