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DER No. 4

Chemical Name: Propanil
24-Month Carcinogenicity Study in Mice
Sponsor Name: Propanil Task Force Year of Study: 1994
MRID No. 43391701 HED Doc. No.

EPA Reviewer: W. Greear,M.P.H., D.A.B.T. _____, Date _____
Review Section III, Toxicology Branch I (7509C)
EPA Secondary Reviewer:
M. Copley, D.V.M., D.A.B.T. _____, Date _____
Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Feeding - Mouse
OPPTS 870.4200 [§83-2]

DP BARCODE: D206275, D216899
P.C. CODE: 028201
SUBMISSION CODE: S471356, S477999
TOX. CHEM. NO.: 325

TEST MATERIAL (PURITY): Propanil (97.1% w/w)

SYNONYMS: not provided

CITATION: Tompkins, E. (1994) 24-Month dietary oncogenicity study in mice with Propanil. WIL Research Laboratories, Inc., 1407 George Road, Ashland, Ohio 44805-9281. Laboratory study number WIL-141011, September 9, 1994. MRID 43391701. Unpublished.

SPONSOR: Propanil Task Force, S. Victory Lane, Suite 201, Liberty, Missouri 64068.

EXECUTIVE SUMMARY: In a 24-month oncogenicity study (MRID 43391701) Propanil (97.1% w/w a.i.) was administered to 80 Crl:CD-1®(ICR)BR mice/sex/dose in the feed at dose levels of 0, 500, or 1000 ppm (males: 0, 74.9, and 150.0 mg/kg/day; females: 0, 88.6 and 174.1 mg/kg/day) for up to 104 weeks. Twenty mice/sex/dose were designated for interim sacrifice at week 52.

High-dose males and females had significantly ($p < 0.01$) lower body weight gain during week 1 of the study, resulting in slightly lower mean body weights in high-dose animals as compared to controls beginning at week 1 and continuing through weeks 66 and 61 for males and females, respectively. These differences were occasionally statistically significant, but were always within 5% of controls and were not considered toxicologically significant. Dose-related increases in blue coloration of the extremities (0/0, 22/5, and 205/19 observations/# animals in control, low-, and high-dose males; 0/0, 17/3, and 142/15 observations/# animals in control, low-, and high-dose females) and corresponding increases in mean methemoglobin values were observed in treated mice when compared to controls. Methemoglobin differences were statistically significant ($p<0.05$ or $p<0.01$) in 500 ppm males (7-fold increase) and 1000 ppm males (15-fold increase) and females (12-fold increase). Mean

erythrocyte, hemoglobin, and hematocrit values were lower in high-dose males compared to controls at week 104; however, the differences were not statistically significant. An increase in mean reticulocyte count and MCV was observed in 1000 ppm males, suggesting the presence of a compensatory mechanism. Mean Heinz body counts were significantly increased in 500 ppm ($p<0.05$) and 1000 ppm ($p<0.01$) males when compared to controls at week 104; however, the increase was of small magnitude.

The systemic toxicity LOEL for this study is 500 ppm (74.9 mg/kg/day for males and 88.6 mg/kg/day for females) based on methemoglobinemia and blue coloration of the extremities. The systemic toxicity NOEL is not identified.

The carcinogenic potential of Propanil was evidenced by an increased incidence of malignant lymphomas of the spleen in females. Lymphomas were observed in 4/61 control, 4/61 low-dose, and 13/61 high-dose females. The increase was statistically significant ($p<0.05$) in 1000 ppm female mice when compared to controls.

This oncogenicity toxicity study in mice is acceptable and satisfies the guideline requirement for an oncogenicity feeding study (83-2) in mice. However, a study deficienciencies exist: the testing of only two doses and a control and no identification of a NOEL for systemic toxicity.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Propanil

   Description: light brown to dark purple granular solid
   Lot/Batch #: 01
   Purity: 97.1% w/w a.i.
   Stability of compound: considered stable for duration of study
   CAS No.: None

2. Vehicle and/or positive control

   Purina® Certified Rodent Chow #5002 was used as carrier and negative control. No positive control was used in this study.

3. Test animals

   Species: mouse
   Strain: Crl:CD-1®(ICR)BR

Age and weight at study initiation: 43 days; males, 21.1-31.3 g; females, 18.1-26.8 g
Source: Charles River Breeding Laboratories, Inc., Portage, Michigan.
Housing: individually in wire-mesh, suspended cages.
Diet: Purina® Certified Rodent Chow #5002, available ad libitum.
Water: Tap water, available ad libitum from an automatic watering system.
Environmental conditions:
Temperature: 67-77°F
Humidity: 22-88%
Air changes: not specified
Photoperiod: 12-hour light/dark
Acclimation period: 10 days

B. STUDY DESIGN

1. In life dates

Start: November 18, 1991; end: November 17, 1993

2. Animal assignment

Animals were assigned to one control or one of two treated groups of 80 rats each as shown in Table 1. Twenty mice/sex/group were designated for interim sacrifice at 52 weeks. The other 60 mice/sex/group were designated for terminal sacrifice at week 104. All mice were weighed and examined for physical abnormalities one week before dosing. Individual body weights and animal numbers for all suitable animals were entered into WIL's Computer Data Management system for randomized group assignment based on body weight stratification in a block design.

TABLE 1. Study design

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg/day)		Main Study 104 weeks		Interim Sac. 52 weeks	
		Male	Female	Male	Female	Male	Female
Control	0	0	0	60	60	20	20
Low	500	74.9	88.6	60	60	20	20
High	1000	150.0	174.1	60	60	20	20

Data taken from pp. 19 and 31, MRID 43391701.

3. Dose selection rationale

No information concerning dose selection rationale was presented.

4. Diet preparation and analysis

Experimental diets were prepared weekly during the study. Propanil was ground in an Ika-Werk grinder for 1 minute before weighing. The Propanil was then weighed into tared weighing vessels for each dose and mixed with 1 kg. rodent feed in a Hobart mixer for 10 minutes. A predetermined amount of rodent feed was weighed and placed in a V-twin shell mixer. The premix was then added and the test diet was mixed for 15 minutes to produce a batch of homogeneous test diet. The diets were stored at room temperature for weeks 0 to 4 and were stored frozen from week 5 through the end of the study. Three samples (top, middle, and bottom) from each of the three dose level diets were collected prior to study initiation for homogeneity analysis. Two sets of samples were analyzed for homogeneity and 14-day stability, and the third set was frozen. Stability was examined from diets from weeks 3, 5, and 8. Diet preparations from weeks 0, 1, 2, 3, 7, 11, 24, 37, 50, 63, 76, 89, and 102 were analyzed for concentration.

Results -
Homogeneity Analysis - Samples from the top, middle, and bottom of 500 ppm diets ranged from 94.8 to 97.9% of target (96.3% overall mean) and for 1000 ppm diets ranged from 89.7 to 94.6% of target (92.2% overall mean).

Stability Analysis - After storage for 7 or 14 days at room temperature, mean concentration from the 500 and 1000 ppm diets were within 10% of their original measured concentrations.

Concentration Analysis - Mean dietary concentrations of Propanil were 486 ppm (97.1% of nominal 500 ppm, range 461-509 ppm) and 966 ppm (96.6% of nominal 1000 ppm, range 862-1015 ppm). All diet preparations were within ±15% of targets.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

All statistical analyses were conducted using two-tailed tests for significance levels of 5% and 1%. Males and females were considered separately. Body weight, body weight change, food consumption, hematological parameters, and abso-

lute and relative organ weight data were analyzed by a one-way analysis of variance followed by a two-tailed Dunnett's test. Clinical laboratory data for infrequently occurring cell types and leukocyte differential data for animals euthanized *in extremis* were not subjected to statistical analysis. Terminal mortality data were analyzed using the one-tailed Fisher's Exact Test.

Tumor incidence data in control and treated groups were analyzed using the method of Peto (one-tailed) and Fisher's Exact Test (one-tailed).

C. METHODS

1. Observations

All mice were observed twice daily for mortality and moribundity, and daily for significant clinical changes. A detailed physical examination was made weekly.

2. Body weight

Animals were weighed weekly, beginning one week prior to Propanil administration.

3. Food consumption and compound intake

Individual food consumption was determined weekly for the first 14 weeks, and every two weeks thereafter. Food intake was calculated as g/animal/day. Mean compound consumption, calculated from individual consumption and body weight, was reported in terms of mg Propanil/kg body weight/day. The study authors did not calculate food efficiency.

4. Ophthalmoscopic examination

An ophthalmoscopic examination is not required in an oncogenicity study based on Subdivision F Guidelines and was not performed.

5. Blood was collected for hematology analysis from the vena cava of animals at the time of necropsy (week 52 or week 104). At each scheduled termination, blood samples from 10 mice/sex/group (lowest numbered) were used to measure standard hematology parameters and samples from 10 mice/sex/group (highest numbered) were used for methemoglobin measurements. Additionally, reticulocyte and Heinz body counts were done at week 104 on 10 mice/sex/group. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)	X	Leukocyte differential count**
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count	X	Reticulocyte count
	Blood clotting measurements	X	Heinz body count*
	(Thromboplastin time)	X	Methemoglobin*
	(Clotting time)		
	(Prothrombin time)		

* Performed at week 104 only.
**Minimal requirement for oncogenicity studies, but only on control and high-dose unless effects are observed based on Subdivision F Guidelines.

b. Clinical chemistry is not required in an oncogenicity study based on Subdivision F Guidelines and was not performed.

6. Urinalysis is not required in an oncogenicity study based on Subdivision F Guidelines and was not performed.

7. Sacrifice and pathology

Complete necropsies were conducted on all mice dying spontaneously, euthanized *in extremis* or killed at interim (week 52) or terminal (week 104) necropsies. Animals requiring euthanasia and those killed at week 52 or 104 were killed by exsanguination under carbon dioxide anesthesia. The CHECKED (X) tissues were collected, preserved in 10% formalin, embedded in paraffin blocks, and stained with hematoxylin and eosin. The [XX] organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Oral cavity	X	Aorta*	XX	Brain*•
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL		
X	Colon*	XX	Kidneys*•	X	Adrenal gland*
X	Rectum*	X	Urinary bladder*		Lacrimal gland
X	Liver*•	XX	Testes*•	X	Mammary gland*
X	Gall bladder	X	Epididymides*	X	Parathyroids
X	Pancreas*	X	Prostate	X	Thyroids*
		X	Seminal vesicle		
	RESPIRATORY	XX	Ovaries*		OTHER
	Trachea*	X	Uterus*		
X	Lung*		Cervix	X	Bone
X	Nose			X	Skeletal muscle*
	Pharynx			X	Skin*
	Larynx			X	All gross lesions and masses*
				X	Femur

*Required for oncogenicity studies based on Subdivision F Guidelines.
•Organ weight required in oncogenicity studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

A dose-related blue coloration of the extremities was observed in 500 and 1000 ppm males and females, generally during the second year of the study. This coloration was observed 22 times in 5 mice and 205 times in 19 mice for 500 and 1000 ppm males, respectively, and 17 times in 3 mice and 142 times in 15 mice for 500 and 1000 ppm females, respectively. The effect was not observed in control animals and is consistent with the fact that propanil elevates blood methemoglobin levels. Unkempt appearance was observed in 9 control males, 16 500 ppm males, and 16 1000 ppm males. The author did not consider this to be treatment-related since no dose-response was present and it was observed prior to death in the absence of an increase in mortality. No other treatment-related effects were observed.

2. Mortality

No statistically significant differences in survival were observed between control and propanil treated mice. Survival at study termination (week 104, excluding animals sacrificed at interim necropsy) was 25/61, 27/63, and 22/61 for control, low-, and high-dose males, and 30/61, 25/61, and 22/61 for control, low-, and high-dose females.

B. BODY WEIGHT

Body weights and body weight gains are presented in Table 2 for male mice and in Table 3 for female mice. No effects on mean body weight or body weight gain were observed in 500 ppm males or females. High-dose males and females had significantly ($p < 0.01$) lower body weight gain during week 1 of the study, resulting in slightly lower mean body weights in high-dose animals as compared to controls beginning at week 1 continuing through weeks 66 and 61 for males and females, respectively. These differences were occasionally statistically significant and were always within 5% of controls. The last statistically significant difference was observed in 1000 ppm males at week 66 and in 1000 ppm females at week 61. By week 104, body weights for high-dose males and females were similar to controls.

TABLE 2. Selected body weights (g) and body weight gains (g) of male mice given Propanil in the diet for 2 years

Week of Study	0 ppm	500 ppm	1000 ppm
Absolute Body Weights[1]			
0	26.5 ± 2.02	26.5 ± 1.99	26.5 ± 2.00
1	27.3 ± 2.04	27.3 ± 1.90	26.9 ± 2.03
25	34.8 ± 3.22	35.0 ± 2.70	34.0 ± 2.91
52	36.6 ± 3.65	36.8 ± 3.54	35.8 ± 2.95
75	37.0 ± 3.19	36.7 ± 3.59	36.0 ± 3.32
104	36.1 ± 2.97	36.5 ± 3.56	35.7 ± 3.19
Body Weight Gains			
0-1	0.8	0.8	0.4**
24-25	-0.2	-0.1	-0.1
51-52	0.5	0.6	0.6
74-75	0.1	0.2	0.2
103-104	0.1	0.2	0.5

Data taken from Tables 5 & 6, pp. 82-167, MRID 43391701.

[1]Mean ± S.D.
**P<0.01

TABLE 3. Selected Body weights (g) and body weight gains (g) of female mice given Propanil in the diet for 2 years

Week of Study	0 ppm	500 ppm	1000 ppm
Absolute Body Weights[1]			
0	22.5 ± 1.59	22.5 ± 1.58	22.5 ± 1.59
1	23.4 ± 1.66	23.4 ± 1.61	22.9 ± 1.71
25	30.0 ± 2.68	30.0 ± 2.48	29.6 ± 2.63
52	32.6 ± 3.64	32.3 ± 3.30	31.4 ± 3.08
75	33.2 ± 3.65	33.0 ± 3.20	32.5 ± 3.09
104	32.6 ± 3.58	32.7 ± 3.03	33.0 ± 2.83
Body Weight Gains			
0-1	0.9	0.9	0.5**
24-25	0.2	0.1	0.0
51-52	0.0	0.1	0.0
74-75	0.2	0.5	0.7
103-104	0.4	-0.3	0.3

Data taken from Tables 5 & 6, pp. 82-167, MRID 43391701.
[1]Mean ± S.D.
**P<0.01

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Food consumption for male and female mice at selected weeks during the study is presented in Table 4. Statistically significantly ($p<0.05$ or $p<0.01$) lower mean food consumptions were observed during the first year of the study in 1000 ppm males and females. No treatment-related differences were observed in 500 ppm mice. During the second year of the study, no consistent differences were observed between treated and control animals, and the effects observed during year 1 are not considered toxicologically significant.

TABLE 4. Food consumption (g/mouse/day) by mice given Propanil in the diet for 2 years

Week of Study	0 ppm	500 ppm	1000 ppm
Food consumption	Males		
0-1	5.3±0.48	5.4 ± 0.49	5.3 ± 0.48
23-24	5.0 ± 0.45	5.0 ± 0.58	4.7 ± 0.50**
51-52	4.9 ± 0.65	5.0 ± 0.69	4.8 ± 0.58
73-74	5.3 ± 0.62	5.3 ± 0.60	5.2 ± 0.63
103-104	5.0 ± 0.50	5.0 ± 0.71	5.0 ± 0.52
Females			
0-1	5.5 ± 0.63	5.5 ± 0.56	5.3 ± 0.58*
23-24	5.3 ± 0.72	5.3 ± 0.91	5.1 ± 0.65*
51-52	5.1 ± 0.92	5.2 ± 1.06	5.1 ± 0.67
73-74	5.2 ± 0.57	5.1 ± 0.68	5.1 ± 0.55
103-104	4.9 ± 0.83	5.6 ± 0.56**	5.1 ± 0.72

Data taken from Table 7, pp. 168-191, MRID 43391701.
[1]Mean ± S.D.
*p ≤ 0.05, **p ≤ 0.01.

2. Compound consumption

The overall average dose received for each of the treated groups is given in Table 1.

D. BLOOD WORK

1. Hematology

Hematology data are presented in Table 5. Dose-related increases in mean methemoglobin values were observed at weeks 52 and 104 in treated mice when compared to controls. Values were increased 5- and 10-fold over controls in low-dose males at weeks 52 and 104, respectively; 8- and 15-fold over controls in high-dose males at weeks 52 and 104, respectively; 7- and 3-fold over controls in low-dose females at weeks 52 and 104, respectively; and 12- and 5-fold over controls in high-dose females at weeks 52 and 104, respectively. Differences were statistically significant ($p \leq 0.05$ or $p \leq 0.01$)

TABLE 5. Selected mean hematological values in mice given Propanil in the diet for 2 years[1]

Hematological Values	0 ppm		500 ppm		1000 ppm	
	Males					
	week 52	week 104	week 52	week 104	week 52	week 104
Methemoglobin (%)	1.4±1.26	1.1±0.91	6.3*±1.98	10.6**±9.43	11.2**±5.75	16.6**±7.30
RBC ($10^6/\mu L$)	9.00±1.232	9.36±1.159	8.73±0.424	9.69±1.596	8.46±0.551	8.09±1.600
Hemoglobin (g/dL)	13.8±1.83	14.6±2.01	13.4±0.64	14.7±2.54	13.1±0.95	12.8±1.92
Hematocrit (%)	47.8±7.73	48.5±6.33	44.0±1.95	48.9±8.31	43.4±3.05	42.9±5.90
MCV (μ^3)	53.1±3.32	51.9±3.59	50.4±1.73	50.5±2.31	51.3±2.48	53,9±5.84
Reticulocyte (%)	---	1.5±0.76	---	1.9±1.12	---	4.6**±3.67
Heinz body (%)	---	0.0±0.00	---	0.1*±0.06	---	0.1**±0.11
Females						
Methemoglobin (%)	0.9±1.03	1.8±1.67	6.4±4.99	5.1±4.62	10.5**±8.61	8.7**±2.92
RBC ($10^6/\mu L$)	8.86±0.634	8.43±1.385	8.75±0.926	8.94±0.604	8.90±0.638	8.75±0.753
Hemoglobin (g/dL)	14.0±0.95	13.0±1.50	13.7±0.96	13.6±0.98	13.8±0.65	13.5±1.16
Hematocrit (%)	46.8±4.19	43.6±5.41	46.7±4.22	45.6±3.41	46.7±2.60	44.9±4.79
MCV (μ^3)	52.8±1.54	52.2±5.02	53.5±3.14	51.0±1.96	52.6±2.36	51.2±2.48
Reticulocyte (%)	---	3.4±2.50	---	3.4±1.14	---	4.0±1.09
Heinz body (%)	---	0.0±0.06	---	0.0±0.05	---	0.0±0.07

Data taken from Table 9, pp. 216-223, MRID 43391701.
[1]Mean ± S.D.
*$p \leq 0.05$, **$p \leq 0.01$.

in 500 ppm males and 1000 ppm males and females. The effect is considered biologically significant and treatment-related in males and females at both doses even though statistical significance was not achieved in the 500 ppm females. Mean erythrocyte, hemoglobin, and hematocrit values were lower in high-dose males compared to controls at week 104; however, the differences were not statistically significant. A statistically significant ($p<0.01$) increase in mean reticulocyte count and MCV was observed in 1000 ppm males, suggesting the presence of a compensatory process. These effects were not observed in other treatment groups. Mean Heinz body counts were significantly increased in 500 ppm ($p<0.05$) and 1000 ppm ($p<0.01$) males when compared to controls at week 104; however, the increase is of small magnitude. No other test-material related hematological effects were observed. The mean lymphocyte count of 1000 ppm females was significantly ($p<0.01$) increased at week 104. However, the value was similar to the week 52 control mean and the week 52 mean for the high-dose females and was not considered treatment-related.

E. SACRIFICE AND PATHOLOGY

1. Organ weight

Absolute and relative mean spleen weights were significantly ($p<0.01$) increased in 1000 ppm female mice at week 52, but not at week 104. No significant effects on spleen weight were observed in treated male mice or in 500 ppm females. Spleen weight summary data are presented in Table 6. The only other significant differences in organ weight were increased ($p<0.05$) absolute brain and kidney weights in 500 ppm males at week 52 and increased relative liver weight ($p<0.05$) in 1000 ppm females at week 104. These latter effects are not considered biologically significant in the absence of supporting histopathology and/or a dose-response.

2. Gross pathology

No treatment-related gross pathology was observed. The incidences of lesions observed were similar in treated and control mice and are consistent with aging mice.

3. Microscopic pathology

a. Non-neoplastic - No treatment-related non-neoplastic lesions were observed. The incidences of lesions observed were similar in treated and control mice and are consistent with aging mice.

TABLE 6. Absolute and relative spleen weights[1] from male and female mice given Propanil in the diet for 2 years (g)

Spleen Weights		Male			Female		
		0 ppm	500 ppm	1000 ppm	0 ppm	500 ppm	1000 ppm
Absolute	52 weeks	0.1106±0.085	0.1173±0.056	0.1397±0.062	0.1045±0.024	0.1379±0.058	0.1689**±0.075
	104 weeks	0.1545±0.119	0.1164±0.046	0.1366±0.064	0.2016±0.175	0.1893±0.114	0.2432±0.143
Relative	52 weeks	0.323±0.2703	0.319±0.1568	0.394±0.1726	0.320±0.0811	0.442±0.1982	0.527**±0.2327
	104 weeks	0.425±0.3143	0.322±0.1308	0.389±0.2011	0.611±0.5050	0.582±0.3629	0.722±0.3821

[1]Mean±Standard deviation
Data taken from Table 15, pp. 260-276, MRID 43391701.
**p ≤ 0.01.

b. Neoplastic - There was a significant ($p<0.05$) increase in the incidence of malignant lymphomas in 1000 ppm female mice when compared to controls. Malignant lymphomas were observed in 2/31 control females, 4/36 500 ppm females, and 9/39 1000 ppm females found dead or euthanized *in extremis*. At week 104, malignant lymphomas were observed in 1/30, 0/25, and 3/22 females in the control, 500 ppm, and 1000 ppm groups, respectively. The malignant lymphomas were detected in various tissues; however, the spleen was involved in all females found dead or euthanized *in extremis*, and malignant lymphoma was listed as the cause of death for these animals. Single females in the control and 1000 ppm had malignant lymphomas but not in the spleen. A summary of the lymphoma data for combined necropsies is presented in Table 7.

There was a statistically significant ($p<0.05$) increase in the incidence of hepatocellular adenomas in 1000 ppm males found dead or euthanized *in extremis* when compared to controls. However, the incidence of hepatocellular adenomas in 1000 ppm male survivors to week 104 was lower than controls. When the tumor incidences for unscheduled deaths and terminal sacrifice at week 104 were combined, there was no significant difference between 1000 ppm males and controls. Summary data for hepatocellular adenomas are presented in Table 8. The incidence of combined hepatocellular carcinomas in males was 3/61, 1/63, and 0/61 for the control, 500 ppm, and 1000 ppm groups, respectively.

III. DISCUSSION

A. DISCUSSION

In an oncogenicity study (MRID 43391701), Propanil (97.1% a.i.) was administered in the feed to 80 Crl:CD-1®(ICR)BR mice/sex/dose at concentrations of 0, 500, or 1000 ppm (males: 0, 74.9 or 150.0 mg/kg/day; females: 0, 88.6, or 174.1 mg/kg/day) for 104 weeks. Twenty mice/sex/dose were designated for interim sacrifice at week 52.

Blue coloration of the extremities related to increased methemoglobin values during the second year of the study was observed in males and females in a dose-related manner. Mean blood methemoglobin values were also increased in a dose-related manner at weeks 52 and 104, correlating with the established mechanism of propanil of methemoglobinemia and erythrocyte hemolysis. Decreased red blood cell counts, hemoglobin, and hematocrit were observed in 1000 ppm males at week 104.

TABLE 7. Summary of lymphoma incidence in mice fed Propanil for 2 years[1]		
0 ppm	500 ppm	1000 ppm
Males		
3/61	4/63	1/61
Females		
4/61*	4/61	13/61**

Data taken from p. 33-34, MRID 43391701.
[1]Combined necropsy, excluding interim necropsy, incidence for all tissues.
*Significant trend, method of Peto ($p<0.01$); ** $p<0.05$, Fisher Exact Test

TABLE 8. Summary of hepatocellular adenoma incidence in mice fed Propanil for 2 years			
Time Point	0 ppm	500 ppm	1000 ppm
Males			
Unscheduled death	1/36	3/36	8/39*
Week 104 necropsy	7/25	6/27	3/22
Combined	8/61	9/63	11/61
Females			
Combined	1/61	2/61	1/61

Data taken from p. 34-35, MRID 43391701.
* $p<0.05$, Fisher Exact Test

These decreased erythrocyte parameters were accompanied by increases in reticulocyte count and MCV, suggesting a compensatory process related to methemoglobinemia. A small increase in the incidence of Heinz bodies was observed in both groups of treated males at week 104. Body weight, body weight gain, and food consumption were slightly decreased in 1000 ppm males and females during the first year of the study, but were similar to control means during the second year of the study and are not considered toxicologically significant. Increased absolute and relative spleen weights in 1000 ppm females at week 52 may be treatment-related. However, no splenic lesions were observed and spleen weights were not elevated at terminal necropsy, suggesting little toxicological significance.

Therefore, the systemic toxicity LOEL for this study is 500 ppm (74.9 mg/kg/day for males and 88.6 mg/kg/day for females) based on methemoglobinemia and related blue coloration of the extremities. The systemic toxicity NOEL is not identified.

There was a dose-related increase in the incidence of malignant lymphomas in 1000 ppm females. The study author states that while the 21.3% incidence of malignant lymphomas in the 1000 ppm females was above the incidence in the concurrent control and was generally above the mean of historical controls, it was always in the range of historical control data from 21-24 month studies. However, the reviewer believes that the probability of the lesion being treatment-related cannot be dismissed; the historical control data (presented in MRID 43677801) are from a variety of laboratories utilizing CD-1 mice from a variety of sources and feeding the mice a variety of diets. Any one of these factors may be confounding. Also, the historical control data were collected over a period of 13 years and should not be used for comparison with the current study results. Only data collected over the previous 3 years should be considered, Furthermore, when historical control data from the performing laboratory is examined, a 11.5% incidence of malignant lymphomas was observed in female CD-1 mice after 24 months (1 study). In four 18 month feeding studies of CD-1 mice conducted by the performing laboratory, the incidence of malignant lymphomas in females ranges from 3.6 to 8.3%. Since the incidence of malignant lymphomas in high-dose female mice (21.3%) in this study exceeds the upper range of historical control data from the performing laboratory, it is not possible to rule out a test article-related increase in the incidence of malignant lymphomas of the spleen in the 1000 ppm female mice. The reviewer considers these lymphomas to be treatment-related.

The upper dose is considered adequate. A 90-day range-finding study (March 24, 1983; Protocol Number 78P-079; Report Number 82R-065) supports the use of 1000 ppm as an upper dose for a 24-month dietary study. "Life-threatening" toxic effects (cyanosis, increased mixed function oxidase activity, increased spleen weight, increased extramedullary hemaopoiesis and hemosiderin in the spleen) were observed at 1,600 ppm in the 90-day study.

B. STUDY DEFICIENCIES

This study has deficiencies. The considerable variation in body weights at study initiation may suggest variation in the age of the animals. Also, only two test doses (not three as specified in the Guidelines) and a control were tested, and no systemic NOEL was identified. However, a Data Evaluation Report from a previous 24-month dietary oncogenicity study in CD-1

mice with propanil included doses of 30 and 180 ppm (Report issued December 2, 1983; Study Number 417-400; MRID Number 155215). The 30 ppm dose was identified as a NOEL for systemic toxicity in the 1983 study. While it may be possible to identify 30 ppm as a NOEL for Propanil, this should be done cautiously since the study is over ten years old (allowing for possible genetic drift of inbred mice), was performed in a different testing laboratory, and the toxic endpoints identified are not consistent between the two studies. Effects observed at 180 ppm in the 1983 study, but not in the 1994 study, included bilateral retinal degeneration in both sexes, thyroiditis in females, and centrilobular hepatocytic enlargement in males.