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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

May 21, 2003
TXR 0051450

MEMORANDUM

SUBJECT: Propanil (028201) - Review of repeated dose (30-day) dietary toxicity study in rats

TO: Carmen Rodia
Chemical Review Manager
Special Review and Reregistration Division (7508C)

FROM: Susan L. Makris 5/21/03
Toxicology Branch
Health Effects Division (7509C)

THRU: Alberto Protzel, Branch Senior Scientist 8/27/03
Toxicology Branch
Health Effects Division (7509C)

CC: Richard Griffin, RRB2/HED (7509C)

PC CODE: 028201 **DP BARCODE:** D287611
CASE: 818688 **SUBMISSION NO.:** S627371

CITATION: O'Neill, T.P. (2002) A repeated dose 30-day oral (diet) toxicity study in rats. WIL Research Laboratories, Inc., Ashland, OH. Laboratory study no. WIL-464001, December 27, 2002. MRID 45829301. Unpublished.

SPONSOR: Propanil Task Force II; c/o Edward M. Ruckert; McDermott, Will and Emery; 600 13th Street, NW; Washington, DC 20003-3096

ACTION REQUESTED: Review the repeated dose (30-day) dietary toxicity study in rats, in support of the reregistration eligibility decision for propanil (028201).

EXECUTIVE SUMMARY: In a 30-day repeated dose oral toxicity study (MRID 45829301) Propanil (99.3%; batch 2), was administered in the diet to Crl:CD®(SD)IGS BR (Sprague-Dawley) rats (10/sex/dose) at dose levels of 0, 300, 500, or 700 ppm (equivalent to 0, 25, 41, and 57 mg/kg bw/day for males and 0, 28, 41, and 67 mg/kg bw/day for females). As a result of increasing methemoglobin values, dosing was terminated following 17 days of administration

and recovery from this effect was evaluated. All animals were observed for mortality/moribundity; clinical observation, body weight, and food consumption data were recorded. Clinical pathology evaluations (methemoglobin) were performed for all animals during the pretest period and on study days 0 (prior to test substance administration), 1, 5, 7, 14, 21, and 30. Complete necropsies were conducted on all animals; tissues were not collected for further evaluation.

At 700 ppm, decreased mean body weight gain (males and females) and food consumption (males only) values were observed during study weeks 0-2 or 0-3. Increased mean methemoglobin values were observed in the males and females (144-256% and 400-538%, respectively, relative to controls) beginning at study day 5 and throughout the treatment period. Recovery was noted after treatment was stopped (study day 17); on study day 30, mean methemoglobin was increased 88% (males) and 50% (females), as compared to controls.

At 500 ppm, decreased mean body weight and food consumption values were noted in males during study weeks 0-1 or 0-2. Mean methemoglobin values were increased ($p \leq 0.01$) in males (89-133%) and females (225-313%) beginning at study day 5 and throughout the entire treatment period as compared to the control group. Recovery was noted following the cessation of treatment; on study day 30, mean methemoglobin values were increased 50% (males) and 70% (females) as compared to controls.

At 300 ppm, mean methemoglobin values were increased ($p \leq 0.05$, $p \leq 0.01$) in males (33-67%) and females (75-175%), relative to control values, beginning at study day 5 and throughout the remainder of the treatment period. Recovery was noted following cessation of treatment; at study day 30, mean methemoglobin values were 38% and 50% of control values for males and females, respectively. There were no other signs of toxicity noted at 300 ppm

The acute (1-day) LOAEL is not determined. The acute (1-day) NOAEL is 700 ppm (57 mg/kg/day for males and 67 mg/kg/day for females) (HDT).

The repeated dose (≥5 days) LOAEL is 300 ppm (25 mg/kg/day for males and 28 mg/kg/day for females), based on increased methemoglobin levels. No repeated dose (≥5 days) NOAEL was established.

This 30-day oral toxicity study in the rat is **Acceptable, Non-guideline**. This study does not satisfy any guideline requirement, but provides additional information relevant to the hazard assessment for propanil.

B

EPA Reviewer: Susan L. Makris
Toxicology Banch, Health Effects Division (7509C)
EPA Secondary Reviewer: Judy Facey
Reregistration Branch 2, Health Effects Division (7509C)

Signature: Susan L Makris
Date 5/21/03
Signature: Judy Facey
Date 5/27/03

TXR#: 0051450

DATA EVALUATION RECORD

STUDY TYPE: Repeated Dose (30-Day) Dietary Toxicity Study in Rats; Non-guideline

PC CODE: 028201

DP BARCODE: D287611
SUBMISSION NO.: S627371

TEST MATERIAL (PURITY): Propanil (99.3%)

SYNONYMS: 3',4'-dichloropropionanilide

CITATION: O'Neill, T.P. (2002) A repeated dose 30-day oral (diet) toxicity study in rats. WIL Research Laboratories, Inc., Ashland, OH. Laboratory study no. WIL-464001, December 27, 2002. MRID 45829301. Unpublished.

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At 700 ppm, decreased mean body weight gain (males and females) and food consumption (males only) values were observed during study weeks 0-2 or 0-3. Increased mean methemoglobin values were observed in the males and females (144-256% and 400-538%, respectively, relative to controls) beginning at study day 5 and throughout the treatment period. Recovery was noted after treatment was stopped (study day 17); on study day 30, mean methemoglobin was increased 88% (males) and 50% (females), as compared to controls.

At 500 ppm, decreased mean body weight and food consumption values were noted in males during study weeks 0-1 or 0-2. Mean methemoglobin values were increased ($p \leq 0.01$) in males (89-133%) and females (225-313%) beginning at study day 5 and throughout the entire treatment period as compared to the control group. Recovery was noted following the cessation of treatment; on study day 30, mean methemoglobin values were increased 50% (males) and 70% (females) as compared to controls.

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This 30-day oral toxicity study in the rat is **Acceptable, Non-guideline**. This study does not satisfy any guideline requirement, but provides additional information relevant to the hazard assessment for propanil.

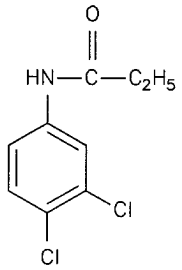
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Propanil

Description:	Light brown, crystalline solid containing small black particles
Lot/Batch #:	Lot No. C048203E. Batch No. 2, Aliquot 21, WIL Log No. 5493A
Purity:	99.3 % a.i.
Storage:	At room temperature, protected from the light
Compound Stability:	Stability was demonstrated for 24-hours of storage at room temperature
CAS # of TGAI:	709-98-8



2. Vehicle and/or positive control: Certified Rodent LabDiet® 5002 (meal), PMI Nutrition International, Inc.

3. Test animals:

Species:	Rat	
Strain:	Crl:CD®(SD)IGS BR	
Age/weight at study initiation:	7.5 weeks old, 202-254 g (males), 159-203 g (females)	
Source:	Charles River Laboratories, Inc., Raleigh, NC	
Housing:	Individually housed, in suspended wire-mesh cages	
Diet:	Certified Rodent LabDiet® 5002 (meal), PMI Nutrition International, Inc., *ad libitum*	
Water:	Municipal water, *ad libitum*, via automated watering system	
Environmental conditions:	**Temperature:**	Nominal: 71 ± 5°F; Actual: 71.1°F to 72.3 °F
	Humidity:	Nominal: 50 ± 20 %; Actual: 39.4% to 45.6%
	Air changes:	Not provided
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	15 days	

B. STUDY DESIGN:

1. In life dates - Start: September 11, 2002 End: October 12, 2002

2. Animal assignment: Animals were assigned to the test groups noted in Table 1, utilizing a computer-generated randomization procedure, based on body weight stratification in a block design.

TABLE 1: Study design

Test Group	Conc. in Diet (ppm)	Dose to Animal (M/F, mg/kg/day) a	# Male	# Female
Control	0	0/0	10	10
Low	300	25/28	10	10
Mid	500	41/41	10	10
High	700	57/67	10	10

a Calculated from body weight and food consumption data.

3. Test substance administration:

The test substance was administered in the diet to treated groups, beginning at study day 0, for 17 consecutive days. Test diet administration was suspended after that time, and treatment groups were maintained on basal diet until study termination (study day 30). Concurrent control rats received basal diet throughout the study duration. The control and test diets were offered *ad libitum* and supplied fresh weekly during the dosing period.

4. Dose selection rationale:

No dose selection rationale was provided. It is noted, however, that in a previous dietary chronic/oncogenicity study in rats that was conducted with 98% propanil (MRID 43303201, TXR 011787), increased mean methemoglobin ($p \leq 0.01$) was observed in 200 ppm (9 mg/kg/day) females on study week 13.

5. Diet preparation and analysis:

Diet was prepared weekly by mixing appropriate amounts of test substance with PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002 meal, using a combination of Hobart and V-blenders. The storage conditions for formulated diets was not specified in the study report. For dose calculation purposes, the purity of the propanil was assumed to be 100%.

A description of homogeneity, stability, and concentration evaluations follows (from MRID 45829301, page 18): Prior to the initiation of test diet administration, samples for homogeneity determination were collected from the top, middle and bottom strata of the 300 and 700 ppm test diets. In addition, samples for stability determinations were collected from the middle strata of these same test diets and stored at room temperature for 10 days. Prior to the initiation of dosing, an additional sample was collected from the middle stratum for the 300 and 700 ppm groups for freeze-thaw stability analysis. Samples for concentration analysis were collected weekly from each test and control diet. Two sets of the concentration samples collected the day prior to test diet administration were frozen overnight; one set was thawed the following day and stored at room temperature for nine days for stability analysis. The other set was stored frozen for 10 days and analyzed for stability. Portions of the concentration samples collected 1 week after study

start were also stored at room temperature for nine days and analyzed for stability.

Results -

Homogeneity Analysis: Samples from the top, middle, and bottom of a set of formulations representative of the low and high dose groups were analyzed prior to study start. The concentrations (top, middle, bottom respectively) for the low dose were 370, 307, and 343; the concentrations for the high dose were 681, 762, and 746. These values ranged from 2 to 23% higher than nominal for the low-dose formulations and from -3 to 9% different from nominal for the high-dose formulations. These results suggest that the mixing procedure may not have achieved homogeneity in the low dose formulations.

Stability Analysis: Following 24 hours of storage at room temperature, sample peak areas were ≥99.6% of initial values, demonstrating stability of propanil under these storage conditions. The stability of formulations were evaluated following 9 to 10 days of storage either frozen or at room temperature. For all analyses, post-storage concentrations were within ±15% of the target concentrations.

Concentration Analysis: The results of three analyses of formulated dose materials demonstrated concentrations within ±6% of the target dose concentrations, with the exception of one low-dose (300 ppm) sample, which was 145% of nominal (i.e., 436 ppm). This formulation was mixed on 9/17/02 and was apparently administered to the 300 ppm animals during study week 1.

The analytical chemistry data indicated that there were problems with the mixing procedure used to formulate the low-dose diet. This was evidenced by 1) the lack of homogeneity in formulations analyzed prior to study start, and 2) a concentration analysis indicating that the low dose (300 ppm) animals received approximately 436 ppm of propanil during week 1. However, homogeneity was demonstrated for the high-dose mixing procedure, and acceptable variance between nominal and actual dosage to the animals (±15%) was noted for the mid and high-dose groups, and for the low-dose formulations that were administered to the animals during study weeks 0 and 2.

C. METHODS:

1. Observations:

1a. Cageside Observations

Animals were inspected twice daily for signs of moribundity and mortality.

1b. Clinical Examinations

Clinical examinations were conducted daily. Detailed physical examinations were conducted for all animals weekly, beginning one week prior to test substance administration.

2. Body weight:

Animals were weighed weekly, beginning approximately one week prior to test article administration. Final body weights (non-fasted) were recorded prior to each schedule necropsy.

3. Food consumption and compound intake:

Food consumption for each animal was determined weekly through study week 5 for males and through study week 4 for females. Mean daily food intake was calculated as g food/animal/day. Mean compound intake (mg/kg bw/day) for each group was determined for each interval. Since test diet administration was terminated on study day 17, calculated compound consumption for study weeks 2-3 and for the overall mean compound consumption (study weeks 0-3) include the first several days of the recovery period (study days 17-21).

4. Clinical chemistry and hematology:

Blood was collected from the lateral tail vein from all study animals during the pretest period and on study days 0 (pre-treatment), 1, 5, 7, 14, 21, and 30 for clinical pathology evalutions. Blood was also collected from the vena cava at termination on study day 30 for hematology evaluations. Animals were not fasted prior to blood collection. The following parameters were examined.

CLINICAL CHEMISTRY	HEMATOLOGY
Methemoglobin	Erythrocyte count (RBC) Hemoglobin Hematocrit Mean corpuscular volume (MCV) Mean corpuscular hemoglobin (MCH) Mean corpuscular hemoglobin concentration (MCHC)

5. Sacrifice and necropsy:

All animals that died and those sacrificed on schedule were subjected to gross pathological examination. Animals at the schedule necropsy (study day 30) were euthanized by isoflurane anesthesia followed by exsanguination. The necropsies included examination of the external surface, all orifices, and the cranial, thoracic, abdominal, and pelvic cavities including the viscera. No organs were weighed, and no tissues were saved.

6. Statistical evaluation:

From MRID 45829301, pages 22-23: "Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test article-treated group to the control group by sex. Each mean was presented with the standard deviation

(S.D.) and the number of animals (N) used to calculate the mean. Statistical analyses were not conducted if the number of animals was two or less." ..... "Body weight, body weight change, food consumption and clinical pathology data were subjected to a parametric one-way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed statistical significance ($p<0.05$), Dunnett's test was used to compare the test article-treated groups to the control group. Clinical pathology values for white blood cell types that occur at a low incidence (i.e., monocytes, eosinophils and basophils) were not subjected to statistical analysis."

II. RESULTS

A. OBSERVATIONS:

1. Clinical signs of toxicity - Clinical findings observed during test substance administration included sporadic incidences of localized hair loss or scabs, chromodacryorhea, soft feces, or malaligned incisors. These findings were not attributed to propanil administration since they occurred in single animals, were observed at similar incidence in control and treated animals, or did not occur in a dose dependant manner.

2. Mortality - One female in the 300 ppm group died during blood collection on study day 7. This death was considered accidental and unrelated to test substance administration. All other animals survived to scheduled termination.

B. BODY WEIGHT AND WEIGHT GAIN: Mean body weight and body weight gain data are summarized in Tables 2A and 2B. Absolute body weight values for the 700 ppm males were significantly ($p \leq 0.05$) decreased at study weeks 2 and 3. Mean body weight gains were generally lower than control for 500 and 700 ppm males during the treatment period (study weeks 0-3). During the recovery period, mean body weight gain in 500 and 700 ppm males was similar to controls. No treatment-related effects on body weight or body weight gain were noted for 300 ppm males.

A treatment-related decrease in mean body weight gain was observed in 700 ppm females during study week 0-1, but during the reminder of the treatment period (study weeks 1-2 and 2-3) and during the recovery period, female body weight gains were similar to controls. No treatment-related effects on body weight or body weight gain were observed in 300 or 500 ppm females.

TABLE 2A. Mean (± S.D) body weights (g) [a]

Study Week	Dose (ppm)			
	0	300	500	700
Male				
-1	184 ± 13.6	179 ± 9.8	183 ± 9.8	184 ± 9.6
0	228 ± 14.8	228 ± 11.5	228 ± 11.3	227 ± 11.1
1	284 ± 16.4	283 ± 18.3	269 ± 15.5	267 ± 20.7
2	322 ± 19.4	320 ± 22.4	300 ± 19.3	295 ± 25.7 * (8)
3	353 ± 22.5	355 ± 27.3	332 ± 23.2	323 ± 28.4 * (8)
4	395 ± 28.3	401 ± 30.1	376 ± 27.7	367 ± 34.2
Female				
-1	156 ± 5.5	157 ± 6.2	157 ± 10.6	157 ± 6.2
0	181 ± 9.7	179 ± 7.7	178 ± 9.9	179 ± 7.6
1	206 ± 12.5	204 ± 7.3	199 ± 14.8	196 ± 11.2
2	221 ± 14.7	223 ± 11.3	213 ± 15.7	210 ± 15.7 (5)
3	238 ± 15.6	236 ± 14.5	228 ± 18.5	225 ± 17.3 (5)
4	256 ± 16.5	257 ± 14.7	248 ± 20.4	246 ± 22.4

a Data obtained from MRID 45829301, Tables 7-8, pages 46-49. N = 9-10
Percent differences from control values are presented in parentheses.
Note: treatment was stopped on study day 17 (during study week 2).
* Statistically different ($p < 0.05$) from control value (Dunnett's test).

TABLE 2B. Mean (± S.D) body weight gains (g) [a]

Study Week	Dose (ppm)			
	0	300	500	700
Male				
-1 - 0	44 ± 6.2	49 ± 4.9	45 ± 3.2	43 ± 6.7
0 - 1	56 ± 6.7	55 ± 7.4	42 ± 6.5 ** (25)	39 ± 11.2 ** (30)
1 - 2	38 ± 5.3	37 ± 7.1	31 ± 5.9 * (18)	28 ± 5.5 ** (26)
2 - 3	31 ± 9.8	35 ± 6.2	32 ± 4.5	29 ± 5.9
3 - 4	42 ± 13.9	46 ± 6.9	43 ± 7.0	44 ± 8.1
0 -2	94 ± 10.0	92 ± 12.8	72 ± 10.2 ** (23)	68 ± 16.4 ** (28)
0 - 3	125 ± 16.8	128 ± 18.0	105 ± 13.9 * (16)	96 ± 18.8 ** (23)
0 - 4	167 ± 24.8	174 ± 21.6	148 ± 19.1 (11)	140 ± 25.1 * (16)
Female				
-1 - 0	24 ± 5.9	22 ± 7.5	21 ± 2.9	22 ± 4.9
0 - 1	25 ± 7.3	25 ± 6.9	20 ± 6.3 (20)	18 ± 7.7 * (28)
1 - 2	15 ± 3.8	18 ± 4.6	15 ± 3.9	14 ± 6.8
2 - 3	17 ± 5.2	14 ± 6.8	15 ± 4.9	15 ± 5.1
3 - 4	18 ± 3.0	21 ± 7.0	20 ± 4.7	21 ± 8.9
0 - 2	41 ± 10.1	44 ± 8.0	35 ± 7.5 (15)	32 ± 11.2 (22)
0 - 3	57 ± 9.4	57 ± 10.4	50 ± 11.0 (12)	47 ± 11.5 (18)
0 - 4	76 ± 10.7	78 ± 12.7	69 ± 13.3 (9)	67 ± 17.1 (12)

a Data obtained from MRID 45829301, Tables 9-10A, pages 46-53. N = 9-10
Percent differences from control values are presented in parentheses.
Note: treatment was stopped on study day 17 (during study week 2).
* Statistically different ($p < 0.05$) from control value (Dunnett's test).
** Statistically different ($p < 0.01$) from control value (Dunnett's test).

C. FOOD CONSUMPTION AND COMPOUND INTAKE :

1. Food consumption - Mean food consumption data are summarized in Table 3. Food efficiency was not calculated. Treatment-related decreases in food consumption were observed in 500 ppm males at weeks 0-1 ($p \leq 0.05$) and in 700 ppm males at weeks 0-1 and 1-2 ($p \leq 0.01$).

In females, the only significant food consumption value consisted of an increase ($p \leq 0.01$) for 700 ppm females during weeks 2-3. This finding was not correlated to increased body weight gain during the same period, and was therefore unlikely to have been a rebound effect from the removal of treated feed on study day 17 (during week 2-3).

TABLE 3. Mean (± S.D) food consumption (g) [a]

Study Week	Dose (ppm)			
	0	300	500	700
Male				
-1 - 0	22 ± 2.1	21 ± 2.9	20 ± 1.9	21 ± 1.9
0 - 1	24 ± 1.5	24 ± 1.7	22 ± 1.2 * (8)	22 ± 1.9 ** (8)
1 - 2	26 ± 1.7	26 ± 1.8	24 ± 1.6	22 ± 2.5 ** (15)
2 - 3	25 ± 2.9	25 ± 2.2	22 ± 3.1	24 ± 4.5
3 - 4	25 ± 2.3	24 ± 2.5	24 ± 0.8	23 ± 3.2
4-5	26 ± 2.5	26 ± 2.2	24 ± 1.0	24 ± 2.5
Female				
-1 - 0	19 ± 2.2	17 ± 2.1	18 ± 1.6	19 ± 2.8
0 - 1	17 ± 3.0	19 ± 2.5	17 ± 1.6	17 ± 2.0
1 - 2	18± 4.2	20± 3.2	18± 1.6	17± 2.5
2 - 3	16 ± 1.5	20 ± 6.1	14 ± 4.5	26 ± 8.7 ** (62)
3 - 4	20 ± 2.1	20 ± 2.2	20 ± 1.7	20 ± 2.2

a Data obtained from MRID 45829301, Tables 11-12, pages 54-56. N = 9-10
Percent differences from control values are presented in parentheses.
Note: treatment was stopped on study day 17 (during study week 2).
* Statistically different ($p < 0.05$) from control value (Dunnett's test).
** Statistically different ($p < 0.01$) from control value (Dunnett's test).

2. <u>Compound consumption</u> The mean compound intake, calculated from individual food consumption and body weight data over a 3-week period (i.e., study weeks 0-1, 1-2, and 2-3) is presented in Table 4. Although test substance administration was terminated on study day 17, which was mid-way through week 2, body weight and food consumption data were not collected on that day. Therefore, the intake values are calculated in part with data from the recovery period. The increased magnitude of the intake calculated for high dose (700 ppm) females, as compared to the males at that treatment level, appears to be related to the significantly increased food consumption noted for females during study weeks 2-3 (Table 3), which may have been related to spillage. This finding has no impact on study interpretation, since it was only evident

at the highest dose tested.

TABLE 4. Mean propanil intake (mg/kg/day) [a]

	Dose (ppm)			
	0	**300**	**500**	**700**
Male	0	25	41	57
Female	0	28	41	67

a Data obtained from MRID 45829301, Tables 13-14, pages 57-58.

D. BLOOD ANALYSES:

1. Clinical Chemistry - Mean methemoglobin values are presented in Table 5. The study did not include data from the week -1 blood collection and analysis.

At study day 1, mean methemoglobin values appeared to be increased as compared to controls in all treated groups, for both males and females. These increases were found to be statistically significant from control for the 700 ppm males ($p \leq 0.05$) and the 500 ppm females ($p \leq 0.01$). However, day 1 control values were decreased from baseline for both males and females, and treated group mean methemoglobin values were not substantially different from control baseline values. Additionally, a clear dose response was not established for either males or females. While this lack of dose response may have been due in part to problems with homogeneity and/or concentration of the dietary formulation for the low-dose group, it was also noted that the study day 1 values appeared to be within the range of variability observed in the control rats on this study. Based upon these findings, it was concluded that no adverse treatment-related changes were noted in methemoglobin values in male or female rats following a single day of dietary administration of propanil.

At study day 5, mean methemoglobin levels were significantly increased from control ($p \leq 0.01$) and baseline ($p \leq 0.01$, $p \leq 0.05$) values for all treatment groups. Mean methemoglobin levels continued to increase at the day 7 and 14 measurements. Treatment was stopped at study day 17, and in general, decreased (but not fully recovered) methemoglobin values were observed at the day 21 and 30 measurements.

TABLE 5. Mean (± S.D) methemoglobin values (%) [a]

Study Day	Dose (ppm)			
	0	300	500	700
Male				
0	0.9 ± 0.74	0.8 ± 0.25	0.6 ± 0.22	0.6 ± 0.15
1	0.6 ± 0.32	1.0 ± 0.69(67)[25]	0.9 ± 0.19 A (50)[50]	1.2 ± 0.28*A (100)[100]
5	0.6 ± 0.16	1.0 ± 0.27**B(67)[25]	1.4 ± 0.24**A (133)[133]	1.8 ± 0.27**A (200)[200]
7	0.9 ± 0.47	1.2 ± 0.19 A (33)	1.7 ± 0.43**A (89)	2.2 ± 0.43**A (144)
14	0.9 ± 0.35	1.4 ± 0.35 A (56)[75]	2.1 ± 0.27**A (133)[250]	3.2 ± 0.76**A (256)[433]
21	1.0 ± 0.27	1.4 ± 0.36**A (40)	1.7 ± 0.26**A (70)	2.2 ± 0.22**A (120)
30	0.8 ± 0.30	1.1 ± 0.67 (38)	1.2 ± 0.14 (50)	1.5 ± 0.13**A (88)
Female				
0	0.6 ± 0.51	0.7 ± 0.19	0.4 ± 0.16	0.5 ± 0.40
1	0.4 ± 0.29	0.7 ± 0.23 (75)[0]	1.0 ± 0.46**A (150)[150]	0.9 ± 0.39 (125)[80]
5	0.6 ± 0.20	1.3 ± 0.35**A (117)	2.3 ± 0.40**A (283)	3.3 ± 0.52**A (450)
7	0.8 ± 0.24	1.8 ± 0.12**A (125)	2.6 ± 0.57**A (225)	4.0 ± 0.43**A (400)
14	0.8 ± 0.32	2.2 ± 0.24**A (175)	3.3 ± 0.36**A (313)	5.1 ± 0.69**A (538)
21	1.2 ± 0.42 A	1.9 ± 0.18 A (58)	2.5 ± 0.34**A (108)	3.1 ± 1.24**A (158)
30	1.0 ± 0.20 B	1.5 ± 0.24**A (59)	1.7 ± 0.23**A (70)	1.5 ± 0.37**A (50)

a Data obtained from MRID 45829301, Tables 15-16, pages 60 and 63. N = 9-10
Percent differences from control values are presented in parentheses ( ). Percent differences from pre-test (study day 0) values are presented in brackets [ ].
Note: treatment was stopped on study day 17 (during study week 2).
* Statistically different ($p < 0.05$) from control value (Dunnett's test).
** Statistically different ($p < 0.01$) from control value (Dunnett's test).
A Statistically different ($p < 0.01$) from the day 0 (pre-dose) value (Dunnett's test).
B Statistically different ($p < 0.05$) from the day 0 (pre-dose) value (Dunnett's test).

2. Hematology - Hematology data are summarized in Table 6. Slight significant ($p \leq 0.05$) alterations in mean hemoglobin (6% decrease for males) and mean cell volume (2% increase for females) were observed at 700 ppm. No other findings were noted.

TABLE 5. Mean (± S.D) hematology values [a]

Analysis (unit) b	Dose (ppm)			
	0	300	500	700
Male				
Red cells (mil/uL)	8.11 ± 0.470	7.98 ± 0.482	7.94 ± 0.563	7.91 ± 0.577
Hemoglobin (g/dL)	16.3 ± 0.58	16.0 ± 0.90	15.8 ± 0.46	15.3 ± 0.82* (6)
Hematocrit (%)	47.6 ± 2.08	46.6 ± 2.97	46.2 ± 2.21	45.3 ± 2.86
MCV (fL)	58.8 ± 1.58	58.5 ± 2.07	58.3 ± 2.24	57.4 ± 1.63
MCH (uug)	20.1 ± 0.80	20.0 ± 0.30	20.0 ± 1.02	19.4 ± 0.93
MCHC (g/dL)	34.2 ± 0.77	34.3 ± 0.71	34.2 ± 1.04	33.8 ± 1.11
Female				
Red cells (mil/uL)	7.35 ± 0.349	7.39 ± 0.262	7.38 ± 0.303	7.06 ± 0.272
Hemoglobin (g/dL)	14.8 ± 0.68	14.6 ± 0.47	14.4 ± 0.48	14.4 ± 0.73
Hematocrit (%)	42.4 ± 1.93	42.3 ± 1.31	41.8 ± 1.73	41.8 ± 1.94
MCV (fL)	57.8 ± 1.27	57.2 ± 1.64	56.6 ± 0.89	59.2 ± 1.20* (2)
MCH (uug)	20.1 ± 0.42	19.7 ± 0.62	19.6 ± 0.40	20.4 ± 0.52
MCHC (g/dL)	34.8 ± 0.29	34.5 ± 0.36	34.6 ± 0.35	34.5 ± 0.42

a Data obtained from MRID 45829301, Tables 15-16, pages 59 and 62. N = 9-10
b Blood collected at termination on study day 30. Units: g/dL = grams/decileter, mil/uL = millions/microliter; uug - picograms; fL = femtoliters
Percent differences from control values are presented in parentheses.
Note: treatment was stopped on study day 17 (during study week 2).
* Statistically different ($p < 0.05$) from control value (Dunnett's test).

G. MACROSCOPIC PATHOLOGY:

No treatment related findings were noted at necropsy of any study animals, including the 300 ppm female that died during blood collection on study day 7, or the animals that were examined at scheduled sacrifice (study day 30). Sporadic findings consisted only of malaligned incisors (one control and one 500 ppm male), discolored lymph node (one 700 ppm male), periocular red material (one control male), and localized hair loss (one 500 ppm female).

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

The study author concluded (MRID 45829301, page 13 and 28) that "Based on the results of this

study, the NOEL for methemoglobinemia for a one day exposure was 300 ppm for both males and females (25 mg/kg/day and 28 mg/kg/day, respectively). No NOEL was determined for subsequent time periods. The LOEL for day 5 and beyond was 300 ppm for both males and females."

B. REVIEWER COMMENTS:

Although the study author established a NOEL at 300 ppm for methemoglobinemia for a one-day dietary propanil exposure, Agency reviewers did not consider any apparent alterations in methemoglobin levels at any dietary treatment level to be indicative of an adverse effect, and on that basis established a NOAEL at 700 ppm (HDT). There is agreement, however, between the study author and Agency reviewers regarding the LOAEL (at the LDT) for increased methemoglobin after repeated (≥5 days) of dietary treatment. The study results are summarized as follows:

> At 700 ppm, decreased mean body weight gain (males and females) and food consumption (males only) values were observed during study weeks 0-2 or 0-3. Increased mean methemoglobin values were observed in the males and females (144-256% and 400-538%, respectively, relative to controls) beginning at study day 5 and throughout the treatment period. Recovery was noted after treatment was stopped (study day 17); on study day 30, mean methemoglobin was increased 88% (males) and 50% (females), as compared to controls.
>
> At 500 ppm, decreased mean body weight and food consumption values were noted in males during study weeks 0-1 or 0-2. Mean methemoglobin values were increased ($p \leq 0.01$) in males (89-133%) and females (225-313%) beginning at study day 5 and throughout the entire treatment period as compared to the control group. Recovery was noted following the cessation of treatment; on study day 30, mean methemoglobin values were increased 50% (males) and 70% (females) as compared to controls.
>
> At 300 ppm, mean methemoglobin values were increased ($p \leq 0.05$, $p \leq 0.01$) in males (33-67%) and females (75-175%), relative to control values, beginning at study day 5 and throughout the remainder of the treatment period. Recovery was noted following cessation of treatment; at study day 30, mean methemoglobin values were 38% and 50% of control values for males and females, respectively. There were no other signs of toxicity noted at 300 ppm.
>
> **The acute (1-day) LOAEL is not determined. The acute (1-day) NOAEL is 700 ppm (57 mg/kg/day for males and 61 mg/kg/day for females) (HDT).**
>
> **The repeated dose (≥5 days) LOAEL is 300 ppm (25 mg/kg/day for males and 28 mg/kg/day for females), based on increased methemoglobin levels. No repeated dose (≥5 days) NOAEL was established.**

This 30-day oral toxicity study in the rat is **Acceptable, Non-guideline**. This study does not satisfy any guideline requirement, but provides additional information relevant to the hazard assessment for propanil.

C. STUDY DEFICIENCIES:

The storage conditions for formulated diets were not specified in the study report. This deficiency in reporting was not considered critical to study interpretation, since the stability of the dietary formulations was confirmed analytically for diets that were stored either frozen or at ambient temperature.

The analytical chemistry data indicated that there were problems with the mixing procedure used to formulate the low-dose diet. This was evidenced by 1) the lack of homogeneity in formulations analyzed prior to study start, and 2) a concentration analysis indicating that the low dose (300 ppm) animals received approximately 436 ppm of propanil during week 1. This error in study conduct did not compromise the interpretation of the data or preclude the establishment of acute and repeated-dose NOAELs.

The study did not include data from the week -1 (pretreatment) blood collection and analysis (i.e., methemoglobin data), since, according to the study author, "negative values were obtained" (MRID 45829301, page 22). While these data might have been useful as background information in the interpretation of methemoglobin values on study days 0 and 1, their absence does not compromise the interpretation or conclusions of the study.

DATA FOR ENTRY INTO ISIS

Repeated dose (30 day) oral study - rodents (non-guideline)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range ppm (mg/kg/day)	Doses ppm (mg/kg/day)	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
028201	45829301	30-day repeated dose (non-guideline)	rat	30-days	oral	dietary	25-67	300, 500, 700 (males: 25, 41, 57 mg/kg/day; females, 28, 41, 67 mg/kg/day)	1-day: 57/67 (M/F) ≥5-days: not determined	1-day: not determined ≥5-days: 25	Hematopoietic system	incr. methemoglobin

13544

R086347

Chemical:	**Propanil**
PC Code:	**028201**
HED File Code	**13000 Tox Reviews**
Memo Date:	**05/21/2003**
File ID:	**TX0051450**
Accession Number:	**412-04-0038**

HED Records Reference Center
12/15/2003