UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

010443

JUL 29 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carbaryl: Review of Mouse Carcinogenicity Study Submitted under SEC. 6(a)(2) of FIFRA.

PC CODE: 056801
DP BARCODE: D192164
CASE: 818954
SUBMISSION: S442405
MRID NUMBER: 427869-01

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer Virginia A Dobozy 7/21/93
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THRU: Yiannakis M. Ioannou, Ph.D., Section Head
Review Section I, Toxicology Branch II
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and

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Toxicology Branch II
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Registrant: Rhone-Poulenc AG Company

Action Requested: Review of Mouse Carcinogenicity Study

Conclusions: The study demonstrated that carbaryl is carcinogenic in mice at doses of 100 ppm (14.73 mg/kg/day) or higher in males and 8000 ppm (1440.62 mg/kg/day) in females.

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Data Summary

Toxicology Branch II has recently reviewed a study titled: "Oncogenicity Study with Carbaryl Technical in CD-1® Mice" (MRID # 427869-01). The major findings were as follows:

Technical carbaryl (99.3% a.i.) was administered in the diet to groups of 80 male and 80 female CD-1® mice at dosages of either 0, 100, 1000 or 8000 ppm for 104 weeks. Systemic toxicity was seen in the high-dose group males and females in the form of clinical signs, reduced body weight gain, decreased RBC parameters, decreased RBC (males) and brain cholinesterase levels, increased liver weight, increased kidney weight and increased incidence of non-neoplastic lesions including intracytoplasmic protein-like droplets in the superficial epithelium of the urinary bladder, cataracts (especially in females) and increased severity of extramedullary hematopoiesis and pigment in the spleen. In the mid-dose group, there were decreases in RBC (males) and brain cholinesterase, increases in intracytoplasmic protein-like droplets in the superficial epithelium of the bladder and an increase in the kidney weight (males at interim sacrifice).

An increased incidence of neoplasms (hemangiomas and hemangiosarcomas) of the vascular system was found in all the treated groups of the males and in the high-dose group females. An increased incidence of renal neoplasms (adenomas, multiple adenomas and carcinomas) was observed in the high-dose group males. An increased incidence of hepatic neoplasms (adenomas, carcinomas and one hepatoblastoma) was seen in the high-dose group females.

The study demonstrated that carbaryl is carcinogenic in mice at doses of 100 ppm (14.73 mg/kg/day) or higher in males and 8000 ppm (1440.62 mg/kg/day) in females.

The systemic No Observed Effect Level (NOEL) was 100 ppm (males: 14.73 mg/kg/day; females: 18.11 mg/kg/day).

The systemic Lowest Observed Effect Level (LEL) was 1000 ppm (145.99 mg/kg/day) in males based on decreases in RBC and brain cholinesterase levels, an increase in kidney weight (interim sacrifice) and bladder pathology and 1000 ppm (180.86 mg/kg/day) in females based on a decrease in brain cholinesterase levels and bladder pathology.

The Maximum Tolerated Dose (MTD) was 8000 ppm (males: 1248.93 mg/kg/day; females: 1440.62 mg/kg/day) based on clinical signs, decreases in body weight gain, alterations in clinical pathology parameters and histopathology changes.

Classification: Minimum - Study deficiencies are described in the DER.

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Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 7/6/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. J M Ioannou 7/12/93
Section I, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Carcinogenicity Study/Mice (83-2)
EPA I.D. NUMBERS:	P. C. CODE: 056801 DP BARCODE: D192164 CASE: 818954 SUBMISSION: S442405 MRID NUMBER: 427869-01
TEST MATERIAL:	Technical Carbaryl
SYNONYMS:	Chemical Name: 1-naphthyl methylcarbamate
STUDY NUMBER:	HWA 656-138
TESTING FACILITY:	Hazleton Washington, Inc. Vienna, Virginia
SPONSOR:	Rhone-Poulenc Ag Company Research Triangle Park, NC
TITLE OF REPORT:	Oncogenicity Study with Carbaryl Technical in CD-1® Mice
AUTHOR(S):	N. Nicki Hamada, Ph.D., D.A.B.T.
REPORT ISSUED:	May 20, 1993
CONCLUSIONS:	Technical carbaryl was administered in the diet to groups of 80 male and 80 female CD-1® mice at dosages of either 0, 100, 1000 or 8000 ppm for 104 weeks. The actual mean mg/kg/day dosages received by the males at 100, 1000 and 8000 ppm were 14.73, 145.99 and 1248.93, respectively. The actual mean mg/kg/day dosages received by the females at 100, 1000 and 8000 ppm were 18.11, 180.86 and 1440.62, respectively. Systemic toxicity was seen in the high-dose group males and females in the form of clinical signs, reduced body weight gain, decreased RBC parameters, decreased RBC (males) and brain cholinesterase levels, increased liver weight, increased kidney weight and increased incidence of non-neoplastic lesions including intracytoplasmic protein-like droplets in the superficial epithelium of the urinary bladder, cataracts (especially females) and increased severity of extramedullary hematopoiesis and

pigment in the spleen. In the mid-dose group, there were decreases in RBC (males) and brain cholinesterase, increases in intracytoplasmic protein-like droplets in the superficial epithelium of the bladder and an increase in the kidney weight (males at interim sacrifice).

An increased incidence of neoplasms (hemangiomas and hemangiosarcomas) of the vascular system was found in all the treated groups of males and in the high-dose group females. An increased incidence of renal neoplasms (adenomas, multiple adenomas and carcinomas) was observed in the high dose group males. An increased incidence of hepatic neoplasms (adenomas, carcinomas and one hepatoblastoma) was seen in the high-dose group females.

The study demonstrated that carbaryl is carcinogenic in mice at doses of 100 ppm (14.73 mg/kg/day) or higher in males and 8000 ppm (1440.62 mg/kg/day) in females.

The systemic No Observed Effect Level (NOEL) was 100 ppm (males: 14.73 mg/kg/day; females: 18.11 mg/kg/day)

The systemic Lowest Observed Effect Level (LEL) was 1000 ppm (145.99 mg/kg/day) in males based on decreases in RBC and brain cholinesterase levels, an increase in kidney weight (interim sacrifice) and bladder pathology and 1000 ppm (180.86 mg/kg/day) in females based on a decrease in brain cholinesterase levels and bladder pathology.

The Maximum Tolerated Dose (MTD) was 8000 ppm (males: 1248.93 mg/kg/day; females: 1440.62 mg/kg/day) based on clinical signs, decreases in body weight gain, alterations in clinical pathology parameters and histopathology changes.

CLASSIFICATION: Minimum - This study satisfies the guideline requirements (83-2) for a carcinogenicity study in mice.

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I. MATERIALS

A. Test Material

Name: Carbaryl
Chemical Name: 1-naphthyl methylcarbamate
Purity: 99.3%
Lot Number: 87191
Description: White powder with small lumps
Storage Conditions: Room temperature

B. Administration: dietary

C. Test Animals

Species: Crl:CD-1®(ICR)BR mice
Source: Charles River Laboratories, Inc., Portage, Michigan
Age: 28 days old when received; 6 weeks at the initiation of treatment
Weight: 25.7 g for the males and 20.1 g for the females at the initiation of treatment
Received: January 23, 1990
Treatment Initiation: February 6, 1990
Gross Necropsies: February 7, 1991 (interim) and February 11, 1992 (terminal)

D. Animal Husbandry and Acclimation

The mice were housed two per cage in stainless steel cages for approximately 1½ weeks after which they were individually housed for the duration of the study. Food (Purina® Certified Rodent Chow® #5002) and water were provided *ad libitum* during the acclimation period. The test material was mixed with this diet for the dosing period. Temperature and relative humidity ranged from 61 to 80.3°F and 20.6 to 89%, respectively. A 12-hour light/12-hour dark cycle was maintained in the animal rooms. Prior to the initiation of treatment, five mice of each sex were randomly selected for serology screening tests including:

Pneumonia Virus of Mice	Reovirus Type 3
Encephalomyelitis Virus	Polyoma Virus
Sendai Virus	Mouse Hepatitis Virus
Lymphocytic Choriomeningitis Virus	Minute Virus of Mice
Ectromelia Virus	Mycoplasma Pulmonis

All of the animals were negative for the above.

E. Administration and Dietary Preparation

<u>Treatment Groups</u>

The animals were assigned to the following groups using a computer-generated weight-randomization procedure:

Group	Dietary Levels (ppm)	Number of Animals Males	Females
1 (Control)	0	80	80
2 (Low Dose)	100	80	80
3 (Mid Dose)	1000	80	80
4 (High Dose)	8000	80	80

An additional 10 mice/sex were designated as sentinels and were used for monitoring any suspected outbreak of disease. One female sentinel was found dead on Week 3; the cause of death could not be identified from gross necropsy.

Diet Preparation

Each level of the test material was premixed in a Waring blender with approximately 200 g of feed. The premix was then added to the required amount of feed for each dosage level and mixed in a Patterson-Kelly twin-shell mixer. Fresh diets were prepared once weekly.

Samples were taken from the top, middle and bottom of the low-, mid- and high-dose diets prior to the initiation of dosing and analyzed for homogeneity.

In previous studies, the stability of the test material in the diets for up to 14 days at room temperature was established for levels of 30 and 200 ppm (HWA 656-136: "Range-Finding Toxicity Study in Mice with Carbaryl Technical") and for levels of 50 and 3000 ppm (HWA 656-137: "Range-Finding Toxicity Study in Rats with Carbaryl Technical"). In this study, samples of the 100 and 8000 ppm diets that had been frozen for approximately 6 months were analyzed for stability.

Concentration analyses were performed on each diet formulation prior to administration on Weeks 1-4. Thereafter, a sample was taken from a randomly selected dose level, frozen and then analyzed monthly.

F. Experimental Design

The study protocol required the following observations and examinations at the indicated times or frequencies.

observations for mortality and moribundity - twice daily
observations for clinical signs of toxicity - once daily
physical examinations - weekly*
body weight - prior to treatment, weekly for Weeks 1-14 and once every two weeks thereafter
food consumption - weekly for Weeks 1-14 and once every fourth week thereafter
hematology - Weeks 53 (10 mice/sex/group) and 105 (10 mice/sex/group)
clinical chemistry (cholinesterase levels only) - Weeks 53 and 105 (10 mice/sex/group)
gross necropsy - all animals that were moribund, found dead or scheduled for the interim (Week 53, 10 mice/sex/group) or terminal (Week 105) sacrifice
histopathology - all tissues from the control, high dose groups and unscheduled deaths only; lungs, liver, kidneys and any gross lesions in the low- and

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mid-dose groups per the original protocol. After the interim sacrifice, the spleen and urinary bladder were also evaluated from the low- and mid-dose groups. After the terminal sacrifice, all tissues from all animals in the low- and mid-dose groups were evaluated.

* The following information was recorded if a mass was palpated: time of onset, location, size (measured at greatest observed diameter), appearance and progression.

G. Pathological Parameters

Hematology

Blood samples were collected via the abdominal aorta under pentobarbital anesthesia. The following evaluations were done:

X Hematocrit (HCT)	__Total plasma protein (TP)
X Hemoglobin (HGB)	X Leukocyte differential count*
X Leukocyte count (WBC)	X Mean corpuscular HGB (MCH)
X Erythrocyte count (RBC)	X Mean corpuscular HGB conc. (MCHC)
X Platelet count	X Mean corpuscular volume (MCV)

* Blood smears were evaluated for leukocyte differential and cell morphology for control and high-dose animals and for animals sacrificed in a moribund condition.

Clinical Chemistry

The only clinical chemistry evaluations done were plasma, brain and erythrocyte cholinesterase.

Gross Necropsy and Organ Weights

Gross necropsies were done on 10 mice/sex/group at the Week 53 (interim) sacrifice, all animals at the week 105 (terminal) sacrifice and any animals that died or were euthanized in a moribund condition during the study. The following organs from all animals at the interim sacrifice and those selected for clinical pathology at the terminal sacrifice were weighed:

liver with gallbladder	adrenals (postfixation)
kidneys	brain (including brainstem)
lungs (with mainstem bronchi)	testes with epididymides
spleen	ovaries (postfixation)

Relative organ weights (organ-to-terminal body weight) and organ-to-brain weight ratios were calculated.

Histopathology

The following tissues were collected and preserved in 10% neutral-buffered formalin:

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Digestive System	Cardiovasc./Hemat. System	Neurologic System
__Tongue	X Aorta*	X Brain*
X Salivary glands*	X Heart*	X Periph. nerve*
X Esophagus*	X Bone marrow*	X Spinal cord (3 levels)
X Stomach	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen*	X Eyes (Optic n.)*
X Jejunum*	X Thymus*	Glandular
X Ileum*	Urogenital System	X Adrenals*
X Cecum*	X Kidneys*	__Lacrimal gland
X Colon*	X Urinary bladder*	X Mammary gland*
X Rectum*	X Testes*	X Parathyroids*
X Liver*	X Epididymides	X Thyroids*
X Gall bladder*	X Prostate	Other
X Pancreas*	X Seminal vesicle	X Bone*
Respiratory System	X Ovaries	X Skeletal muscle*
X Trachea*	X Uterus*	X Skin
X Lung*		X All gross lesions and masses

* EPA Guideline Requirement

The study protocol called for the microscopic examination of the above preserved tissues from all control, high-dose and unscheduled deaths only. Lungs, liver, kidneys and any gross lesions were to be examined from the low- and mid-dose animals. However, during the interim sacrifice, the spleen and urinary bladder were identified as target organs. Therefore, these organs were also examined in the low- and mid-dose groups. At the terminal sacrifice, vascular tumors were found to be treatment-related and therefore all tissues from the low and mid dose animals were examined microscopically.

Non-tumor lesions were graded as to relative severity or degree of involvement on a scale from 1 (minimal) to 5 (severe).

H. Statistical Analyses

A description of the statistical analyses provided in the study report are attached to the DER.

I. Compliance

Signed statements of Quality Assurance and compliance with the Good Laboratory Practice regulations were submitted by the testing facility. The sponsor submitted a Statement of No Data Confidentiality Claims. The study meets or exceeds the criteria under 40 CFR 158.34.

II. RESULTS

A. Diet Analyses and Actual Dosages

Homogeneity

Analyses of samples (Table 1A, page 56 of the study report) from the top, middle and bottom of the diet preparations showed that the diets were homogeneous.

Stability

Analyses of samples (Table 1A, page 56 of the study report) from the low and high dose diets that were frozen for 5 weeks showed that the chemical was stable after such storage. Analyses of samples from the low and high dose diets that were frozen and then stored at room temperature for 14 days demonstrated that the chemical was stable for 7 days, but the low dose was decreased (mean 83.2% of the target value) at 14 days.

Concentration

Analyses of samples (Table 1B, pages 58-69 of the study report) were within 90 - 109% of the target concentration from the beginning of the study until Week 91 when the low-dose diet was 88.4% of the target. On Week 93, no test chemical was found in the low- and mid-dose diets and a "significant amount of non-protocol specified compound" was in the low-dose diet. This error caused the death of 17 animals in the low dose group within one day of feeding (see Survival for additional details). The diets were properly formulated the next day, and the concentrations were within acceptable ranges for the remainder of the study.

Actual Dosages

The range and mean dosages of carbaryl are presented below:

Dose Level (ppm)	Mean Dosage (mg/kg/day)*		Dosage Range (mg/kg/day)	
	Males	Females	Males	Females
100	14.73	18.11	21.85 - 12.90	25.78 - 14.83
1000	145.99	180.86	209.44 - 127.68	268.28 - 153.33
8000	1248.93	1440.62	1802.48 - 1144.69	2280.84 - 1253.32

* Calculated using the formula: (Total Mean Compound Consumption Weeks 14-102 X 4) ÷ (Total Measured Mean Compound Consumption Weeks 14-102 X 4) ÷ 105 Weeks.

B. Survival and Clinical Observations

Survival

Four males in the 8000 ppm group died or were sacrificed in a moribund condition during the first week of the dosing. The cause of death was not stated in the study report. They were replaced by stock-pool animals. On Week 93, the animals in the 100 ppm group were administered what the study report refers to as a "non-protocol specified compound" which caused the death of 17 animals (9 males and 8 females). Table 4 of the 53-week interim report (MRID No. 421889-01) identified the chemical as aldicarb. Cumulative survival is summarized in Table 1.

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Table 1
Cumulative Survival in Mice Treated
with Carbaryl in the Diet for 104 Weeks*

	Dose Levels (ppm)							
	Males				Females			
Week	0	100	1000	8000	0	100	1000	8000
1	80/80 100%	80/80 100%	80/80 100%	80/80 100%	80/80 100%	80/80 100%	80/80 100%	80/80 100%
13	80/80 100%	78/80 98%	80/80 100%	79/80 99%	79/80 99%	80/80 100%	80/80 100%	74/79▲ 94%
26	79/79▲ 100%	78/80 98%	80/80 100%	79/80 99%	79/80 99%	80/80 100%	80/80 100%	73/79 92%
52	76/79 96%	75/80 95%	79/80 99%	78/80 98%	73/80 91%	80/80 100%	76/80 95%	71/78▲ 91%
78▼	53/69 77%	57/70 81%	56/70 80%	56/70 80%	59/70 84%	62/70 89%	58/70 83%	49/68 72%
104	37/69 54%	32/70 46%	37/70 53%	31/70 44%	35/70 50%	31/70 44%	32/70 46%	33/68 49%

* Extracted from Table 2 (pages 71-79 of the study report).
▲ Possible adjustment for accidental death; no explanation offered in Table 2.
▼ Ten animals/sex/group were sacrificed on Week 53 of the study.

Apparently one male (control) and two females (8000 ppm group) were lost due to accidental deaths. No further explanation is offered in the study report.

Clinical Observations

The study report states that the animals in the 8000 ppm group, especially the females, were observed to be thin and to have a hunched posture during the first three weeks of treatment. The signs subsided after six weeks and were then observed again in the last six months of the study. Other clinical signs frequently recorded in the 8000 ppm animals were languid appearance, urine stains (especially in males) and opaque eyes (last three months in females). Table 2 summarizes the incidence of those clinical signs which occurred more frequently in the treated groups.

Table 2
Incidence[a] of Clinical Signs in Mice
Treated with Carbaryl in the Diet for 104 Weeks[b]

	Dose Levels (ppm)							
	Males				Females			
	0	100	1000	8000	0	100	1000	8000
Hunched	7	7	12	22	12	11	15	54
Thin	3	3	2	11	8	3	9	41
Languid	4	1	7	16	3	4	5	18
Soft feces	2	3	2	6	1	0	1	2
Low Body Temperature	0	3	3	6	2	1	7	6
Urine stains	21	29	33	57	5	9	8	30
Rough haircoat	20	31	20	43	14	20	19	36
Squinted	3	3	4	7	0	6	1	4
Opaque Eye(s)	9	12	8	15	11	13	9	25
Red (VBA)	4	4	4	12	1	3	5	8

VBA = Various body areas
[a] The number of animals in which the sign was reported during the study.
[b] Extracted from Table 3A (pages 81-82 of the study report).

C. Body Weight and Body Weight Gain

Body Weight

Mean body weights were significantly decreased in all the treated males (except Week 1 for the 1000 ppm group) and in the 8000 ppm females for the first four weeks of the study and then again at Week 8. Thereafter, there were decreases sporadically in the 8000 ppm males and females. Table 3 summarizes body weights at selected weeks during the study.

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Table 3
Body Weights (G) (at Selected Weeks) of Mice
Treated with Carbaryl in the Diet for 104 Weeks*

	Dose Levels (ppm)							
	Males				Females			
Week	0	100	1000	8000	0	100	1000	8000
0	26.3	25.1	25.7	26.0	20.5	20.1	19.9	20.0
1	28.9	27.7*	28.6	25.2*	22.8	22.8	22.6	19.7*
13	34.1	34.1	34.1	31.2*	28.3	28.9	28.8	26.3*
26	35.8	35.6	35.4	33.3*	30.2	30.3	31.1	28.7*
52	38.7	38.5	37.6	34.2*	32.9	32.8	32.7	29.5*
78	37.9	37.9	38.4	33.5*	34.1	33.7	33.6	29.3*
104	36.7	37.4	37.9	32.4	32.3	33.1	32.5	28.0

* Extracted from Table 4 (pages 86-90 of the study report).
* Significantly different from control value, $p \leq 0.05$

Body Weight Gain

Body weight gain was not discussed in the study report. Mean values have been calculated from the body weight table (Table 4 of the study report) and the treated values are presented as a percentage of the control in Table 4. Mean weight gain in the 8000 ppm group males and females was decreased throughout the study; the percentage of the control value ranged from 62 - 77% and 68 - 90%, respectively.

Table 4
Mean Body Weight Change (G) in Mice
Treated with Carbaryl in the Diet for 104 Weeks[a]

	Dose Levels (ppm)							
	Males				Females			
Week	0	100	1000	8000	0	100	1000	8000
weeks 0-13	7.8	9.0	8.4	5.2	7.8	8.8	8.9	6.3
% control	--	115	108	67	--	113	114	81
weeks 0-26	9.5	10.5	9.7	7.3	9.7	10.2	11.2	8.7
% control	--	111	102	77	--	105	115	90
weeks 0-52	12.4	13.4	11.9	8.2	12.4	12.7	12.8	9.5
% control	--	108	96	66	--	102	103	77
weeks 0-78	11.6	12.8	12.7	7.5	13.6	13.6	13.7	9.3
% control	--	110	109	65	--	100	101	68
weeks 0-104	10.4	12.3	12.2	6.4	11.8	13.0	12.6	8.0
% control	--	118	117	62	--	110	107	68

[a] Calculated by the reviewer from Table 4 (pages 86-90 of the study report).

D. Food Consumption

Food consumption was decreased in the 8000 ppm females throughout the study. In the 8000 ppm males, intake was decreased at the beginning (until Week 11) and the end (from Week 74 until termination) of the study. At Week 78, there were significant decreases in the 1000 and 8000 ppm males. Table 5 summarizes the food consumption data.

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Table 5
Mean Food Consumption (G/WK) in Mice
Treated with Carbaryl in the Diet for 104 Weeks*

	Dose Level (ppm)							
	Males				Females			
Week	0	100	1000	8000	0	100	1000	8000
1	42.5	42.3	41.8	39.6	41.3	41.1	42.3	39.2
% control	--	100	98	93	--	100	102	95
13	38.7	38.5	38.9	38.0	40.4	41.9	41.5	37.5*
% control	--	99	101	98	--	104	013	93
26	36.0	38.8*	38.0*	37.0	39.7	42.5*	42.9*	36.8*
% control	--	108	106	103	--	107	108	93
50	32.5	33.2	32.1	32.7	35.2	35.6	35.4	32.2*
% control	--	102	99	101	--	101	101	91
78	39.7	38.1	37.3*	37.1*	40.3	38.6	39.8	36.1*
% control	--	96	94	93	--	96	99	90
102	33.8	33.5	33.7	32.5	33.5	34.3	34.4	30.4*
% control	--	99	100	96	--	102	103	91

* Extracted from Table 5 (pages 92-94 of the study report); % control calculated by the reviewer.
* Significantly different from control value, $p \leq 0.05$

E. Clinical Pathology

Hematology

There were significant decreases in HCT, HGB and RBC at Week 53 in the 8000 ppm females and at Week 105 in the 8000 ppm males. The statistically significant hematology findings are summarized in Table 6.

Table 6
Selected Hematology Parameters in Mice
Treated with Carbaryl in the Diet for 104 Weeks*

	Dose Levels (ppm)							
	Males				Females			
	0	100	1000	8000	0	100	1000	8000
Week 53								
RBC	8.19	8.22	7.93	7.49	8.45	8.34	8.53	7.69*
HGB	12.1	12.6	12.1	11.6	13.0	12.8	13.3	12.1*
HCT	35.6	37.2	36.3	33.7	38.4	37.9	39.7	35.2*
MCV	43.7	45.4	45.8*	45.0	45.4	45.5	46.5	45.9
Platelet	1678	1644	1530	1843	1408	1303	1383	1779*
Cor. WBC▲	3.1	.0	.0	3.3	1.7	.0	.0	3.6*
Lymphocytes▼	1.8	.0	.0	2.1	1.1	.0	.0	2.7*
Eosinophils▼	.0	.0	.0	.1	.0	.0	.0	.1*
Week 105								
RBC	9.14	8.56	8.20	7.01*	7.83	8.21	7.97	8.27
HGB	13.8	12.7	12.8	11.1*	11.7	12.5	12.4	12.5
HCT	41.4	38.3	38.4	32.9*	36.3	38.0	37.0	37.5
Platelet	1637	1814	1599	1783	845	957	1185	1568*

* Extracted from Table 7 (pages 100-103 of the study report).
* Significantly different from control, $p \leq 0.05$
▲ WBC counts were done on low- and mid-dose animals but differentials were not done to count nucleated RBC's, therefore it appears that corrected WBC was reported as 0.
▼ Differential WBC counts were not done on the low- and mid-dose animals.

Blood or blood smears were collected from animals prior to moribund sacrifice. The study report indicates that the incidences of anemia, thrombocytopenia, leukopenia or leukocytosis were comparable between control and treated groups.

Clinical Chemistry

The RBC cholinesterase values were significantly decreased in the 1000 and 8000 ppm males at Week 53. The brain cholinesterase levels were significantly decreased in the 1000 and 8000 ppm males at Weeks 53 and 105 , in the 1000 ppm group females at Week 53 and in the 8000 ppm females at Weeks 53 and 105. The findings are summarized in Table 7.

Table 7
Cholinesterase Values in Mice Treated
with Carbaryl in the Diet for 104 Weeks[a]

	Dose Levels (ppm)							
	Males				Females			
	0	100	1000	8000	0	100	1000	8000
Week 53								
RBC-CHE	7.3	7.0	5.6*	5.1*	6.3	5.9	5.5	5.5
BR-CHE	86.0	81.3	70.7*	36.9*	84.1	81.1	73.5*	44.6*
Week 105								
RBC-CHE	5.2	4.7	5.1	4.5	4.1	3.3	3.3	3.1
BR-CHE	59.9	59.4	52.0*	35.9*	62.2	58.7	55.2	41.0*

RBC-CHE = Red blood cell cholinesterase; BR-CHE = Brain cholinesterase
[a] Extracted from Table 8 (page 105 of the study report).
* Significantly different from control, $p \leq 0.05$

F. Post-Mortem Findings

Gross Necropsy

The study report indicates that there were no treatment-related changes in the animals that were sacrificed at Week 53 or that were unscheduled deaths. At the terminal sacrifice, the incidence of opaque eyes was increased in the 8000 ppm females. The incidence was 1, 2, 1 and 4 for the 0, 100, 1000 and 8000 ppm females, respectively, and 2, 5, 2, and 16 for the 0, 100, 1000 and 8000 ppm females, respectively.

Organ Weights

Absolute - There was a significant decrease in the weight of the lungs (Week 105) and ovaries (week 53) and a significant increase in the liver with gallbladder (Week 53) in the 8000 ppm females.

Relative - The consistent findings at both Weeks 53 and 105 were an increase in the weight of the liver with gallbladder in the 8000 ppm males and females and an increase in the kidney weight in the 8000 ppm males. Other findings included: increase in weight of lungs in 8000 ppm males and decrease in 1000 ppm females (Week 53); increase in brain with stem in the 8000 ppm males (Week 105); increase in kidneys in 1000 ppm males (Week 53); and decrease in ovaries in 8000 ppm females (Week 53).

Organ-to-Brain Weight - At Week 53, there was an increase in the liver-to-brain weight ratio for the liver in the 8000 ppm males and females; an increase in the kidney-to-brain weight ratio in the 100 and 1000 ppm males and

a decrease in the ovary-to-brain weight ratio in the 8000 ppm females. At Week 105, there was a decrease in the lung-to-brain weight in the 1000 and 8000 ppm females.

The organ weight data in which there were significant changes are summarized in Table 8.

Histopathology

Non-neoplastic Lesions - Interim Sacrifice

The Pathology Report (page 42 of the study report) states that the microscopic findings of these necropsies have been previously reported. The DER (MRID # 421889-01) for the interim report refers to three histopathological changes that were considered to be treatment-related. First, in the urinary bladders of the 1000 ppm males and the 8000 ppm males and females, there were intracytoplasmic protein-like droplets (stained intensely eosinophilic) filling the cytoplasm of the superficial transitional epithelial cells. The finding was graded "minimal to moderately severe". Second, the increased severity of the chronic progressive nephropathy in the 1000 ppm males and the 8000 ppm males and females was considered treatment-related. Third, there was an increase in the incidence of extramedullary hematopoiesis and pigment in the spleen of the 8000 ppm males and females. The pigment was most likely hemosiderin which resulted from the increased splenic turnover of red blood cells and was considered treatment-related to the decrease in erythrocyte values. These evaluations are compatible with the histopathology data found in Table 11B, pages 187-197 of this study report.

Non-neoplastic Lesions - Terminal and Unscheduled Sacrifices

Intracytoplasmic protein-like droplets seen at the interim sacrifice were present in the urinary bladder of the 1000 and 8000 ppm males and females. The incidence and severity of the lesion is summarized in Table 9.

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Table 8
Organ Weight Data on Mice Treated with Carbaryl in the Diet for 104 Weeks[a]

		Dose Levels (ppm)							
		Males				Females			
		0	100	1000	8000	0	100	1000	8000
Week 53									
Body Weight		39.6	40.9	37.1	36.0*	31.7	35.4*	35.6*	32.1
Lung	A[1]	0.24	0.23	0.22	0.25	0.23	0.25	0.22	0.22
	R[2]	.602	.573	.591	[illegible]	.712	.676	[illegible]	.679
	O:B[3]	.452	.463	.441	.502	.439	.455	.420	.428
Kidney	A	.64	.73	.71	.67	.49	.52	.51	.46
	R	1.622	1.780	[illegible]	[illegible]	1.542	1.458	1.439	1.439
	O:B	1.215	[illegible]	[illegible]	1.373	.951	.976	.967	.905
Liver	A	2.23	2.06	2.07	2.64	1.69	1.85	1.88	[illegible]
	R	5.619	5.048	5.586	[illegible]	5.338	5.196	5.280	[illegible]
	O:B	4.231	4.081	4.164	[illegible]	3.304	3.530	3.944	[illegible]
Ovary	A					.042	.034	.049	[illegible]
	R					.1324	.0955	.1373	[illegible]
	O:B					.0818	.0633	.0918	[illegible]
Week 105									
Body Weight		37.3	37.6	38.0	[illegible]	32.4	34.0	32.6	[illegible]
Lung	A	.27	.28	.30	.25	.38	.30	.24	[illegible]
	R	.714	.733	.817	.758	1.200	.935	.778	.783
	O:B	.546	.564	.608	.506	.733	.578	[illegible]	[illegible]
Brain	A	.49	.50	.48	.50	.51	.51	.52	.48
	R	1.314	1.314	1.309	[illegible]	1.554	1.601	1.659	1.704
Kidney	A	.77	.77	.80	.82	.49	.50	.54	.50
	R	2.038	2.016	2.143	[illegible]	1.515	1.371	1.702	[illegible]
	O:B	1.562	1.535	1.661	1.651	.982	.991	1.030	1.038
Liver	A	2.05	2.26	2.14	2.51	2.05	2.05	1.81	2.09
	R	5.434	5.905	5.735	[illegible]	6.258	6.332	5.740	[illegible]
	O:B	4.156	4.585	4.436	5.059	4.050	4.025	3.483	4.324

[a] Extracted from Tables 10 A and 10B (pages 144-163 of the study report).
* Significantly different from control value, $p \leq 0.05$.
1) Absolute organ weight; 2) Relative organ weight; 3) organ-to-brain weight ratio

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Table 9
Incidence and Severity of Intracytoplasmic Protein-like Droplets in the Urinary Bladder in Mice Treated with Carbaryl in the Diet for 104 Weeks[a]

	Males		Females	
Group (ppm)	Incidence	Severity	Incidence	Severity
0	0	0	0	0
100	0	0	0	0
1000	13	0.3	10	0.2
800	37	0.9	44	1.2

[a] Extracted from Text Table 6 (page 48 of the study report).

The incidence of unilateral and bilateral cataracts was increased in the 800 ppm males and females as summarized in Table 10.

Table 10
Incidence of Cataracts in Mice
Treated with Carbaryl in the Diet for 104 Weeks[a]

	Males			Females		
Group (ppm)	Unilat	Bilat	No. with Lesion	Unilat	Bilat	No. with Lesion
0	8	8	16	15	6	21
100	8	7	15	4	7	11
1000	11	7	18	11	4	15
8000	14	15	29	13	18	31

[a] Extracted from Text Table 7 (page 48) of the study report.

In the 8000 ppm group males and females, the severity of extramedullary hematopoiesis and pigment in the spleen was increased as observed at the interim sacrifice. Chronic progressive nephropathy, which appeared to be a treatment-related finding at Week 52, was found at comparable incidences between the treated and control groups at the terminal sacrifice.

Neoplastic Lesions - Interim, Terminal and Unscheduled Necropsies

Neoplastic changes were found in the vascular system, kidney and liver.

Vascular System

There was an increased incidence of vascular neoplasms (hemangiomas and hemangiosarcomas) in all the treated males and in the 8000 ppm females at the terminal and unscheduled necropsies. There was no evidence of these changes at

the Week 52 necropsy. The tumors were most frequently found in the liver and spleen. Of 40 tumors reported in the treated males, 24 were found in the liver and spleen; of ten reported in the 8000 ppm females, 8 were found in these organs. Table 11 summarizes the incidence of the tumors in each of the groups.

Table 11
Incidence of Vascular Tumors in Mice
Treated with Carbaryl in the Diet for 104 Weeks[a]

	Dose Levels (ppm)							
	Males				Females			
	0	100	1000	8000	0	100	1000	8000
Number of Animals Examined	80	80	80	80	80	80	80	80
No. Animals with Hemangiomas	0	1	1	3	1	0	1	0
No. Animals with Hemangiosarcomas	2	5	9	7	2	3	3	9
Total Number of Animals with Vascular Tumors	2	6	10	10	3	3	4	9
Total Number of Vascular Tumors	2	9	13	18	5	6	5	10

[a] Extracted from Text Tables 1 and 2 (pages 43-44 of the study report).

According to the study report, the tumors were morphologically compatible with vascular tumors described in mice. A detailed description is found in the PATHOLOGY REPORT (page 45 of the study report). Hemangiosarcoma was the cause of death for the following number of animals.

Group (ppm)	Males	Females
0	0	1
100	2[a]	3
1000	2	1
8000	2	8[b]

[a] One of these animals died after a misdosing at Week 93.
[b] Ovarian hematocyst and pulmonary carcinoma were also present in two of these animals.
Extracted from Text Table 3 (page 46 of the study report).

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Renal Neoplasms

There was an increased incidence of adenomas, multiple adenomas and carcinomas of the kidney in the 8000 ppm males. The carcinomas were observed grossly as unilateral kidney masses ranging from 0.7 to 1.5 cm in diameter. Microscopically, they were comprised of moderately differentiated renal tubular epithelium. No evidence of metastasis was observed. The renal tubular adenomas were not seen on gross examination. No renal tumors were observed in the treated females at any of the necropsies. The incidence of renal neoplasms is summarized in Table 12.

Table 12
Incidence of Renal Neoplasms in Male Mice
Treated with Carbaryl in the Diet for 104 Weeks[a]

	Dose Levels (ppm)			
	0	100	1000	8000
Number of Animals Examined	79	80	80	80
No. Mice with Renal Adenomas	0	0	0	2
No. Mice with Multiple Renal Adenomas	0	0	0	1
No. Mice with Renal Carcinomas	0	0	0	3
Total Number of Mice with Renal Tumors	0	0	0	6

[a] Extracted from Text Table 4 (page 46 of the study report).

Hepatic Neoplasms

The incidence of hepatic neoplasms (adenomas, carcinomas and one hepatoblastoma) was increased in the 8000 ppm females. Neoplasms of the liver were observed in the treated males; the incidence was comparable in the treated and control groups. The adenomas and carcinomas were comprised of moderate to well-differentiated hepatocytes; there was no evidence of metastasis. The hepatoblastoma was comprised of poorly differentiated basophilic cells with hyperchromatic nuclei and scant cytoplasm. The incidence of hepatic neoplasms in males and females is summarized in Table 13.

Table 13
Incidence of Hepatic Neoplasms in Mice
Treated with Carbaryl in the Diet for 104 Weeks[a]

	Dose Levels (ppm)							
	Males				Females			
	0	100	1000	8000	0	100	1000	8000
Number of Mice Examined	79	80	80	80	79	80	79	80
No. of Mice with Hepatic Adenomas	11	7	11	5	0	0	1	6
No. of Mice with Multiple Hepatic Adenomas	1	0	2	3	0	0	0	1
No. of Mice with Hepatic Carcinomas	6	7	3	8	1	1	1	2
No. of Mice with Multiple Hepatic Carcinomas	0	0	0	0	0	0	0	1
No. of Mice with Hepatoblastomas	0	0	0	0	0	0	0	1
No. of Mice with Hepatic Neoplasms	18	14	16	16	1	1	2	11

[a] Extracted from Text Table 5 (page 47 of the study report).

III. STUDY DEFICIENCIES

A. The following procedures affected the survival data.

1. The low dose animals were misdosed with an acutely toxic compound at Week 93 of the study which caused the death of 9 males and 8 females.

2. Four males in the 8000 ppm group died or were sacrificed in a moribund condition the first week of dosing. They were replaced by stock animals and their deaths were not accounted for in the survival analyses.

3. Three animals (one control male and two 8000 ppm females) were lost due to accidental deaths. No explanation was provided in the study report. The number in the group was reduced and therefore, these animals were essentially deleted from the survival data.

B. Explanation was not offered for the following data or statements in the study report.

1. Some tissues were not evaluated histopathologically, however the study report indicates that the number did not preclude the evaluation of the test compound. A further explanation of which tissues not examined should have been provided.

2. Corrected WBC's in Table 7 (page 101 of the study report) are reported as 0 for the low dose males and females, whereas values for WBC's are provided.

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3. Eosinophilic protein-like intracytoplasmic droplets were observed in the superficial epithelium of the urinary bladder in the mid- and high-dose groups. The study report offers no possible pathogenesis or significance for this finding. Additionally, there was no postulation about the cause of the cataracts in the high-dose group (especially females).

4. Historical control data on liver, renal and vascular tumors in male and female mice were not provided.

IV. DISCUSSION

Technical carbaryl was administered in the diet to groups of 80 male and female CD-1® mice at dosages of either 0, 100, 1000 or 8000 ppm for 104 weeks to study the carcinogenicity potential of the chemical. The actual mean mg/kg/day dosages received by the males at 100, 1000 and 8000 ppm were 14.73, 145.99 and 1248.93, respectively. The actual mean mg/kg/day dosages received by the females at 100, 1000 and 8000 ppm were 18.11, 180.86 and 1440.62, respectively. The mice were monitored throughout the study for mortality, clinical signs of toxicity, body weight and food consumption. Hematology and clinical chemistry (cholinesterase levels) were done at Weeks 53 and 105. Ten animals/sex/group were sacrificed at Week 53 (interim sacrifice) and the remainder of the animals were sacrificed at Week 105 (terminal sacrifice). The study protocol indicated that histopathological examinations should be conducted on all tissues from the control and high-dose groups and on all animals that were unscheduled deaths. However, the spleen and urinary bladder were identified as target organs at the interim sacrifice, therefore these tissues from the low-and mid-dose groups were also examined. At the terminal sacrifice, neoplasms were found to be treatment-related, therefore tissues from all the groups were examined microscopically.

The percentages of treated animals which survived to study termination were relatively comparable to the control group. Seventeen animals (9 males and 8 females) died at Week 93 when they were given an acutely toxic compound in place of the test chemical. It is unlikely that this error influenced the outcome of the study for the following reasons: (1) it occurred at Week 93, too late in the study to influence the development of tumors; (2) only one dose of the chemical was administered; and there were no signs of systemic toxicity in this group except for the deaths.

The following clinical signs were observed in the 8000 ppm group (especially females): hunched appearance, thin, languid, soft feces, urine stains, low body temperature, rough haircoat, squinted, opaque eyes (females) and red at various body areas.

Mean body weights were significantly decreased in all the treated males (except for Week 1 for the 1000 ppm group) and in the 8000 ppm group females for the first four weeks of the study and then again at Week 8. Thereafter, there were sporadic decreases in the 8000 ppm group males and females. Body weight gain was decreased in both the males and females in the 8000 ppm group throughout the study. At Week 13, there was a decrease in body weight gain of 33% in males and 19% in females of the 8000 ppm group. Overall, body weight gain was 62% of the control value in males and 68% in females. Food

consumption was decreased in the 8000 ppm group females throughout the study. In the 8000 ppm group males, food consumption was decreased at the beginning (until Week 11) and at the end (from Week 74 until termination) of the study. The decrease in food consumption cannot account for the decrease of body weight gain in the 8000 ppm group males and females.

There were significant decreases in the hemoglobin, hematocrit and red blood cell count at Week 53 in the 8000 ppm group females and at Week 105 in the 8000 ppm group males indicating that the chemical has an effect on red cell mass. RBC cholinesterase values were significantly decreased in the 1000 and 8000 ppm group males at Week 53 (77 and 70% of the control, respectively). The brain cholinesterase levels were significantly decreased in the 1000 and 8000 ppm group males at Weeks 53 (82 and 43% of the control, respectively) and 105 (87 and 60% of the control, respectively), in the 1000 ppm group females at Week 53 (87% of the control) and in the 8000 ppm group females at Weeks 53 and 105 (53 and 66% of the control, respectively).

No treatment-related gross necropsy findings were evident at either the interim, terminal or unscheduled necropsies except for an increase in the incidence of opaque eyes in the 8000 ppm group (males: 4 vs. 1 in the control; females: 16 vs. 2 in the control) at the terminal necropsy.

The weight of the liver was significantly increased in the high dose group at both the interim and terminal sacrifices. At the interim sacrifice, the absolute weight was increased in the 8000 ppm group females, and the relative weight and the liver-to-brain weight ratio were increased in both sexes. At terminal sacrifice, the relative weight was increased in the 8000 ppm group males and females. The increase in the weight of the kidney in the 8000 ppm group males was also a consistent finding at necropsy. The relative weight was increased in the 8000 ppm males at both the interim and terminal sacrifices and in the 1000 ppm group males at the interim sacrifices. The only other consistent organ weight change was a decrease in the weight of the ovaries in the 8000 ppm group females, absolute, relative and ovary-to-brain weight ratio at Week 53. The increased weight of the liver and kidneys is reflective of the increased incidence of neoplasms in these organs; the reason for the decrease in ovarian weight was not evident from histopathology.

Treatment-related non-neoplastic lesions observed at the interim sacrifice included: (1) eosinophilic protein-like droplets in the cytoplasm of the superficial epithelial cells of the bladder in the 1000 ppm group males and 8000 ppm group males and females; (2) an increased severity of chronic progressive nephropathy in the 1000 ppm group males and 8000 ppm group males and females; (3) an increased incidence of extramedullary hematopoiesis and pigment in the spleen of the 8000 ppm males and females. The latter finding is compatible with the decrease in erythrocyte parameters in the 8000 ppm group females at Week 53.

Treatment-related non-neoplastic lesions observed at the terminal and unscheduled necropsies included the same intracytoplasmic droplets in the bladder as seen at the interim sacrifice in the 1000 and 8000 ppm males and females. Unilateral and bilateral cataracts were seen with increased frequency in the 8000 ppm group males and females. The severity of extramedullary

hematopoiesis and pigment in the spleen was increased in the 8000 ppm group males and females.

Neoplastic changes at the interim, terminal and unscheduled necropsies involved the vascular system, kidney and liver. There was an increase in the incidence of hemangiomas and hemangiosarcomas in all of the treated males and in the 8000 ppm females at the terminal and unscheduled necropsies but not at the interim necropsy. The incidence of both tumors in males in the 0, 100, 1000 and 8000 ppm groups was 2, 9, 13, and 18, respectively. The incidence in females in the 0, 100, 1000 and 8000 ppm groups was 5, 6, 5, and 10, respectively. (The number of animals per group is identical.)

There was an increased incidence of adenomas, multiple adenomas and carcinomas of the kidney in the 8000 ppm group males. A total of six renal tumors were found in this group as compared to none in either the treated or control groups.

The incidence of hepatic neoplasms (adenomas, carcinomas and one hepatoblastoma) was increased in the 8000 ppm group females. The incidence in the 0, 100, 1000 and 8000 ppm groups was 1, 1, 2 and 11, respectively.

The findings of the study are summarized in Table 14.

Table 14
Summary of Findings in Mice Treated with Carbaryl in the Diet for 104 Weeks

	Dose Levels (ppm)							
	Males				Females			
	0	100	1000	8000	0	100	1000	8000
Clinical Signs				↑				↑
Body Weight Gain				↓				↓
RBC Mass				↓				↓
RBC-CME			↓	↓				
BR-CME			↓	↓			↓	↓
Kidney Weight			↑	↑				↑
Liver Weight				↑				↑
Bladder Lesion			↑	↑			↑	↑
Cataracts				↑				↑
Splenic EMH & Pigment				↑				↑
Vascular Tumors		↑	↑	↑				↑
Renal Tumors				↑				
Hepatic Tumors								↑

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V. CONCLUSIONS

Technical carbaryl was administered in the diet to groups of 80 male and 80 female CD-1® mice at dosages of either 0, 100, 1000 or 8000 ppm for 104 weeks. The actual mean mg/kg/day dosages received by the males at 100, 1000 and 8000 ppm were 14.73, 145.99 and 1248.93, respectively. The actual mean mg/kg/day dosages received by the females at 100, 1000 and 8000 ppm were 18.11, 180.86 and 1440.62, respectively. Systemic toxicity was seen in the high-dose group males and females in the form of clinical signs, reduced body weight gain, decreased RBC parameters, decreased RBC (males) and brain cholinesterase levels, increased liver weight, increased kidney weight and increased incidence of non-neoplastic lesions including intracytoplasmic protein-like droplets in the superficial epithelium of the urinary bladder, cataracts (especially females) and increased severity of extramedullary hematopoiesis and pigment in the spleen. In the mid-dose group, there were decreases in RBC (males) and brain cholinesterase, increases in intracytoplasmic protein-like droplets in the superficial epithelium of the bladder and an increase in the kidney weight (males at interim sacrifice).

An increased incidence of neoplasms of the vascular system (hemangiomas and hemangiosarcomas) was found in all the treated groups of males and in the high-dose group females. An increased incidence of renal neoplasms (adenomas, multiple adenomas and carcinomas) was observed in the high dose group males. An increased incidence of hepatic neoplasms (adenomas, carcinomas and one hepatoblastoma) was seen in the high-dose group females.

The study demonstrated that carbaryl is carcinogenic in mice at doses of 100 ppm (14.73 mg/kg/day) or higher in males and 8000 ppm (1440.62 mg/kg/day) in females. The data will be presented to the HED Carcinogenicity Peer Review Committee for evaluation of the carcinogenic potential of Carbaryl.

The systemic No Observed Effect Level (NOEL) was 100 ppm (males: 14.73 mg/kg/day; females: 18.11 mg/kg/day)

The systemic Lowest Observed Effect Level (LEL) was 1000 ppm (145.99 mg/kg/day) in males based on decreases in RBC and brain cholinesterase levels, an increase in kidney weight (interim sacrifice) and bladder pathology and 1000 ppm (180.86 mg/kg/day) in females based on a decrease in brain cholinesterase levels and bladder pathology.

The Maximum Tolerated Dose (MTD) was 8000 ppm (males: 1248.93 mg/kg/day; females: 1440.62 mg/kg/day) based on clinical signs, decreases in body weight gain, alterations in clinical pathology parameters and histopathology changes.

VI. CLASSIFICATION - Minimum - See DEFICIENCIES

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