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

010092

MAR 2 2 1993

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Carbaryl (1-naphthyl N-methylcarbamate)
53-Week Interim Report, Mouse Carcinogenicity Study
52-Week Interim Report, Rat Feeding/Carcinogenicity Study

FROM: Ray Landolt 3/16/93
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)

Barcode D175180
Chem. No.: 056801
MRID No. 421889-01
MRID No. 421889-02

TO: Larry Schnaubelt, PM 72
Reregistration Branch
Special Review and Reregistration Division (H7508W)

THRU: Mike Ioannou, Section Head Stephen C. Dapson for 3/16/93
Review Section I
Toxicology Branch II
Health Effects Division (H7509C)
and
Marcia van Gemert, Chief Marcia van Gemert 3/17/93
Toxicology Branch II
Health Effects Division (H7509C)

Registrant: Rhone-Poulenc AG Company, letter of January 29, 1992.

Action Requested: The registrant has submitted the interim reports for (83-2) Mouse Carcinogenicity Study (MRID No.421889-01) and Rat Chronic Feeding/Carcinogenicity study (MRID No.421889-02) conducted with carbaryl in response to the data call notice of May 14, 1991. These two studies are to replace the two rodent feeding/carcinogenicity studies found deficient with HED review by R.B. Jaeger (DER 007191) of December 27, 1988.

Conclusion: The Toxicology reviews of these two interim reports are attached. In addition, appended to these reports are the preliminary histopathological findings of adverse effects of those animals examined from the terminal sacrifice of each study. This selected information from these two studies was submitted with Rhone-Poulenc letters of July 21, 1992 and December 2, 1992.

1 of 32

53-Week Interim Report of Oncogenicity Study with Carbaryl in Mice

Conclusions: Classification of Data - Supplementary

Deficiency: Pending the final report to be submitted by Rhone-Poulenc.

Cholinesterase NOEL = 100 ppm (males 12.4 and females 15.5 mg/kg/day)
LEL = 1000 ppm (males 119.3 and females 152.6 mg/kg/day) with a significant decrease in erythrocyte in males by 23% and brain cholinesterase activity in males and females by 18 and 13%, respectively.

Systemic NOEL = 100 ppm (males 12.4 and females 15.5 mg/kg/day)
LEL = 1000 ppm (males 119.3 and females 152.6 mg/kg/day) with increased kidney relative to body weight (18%) increased kidney relative to brain weight (18%) in males, nephrophathy (males), and transitional epithelial intracytoplasmic pigment of the urinary bladder (males).

52-Week Interim Report of Chronic Feeding/Oncogenicity Study with Carbaryl in Rats

Conclusion: Classification of Data - Supplementary

Deficiency: Pending the final report to be submitted by Rhone-Poulenc.

Cholinesterase NOEL = 250 ppm (males 10.4 and females 13.5 mg/kg/day)
LEL = 1500 ppm (males 62.1 and females 82.8 mg/kg/day) with a significant decrease in RBC (19 to 26%) and brain (10 to 13%) cholinesterase activity in males and females.

Systemic NOEL = 250 ppm (males 10.4 and females 13.5 mg/kg/day)
LEL = 1500 ppm (males 62.1 and females 82.8 mg/kg/day) with a significant decrease in body weight by 9% for females and urine dark yellow in appearance for both sexes.

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Reviewed by: Ray Landolt 3/3/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Mike Ioannou 3/3/93
Section I, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

010092

Study Type: Feeding/Carcinogenicity - Rat (83-2)

Barcode D175180
Tox.Chem.No.056801
MRID 421889-02

Test Material: 1-naphthyl methylcarbamate

Common Name: Carbaryl

Classification: Carbamate Insecticide

Title of Study: 52-Week Interim Report with 4-Week Recovery of a Combined Chronic Toxicity and Oncogenicity Study with Carbaryl Technical in Sprague Dawley Rats

Study Number: 656-139

Study Date: December 12, 1991

Sponsor: Rhone-Poulenc Ag Company

Testing Facility: Hazleton Washington, Inc

Author: N. Nicki Hamada

Quality Assurance: Karen E Butler

Conclusions: Classification of Data - Supplementary

Deficiency: This study will be evaluated in it's entirety when submitted by Rhone-Poulenc

Cholinesterase NOEL = 250 ppm (males 10.4 and females 13.5 mg/kg/day
LEL = 1500 ppm (males 62.1 and females 82.8 mg/kg/day) with a significant decrease in RBC (19 to 26%) and brain (10 to 13%) cholinesterase activity in males and females.

Systemic NOEL = 250 ppm (males 10.4 and females 13.5 mg/kg/day)
LEL = 1500 ppm (males 62.1 and females 82.8 mg/kg/day) with a significant decrease in body weight by 9% for females and urine dark yellow in appearance for both sexes.

Addendum to this study was submitted with Rhone-Poulenc letter of December 2, 1992 of preliminary histopathological findings from the terminal sacrifice. "These data indicate a possible increased incidence of bladder (males), thyroid (males), and liver (females) tumors in the high dose treatment group". In addition, "an increased incidence of sciatic nerve and skeletal muscle degeneration in the high dose treatment group in comparison to controls" was reported (MRID 425744-01, copy attached).

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A. Materials

1. Test material - Carbaryl technical, a white to tan powder, of Lot No. 12CNG32 and purity of 99.6% was used in this study.

2. Animals - Male (193.9 to 278.9g) and female (154 to 205.4g) 6-week-old Crl:CD®BR rats from Charles River Laboratories were used.

B. Study Design:

1. Allocation of Animals:

Test group	Dose Level Diet (ppm)	Interim Sacrifice 53 week Male	Female	57 week* Male	Female	Main study 104 weeks Male	Female
1 (Control	0	10	10	10	10	70	70
2 (Low)	250	10	10	0	0	70	70
3 (Mid)	1500	10	10	0	0	70	70
4 (High)	7500	10	10	10	10	70	70

Recovery Group*- In order to determine the extent of recovery following 53 weeks on study, 10 rats/sex of the high dose level were placed on control diet for 4-weeks. These animals with 10 rats/sex of the control groups were sacrificed on week 57 for complete necropsy, organ weights, clinical pathology and histology evaluation.

All animals were housed individually with temperature (66 to 80°F), and relative humidity (38 to 86%) controlled to provide a uniform environment. A 12-hour light-dark cycle was provided. Purina® Certified Rodent Chow® #5002 and tap water were available ad libitum. A sentinel group of 10 rats/sex were included to assess the health status of the animals over their lifetime. Prior to initiation of this study a viral screen was conducted on 10 rats/sex with negative results for the presence of sendai, reovirus type 3, polyoma, mycoplasma pulmonis, sialodacryoadenitis, pneumonia, minute virus of mice, GD VII, H-1, mouse adenovirus, and lymphocytic choriomeningitis virus.

2. Diet - A premix was prepared for each level then added to the required amount of food for each dietary level to be fed fresh weekly. Dietary levels were analyzed by reverse phase high performance liquid chromatography for homogeneity, stability, and to verify targeted concentration. Stability of carbaryl in the diet at 7500 ppm was determined to be within 98-104% of the target concentration for 14 days at room temperature.

Diet analysis as Percent of the Targeted Dose

Targeted Dose (ppm):	250	1500	7500
Homogeneity	96-100	97-105	92-104
Dietary Concentration - Week 1 to 52	88-108	95-106	92-107

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3. Statistical analysis of absolute body weights at weeks 1-14, 17, 21, 25, 27, 29, 33, 37, 41, 45, 49, and 53, weekly food consumption, clinical pathology, and organ weights of the controls were compared statistically to data from the same sex of each treated group. Group comparisons were performed at the 5.0% two-tailed probability level.

C. Method and Results:

1. Observations of all animals were made twice daily for mortality and signs of toxicity. Detailed physical examinations, to include mass development, were performed weekly.

(a) An increased incidence of alopecia of the front limb and feet was observed for group 4 females accompanied by "urine stains" among males and females of group 4. A decreased incidence of these observations was apparent in the recovery group. No increased incidence of mass development was observed relative to the dietary levels fed.

(b) Survival for the test groups ranged between 96-99% for the males and 98-100% for the females as compared to the survival of the male and female controls of 97 and 93%, respectively during the initial 52 weeks of the study. "The high mortality in the female control group could not be determined."

Survival Rate per Dietary Level (ppm)

Week	13		26		39		52	
Dose (ppm)	Male	Female	Male	Female	Male	Female	Male	Female
Control	90/90	90/90	89/89	90/90	88/89	88/90	85/88	84/90
250	79/80	80/80	78/80	80/80	77/80	80/80	77/80	80/80
1500	80/80	80/80	80/80	80/80	80/80	79/80	79/80	79/80
7500	90/90	89/90	90/90	89/90	88/90	88/90	86/89	88/90

(c) Ophthalmoscopic examinations were performed by indirect ophthalmoscopy on all animals initially, during week 52 for those animals scheduled for the interim sacrifice and for the recovery group on week 56. No ophthalmoscopic observations were reported for the interim sacrifice or recovery group relative to the dietary levels fed.

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2. Body weights were recorded initially, weekly during weeks 1-14, and once every second week thereafter. A significant ($p<0.05$) decrease in body weight was reported for group 3 females during weeks 13 and 53 by 9 and 8%, respectively. Body weight of the high dose males was significantly ($p<0.05$) decreased by 40% during weeks 13 and 53. Body weight of the high dose females was significantly ($p<0.05$) decreased by 52 and 65% during weeks 13 and 53, respectively. The following table summarizes actual(g) and percent change in body weight as compared to the controls during weeks 13 and 53.

	Control		250 ppm		1500 ppm		7500 ppm	
Week	Male	Female	Male	Female	Male	Female	Male	Female
13	322	138	310	136	305	126	193	66
			-4	-1	-5	-9*	-40*	-52*
53	489	252	481	249	472	232	291	89
			-2	-1	-3	-8*	-40*	-65*

* Significantly different from control value, $p<0.05$.

Body weight gain of the recovery group males and females increased by 4 and 9%, respectively as compared to the high dose animals of the main study.

3. Food consumption was recorded initially, weekly during weeks 1 to 14, and once every fourth week thereafter. Food consumption of groups 2, 3, and 4 was not significantly different from the controls for any weekly interval. However, total food consumption over the 50 week period was significantly ($p<0.05$) decreased for group 3 females by 28% and for group 4 males and females by 17%. The following table summarizes the per cent decrease in food consumption for the 7500 ppm level. Food consumption for group 2 was comparable to the controls.

Week	13		26		38		50	
Dose (ppm)	Male	Female	Male	Female	Male	Female	Male	Female
7500	18	19	14	16	13	20	16	20

Food consumption of the recovery group males and females increased by 15 and 31%, respectively as compared to the high dose animals of the main study.

4. The following table from this report summarizes the mean compound consumed (mg/kg/day) during the initial 50 weeks of this study.

	Males				Females		
Group:	2	3	4		2	3	4
ppm:	250	1500	7500		250	1500	7500
Weeks[a] 1-13	16.4	99.8	520.8	Mean	19.2	119.0	622.8
	4.08	25.21	91.96	S.D.	3.64	24.67	82.59
Weeks[b] 14-50	10.4	62.1	354.0	Mean	13.5	82.8	487.3
	1.49	8.00	31.30	S.D.	1.72	8.59	32.34

[a] Weeks 1-13 calculated weekly (13 means/group).
[b] Weeks 14-50 calculated once every fourth week (10 means/group).

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5. Clinical Pathology - Blood was collected by orbital sinus puncture for clinical analysis from 10 rats/sex/group during weeks 27 and 53. Also blood was collected from the control and high dose recovery group during week 57.

(a) Hematology - The checked (*) parameters are recommended by Subdivision F Guidelines of November 1989. Cell morphology, leukocyte differential and reticulocyte count were determined for the control and group 4 animals. The following parameters were evaluated in this study.

* Hematocrit (HCT)	* Platelet count
* Hemoglobin (HGB)	Mean cell volume (MCV)
* Leukocyte count (WBC)	Mean cell hemoglobin (MCH)
* Erythrocyte count (RBC)	Mean cell hemoglobin concentration (MCHC)

Except for a significant ($p<0.05$) decrease in WBC (19%) for group 3 males and decreased MCHC (2%) for group 4 females, hematologic findings were limited to the group 4 males. A significant ($p<0.05$) increase in HGB (13%), HCT (11%), MCV (4%), MCH (5%), and MCHC (2%) accompanied by a decrease in WBC (25%), corrected WBC (25%), and lymphocyte count (27%) were reported for group 4 males. These hematologic changes are considered to be of questionable significance. The following table summarizes the significant ($p<0.05$) hematologic changes reported for the interim sacrifice and recovery groups.

Percent Change in Significant ($p<0.05$) Hematology Parameters

			Male			Female		
Increased	Week	Group:	2	3	4	2	3	4
HGB	57				13			
HCT	57				11			
MCV	27				4			
MCH	27				6			
	53				5			
MCHC	27				2			
	57				3			
Decreased								
MCHC	57							2
WBC	27			19	25			
Cor WBC	27				25			
Lymph	27				29			
	53				27			

(b) Clinical Chemistry - The checked (*) parameters are recommended by Subdivision F Guidelines of November 1989. Except for alkaline phosphatase and lactic dehydrogenase recommended by Subdivision F, the following parameters were evaluated.

* Calcium	* Albumin	* Aspartate aminotransferase (AST)
* Chloride	* Creatinine (CREAT)	* Total bilirubin (T BILI)
* Sodium	* Urea nitrogen (BUN)	Globulin
* Phosphorus	* Creatine kinase (CK)	Alanine aminotransferase (ALT)
* Potassium	* Total protein (T PROT)	
* Glucose	* Total Cholesterol (T CHOL)	

Cholesterol values were significantly ($p<0.05$) increased for the group 4 males and females by 39 and 68%, respectively being reversible during week 57. The statistical significance of the decrease in creatinine, creatine kinase, aspartate aminotransferase, alanine aminotransferase, accompanied by increased total protein and sodium values is questionable. The following table summarizes the significant ($p<0.05$) clinical chemistry changes for the interim sacrifice and recovery groups.

Percent Change in Significant ($p<0/05$) Clinical Chemistry Parameters

			Male			Female		
Increased	Week	Group:	2	3	4	2	3	4
T CHOL	27				39			68
	53							62
T PROT	57				8			
Sodium	53				1			
Decreased								
AST	27				21			37
	53							48
ALT	27							49
	53							46
CK	53				63			

(c) Urinalyses - The checked (*) parameters are recommended by Subdivision F guidelines of November 1989.

* Appearance	* Bilirubin	* Volume	* Specific Gravity
* Occult blood	* Urobilirubin	* Ketones	pH
* Protein	* Sediment	* Glucose	

A dose related increased incidence of urine dark yellow to brown in appearance was observed for the group 3 and 4 males and females during weeks 27 and 53. By week 57 urine from group 3 animals was comparable to the controls with a decreased incidence of coloration for group 4 males and females. The other parameters evaluated during this period were comparable between the control and test levels except for an increased incidence of erythrocytes in the urine of group 4 during weeks 53 and 57.

(d) Cholinesterase - Blood for plasma and erythrothcyte cholinesterase activity was collected from the orbital sinus of 10 animals/sex/group initially, then during weeks 26 and 52. Cholinesterase activity was also determined during week 56 for 10 animals/sex of the control and group 4 recovery groups. Animals were not fasted prior to cholinesterase sampling.

Cholinesterase activity of group 3 males and females was significantly ($p<0.05$) depressed for erythrocyte (19-26%) and brain (10-13%) during week 52 of the study. Group 4 male plasma, erythrocyte, and brain cholinesterase values were significantly ($p<0.05$) decreased by 40, 22, and 28%, respectively during week 52 being reversible by week 56. Group 4 female plasma, erythrocyte, and brain cholinesterase values were decreased significantly ($p<0.05$) by 56, 36, and 31%, respectively during week 52 being reversible by week 56. Cholinesterase values between males and females of group 2 and controls were comparable during weeks 26 and 52. The following table summarizes the significant ($p<0.05$) decrease in plasma, erythrocyte and brain cholinesterase activity for the scheduled sacrifice.

Percent Decrease in Cholinesterase Activity as Compared to Control

			Male			Female		
Cholinestarese	Week	Group:	2	3	4	2	3	4
Plasma	26				27			53
	52				40			56
Erythrocyte	26							25
	52			19	22		26	36
Brain	53			10	28		13	31

6. Terminal Observations - All animals of the scheduled sacrifice were weighed, anesthetized with sodium pentobarbital, and exsanguinated for examination of the following tissues of the control and high dose interim and recovery groups. In addition, gross lesions, lungs, liver and kidneys were examined from the low and mid dose. The checked (*) parameters are recommended by Subdivision F Guidelines of November 1989. The checked (**) organs were weighed from 10 animals/sex/group during week 53 and from the control and high dose during week 57.

* Trachea
* Salivary glands
* Esophagus
* Stomach
* Duodenum, jejunum & ileum
* Colon, cecum & rectum
** Adrenals
** Lung
* Pancreas
* Pituitary
** Liver
Aorta
* Heart
* Bone marrow
* Lymph nodes-mandibular & mesenteric
* Thymus
** Spleen
** Kidneys
* Urinary bladder
Seminal vesicle
Prostate
** Testes & epididymides
** Brain with medulla/pons
Sciatic nerves
* Spinal cord (3 levels)
* Eyes
* Thyroids (parathyroids)
* Skeletal muscle
* Skin
* Mammary gland
** Ovaries
Lesions and tumors
Bone
* Uterus, vagina & cervix

(a) Macroscopic observations - No gross pathologic findings were reported for the unscheduled deaths, interim sacrifice or recovery groups relative to the dietary level fed.

(b) Organ weights The following table summarizes the statistically ($p<0.05$) significant changes in organ weights as compared to the control values.

Percent Change	Group:	Males 2	Males 3	Males 4	Females 2	Females 3	Females 4
Decrease in terminal body weight -	interim			28			42
	recovery			23			24
Decrease in Absolute Organ weight							
Kidney	interim						13
Liver	interim						16
Spleen	recovery			20			
Lung	recovery			13			
Increase in Organ to Body weight							
Lung	interim			26			59
	recovery						22
Brain	interim			35			75
	recovery			31			28
Spleen	interim						48
	recovery						29
Kidney	interim			50			49
	recovery			28			18
Liver	interim			44		13	46
	recovery			27			25
Testis/epididymides	interim			38			
	recovery			26			
Adrenals	interim			46			53
	recovery						
Ovary	interim						63
	recovery						49
Decrease in Organ to Brain weight							
Kidney	interim						14
Liver	interim						17
Lung	recovery			15			
Spleen	recovery			21			

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(b) Organ weights (con't)

Interim absolute and organ to brain weights of kidney and liver were significantly ($p<0.05$) decreased for group 4 females.

Recovery absolute and organ to brain weights of spleen and lung were significantly ($p<0.05$) decreased for group 4 males.

(c) Histopathology Findings

Summary Incidence of Selected Histopathology Findings

Sex	Male				Female			
Dose (ppm)	0	250	1500	7500	0	250	1500	7500
Unscheduled death								
Thyroid - No.exam.	5	3	1	4	6	0	1	2
Adenoma,"C" cell	1	0	0	0	0	0	0	0
Urinary bladder - No. exam.	4	3	1	4	6	0	1	2
Papilloma, transitional cell	0	0	0	0	1	0	0	0
Liver - No. exam.	5	3	1	4	6	0	1	2
Intracytoplasmic hyaline inclusion	0	0	0	1	0	0	0	0
Interim sacrifice								
Thyroid - No. exam.	9	0	0	9	9	0	0	10
Hyperplasia, "C" cell	1	0	0	1	0	0	0	0
Liver - No. exam.	9	10	10	9	10	10	10	10
Hepatocyte, hypertrophy	0	0	0	1	0	0	0	1
Centrilobular hypertrophy	0	0	0	0	0	0	0	1
Intracytoplasmic hyaline inclusion	0	0	0	4	0	0	0	0
Kidney - No. exam.	9	10	10	9	10	10	10	10
Hyperplasia, transitional epithelium	0	0	0	1	0	0	0	1

In the above table, histopathological findings of the thyroid, urinary bladder, and kidney were added to the findings discussed in this report for the liver, as possible treatment related effects.

The incidence of hepatocellular intracytoplasmic hyaline inclusion reported for the group 4 males in the interim sacrifice and unscheduled deaths was not observed in the recovery group 4 males or females. No histopathologic findings were reported for the recovery group relative to the dietary level fed for 52 weeks.

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(d) Histopathology Findings (con'c)

Addendum to this study (MRID 425744-01) was submitted with Rhone-Poulenc letter of December 2, 1992. The registrant concluded that "These findings represent preliminary and unaudited data, it is not possible to reach meaningful conclusions regarding this study prior to an evaluation of the final report."

These data consist of selected histopathological findings that "indicate a possible increased incidence of bladder (males), thyroid (males), and liver (females) tumors in the high dose treatment group" In addition, "an increased incidence of sciatic nerve and skeletal muscle degeneration in the high dose treatment group in comparison to controls" was reported.

The incidence of selected findings in the terminal sacrifice of the control and 7500 ppm level, from the attached Histopathology Incidence Summary submitted by Rhone-Poulenc, are presented in the following table.

Sex	Male		Female	
Dose (ppm)	0	7500	0	7500
Urinary bladder - No. examined	70	71	69	69
Transitional Cell Papilloma	0	9	1	7
Transitional Cell Carcinoma	0	2	0	6
Thyriod - No. examined	71	71	70	70
Follicular Cell Adenoma	0	8	0	1
Follicular Cell Carcinoma	0	1	1	0
Liver - No. examined	70	71	70	70
Hepatocellular Adenoma	0	1	1	7
Hepatocellular Carcinoma	0	1	0	0
Kidney - No examined	70	71	70	69
Hyperplasia, transitional epithelium	2	17	10	10
Transitional Cell Carcinoma	0	1	0	0
Tubule Cell Adenoma	0	1	0	0
Tubule Cell Carcinoma	0	1	0	1
Sciatic Nerve - No. examined	71	69	70	68
Degeneration	44	59	34	61
Skeletal Muscle - No. examined	71	71	70	69
Degeneration	4	13	0	6

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D. Conclusions: Classification of Data - Supplementary

Deficiency: This study will be evaluated in it's entirety when submitted by Rhone-Poulenc

Cholinesterase NOEL = 250 ppm (males 10.4 and females 13.5 mg/kg/day
LEL = 1500 ppm (males 62.1 and females 82.8 mg/kg/day) with a significant decrease in RBC (19 to 26%) and brain (10 to 13%) cholinesterase activity in males and females.

Systemic NOEL = 250 ppm (males 10.4 and females 13.5 mg/kg/day)
LEL = 1500 ppm (males 62.1 and females 82.8 mg/kg/day) with a significant decrease in body weight by 9% for females and urine dark yellow in appearance for both sexes.

010092

425744-01

CHRONIC TOXICITY/ONCOGENICITY STUDY WITH CARBARYL TECHNICAL IN RATS PRELIMINARY DATA

RHONE POULENC AG COMPANY
P.O. BOX 12014, 2 T.W. ALEXANDER DRIVE
RESEARCH TRIANGLE PARK, NC 27709
DECEMBER 2, 1992

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CARBRYL

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____ Identity of product inert ingredients.

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Reviewed by: Ray Landolt 3/10/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Mike Ioannou 3/10/93
Section I, Toxicology Branch II (H7509C)

010092

DATA EVALUATION REPORT

Study Type: Carcinogenicity - Mouse (83-2)

Barcode D 175180
Tox Chem No.056801
MRID No. 421889-01

Test Material: 1-naphthyl methylcarbamate

Common Name: Carbaryl

Classification: Carbamate Insecticide

Title of Study: 53-Week Interim Report of Oncogenicity Study with Carbaryl Technical in CD-1® Mice

Study Number: HWA 656-138

Study Date: December 17, 1991

Sponsor: Rhone-Poulenc Ag Company

Testing Facility: Hazleton Washington, Inc

Author: N. Nicki Hamada

Quality Assurance: Ian S. Puente

Conclusions: Classification of Data - Supplementary

Deficiency: Pending the final report to be submitted by Rhone-Poulenc.

Cholinesterase NOEL = 100 ppm (males 12.4 and females 15.5 mg/kg/day)
LEL = 1000 ppm (males 119.3 and females 152.6 mg/kg/day) with a significant decrease in erythrocyte in males by 23% and brain cholinesterase activity in males and females by 18 and 13%, respectively.

Systemic NOEL = 100 ppm (males 12.4 and females 15.5 mg/kg/day)
LEL = 1000 ppm (males 119.3 and females 152.6 mg/kg/day) with increased kidney relative to body weight (18%) increased kidney relative to brain weight (18%) in males, nephrophathy (males), and transitional epithelial intracytoplasmic pigment of the urinary bladder (males).

Addendum to this study was submitted with Rhone-Poulenc letter of July 21, 1992 of preliminary histopathological findings from the terminal sacrifice.

These findings indicate "an increased incidence of renal (male), hepatocellular (female), and vascular (male and female) neoplasia in the treatment groups". In addition, unilateral and bilateral cataracts were reported for the high dose males and females". The animals were misdosed with aldicarb at week 93 of the study.

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A. Materials:

1. Test material - Carbaryl technical, a white powder, of Lot No. 87191 and purity of 99.3% was used in this study.

2. Animals -Male (23.2 to 27.9g) and female (18.0 to 23.0g) 29-day-old Crl:CD-1® (ICR)BR mice from Charles River Laboratories were use in this study.

B. Study Design:

1. Allocation of Animals:

Test Group	Dose Level Diet (ppm)	12-Month Sac. Male	12-Month Sac. Female	24 Month Sac. Male	24 Month Sac. Female
1. Control	0	10	10	70	70
2. Low (LDT)	100	10	10	70	70
3. Mid (MDT)	1000	10	10	70	70
4. High (HDT)	8000	10	10	70	70

All animals were housed individually with temperature (61 to 77°F) and relative humidity (32 to 89%) controlled to provide a uniform environment. A 12-hour light-dark cycle was provided. Purina® Certified Rodent Chow® #5002 and tap water were available ad libitum A sentinel group of 10 mice/sex was included to assess the health status of the animals over their lifetime. No evidence of disease noted.

Prior to the initiation of this study a viral screen was conducted on 5 mice/sex with negative results for the presence of pneumonia, encephalomyelitis, sendai, lymphocytic choriomeningitis, ectromelia, reovirus type 3, polyoma, mouse hepatitis, minute virus of mice and mycoplasma pulmonis.

2. Diet - A premix was prepared for each level, added to the required amount of food for the dietary level to be fed fresh weekly. Dietary levels were analyzed by reverse phase high performance liquid chromatography for homogeneity, stability, and to verify targeted concentration. Carbaryl was determined to be stable in rodent diet for 14-days at room temperature for levels of 30 to 2000 ppm. Stability of the 8000 ppm diet was not determined. A homogeneous mix was reported for all levels fed.

Diet Analysis as Percent of the Targeted Dose

Targeted Dose (ppm):	100	1000	8000
Homogeneity	101-103	101-107	92-106
Dietary Concentration - Week 1 to 52	90-106	95-107	93-106

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3. Statistical Analysis was performed on absolute body weights at weeks 1, 2, 3, 4, 8, 12, 16, 18, and 52, food consumption at weeks 13, 26 and 50, clinical pathology and organ weights. Control values were compared statistically to data from the same sex of each treated group. Test for homogeneity of variance and ANOVA were evaluated at the 5.0% one-tailed probability level. Group comparisons were evaluated at the 5.0% two-tailed probability level.

C. Methods and Results:

1. Observations of all animals were made twice daily for mortality and signs of toxicity. Detailed physical examinations were performed weekly.

   (a) An increased incidence of clinical signs of toxicity was observed for Group 4 males and females to include tremors, hunched and weak in appearance, rough haircoat, urine stains, and few feces.

   (b) Survival for Group 4 females decreased to 94% after 8 weeks. Following 50 weeks, survival/adjusted survival for Groups 1-4 was 97, 95, 99, and 98% for males, and 93, 100, 96, and 90% of females.

Survival Rate per Dietary Level (ppm)

Week	13		26		38		50	
Dose (ppm)	Male	Female	Male	Female	Male	Female	Male	Female
Control	80/80	79/80	79/79	79/80	78/79	76/80	77/79	74/80
100	78/80	80/80	78/80	80/80	77/80	80/80	76/80	80/80
1000	80/80	80/80	80/80	80/80	79/80	78/80	79/80	77/80
8000	79/80	74/79	79/80	73/79	79/80	72/79	78/80	71/79

2. Body weights were recorded initially, weekly during weeks 1 to 14, and then once every second week thereafter. During the first 4 weeks a significant ($p<0.05$) decrease in body weight was recorded for Group 4 males and females by 81 and 70%, respectively as compared to controls. The following table summarizes actual(g) and percent change in body weight as compared to the controls during weeks 13 and 52.

Week	Control		100		1000		8000	
	Male	Female	Male	Female	Male	Female	Male	Female
13	7.8	7.8	9.0	8.8	8.4	8.9	5.2	6.3
			+15.3	+13.0	+8.0	+14.0	-33.3*	-19.0*
52	12.4	12.4	13.4	12.7	11.9	12.8	8.2	9.5
			+8.0	+2.0	-4.0	+3.0	-34.0*	-23.0*

* Significantly different from the control values, $p<0.05$.

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3. Food consumption was recorded weekly for 4 weeks, then every fourth week.

No significant change was recorded for males related to the dietary levels fed during the 50 weeks of the study. A significant ($p<0.05$) decrease in dietary intake of 7 to 9% was reported for Group 4 females during weeks 13, 26, and 50.

4. Mean compound consumed (mg/kg/day) is summarized in the following table.

	100 ppm		1000 ppm		8000 ppm	
Week	Male	Female	Male	Female	Male	Female
13	16.2	20.8	163.1	207.6	1392.2	1621.8
50	12.4	15.5	119.3	152.6	1062.7	1217.6

5. Clinical Pathology - Blood samples for hematology and clinical chemistry were obtained via abdominal aorta under sodium pentobarbital anesthesia from 10 mice/sex/group. Clinical pathology data from animals sacrificed in extremis were collected to be included in the final report.

(a) Hematology - The following parameters were evaluated for all groups. Cell morphology and corrected leukocyte count were determined for the control and high dose animals.

Erythrocyte count	Leukocyte count	Mean cell volume
Hematocrit	Leukocyte differential count	Mean cell hemoglobin
Hemoglobin	Mean cell hemoglobin concent.	Platelet count

A significant ($p<0.05$) decrease in erythtocyte (9%), hemoglobin (7%), and hematocrit (8%) values for Group 4 females was associated with decreased body weight. A significant ($p<0.05$) increase in platelet count (26%) was reported. In addition, corrected leukocyte, lymphocyte, and eosinophil values for Group 4 females increased in excess of 100%. Erythrocyte cellular morphology revealed a slight increase in the incidence of echinocytosis (spiny surface projections) in Group 4 males and females.

The following table summarizes percent change in significant ($p<0.05$) hematology findings reported during week 53 of this study.

		Males			Females		
	Group:	2	3	4	2	3	4
Decreased:	Erythrocyte						9
	Hemoglobin						7
	Hematocrit						8
Increased:	Mean cell volume		5				
	Platelet						26
	Corr. leukocyte						>100
	Lymphocyte						>100
	Eosinophil						>100

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(b) Clinical chemistry evaluations were limited to cholinesterase (ChE) determination for plasma, erythrocyte and brain activity.

Group 3 male cholinesterase values decreased significantly ($p<0.05$) for erythrocyte and brain activity by 23 and 18%, respectively.

Group 3 female brain cholinesterase activity decreased significantly ($p<0.05$) by 13%.

Group 4 male cholinesterase values decreased significantly ($p<0.05$) for erythrocyte and brain activity by 30 an 57%, respectively.

Gruop 4 female brain cholinesterase activity decreased significantly ($p<0.05$) by 47%.

The following table summarizes the significant ($p<0.05$) decrease in plasma, erythrocyte, and brain cholinesterase activity during week 53.

Percent Decrease in Cholinestease Activity as Compared to Control

		Male			Female		
Cholinesterase	Group:	2	3	4	2	3	4
Plasma							
Erythtocyte			23	30			
Brain			18	57		13	47

3. Terminal Findings - For the interim sacrifice of 10 mice/sex/group

Tissues in the following table were prepared for histopathological evaluation from the control and high dose. In the mid and low dose levels, tissues of liver, kidney, lung, spleen, urinary bladder and gross lesions were examined microscopically. The checked (X) tissues are recommended by Subdivision F guidelines of November 1989. The checked (XX) tissues were weighed.

XX	Brain	X	Skin	X	Aorta
X	Salivary glands	X	Heart	X	Sciatic nerve
X	Esophagus	X	Bone marrow	X	Spinal cord (3 level)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	XX	Spleen		Eyes
X	Jejunum	X	Thymus	X	Treachea
X	Ileum	XX	Lungs	XX	Adrenals
X	Caecum	XX	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder	X	Mammary gland
X	Rectum	XX	Testes	X	Parathyroids
XX	Liver	XX	Epididymides	X	Thyroids
XX	Gallbladder		Prostate	X	Skeletal muscle
X	Pancreas		Seminal vesicle	X	All gross lesions
X	Uterus	XX	Ovaries		and masses

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3. Terminal findings (cont')

(a) Macroscopic observations - No gross pathologic findings were observed for the unscheduled deaths relative to the dietary levels fed. Enlarged livers were observed for 2/10 males and 2/10 females of the Group 4 interim sacrifice animals.

(b) Organ weights - The following table summarizes the significant ($p<0.05$) changes in organ weights as compared to the control values.

Percent Change	Males			Females		
Group:	2	3	4	2	3	4
Terminal body weight - decreased			9			
increased				12	12	
Absolute organ weight						
Decreased-ovary						33
Increased-liver with gall bladder						24
Organ to body weight ratios						
Decreased-ovary						35
lung					12	
Increased-liver with gall bladder			21			23
kidney		18	15			
lung			14			
Organ to brain weight ratios						
Decreased-ovary						33
Increased-liver with gall bladder			18			25
kidney	18	18				

Group 4 female ovary, absolute, relative to body and brain weights were significantly ($p<0.05$) decreased by 33, 35, and 33%, respectively. Group 4 female liver, absolute, relative to body and brain weights were significantly ($p<0.05$) increased by 24, 23, 25%, respectively.

Group 4 male liver, relative to body and brain weights were significantly ($p<0.05$) increased by 21 and 18%, respectively.

A significant ($p<0.05$) change in relative to body weights was reported for Group 3 with increased kidney (18%) for males and decreased lung (12%) weights for females. Group 4 male lung and kidney, relative body weights were increased significantly ($p<0.05$) by 14, and 15%, respectively.

Group 2 and 3 male kidney, relative to brain weights were significantly ($p<0.05$%) increased by 18%.

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(c) Histopathology - Observations of the urinary bladder of Group 3 males and Group 4 males and females were characterized by intracytoplasmic protein-like droplets (stained intensely eosinophilic) filling the cytoplasm of the superficial transitional epithelial cells. This treatment-related finding was graded "minimal to moderately severe".

An increased incidence of chronic progressive nephropathy in the Group 3 males and Group 4 males and females, while a common finding in 53-week-old CD-1® mice, was considered treatment related due to the severity of this lesion.

An increased incidence of extramedullary hematopoiesis and pigment was observed in the spleens of Group 4 males and females. This splenic pigment, compatible with hemosiderin, was associated with increased splenic turnover of red blood cells and considered treatment related to the decrease in erythrocyte values.

Protocol Deviations: Protocol-specified histopathological evaluations were not performed on unscheduled deaths at the 52-week interval. They will be included in the terminal histopathology report.

Summary Incidence of Selected Histopathology Findings

Sex	Male				Female			
Dose in ppm	0	100	1000	8000	0	100	1000	8000
Uninary Bladder-No. of tissues examined	10	10	10	10	10	10	10	10
Transitional epithelial Intracytoplasmic Pigment	0	0	6	10	0	0	0	10
Kidney-No. of tissues examined	10	10	10	10	10	10	10	10
Nephropathy, chronic progressive	6	7	10	9	9	9	10	10
Spleen-No. of tissues examined	10	10	10	10	10	10	10	10
Pigment	0	1	1	9	1	1	2	8
Extramedullary hematopoiesis	7	7	7	10	7	7	7	9
Liver-No. of tissues examined	10	10	10	10	10	10	10	10
Necrosis	0	0	0	0	0	1	2	0
Pigment	0	0	0	1	0	0	0	1
B Hepatocellular Adenoma	0	0	1	0	0	0	0	0
Eye-No. of tissues examined	10	0	0	10	10	0	0	10
Cataract	0	0	0	1	0	0	0	0

In the above table, histopathology findings of the liver and eye were added to the findings discussed in this report for the urinary bladder, kidney and spleen as possible treatment related effects.

(c) Histopathology (con't)

Addendum to this study (MRID 424112-01) was submitted with Rhone-Poulenc letter of July 21, 1992 of the preliminary histopathological findings from the terminal sacrifice of study No. 656-138.

"An increased incidence of renal(male), hepatocellular(female), and vascular(male and female) neoplasia was reported in the treatment groups". In addition, "an increased incidence of unilateral and bilateral cataracts of the high-dose males and females" was reported. The animals were misdosed with aldicarb at week 93 of the study (copy attached).

Summary Incidence of Selected Neoplastic Findings in the Terminal Sacrifice

Sex	Male				Female			
Dose in ppm	0	100	1000	8000	0	100	1000	8000
Renal-No. of tissues examined:	69	70	69	70	0	0	0	0
Adenoma	0	0	0	2	0	0	0	0
Multi Adenoma	0	0	0	1	0	0	0	0
Carcinoma	0	0	0	3	0	0	0	0
Total tumor-bearing animals				6/70				
Liver-No. of tissues examined:	0	0	0	0	59	70	69	70
Adenoma	0	0	0	0	0	0	1	6
Multi Adenoma	0	0	0	0	0	0	0	1
Carcinoma	0	0	0	0	1	1	1	2
Multi Carcinoma	0	0	0	0	0	0	0	1
Hepatoblastoma	0	0	0	0	0	0	0	1
Total tumor-bearing animals					1/69	1/70	2/69	11/70
Vascular-No. of tissues examined:	70	70	70	70	70	70	70	70
Hemangioma	0	0	0	3	1	0	1	0
Hemangiosarcoma	2	5	9	7	2	3	2	9
Total tumor-bearing animals	2/70	5/70	9/70	10/70	3/70	3/70	3/70	9/70

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D. Conclusions: Classification of Data - Supplementary

Deficiency: Pending the final report to be submitted by Rhone-Poulenc.

Cholinesterase NOEL = 100 ppm (males 12.4 and females 15.5 mg/kg/day)
LEL = 1000 ppm (males 119.3 and females 152.6 mg/kg/day) with a significant decrease in erythrocyte in males by 23% and brain cholinesterase activity in males and females by 18 and 13%, respectively.

Systemic NOEL = 100 ppm (males 12.4 and females 15.5 mg/kg/day)
LEL = 1000 ppm (males 119.3 and females 152.6 mg/kg/day) with increased kidney relative to body weight (18%) increased kidney relative to brain weight (18%) in males, nephrophathy (males), and transitional epithelial intracytoplasmic pigment of the urinary bladder (males).

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CARBARYL

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