UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

February 12, 2002

MEMORANDUM

SUBJECT: Carbaryl - Report of the Cancer Assessment Review Committee

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Virginia Dobozy, Toxicologist
Jeff Dawson, Risk Assessor
Reregistration Branch 1
Health Effects Division (7509C)

And

Anthony Britten, Chemical Review Manager
Reregistration Branch 111
Special Review and Reregistration Division (7508C)

The Cancer Assessment Review Committee met on November 7, 2001 to evaluate the carcinogenic potential of Carbaryl. Attached please find the Final Cancer Assessment Document.

cc: K. Dearfield
R. Hill
Y. Woo
J. Pletcher

TXR # 0050464

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF
CARBARYL (SECOND REVIEW)
PC Code: 056801

FINAL REPORT

February 12, 2002

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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DATA PRESENTATION: Virginia Dobozy, Veterinary Medical Officer

DOCUMENT PREPARATION: Virginia Dobozy for Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke	
William Burnam	
Marion Copley	
Kerry Dearfield	
Vicki Dellarco	
Virginia Dobozy	
Tim McMahon	
Nancy McCarroll	
Esther Rinde	
Joycelyn Stewart	
Clark Swentzel	
Linda Taylor	
Yin-Tak-Woo	See attached sheet

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John M. Pletcher, Pathology Consultant	See attached sheet
Lori Brunsman, Statistical Analysis	

CARBARYL CANCER ASSESSMENT DOCUMENT FINAL REPORT

DATA PRESENTATION: Virginia Dobozy, Veterinary Medical Officer

DOCUMENT PREPARATION: Sanjivani Diwan, Executive Secretary

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John M. Fletcher, Pathology Consultant
Lori Brunsman, Statistical Analysis

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EXECUTIVE SUMMARY

The carcinogenic potential of carbaryl was evaluated earlier by the Carcinogenicity Peer Review Committee (CPRC, 1994). The Committee concluded that carbaryl induced tumors at multiple sites in the rat and mouse at doses considered to be excessively toxic. Only hemangiosarcomas in the CD-1 male mouse occurred at a dose which was considered adequate and not excessive. The Committee concluded that carbaryl should be classified as a Group C - possible human carcinogen. Both the low-dose extrapolation (Q_1*) approach and a margin of exposure (MOE) approach were suggested as methods of quantifying the cancer risk in humans. The CPRC requested additional studies to direct the selection of the more appropriate method of quantification and provide insight into the tumors at excessively toxic doses. Additional submitted metabolism studies were evaluated by the Metabolism subgroup of the Cancer Assessment Review Committee (CARC, 1998) and were considered inadequate evidence to divert from the default linear low-dose extrapolation approach for risk assessment.

On November 7, 2001, the CARC reconsidered the cancer classification of carbaryl by evaluating the results of a Pathology Working Group (PWG) re-reread of the tumor pathology slides from the rat and mouse carcinogenicity studies (refer to CPRC, 1994 for details of the doses administered). The Committee also reviewed additional data including the results of a p53 knockout mouse study, new historical control data on vascular tumors in CD-1 mice and various mechanistic studies in both rats and mice.

The CARC concluded that carbaryl was carcinogenic to male mice at doses which were adequate and not excessive. Tumors in male and female rats and female mice, as well as other tumors in male mice, occurred at excessively toxic high dose levels. However, preneoplastic lesions in the target organs in male rats occurred at the mid dose level which was below the dose adequate for testing the carcinogenic potential of carbaryl. The findings of the rat combined chronic toxicity/carcinogenicity study are discussed below.

1. The reanalyses of rat tumor data showed that male rats had significant increasing trends and significant differences in pair-wise comparisons of the 7500 ppm dose group with the controls for thyroid follicular cell adenomas and combined adenomas/carcinomas, as well as for urinary bladder transitional cell papillomas, carcinomas, and combined papillomas/carcinomas, all at $p<0.01$. The increase in the incidence of combined thyroid follicular cell adenomas/carcinomas at 7500 ppm was driven by the adenomas. At 7500 ppm, the incidences of thyroid follicular cell adenomas, as well as combined urinary transitional cell papillomas and carcinomas, exceeded their respective range for the historical controls. The female rats had a significant increasing trend ($p<0.01$) and a significant increase by pair wise comparison of the 7500 ppm dose group with the controls for hepatocellular adenomas ($p<0.05$). The re-read of tumor data by the PWG showed that the female rats had a significant increasing trend for urinary bladder transitional cell papillomas, carcinomas and combined papillomas/carcinomas, all at $p<0.01$. There were significant differences in the pair-wise comparisons of the 7500 ppm dose group with the controls for urinary bladder transitional cell papillomas ($p<0.05$), carcinomas ($p<0.05$), and combined carcinomas/papillomas ($p<0.01$). The incidences of hepatocellular adenomas,

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urinary bladder transitional cell papillomas and urinary transitional cell carcinomas exceeded the respective ranges for the historical controls. The CARC noted that at the week 53 necropsy, transitional epithelial hyperplasia, a preneoplastic stage, was observed in the urinary bladder of mid dose tested (MDT) males and highest dose tested (HDT) males and females. After the 4-week recovery period, this change was still present in HDT males and females. At the terminal necropsy, the transitional cell hyperplasia was observed in HDT males and females, along with an increased incidence of squamous cell metaplasia, high mitotic index and atypia.

The HDT was judged to be excessive based on a significant ($p<0.5$) decrease in body weight gains during week 13 for males and females by 40% and 52%, respectively, as compared to controls. Decreased food efficiency and alterations in hematology and clinical chemistry values were also reported in both sexes at the high dose level. By weeks 52-53, plasma, RBC and brain cholinesterase (ChE) activities were significantly ($p<0.05$) decreased in males by 40%, 22% and 28%, respectively, and in females by 56%, 36% and 37%, respectively, as compared to controls. By week 104, plasma, RBC and brain ChE activities were significantly decreased in males by 42%, 30% and 9%, respectively, and in females by 46%, 38% and 22%, respectively.

The MDT was judged to be below the adequate dose for testing the carcinogenic potential of carbaryl. At this dose, there was no effect on body weight/body weight gain and only minor ChE inhibition (less than 20% inhibition of plasma, RBC and brain ChE in males and females at week 53, except for 26% inhibition of RBC in females; at week 105, only female RBC and brain ChE were decreased (22% and 16%, respectively). The CARC noted that the MDT male rats had transitional cell hyperplasia of the bladder, a preneoplastic lesion, at the week 53 necropsy. If the dose had been adequate, bladder tumors seen at the HDT may have occurred at the MDT.

2. The reanalyses of mouse tumor data showed that male mice had significant increasing trends in kidney tubule cell adenomas ($p<0.05$), carcinomas ($p<0.05$) and combined adenomas/carcinomas ($p< 0.01$). In mice, hemangiomas in the liver and spleen can progress to hemangiosarcomas (Pletcher, JM, personal communication). Therefore, the incidence of hemangiomas and hemangiosarcomas at various sites was combined and analyzed. There were significant differences ($p< 0.05$) in the pair-wise comparison of the ≥ 100 ppm (all doses tested) with the controls for hemangiosarcomas and in combined hemangiomas/hemangiosarcomas at 1000 and 8000 ppm. In addition, a significant difference in the pair-wise comparison of the 8000 ppm dose group with controls was noted for combined kidney tubule cell adenomas/carcinomas ($p< 0.05$).

The female mice had significant increasing trend in hepatocellular adenomas ($p< 0.01$), combined hepatocellular adenomas/carcinomas ($p< 0.01$), hemangiosarcomas($p<0.01$), and combined hemangiomas/hemangiosarcomas ($p<0.05$). There were also significant differences in the pair-wise comparison of the 8000 ppm dose group with the controls for hepatocellular adenomas ($p<0.05$), combined hepatocellular adenomas/carcinomas/hepatoblastomas ($p<0.01$), and hemangiosarcomas ($p<0.05$).

Appropriate historical control data for various types of tumors were not available for comparison (CPRC, 1994). However, based on recently submitted historical control data on vascular tumors in the liver and spleen (sites for most hemangiomas/hemangiosarcomas), the incidence of hemangiosarcomas exceeded the range for the historical controls in both male and female mice.

The CARC considered the dosing at the HDT in male and female mice to be excessive because the decrease in body weight gain, clinical signs and ChE inhibition, and histopathological changes in various organs were indicative of excessive toxicity. **The CARC concluded that the malignant vascular tumors (hemangiosarcomas) in male mice occurred at doses which were adequate and not excessive. In females these tumors occurred only at the highest dose which was excessively toxic. Nevertheless, the findings in female mice were supportive of vascular tumors in male mice.**

Carbaryl produces epoxides and its genotoxicity is manifested as chromosomal aberrations in cultured mammalian cells while older *in vivo* studies indicate negative results for aberrations. More recent studies with cultured cells have demonstrated effects on microtubule assembly, karyokinesis and cytokinesis as well as stress genes associated with oxidative damage.

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified carbaryl into the category **"Likely to be carcinogenic to humans"** based on the following weight-of-the-evidence considerations:

1. Carbaryl induced a statistically significant increase in urinary bladder tumors in male and female rats, thyroid tumors in male rats and liver tumors in female rats. These tumors were induced at an excessively toxic dose (7500 ppm) and, therefore, were not relevant for human cancer risk assessment. However, there was evidence of preneoplastic lesions in the bladder in males at 1500 ppm, a dose which was below the adequate dose for testing the carcinogenic potential of carbaryl. In mice, a treatment-related increase in malignant vascular tumors (hemangiosarcomas) was noted in males at all doses, both excessive and adequate, whereas in females, this same tumor type was seen only at excessive doses.

2. Carbaryl is clastogenic in *in vitro* studies with effects on aberrations; aneuploidy-associated events are also observed and further, a single report from the published literature suggests that carbaryl may induce oxidative stress. These types of effects may contribute to carbaryl-induced tumors. Nevertheless, carbaryl is negative for micronucleus induction in one mouse strain, not clastogenic in Syrian hamsters, and negative in a p53 knockout transgenic mouse bioassay.

VI. QUANTIFICATION OF CARCINOGENICITY

The Committee recommended a low-dose linear extrapolation approach using all dose levels for the quantification of human cancer risk based on the most potent vascular tumors in mice. This approach was supported by the lack of confirmation of a mode of action.

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I. INTRODUCTION

On November 7, 2001, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Program met to reevaluate the carcinogenic potential of carbaryl **in light of the data submitted since 1994 which could influence the classification of the carcinogenic potential of carbaryl. These include the following**: 1) a 1996 Pathology Working Group reexamination of the slides from the rat and mouse carcinogenicity studies (D233467); 2) a reexamination of the interim necropsy slides (MRID 45365503); 3) a p53 knockout mouse study; 4) new historical control data on vascular tumors in CD-1 mice; and 5) various mechanistic studies in both rats and mice.

Dr. Virginia Dobozy of the Reregistration Branch 1 discussed the results of the reexamination of tumor pathology data from rat and mouse carcinogenicity studies (D233467), treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data and the adequacy of the dose levels tested. She also presented toxicology, metabolism, mutagenicity and mechanistic data as well as structure-activity relationships of the related compounds.

II. BACKGROUND INFORMATION

Carbaryl (1-naphthyl methylcarbamate) is one of the most widely used broad-spectrum insecticides in agriculture, professional turf management, professional ornamental production, and in residential pet, lawn and garden markets. Carbaryl is a carbamate insecticide; it's mode of toxic action is through ChE inhibition (ChEI).

The carcinogenic potential of carbaryl was evaluated by the CPRC on October 27 and December 8, 1993 (CPRC, 1994). The Committee concluded that carbaryl induced tumors at multiple sites in the rat and mouse at doses considered to be excessively toxic. Only hemangiosarcomas in the CD-1 male mouse occurred at a dose which was considered adequate and not excessive. The Committee concluded that carbaryl should be classified as a Group C - possible human carcinogen. Both the low-dose extrapolation (Q_1*) approach and a margin of exposure (MOE) approach were suggested as methods of quantifying the cancer risk in humans. In addition, a RfD approach was suggested to provide the most sensitive non-cancer health endpoint for comparison to the linear and MOE approaches. The Committee requested additional metabolism studies and a genotoxicity study to: 1) direct the selection of the more appropriate quantitative approach; and 2) provide insight into the significance of tumors seen only at excessively toxic doses.

Additional metabolism studies were submitted and evaluated by the Metabolism Subgroup of the CARC (October 5, 1998). The subgroup concluded that the available metabolism studies were not adequate evidence to divert from the default linear approach for risk quantification.

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III. REEVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study with Carbaryl in Sprague-Dawley Rats

A. Experimental Design

In a combined chronic toxicity and carcinogenicity study (MRID 42918801), carbaryl technical (99% a.i.) was administered in the diet to Sprague-Dawley rats (70/sex/dose) at dose levels of 0, 250, 1500 or 7500 ppm (0, 10, 60 or 350 mg/kg/day in males and 0, 13, 79 or 486 mg/kg/day in females, respectively) for 104 weeks (MRID 42918801). An additional 10 rats/sex/group were administered carbaryl at the same doses for 52 weeks and then sacrificed. Another 10 rats/sex/group were administered 0 or 7500 ppm of carbaryl for 52 weeks and then basal diet for another four weeks prior to sacrifice.

B. Discussion of Tumor Data

In 1997, the registrant submitted a reevaluation of the slides from the rat combined chronic toxicity/carcinogenicity study by an acceptable PWG which was performed in accordance with PR Notice 94-5 (copy attached) (D233467; HED document 012394). Because of this reexamination of tumor histopathology data, the CARC decided not to review the initial tumor findings.

The results of statistical analyses of tumor data based on the reevaluation of histopathology findings by the PWG are presented below. All tumor counts have been evaluated by HED using the Exact test for trend and the Fisher's Exact test for pair-wise comparisons since specific animal numbers were not given in the PWG report for those animals where changes were noted from the original study pathology report.

The reanalyses of tumor data showed that male rats had significant increasing trends and significant differences in pair-wise comparisons of the 7500 ppm dose group with the controls for thyroid follicular cell adenomas and combined adenomas/carcinomas, and urinary bladder transitional cell papillomas, carcinomas, and combined papillomas/carcinomas, all at $p<0.01$. At 7500 ppm, the incidences of thyroid follicular cell adenoma (13%) as well as urinary transitional cell papillomas (21%) and carcinomas (15%; refer to Tables 1 and 2, respectively) exceeded the respective ranges for the historical controls (thyroid: follicular cell adenoma: 0% - 12%; urinary bladder: transitional cell papilloma: 0 % - 1.1% and transitional cell carcinoma: 0% - 1.4%; refer to Table 5). Interstitial cell tumors of the testes were statistically analyzed and the incidences were not found to be statistically significant (CPRC, 1994).

As determined during the earlier review (CPRC, 1994), the thyroid follicular cell tumor incidence in the females was not statistically different from controls. However, based on the reread of tumor data, the female rats had a significant increasing trend ($p<0.01$) and a significant increase by pair wise comparison of the 7500 ppm dose group with the controls for hepatocellular adenomas ($p<0.05$; Table 3). The reread of tumor data by the PWG

showed that the female rats had significant increasing trend for urinary bladder transitional cell papillomas, carcinomas and combined papillomas/carcinomas, all at $p<0.01$. There were significant differences in the pair-wise comparisons of the 7500 ppm dose group with the controls for urinary bladder transitional cell papillomas ($p<0.05$), carcinomas ($p<0.05$), and combined carcinomas and/or papillomas ($p<0.01$; See Table 4). Thus, at 7500 ppm, the incidences of hepatocellular adenomas (11%), urinary bladder transitional cell papillomas (9%) and urinary transitional cell carcinomas (6%) exceeded the respective ranges for the historical controls (hepatocellular adenomas; 0%-6.3%; urinary bladder: transitional cell papilloma: 0 % - 1.4% and transitional cell carcinoma: 0% - 0%; see Table 5). The statistical analyses of the liver tumors in the original analysis were based on Peto's prevalence test since there was a statistically significant negative trend for mortality in female rats with increasing doses of carbaryl.

Table 1. Carbaryl - Charles River Sprague-Dawley Crl:CD®BR Rat Study: **1996 PWG Re-Read**

Male Thyroid Follicular Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)-Brunsman, 2001

	Dose (ppm)			
	0	250	1500	7500
Adenomas	0/66	2/66	0/68	9/68
(%)	(0)	(3)	(0)	(13)
p =	0.0001**	0.2481	1.0000	0.0017**
Carcinomas	0/66	0/66	1/68	0/68
(%)	(0)	(0)	(1)	(0)
p =	0.7463	1.0000	0.5075	1.0000
Combined	0/66	2/66	1/68	9/68
(%)	(0)	(3)	(1)	(13)
p =	0.0001**	0.2481	0.5075	0.0017**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 2. Carbaryl - Charles River Sprague-Dawley Crl:CD®BR
Rat Study: **1996 PWG Re-Read**

Male Urinary Bladder Transitional Cell Tumor Rates* and
Exact Trend Test and Fisher's Exact Test Results (p values)-Brunsman, 2001

	Dose (ppm)			
	0	250	1500	7500
Papillomas (%)	0/66 (0)	0/66 (0)	0/68 (0)	14/68 (21)
p =	0.0000**	1.0000	1.0000	0.0000**
Carcinomas (%)	0/66 (0)	0/66 (0)	0/68 (0)	10/68 (15)
p =	0.0000**	1.0000	1.0000	0.0008**
Combined (%)	0/66 (0)	0/66 (0)	0/68 (0)	24/68 (35)
p =	0.0000**	1.0000	1.0000	0.0000**

*Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 3. Carbaryl - Charles River Sprague-Dawley Crl:CD®BR Rat Study

Female Hepatocellular Tumor Rates* and
Peto's Prevalence Test Results (p values)-CPRC, 1994

	Dose (ppm)			
	0	250	1500	7500
Adenomas (%)	1[a]/54 (2)	0/56 (0)	3/61 (5)	7/65 (11)
p =	0.002**	-	0.194	0.016*

*Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]First adenoma observed at week 78, dose 0 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 4. Carbaryl - Charles River Sprague-Dawley Crl:CD®BR Rat Study: **1996 PWG Re-Read**

Female Urinary Bladder Transitional Cell Tumor Rates* and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	250	1500	7500
Papillomas (%)	1[a]/86 (1)	0/79 (0)	0/77 (0)	8/86 (9)
p =	0.0001**	0.5212	0.5276	0.0171*
Carcinomas (%)	0/86 (0)	0/79 (0)	0/77 (0)	5[b]/86 (6)
p =	0.0011**	1.0000	1.0000	0.0294*
Combined (%)	1/86 (1)	0/79 (0)	0/77 (0)	13/86 (15)
p =	0.0000**	0.5212	0.5276	0.0006**

*Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 42.

[a]First papilloma observed at week 42, dose 0 ppm.

[b]First carcinoma observed at week 98, dose 7500 ppm.
Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 5 summarizes the historical control tumor incidence (%) reported for Sprague Dawley rats at Hazleton Washington from March 1985 to May 1992. These data were used by the CARC for comparison to the reanalyzed tumor data. Historical control data were not provided for the incidence of liver tumors in males or thyroid tumors in females (CPRC, 1994).

Table 5. Historical Control Tumor Incidence for Liver, Thyroid, Kidney, and Urinary Bladder Tumors in Males and Females in Sprague Dawley Rats[a] (CPRC, 1994)

	Males	Females
Liver		
Hepatocellular adenoma	-	0 - 6.3
Hepatocellular carcinoma	-	0 - 4.0
Thyroid		
Follicular cell adenoma	0 - 12.0	-
Follicular cell carcinoma	0 - 8.0	-
Kidney		
Tubule cell adenoma	0 - 0	0 - 0
Tubule cell carcinoma	0 - 2.0	0 - 0
Urinary Bladder		
Transitional cell papilloma	0 - 1.1	0 - 1.4
Transitional cell carcinoma	0 - 1.4	0 - 0

[a]Average historical control tumor incidence and range (%) reported in 27 studies using Sprague-Dawley rats at Hazleton Washington from March 1985 to May 1992. Studies were 104 weeks in duration.

C. Non-Neoplastic Lesions

Statistical evaluation of mortality indicated no significant incremental changes with increasing doses of carbaryl in male rats. Female rats showed statistically significant decreasing trend in mortality with increasing doses of carbaryl (CPRC, 1994). Clinical signs of toxicity observed for the high dose males and females included chromodacryorrhea, alopecia of the front limbs and urine stains. Body weight gains at the 7500 ppm level were decreased at the 13, 52 and 104 week intervals in males to 60%, 60% and 47% of control levels, respectively, and in females to 48%, 35% and 31%, respectively. Body weight gain of females fed the 1500 ppm level decreased significantly at the 13, 53 and 104 week intervals to 91%, 92% and 82% of control values, respectively. Food consumption at the 7500 ppm level was decreased by the 13,

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52 and 102 week intervals in males and females to 83-86%, 79-84% and 88-96%, respectively, of the control values.

Hematological findings were limited to decreases in leukocytes and lymphocytes in male and female rats at the 7500 ppm level. Significant increases in cholesterol and BUN were observed accompanied by significant decreases in AST, ALT and CK values in male and female rats at the 7500 ppm level. By weeks 52-53, plasma, RBC and brain ChE activities were significantly ($p<0.05$) decreased in males by 40%, 22% and 28%, respectively, and in females by 56%, 36% and 37%, respectively, as compared to controls. By week 104, plasma, RBC and brain ChE activities were significantly decreased in males by 42%, 30% and 9%, respectively, and in females by 46%, 38% and 22%, respectively. In addition, by week 53 at the 1500 ppm level, RBC and brain ChE activities were decreased significantly in males by 19% and 10%, respectively, and in females by 26% and 13%, respectively. By week 105, RBC and brain ChE activities in female rats fed the 1500 ppm level were decreased significantly by 22% and 16%, respectively. ChE activities (plasma, RBC and brain) of the recovery animals returned to control values when measured at week 53.

At week 53, absolute organ weights of lung, liver, spleen and kidneys were decreased and organ/body weight ratios were significantly increased in male and female rats at the HDT. At terminal sacrifice, absolute weights of adrenal, spleen, liver and kidneys were decreased in the high dose male and female rats, with significant increases in organ/body weight ratios for these organs in males and females. The incidence of unilateral and bilateral cataracts was significantly increased in HDT males and females at week 104 of the study.

Non-neoplastic histopathological findings were limited to the liver, urinary bladder, lung, kidney, thyroid and sciatic nerve. **On the registrant's re-examination of histopathology slides from the week 53 sacrifice (MRID 45365503), there was evidence of preneoplastic changes in the urinary bladder in males and females and thyroid gland in males.** An increased incidence of transitional epithelial hyperplasia (a preneoplastic stage) was noted in the urinary bladder of the MDT males and HDT males and females. After the 4-week recovery period, this change was still present in the HDT males and females. In all cases, the hyperplasia was diffuse and affected the entire surface of the urothelium. There were no signs of inflammatory infiltration. There was an increased incidence of hyperplasia of the cuboidal epithelium lining the papillary surface of the renal pelvis in the MDT and HDT males at the week 53 sacrifice. After the 4-week recovery period, no difference between treatment and controls was observed. Female rats were unaffected. Follicular cell hypertrophy was observed in the thyroid of male rats from all groups, including the controls, at the week 53 sacrifice. Although there was an increased incidence in HDT group as compared to the control, there was no dose-related effect. At week 57, 3/9 HDT males were affected vs 0/9 controls. There was an increased incidence of hepatocellular hypertrophy in the liver of the MDT males and HDT males and females at the week 53 necropsy. The hypertrophy was located in the centrilobular area, except in two HDT males in which the change was diffuse. The severity was minimal, being graded slight to mild. After the 4-week recovery period,

there was no evidence of a difference between treated and control animals.

At the terminal sacrifice, non-neoplastic lesions were limited to the HDT group. There was an increased incidence of hepatocyte hypertrophy observed in male and female rats accompanied by an increased incidence of eosinophilic foci in the liver in female rats. In the urinary bladder, an increased incidence of transitional cell hyperplasia in males and females was observed, along with increased incidence of squamous metaplasia, high mitotic index and atypia. An increased incidence of focal pneumonitis in males and females was reported. An increased incidence of transitional cell hyperplasia of the kidney was observed in males. An increased incidence of thyroid follicular cell hypertrophy was observed in males and females. Degeneration of the sciatic nerve was observed in males and females with increased incidence of degeneration of skeletal muscle.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

The HDT was considered by the CPRC (1994) to be excessive based on a significant ($p<0.5$) decrease in body weight gains during week 13 for males and females by 40% and 52%, respectively, as compared to controls. Decreased food efficiency and alterations in hematology and clinical chemistry values were also reported in both sexes at the high dose level. By weeks 52-53, plasma, RBC and brain ChE activities were significantly ($p<0.05$) decreased in males by 40%, 22% and 28%, respectively, and in females by 56%, 36% and 37%, respectively, as compared to controls. By week 104, plasma, RBC and brain ChE activities were significantly decreased in males by 42%, 30% and 9%, respectively, and in females by 46%, 38% and 22%, respectively. In addition, by week 53 at the 1500 ppm level, RBC and brain ChE activities were decreased significantly in males by 19% and 10%, respectively, and in females by 36% and 13%, respectively. By week 105, RBC and brain ChE activities in female rats fed the 1500 ppm level decreased significantly by 22% and 16%, respectively. On the registrant's re-examination of histopathology slides from the week 53 sacrifice (MRID 45365503), at 1500 and 7500 ppm, preneoplastic changes were noted in the urinary bladder of both sexes and in the thyroid gland in males. The urinary changes were not reversible in the HDT recovery group of both sexes. The severity of hepatocellular hypertrophy in the liver of the MDT males and HDT males and females seen at the week 53 necropsy was graded slight to mild and after the 4-week recovery period, there was no evidence of a difference between treated and control animals. The CARC concluded that the HDT tested was excessive. The MDT was judged to be below adequate for testing the carcinogenic potential of carbaryl. At this dose, there was no effect on body weight/body weight gain and only minor ChEI (less than 20% inhibition of plasma, RBC and brain ChE in males and females at week 53, except for 26% inhibition of RBC in females; at week 105, only female RBC and brain ChE were decreased (22% and 16%, respectively). The CARC noted that the MDT male rats had transitional cell hyperplasia of the bladder, a preneoplastic lesion, at the week 53 necropsy. If the dose had been adequate, bladder tumors seen at the HDT may have occurred at the MDT.

2. Carcinogenicity Study in Mice

a. Experimental Design

In a carcinogenicity study (MRID 42786901), carbaryl technical (99.3% ai)was fed to 70 CRL:CD-1®(ICR)BR mice/sex/group at levels of 0, 100, 1000, or 8000 ppm for 24 months . An additional group of 10 mice/sex/group was designated for interim sacrifice after 12 months of treatment. These doses were equivalent to 0, 15, 146 or 1249 mg/kg/day for males and 0, 18, 181 or 1441 mg/kg/day for females. All of the 100 ppm dose group animals were accidentally dosed with aldicarb, a "non-protocol specified compound," on one day during week 93. Seventeen animals died as a result of this error (9 males and 8 females).

b. Discussion of Tumor Data

In 1997, the registrant submitted a reevaluation of the slides from the mouse carcinogenicity study by an acceptable PWG which was performed in accordance with PR Notice 94-5 (D233467; HED document 012394). Because of this reexamination of tumor histopathology data, the CARC decided not to review the initial tumor findings.

The results of statistical analyses of tumor data based on the reevaluation of histopathology findings by the PWG are presented below. All tumor counts have been evaluated by the HED using the Exact test for trend and the Fisher's Exact test for pair-wise comparisons since specific animal numbers were not given in the PWG report for those animals where changes were noted from the original study pathology report. Appropriate historical controls data for tumors other than vascular tumors were not available for comparison.

The reanalyses of tumor data showed that male mice had significant increasing trends in kidney tubule cell adenomas ($p < 0.05$), carcinomas ($p < 0.05$) and combined adenomas/carcinomas ($p < 0.01$). A significant difference in the pair-wise comparison of the 8000 ppm dose group with controls for combined kidney tubule cell adenomas/carcinomas was also observed ($p < 0.05$). In mice, hemangiomas in the liver and spleen can progress to hemangiosarcomas (Pletcher, JM, personal communication). Therefore, the incidences of hemangiomas and hemangiosarcomas at various sites were combined and analyzed. There were significant differences in the pair-wise comparisons of the ≥100 ppm dose groups (all doses tested) with the controls for hemangiosarcomas and for combined hemangiomas/ hemangiosarcomas at 1000 and 8000 ppm ($p < 0.05$). See Tables 6 and 7 for incidences of kidney and vascular tumors, respectively, in male mice. Appropriate historical control data for tumors other than vascular tumors were not available for comparison (CPRC, 1994). However, based on recently submitted data on vascular tumors, the incidence of hemangiosarcoma (11%-12%%) exceeded the range for the historical controls (Liver: 0%-8%; Spleen: 0%-4.2%).

The female mice had a significant increasing trend in hepatocellular adenomas ($p< 0.01$), combined hepatocellular adenomas/carcinomas/hepatoblastomas ($p< 0.01$),

hemangiosarcomas ($p< 0.01$), and combined hemangiomas/hemangiosarcomas ($p< 0.05$). There were also significant differences in the pair-wise comparison of the 8000 ppm dose group with the controls for hepatocellular adenomas ($p < 0.05$), combined hepatocellular adenomas/ carcinomas/hepatoblastomas ($p <0.01$), and hemangiosarcomas ($p < 0.05$). See Tables 8 and 9 for tumor analysis results. The incidence of hemangiosarcomas (15%) exceeded the range for the historical controls (Liver: 0%-8%; Spleen: 0%-4.2%).

Table 6. Carbaryl - Charles River CD-1 Mouse Study:
1996 PWG Re-Read

Male Kidney Tubule Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	100	1000	8000
Adenomas (%)	0/66 (0)	0/66 (0)	0/69 (0)	3[a]/68 (4)
p =	0.0156*	1.0000	1.0000	0.1278
Carcinomas (%)	0/66 (0)	0/66 (0)	1/69 (1)	3[b]/68 (4)
p =	0.0200*	1.0000	0.5111	0.1278
Combined (%)	0/66 (0)	0/66 (0)	1/69 (1)	6/68 (9)
p =	0.0004**	1.0000	0.5111	0.0152*

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First adenoma observed at week 76, dose 8000 ppm.
[b]First carcinoma observed at week 105, dose 8000 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 7. Carbaryl - Charles River CD-1 Mouse Study:
1996 PWG Re-Read

Male Vascular Tumor Rates* and Exact Trend Test
and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	100	1000	8000
Hemangiomas	1/66	1/66	2/69	2/68
(%)	(2)	(2)	(3)	(3)
p =	0.2771	0.7519	0.5168	0.5113
Hemangiosarcomas	1/66	7/66	8/69	8/68
(%)	(2)	(11)	(12)	(12)
p =	0.1240	0.0310*	0.0195*	0.0184*
Combined	2/66	7/66	10/69	10/68
(%)	(3)	(11)	(14)	(15)
p =	0.0812	0.0822	0.0186*	0.0174*

*Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 8. Carbaryl - Charles River CD-1 Mouse Study:
1996 PWG Re-Read

Female Hepatocellular Tumor Rates* and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	100	1000	8000
Adenomas (%)	1/63 (2)	0/70 (0)	1/66 (2)	7[a]/61 (11)
p =	0.0004**	0.4737	0.7402	0.0276*
Carcinomas (%)	0/63 (0)	1/70 (1)	1/66 (2)	2/61 (3)
p =	0.1078	0.5263	0.5116	0.2400
Hepato-blastomas (%)	0/63 (0)	0/70 (0)	0/66 (0)	1/61 (2)
p =	0.2346	1.0000	1.0000	0.4919
Combined (%)	1/63 (2)	1/70 (1)	2/66 (3)	10/61 (16)
p =	0.0000**	0.7249	0.5176	0.0036**

*Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 9. Carbaryl - Charles River CD-1 Mouse Study:
1996 PWG Re-Read

Female Vascular Tumor Rates* and Exact Trend Test
and Fisher's Exact Test Results (p values)-Brunsman, 2001

	Dose (ppm)			
	0	100	1000	8000
Hemangiomas (%)	2/63 (3)	1/70 (1)	1[a]/66 (2)	0/61 (0)
p =	0.1295	0.4603	0.4824	0.2561
Hemangiosarcomas (%)	2/63 (3)	4/70 (6)	3/66 (5)	9[b]/61 (15)
p =	0.0058**	0.3915	0.5221	0.0236*
Combined (%)	4/63 (6)	4[c]/70 (4)	4/66 (6)	9/61 (15)
p =	0.0220*	0.5806	0.6148	0.1082

*Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First hemangioma observed at week 93, dose 1000 ppm.
[b]First hemangiosarcoma observed at week 74, dose 8000 ppm.
[c]One animal in the 100 ppm dose group had both an hemangioma and an hemangiosarcoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p<0.05$. If **, then $p<0.01$

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In a recent submission (MRID 45365501), the Registrant provided historical control data on the incidence of hemangiomas and hemangiosarcomas in the liver and spleen of male mice since the majority of tumors were found in these two organs. These data were collected from various laboratories including Corning Hazleton Virginia (1988-1993; 25 studies), Corning Hazleton Wisconsin (1986-1993; 11 studies), Charles River Laboratories, (1978-1984 ; number of studies not reported and 1981-1991; 13 studies), Pharmaco LSR (1986-1993; 27 studies) and publications by Maita et al., 1988 [1982-1987; 11 studies] and Chandra and Frith, 1992 [1983-1990; 11 studies]). The registrant stated that vascular tumors in other organs are rare and generally not listed and/or routinely histologically evaluated.

The vascular tumors in female mice were seen at various sites including liver, spleen, heart and uterus. The data on vascular tumors for female CD-1 mice in the present study showed that with the exception of one hemangiosarcoma in 100 ppm dose group located in the heart and one hemangioma in the uterus in the control female, the majority of vascular tumors were seen in the liver and spleen. It was difficult to compare the incidences of vascular tumors with historical control data due to lack of site specific break down of the submitted vascular tumor data in the revised PWG evaluation. Therefore, for the purposes of comparison, the CARC used the historical control data from the above sources. The historical control ranges for hemangiomas and hemangiosarcomas were as follows:

Liver

Hemangiomas	0 - 4%
Hemangiosarcomas	0 - 8%

Spleen

Hemangiomas	0 - 4%
Hemangiosarcomas	0 - 4.2%

The increased incidences of hemangiosarcomas at all doses in male mice were above concurrent controls and exceeded the historical control range.

c. Non-neoplastic lesions and other findings

Statistical evaluation of mortality indicated no significant incremental changes with increasing doses of carbaryl in male and female mice.

Clinical signs of toxicity were observed for the high dose males and females. These signs included tremors, hunched posture, languid appearance and urine stains. Eyes of females at the high dose were opaque in appearance during the last three months of the study. There was a corresponding increased incidence of unilateral and bilateral cataracts in the mid-dose males and high-dose males and females.

During week 93, the animals in the 100 ppm group were fed what the study report refers to as a "non protocol specified compound" which caused the death of 17 animals (9 males and 8

females). This "compound" was identified as aldicarb in Table 4 of the preliminary report of adverse histopathological findings received from Rhone-Poulenc July 21, 1992 (DER 10092).

Mean body weight gain of the 8000 ppm group decreased during the 104-week study in males and females to 62-77% and 68-90% of the control values, respectively, accompanied by a 7-10% decrease in food consumption. There were significant decreases in HCT, HGB and RBC values in high dose females and males by weeks 53 and 104, respectively.

Absolute liver weight of the 8000 ppm females increased significantly by week 53. Relative liver weight of high dose males and females was significantly increased at the 53- and 104-week intervals. Liver-to-brain weight ratio of the 8000 ppm males and females was significantly increased by week 53. Kidney-to-body weight ratio was significantly increased in the mid- and high-dose males at week 53 and in high-dose males and females by week 104.

The urinary bladders of the mid- and high-dose males and females were characterized by intracytoplasmic protein-like droplets (stained intensely eosinophilic) filling the cytoplasm of the superficial transitional epithelial cells at the interim and terminal sacrifice.

The increased incidence of chronic progressive nephropathy in the mid-dose males and high-dose males and females at the interim sacrifice was comparable between the test and control animals at the terminal sacrifice.

An increased incidence of extramedullary hematopoiesis and pigment in the spleens of the high-dose males and females at the interim and terminal sacrifice was considered treatment-related due to the decrease in erythrocyte values.

In addition, brain ChE of the high dose males and females was depressed during weeks 53 and 104 by 34% to 57%, accompanied by a 30% decrease in erythrocyte ChE activity in males during week 53. At the mid-dose, brain ChE activity decreased by 13 to 18% during week 53 for males and females and during week 104 for females accompanied by a 23% decrease in erythrocyte ChE activity for males during week 53.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The HDT, 8000 ppm, was considered by the CPRC (1994) to be excessive for carcinogenicity testing based on significantly decreased body weight gain in males (33%) and females (19%) during week 13, a significant decrease in erythrocyte and brain ChE activity, clinical signs of toxicity and histopathological changes of the bladder, kidneys, and spleen in both sexes. These adverse effects, when considered together, indicated to the CPRC that the high dose was excessive. The CPRC agreed that the statistically significant increases in hemangiosarcomas, and combined hemangiomas/hemangiosarcomas in male mice at the mid-dose level were related to carbaryl administration and relevant to human risk assessment. The Metabolism subgroup could not determine if there was a change in metabolism in mice at 1000 ppm, a dose that was considered adequate (CPRC, 1994) and caused tumors. The CARC considered the dosing at the highest dose in male and female mice to be excessive due to the decrease in body weight gain, clinical signs and cholinesterase inhibition, and histopathological changes in various organs were indicative of excessive toxicity. **The CARC concluded that the vascular tumors**

in male mice also occurred at doses which were not excessive. In addition, the findings in female mice were supportive of vascular tumors in male mice.

3. Carcinogenicity and Other Studies in p53 Knockout Mice

In a special, non-guideline study (MRID 45281801), heterozygous p53-deficient (knockout) male mice (20/group) were administered carbaryl in the diet at concentrations of 0, 10, 30, 100, 300, 1000 and 4000 ppm (approximately 0, 1.8, 5.2, 17.5, 51.2, 164.5 and 716.6 mg/kg/day, respectively) for six months. The doses selected for this study were based on two 28-day studies (MRID 45236603) in wild-type mice in which body weight decreases were observed at 4000 and 8000 ppm concentrations of carbaryl in the diet. A validation study (MRID 45281802) demonstrated that vascular tumors occur in heterozygous p53-deficient mice within 6 months of administration of a known genotoxic carcinogen (urethane). These studies were conducted to demonstrate that carbaryl is a non-genotoxic carcinogen. In the standard mouse carcinogenicity study (MRID 42786901) at dietary concentrations of 0, 100, 1000 or 8000 ppm, there was an increased incidence of vascular neoplasms (hemangiomas and hemangiosarcomas) in all treated males and in the 8000 ppm group females. There was an increased incidence of adenomas, multiple adenomas and carcinomas of the kidney in the 8000 ppm group males. The incidence of hepatic neoplasms (adenomas, carcinomas and one hepatoblastoma) was increased in the 8000 ppm group females. At meetings on October 27 and December 8, 1993, the HED Cancer Peer Review Committee concluded that the 8000 ppm dose was excessive. Therefore, the relevance of tumors at this dose was questionable.

In the p53 knockout mouse study with carbaryl, there was a slight decrease in body weight and food consumption in the 4000 ppm group. No other treatment-related effects were observed, except globular deposits in the urinary bladder were observed in a high proportion of the mice treated at 100 ppm of carbaryl and above with a dose-related increase in incidence and severity. There was no evidence of local irritation or hypertrophy of the bladder epithelium. There was no evidence of neoplastic or preneoplastic changes in the vascular tissue of any organs examined.

The study is classified **Acceptable (non-guideline)**. This is a special study not submitted to fulfill a data requirement.

The CARC concluded that the study was properly conducted and the conclusions seem reasonable. A study with heterozygous p53-deficient mice exposed to a known genotoxic carcinogen demonstrated that the model is valid for vascular tumors. However, the p53 knockout model has not been validated by EPA.

IV. TOXICOLOGY

1. Metabolism

In a rat metabolism study (MRID # 43332101), ^{14}C-Carbaryl was administered orally in carboxymethylcellulose or intravenously in sodium phosphate buffer (pH 6.8) to groups (5 sex/dose) of male and female Sprague-Dawley rats at nominal doses of 1 mg/kg (single and

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repeated low oral doses; intravenous dose) and 50 mg/kg (single high oral dose). Absorption was essentially complete for all dose groups of male and female rats. At 168 hours post-dose, there were negligible percentages of the dose found in any tissue examined. On a µg/g tissue basis, kidney and blood were found to contain the highest concentrations of residual radioactivity, with female rats showing slightly higher values than males. Excretion of carbaryl derived radioactivity was largely through urine, where 88-95% of the dose was recovered for all dose groups. There were no significant dose- or sex-related differences in excretion.

Conjugated metabolites of carbaryl identified in this study included the glucuronic acid conjugate of dihydro-dihydroxy carbaryl (2.2% of the dose), the S(N-acetylcysteine) conjugate of dihydro-hydroxy carbaryl (3.7% of the dose), naphthyl glucuronide (2.0% of the dose), and naphthyl sulfate (6.4% of the dose). Non-conjugated metabolites identified were 1-naphthol, 5-hydroxycarbaryl, 5,6-dihydro-5,6-dihydroxycarbaryl, 4-hydroxycarbaryl, and N-(hydroxymethyl)-hydroxycarbaryl. These accounted for 14.5%, 12.8%, 8.2%, 6.3%, and 5.7% of the administered dose, respectively. Three new metabolites were identified in this study which were the N-(hydroxymethyl)-hydroxycarbaryl metabolite, hydroxy-desmethylcarbaryl (0.5% of the dose), and the S-(N-acetylcysteinyl)-dihydro-dihydroxycarbaryl conjugate. Based on these data, a metabolic scheme for carbaryl was proposed. This study is classified as **acceptable/guideline** and **satisfies** the data requirements for a metabolism study in rats under Subdivision F guideline §85-1.

Metabolism - Special Study in the Rat

In a rat metabolism study (MRID No. 44402501), 1-naphthyl-^{14}C-labeled carbaryl (ca 100% a.i.) was administered to 15 month old male Iffa Credo CD (Sprague-Dawley derived) rats (5 animals/group) as a single oral gavage dose of 50 mg/kg (group A) or as a daily oral dose of 2 mg/kg for 7 days following a 83-day dietary administration with non-radioactive carbaryl (25 animals/group) at 0 (group B), 250 (group C), 1500 (group E), or 7500 ppm (group D). This study was designed to "investigate the mechanisms that caused the appearance of an increased incidence of tumors during the final year of a chronic dietary feeding study in the rat at the high dose level of 7500 ppm."

In all dietary dosing regimens, urinary and fecal excretion totaled 96-103% of the administered dose. Most of the radioactivity was eliminated in the urine and feces within 24 hours after dosing. In the group A, 86% and 11% of the test compound administered was excreted in the urine and feces, respectively, over a 7-day period after a single dose via gavage of radio labeled carbaryl at 50 mg/kg. In the groups B-E (0, 250, 1500, and 7500 ppm), 3 days after the 7th consecutive administration of radio labeled carbaryl, 79-89% and 7-10% of the total administered dose (sum of the 7 daily doses) were excreted in the urine and feces, respectively. Tissue distribution study showed that the levels of radioactivity in the tissues of the animals from group A were 0.4% of the administered dose at sacrifice (168 hours after dosing). In groups B-E, the levels of radioactivity in the tissues ranged from 0.4-0.8% of the administered dose 3 days after the 7th dose of radio labeled carbaryl at 2 mg/kg. This indicates that the potential for bioaccumulation of carbaryl in rats is minimal.

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HPLC analysis of carbaryl metabolites in 24-hour urine samples showed a total of 23 components. Four components identified by LC/MS technique were as follows: UMET/8 (trans-5,6-dihydro-5,6-dihydroxy-1-naphthyl N-methylcarbamate) (accounted for 3.75-6.38% of the dose); UMET/11 (glucuronide of dihydro-dihydroxy-1-naphthyl N-methylcarbamate) (18.55%-28.46% of the dose); UMET/18 (α-naphthyl β-D-glucuronide sodium salt or α-naphthyl sulfate potassium salt (15.69-21.75 % of the dose); and UMET/23 (naphthyl sulfate) (17.78%-30.01% of the dose).

A total of 20 components was detected in the 24-hour feces by HPLC analysis. One component (FMET/15) was identified as parent and accounted for 0.2-1.4% of the administered dose by LC/MS technique. The remaining 19 components were not identified because the levels of radioactivity in these components were too low.

There were 2 major metabolites in the tissues from groups B-E at 6 hours after administration of ^{14}C-carbaryl. These metabolites were confirmed by LC/MS analysis as naphthyl sulfate (found in plasma, kidney, and urinary bladder) and naphthyl glucuronide (found in the kidney and urinary bladder). Quantitative identification for these metabolites was not available because the levels of radioactivity in these tissues were too low.

The sulfate conjugation pathway appears to be saturable following a subchronic (83-day) feeding of carbaryl at a high dose (group D, 7500 ppm). This saturation of the sulfate conjugation pathway is seen in the urinary levels of UMET/23 (naphthyl sulfate) between the dose groups following the 83-day dietary administration of non-radioactive carbaryl. The level of radioactivity associated with UMET/23 (naphthyl sulfate) was higher (23-27% of the dose) in 0, 250, and 1500 ppm dose groups and lower (12% of the dose) in the 7500 ppm group. On the other hand, the level of radioactivity associated with UMET/11 (naphthyl glucuronide) was lower (15-21% of the administered dose) in 0, 250, and 1500 ppm dose groups and higher (28%) in the group 7500 ppm group.

Statistically significant decreases ($p<0.05$ or $p<0.01$) in body weight (9-20%) when compared to the control group were observed only in the 7500 ppm group as early as study day 14 and sustained throughout the remainder of the study. In the 7500 ppm group, the statistically significant decreases ($p<0.05$ or $p<0.01$) in food consumption were observed at week 1 (74%), week 2 (61%), week 3 (40%), and weeks 4-11 (19-31%). In the 1500 ppm group, the statistically significant decreases ($p<0.05$) in food consumption were observed at week 5 (8%), week 10 (21%), and week 11 (12%).

Significant increases (statistical analyses were not performed) in kidney, spleen, and thyroid weights were observed in the 1500 or 7500 ppm groups when compared to the control group. Absolute and relative liver weights increased 18% and 39%, respectively, at 7500 ppm. Absolute spleen weight increased 30% at 7500 ppm and relative spleen weight increased 24% and 30% at 7500 and 1500 ppm, respectively. Absolute thyroid weight increased 63% and 69% at 7500 and 1500 ppm, respectively, and relative thyroid weight increased 103% and 121% at 7500 and 1500 ppm, respectively. Statistically significant increases ($p<0.01$) in total

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glutathione concentrations (higher by 79% per g of liver or 102% per g of protein) were observed at 7500 ppm only, compared to the controls.

The incidences of hepatocellular adenoma (benign) were 1/5, 0/5, 0/5, and 2/5 at 0, 250, 1500, and 7500 ppm, respectively. Although the authors concluded that "there was no treatment-related change in the incidence of tumors under carbaryl treatment," definite conclusion cannot be made from this finding based on the limited number of animals used.

Significant treatment-related changes were noted in liver, thyroid glands, and kidneys at 7500 ppm only. The incidences of centrilobular hypertrophy of the hepatocytes, pericholangitis (an inflammatory cell infiltrate around biliary ducts), and bile duct hyperplasia were 5/5, 3/5, and 3/5, respectively. The incidences of follicular cell hypertrophy of the thyroid glands were 0/5, 3/5, 5/5, and 5/5 and the incidences of transitional cell hyperplasia of the renal pelvis were 0/5, 0/5, 1/5 and 2/5 at 0, 250, 1500, and 7500 ppm, respectively.

This metabolism study in the rat is classified acceptable for its intended purpose of investigating "the mechanisms that caused the appearance of an increased incidence of tumors during the final year of a chronic dietary feeding study in the rat at the high dose level of 7500 ppm." Although the study supplies some information to the Agency, this study **does not satisfy** the guideline requirement for a metabolism study (85-1) in rats.

Metabolism - Special Study in the Mouse

In a special metabolism study (MRID 45236604), five groups of 9-10 male CD-1 mice received diet containing 0, 10, 100, 1000 or 8000 ppm of non-radio labeled carbaryl for a period of 14 days followed by a single oral dose of radio labeled material (1-naphthyl-^{14}C carbaryl) by gavage on the 15th day. Urine and feces were collected at 24-hour intervals following dosing. All animals were euthanized 168 hours after administration of the dose. The amount of radioactivity (as a percentage of administered dose) in the urine, feces, blood and residual carcass was determined. The cages were washed with distilled water every 24 hours and the amount of radioactivity determined. Urine samples were pooled according to time period for metabolite quantification using HPLC. Aliquots of pooled urine (Day 1) from the mice treated at 8000 ppm were subjected to enzymatic hydrolysis. The study was conducted to investigate the mechanism that caused an increase in tumors in CD-1 mice at dose levels of 1000 and 8000 ppm in the carcinogenicity study.

The total recovery of radioactivity, expressed as a percentage of the total administered dose, ranged from 88.75% to 100.85% (mean 96.23%) at 0 ppm, from 68.24% to 93.42% (mean 88.66%) at 10 ppm, from 95.49% to 103.56% (mean 99.95%) at 100 ppm, from 98.90% to 103.94% (mean 100.99%) at 1000 ppm and from 93.67% to 103.80% (mean 98.29%) at 8000 ppm. Elimination of radiolabel in the urine was greater than the feces for all five groups. The mean levels of elimination via the urine for the groups were respectively: 0 ppm, 68.55%; 10 ppm, 55.78%; 100 ppm, 64.57%; 1000 ppm, 68.89%; and 8000 ppm, 69.25%. The mean levels of recovery in the feces were respectively: 0 ppm, 12.21%; 10 ppm, 15.44%; 100 ppm, 18.40%; 1000 ppm, 16.47%; and 8000 ppm, 18.64%. The majority of the radioactivity in the urine and

feces was eliminated in the first 48 hours post dosing. Very low levels of radioactivity were recovered from the residual carcass and cardiac blood. The mean recovery in the residual carcass was as follows: 0 ppm, 0.45%; 10 ppm, 0.50%; 100 ppm, 0.82%; 1000 ppm, 0.34%; and 8000 ppm, 0.24%. The mean recovery of radioactivity in the blood was less than 0.01% for most groups.

A total of 21 components were observed in the pooled urine samples over 96 hours. The four major components for all dose groups were: UMET/1, UMET/12, UMET/16 and UMET/18; however, there were some differences in which component ranked highest in the dose groups. The four components were identified as dihydro, dihydroxy-naphthyl sulphate (UMET/1), hydroxy-carbaryl glucuronide + RPA 110355[1] (UMET/12), α-naphthyl sulphate (UMET/16) and α-naphthyl β-D-glucuronide (UMET/18).

Following the incubation of urine samples at 37°C overnight in the absence of enzyme preparations, none of the four fractions was found to be thermo-sensitive. Incubation with the sulphatase enzyme resulted in the slight reduction of UMET/1 level, the disappearance of UMET/12 and the marked decrease in UMET/16 and UMET/18. Two metabolites, UMET/20 (4-hydroxy carbaryl) and UMET/25(1-naphtol) appeared. The addition of D-saccharolactone, a β-glucuronidase inhibitor, to the incubation medium containing the sulphatase preparation led to the total disappearance of UMET/16 (trace amounts) and the appearance of UMET/25; UMET/12 and UMET/18 were unchanged and UMET/1 was slightly decreased. The incubation of β-glucuronidase from bovine liver resulted in the decrease of UMET/12 and UMET/18 and the appearance of UMET/20 and UMET/25. No significant changes were noted in UMET/1 and UMET/16. Following the incubation of urine samples at 37°C and pH 6.8 the profile resembled that obtained during the metabolic profiling of urine samples from the 8000 ppm group. The incubation of β-glucuronidase from *E. coli* resulted in the marked decrease of UMET/12 and UMET/18 and the appearance of UMET/20 and UMET/25.

This metabolism study in the mouse is classified Acceptable (non-guideline). It is a special study designed to investigate the mechanism that caused an increase in tumors in CD-1 mice at dose levels of 1000 and 8000 ppm in the carcinogenicity study and not intended to comply with specific guidelines .

The CPRC (CPRC, 1994) classified carbaryl as a Group C based on a statistically significant increase in hemangiomas and combined hemangiomas/hemangiosarcomas in male mice at a dose which was adequate and not excessive (1000 ppm). There were additional tumors at the highest dose (8000 ppm in mice and 7500 ppm in rats) that was considered excessive. The Committee requested additional metabolism studies to help direct the selection of the more appropriate quantitative approach for cancer risk and provide insight into the significance of the tumors seen only at excessively toxic doses. On September 3, 1998, the Metabolism subgroup of the CARC reviewed these studies and concluded that the method used in a DNA binding study had limited sensitivity and it could not be determined if the radioactivity from the test

[1] Trans-5,6-dihydro-5,6-dihydroxy-1-naphthyl-N-methylcarbamate

material binds to DNA or not. The P-450 study demonstrated that carbaryl is a weak "phenobarbital-type" inducer in mice. There were no studies to examine the effect of increasing doses on carbaryl metabolism. The subgroup determined that to establish a nonlinear approach for risk estimation one should examine if there was a change in metabolism in mice at 1000 ppm, a dose that was adequate and caused tumors. The subgroup also emphasized, however, that alteration in metabolism, by itself, is not sufficient evidence of a nonlinear mode of action. The registrant postulated that at high doses there were alterations in the metabolism, distribution and excretion pattern of carbaryl, including formation of epoxides, depletion of glutathione, increase in the half-life of the epoxide intermediates and increased tissue concentrations of carbaryl. Concerning the rat studies, the subgroup concluded that while there was an apparent shift in the metabolic pathway from sulfation to glucuronidation at the high dose (7500 ppm), there was no clear evidence of new metabolites being formed. There was no evidence of a correlation between the metabolic shift and increased toxicity or tumor formation. Thus, the subgroup concluded that the data from the available metabolism studies were not adequate evidence to divert from the default linear approach for risk quantitation.

2. <u>Mutagenicity</u>:

During the earlier meetings (on October 27, and December 8, 1993), the CPRC (1994) recommended that an *in vivo* cytogenetic assay in rodents be conducted to provide insight into the structural and/or numerical aberrations, which were observed in the gene mutation assay and reported in the open literature. In response to CPRC's request, a mouse micronucleus assay (MRID 44069301) was submitted to fulfill the guideline requirement but it was classified as unacceptable.

A recent review of the data from the submitted studies and the published literature were in general agreement and show that carbaryl is clastogenic *in vitro*. The wide variety of induced aberrations (both simple and complex) was consistent between the submitted study and the open literature. However, there are inconsistencies relative to the requirement for S9 activation.

Nevertheless, the two *in vivo* studies for micronuclei induction or chromosome aberrations were negative. Similarly, the 6-month p53 knockout transgenic mouse bioassay (see Section III.3) was negative up to a high level (4000 ppm, ≈720 mg/kg/day) that approached the limit dose for a mouse carcinogenicity assay. Carbaryl was also negative for DNA binding in the livers of mice treated with 8000 ppm for 2 weeks but the study was considered to be of limited sensitivity by the CARC Metabolism Subgroup (HED Document No. 012892). The same Subgroup identified epoxide intermediates of carbaryl which were found to be conjugated to glucuronide, "rapidly metabolized and excreted as any endogenous epoxide would be".

Overall, these findings indicate that carbaryl produces epoxides and its DNA reactivity is manifested as chromosomal aberrations in cultured mammalian cells. Other *in vitro* studies indicate carbaryl's effects on karyokinesis and cytokinesis, as well as stress genes associated with oxidative damage. Based on these considerations, it was concluded that there is a concern for mutagenicity, which is somewhat lessened because of the lack of an effect in *in vivo* mutagenicity studies.

GENE MUTATIONS

Mutagenicity - Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay (Ames test)

In a Salmonella/mammalian activation gene mutation assay (MRID 41370303), carbaryl technical (99.3%) was initially evaluated in the Salmonella typhimurium/microsome mutagenicity assay over a concentration range of 5 to 1000 µg/plate. The test material was not mutagenic, however the highest assayed dose was cytotoxic in S. typhimurium strains TA98 and TA100, but not in strains TA1535, TA1537, or TA1538. Accordingly, the assay was repeated with six concentrations (10 to 2000 µg/plate +/-S9). Results from the repeat assay indicated that 2000 µg/plate +/-S9 was cytotoxic in strains TA98 and TA100, and the remaining doses were not mutagenic. It is concluded, therefore, that carbaryl technical was assayed to an appropriately high concentration with no evidence of mutagenicity in a well-conducted study. The study is classified as **acceptable/guideline** and **satisfies** the guideline requirements (§84-2) of bacterial reverse mutation test.

Mutagenicity - Mammalian Cells in Culture Gene Mutation Assay in Chinese Hamster Ovary (CHO) Cells

In a mammalian cells in culture gene mutation assay in Chinese Hamster Ovary (CHO) Cells (MRIDs 41370302, 41420201), carbaryl technical (99.3%) was evaluated in two nonactivated and three S-9 activated Chinese hamster ovary (CHO) cell forward mutation assays. The findings from both nonactivated assays were in good agreement and indicated that over a concentration range of 1 to 300 µg/mL, the test material did not induce a mutagenic response. Doses ≥200 µg/mL were severely cytotoxic (<10% cell survival), and <50% of the cells survived exposure to ≥50 µg/mL. Carbaryl was less cytotoxic in the presence of S9 activation as indicated by increased survival at comparable levels in the preliminary cytotoxicity test (e.g., 29.5% survival at 62.5 µg/mL -S9 as compared with 95.7% survival at 62.5 µg/mL +S9) and the initial mutation assay (e.g., 18.1% survival at 100 µg/mL -S9 as compared with 46.8% at 100 µg/mL +S9). There was no definitive evidence of increased mutation frequencies (MFs) in this trial. The second S9-activated trial was aborted because of excessive cytotoxicity at test material levels of ≥10 µg/mL. Results from the third S9-activated trial (dose range: 1 to 80 µg/mL) showed severe cytotoxic effects at levels ≥60 µg/mL; no evidence of mutagenic effect was seen at the remaining doses.

The results of the assays provide no clear indication of a mutagenic response, however, the study does not fully support a negative conclusion. The conflicting cytotoxicity data for the S9-activated assays provide no assurance that the final S9-activated mutation assay was conducted over an appropriate dose range. The study is classified as **unacceptable/guideline** and **does not satisfy** the guideline requirements (§84-2) for an *in vitro* mammalian cell gene mutation test.

CHROMOSOME ABERRATIONS

Mutagenicity - Mammalian Cells in Culture Cytogenetic Assay

Carbaryl (technical) was assayed for clastogenic effects in both the presence and absence of S9 activation using Chinese hamster ovary (CHO) cells (MRID 41370301). Because of severe cell cycle delay, which was more pronounced without S9 activation, a 20-hour cell harvest was selected to evaluate seven nonactivated doses ranging from 5 to 100 μg/mL. In the presence of S9 activation, cells exposed to carbaryl at doses of 25, 50, 75, 100, 150, 200, 250, and 300 μg/mL were harvested 30 hours post treatment. Results indicated that the nonactivated test material was more cytotoxic than the S9-activated test material (*i.e.*, few metaphases were recovered at 75 and 100 μg/mL , and moderate to slight cytotoxic effects were seen at doses ≥10.0 μg/mL). With the exception of a single rare complex aberration (quadriradial) scored at the 50.0-μg/mL dose level, there was no evidence of a clastogenic effect. By contrast, in the S9-activated assays, all scored doses (150, 200, 250, and 300 μg/mL) at both harvest times induced significant ($p \leq 0.01$) increases in the percentage of cells with aberrations. The majority of S9-activated doses (both harvests) also induced significant ($p \leq 0.01$) increases in the percentage of cells with >1 aberration. At both the 20- and 30-hour harvest times, cytotoxicity (*i.e.*, reduced monolayers, dead cells, and/or reduced mitotic cells) were observed at levels ≥200 μg/mL. Induced structural damage included simple (*i.e.*, chromatid and chromosome breaks) and complex aberrations (*i.e.*, triadials, quadriradials, complex rearrangements, dicentrics and rings). The data show little or no dose responsiveness and the lowest reactive level of carbaryl was not determined. It was concluded, however, that the study was technically sound and, therefore, **acceptable/guideline**. The study **satisfies** the Guideline requirements (§84-2) for an *in vitro* mammalian cell chromosomal aberration test.

Mutagenicity - Mouse Micronucleus Test

In a mouse micronucleus assay (MRID No: 44069301), groups of five male and five female CD-1 mice received single oral gavage administrations of 50, 100 or 200 mg/kg carbaryl (99.9%) once daily for 2 days. Based on analytical determinations, average daily doses were ≈34, 79 or 180 mg/kg. Mice were sacrificed at 24 and 48 hours postadministration of the second dose and harvested bone marrow cells were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs). The test material was delivered as suspensions prepared in 0.5% carboxymethyl cellulose.

The minimal toxicity (i.e., lethargy which lasted for 2 hours) in the absence of cytotoxicity to the target cells does not support the testing of the maximum tolerated dose (MTD). The positive control induced the expected high yield of MPEs in males and females. Carbaryl did not induce a clastogenic or aneugenic effect in either sex at any dose or sacrifice time. However, there was no convincing evidence that the MTD was achieved. The study is classified as **unacceptable/guideline** and **does not satisfy** the guideline requirements(§84-2; OPPTS 870.5385) for *in vivo* cytogenetic mutagenicity data.

OTHER MUTAGENIC EFFECTS

Mutagenicity - UDS Assay

In a UDS Assay in primary rat hepatocytes (MRID 41370301), under the conditions of two independent trials, six doses of carbaryl technical (99.3%) ranging from 0.5 to 25.0 µg/mL in the first assay and six doses ranging from 5.0 to 25.0 µg/mL in the repeat assay did not induce an appreciable increase in the net nuclear grain counts of treated rat hepatocytes. Doses >25.0 µg/mL were severely cytotoxic; reduced cell survival (≈25%) was observed at 25.0 µg/mL in both assays. Although an increase in the percentage of cells with ≥ 6 grains per nucleus was seen in the initial test, the increase was confined to a single dose (10 µg/mL) and was not dose-related or reproducible. The study demonstrated that carbaryl is not genotoxic in this test system at doses of 5.0 to 25.0 µg/mL. The study is classified as **acceptable/guideline** and **satisfies** the guideline requirements (§84-2) for a unscheduled DNA synthesis in mammalian cells in culture.

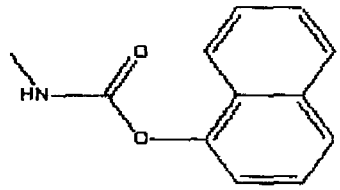
STUDIES FROM THE OPEN LITERATURE

Studies in the open literature indicate that Carbaryl is not mutagenic in bacteria but produced conflicting results in Chinese hamster V79 gene mutation assays [negative in the study of Onfelt and Klasterska (1984) but weakly positive minus S9 metabolic activation as reported by Ahmed et al. (1977)]. Nonactivated carbaryl induced aneuploidy and sister chromatid exchanges in V79 cells; the addition of S9 or an excess of glutathione eliminated these responses (Onfelt and Klasterska 1983, 1984). In the former study, multiple chromatid exchanges (quadriradials and complex rearrangements) plus chromosome breaks were also induced by 100 mM carbaryl; this effect was largely abolished by the simultaneous addition of S9 or glutathione. There are positive data for DNA damage in a human lymphoblastoid cell line (induction of CYP1A1 genes); carbaryl also activated other stress genes known to be sensitive to oxidative damage (Delescluse *et al.*, 2001). Also, carbaryl causes depolymerization of spindle microtubules and an apparent uncoupling of karyokinesis and cytokinesis in cultured V79 cells (Renglin *et al*, 1988, 1989).

In contrast to the *in vitro* data, carbaryl administered by oral gavage at 1/3 of the LD_{50} (146 mk/kg/day) for 2 consecutive days was negative for micronuclei induction in Swiss albino male mice (Usha Rani et al., 1980). Carbaryl was also negative for the induction of chromosome aberrations in bone marrow cells of Syrian hamsters treated with 1/10, 1/5 and ½, of the LD_{50} and the LD_{50} (Dzwonkowska and Hubner, 1986).

3. Structure-Activity Relationship

The chemical structure of carbaryl is as follows:



Carbaryl is structurally related to nine other carbamate insecticides possessing the carbamic acid moiety. The chemicals include aldicarb, bendiocarb, carbofuran, cloethocarb, methiocarb, methomyl, mexacarbate, propoxur and trimethacarb. The CPRC considered these data and concluded that none of these analogues provided good structure activity relationship (SAR) support for the tumor response seen with carbaryl. Propoxur was discussed as a possible analogue for SAR support as it induced urinary bladder tumors (same target as seen at the excessive dose in the carbaryl rat study). However, propoxur was not considered relevant in that there is little evidence that propoxur has genotoxic activity and the induction of urinary bladder tumors appears to have a different mechanism than that considered for carbaryl.

4. Subchronic and Chronic Toxicity

a) Subchronic Toxicity

There are no acceptable subchronic toxicity studies in rodents or nonrodents; however there is an acceptable subchronic neurotoxicity study.

In a subchronic neurotoxicity study (MRID 44122601), 12 Crl:CD(SD)BR rats/sex/group were administered technical Carbaryl (99.1%) by gavage at doses of 0, 1, 10 or 30 mg/kg/day for 13 weeks. Cholinesterase (RBC, whole blood, plasma and brain) determinations were done on an additional three groups of five rats/sex/group at Weeks 4, 8 and 13. Neurobehavioral screening, consisting of Functional Observational Battery (FOB) and motor activity evaluations, was performed prior to treatment and during Weeks 4, 8 and 13. At terminal sacrifice, six animals/sex/dose were anesthetized and perfusion fixed *in situ* for neuropathological evaluation.

There were no deaths during the study. There was an increased incidence of clinical signs of toxicity, including slight and moderate salivation and tremors, in the 30 mg/kg/day males and females. Body weight over the course of the study was statistically significantly decreased in the 30 mg/kg/day males (14%) and females (15%). Body weight gain for these groups was decreased 27% in males and 37% in females, compared to controls. Food consumption was decreased during most of the study for the 30 mg/kg/day males and females. Males and females in the 30 mg/kg/day group had a statistically significant decrease in RBC (M:42-46%; F:52-55%), whole blood (M: 49-51%; F: 59-63%) and plasma cholinesterase values (M: 63-69%; F: 63-69%) at most of the testing periods. Males and females in the 10 mg/kg/day group had a

statistically significant decrease in RBC (M: 26-38%; F: 17-24%); whole blood (M: 30-41%; F: 21-26%) and plasma cholinesterase values (M:43-48%; F: 23-30%). There was a statistically significant decrease in brain cholinesterase in males and females in the 10 mg/kg/day (M: 27-61%; F: 20-58%) and 30 mg/kg/day (M: 36-80%; F: 50-73%) groups. For the 1 mg/kg/day males, there were statistically significant decreases in whole blood (13%) at week 13 and for plasma (20%) at week 8. These changes are not considered toxicologically significant since they occurred infrequently and were relatively minor effects.

Multiple qualitative and quantitative FOB parameters were affected in the 10 and 30 mg/kg/day males and females, including the following: slight tremors, gait alterations, pinpoint pupils, increased salivation, reduced extensor thrust, decreased pinna reflex, reduced number of rearings, decreased vocalizations, decreased body temperature and decreased forelimb grip. Reduced number of defecations was observed only at 30 mg/kg/day. There was an occasional alteration at the 1 mg/kg/day dose. At week 8, males had a very slight increase in the incidence of pinpoint pupils (incidence in control, 1, 10 and 30 mg/kg/day groups was 0/12, 1/12, 6/12 and 10/12, respectively). A statistically significant decrease in forelimb grip was observed at week 4 in males (values for control, 1, 10 and 30 mg/kg/day groups were 1060.8, 943.8, 943.8 and 950.0, respectively). The number of defecations was statistically reduced in females at week 13 (mean number of defecations in control, 1, 10 and 30 mg/kg/day groups were 1.4, 0.2, 0.5 and 0.0, respectively). The toxicological significance of these effects in the 1 mg/kg/day group is questionable since the incidence was either low or there was no dose-response relationship.

Motor activity was statistically significantly decreased in the 30 mg/kg/day males at Week 4 and the 30 mg/kg/day females at Weeks 4 and 8.

On necropsy, there was an increased incidence of dark areas in the meninges of the 30 mg/kg/day males; these animals had an increased incidence of hemorrhage on microscopic examination. One female in the 30 mg/kg/day group also had retinal atrophy. There were no differences in brain length or width measurements.

The LOAEL for neurotoxicity was 10.0 mg/kg/day based on an increased incidence of FOB changes; the NOAEL was 1.0 mg/kg/day. The LOAEL for cholinesterase inhibition was 10.0 mg/kg/day based on statistically significant decreases in RBC, whole blood, plasma and brain cholinesterase; the NOAEL was 1.0 mg/kg/day.

The subchronic neurotoxicity study in the rat is classified **acceptable/guideline** and **does satisfy** the guideline requirement for a subchronic neurotoxicity study (OPPTS 870.6200) in the rat.

c) Chronic Toxicity

In a chronic toxicity study (MRID 40166701), carbaryl (99%) was administered in the diet to 6

beagle dogs/sex/group at doses of 0, 125, 400 or 1250 ppm for one year. Nominal doses were 3.1, 10 and 31.3 mg/kg/day.

There were no deaths during the study. With the 1250 ppm females, there was an increased incidence of clinical signs of toxicity, including emesis, lacrimation, salivation and tremors. Mean body weight gain was decreased (50%) in the 1250 ppm females for weeks 0-6. Mean food consumption was decreased (16-24%, not statistically significant) in the 1250 ppm females at multiple time periods during the study. No treatment-related ophthalmoscopic changes were observed. There was a statistically significant increase in white blood cell and segmented neutrophil counts at some of the testing intervals for the 1250 ppm group males. Albumin levels were significantly decreased (9-11%) at all of the testing periods in the 1250 ppm females. Plasma cholinesterase (ChE) levels in males were significantly decreased in the 400 ppm (30-36%↓) and 1250 ppm (58-66%↓) groups at all testing intervals (weeks 5, 13, 26 and 52). Plasma ChE levels in females were significantly decreased at most intervals in the 125 ppm group (12-23%↓), 400 ppm group (9-31%↓) and 1250 ppm group (47-60↓). RBC ChE levels in males were significantly decreased in the 400 ppm group (23-28%↓at weeks 5 and 13) and 1250 ppm group (46-56%↓ for all intervals). RBC ChE levels in females were significantly decreased in the 400 ppm group (29-34% ↓at weeks 5, 13 and 26) and 1250 ppm (29-38% ↓for all intervals). Brain ChE in males was not statistically significantly decreased but biologically decreased in the 400 ppm group (32%↓) and 1250 ppm group (25%↓). Brain ChE in females was significantly decreased (20-36% ↓) in all the groups. No treatment-related effects were seen in urinalysis parameters.

At necropsy, there was a statistically significant increase in the absolute weight of the liver/gall bladder in the 1250 ppm group males. Relative and liver-to-brain weights were also increased but not significantly. There was a dose-related decrease in the absolute, relative and organ-to-brain weights of the pituitary in males, although none of the changes was statistically significant. There was also a significant decrease in the relative weight of the thyroid in this group. However, since there were no accompanying microscopic changes in these organs, the toxicological significance of these organ weight effects is questionable.

The LOAEL for systemic toxicity was 1250 ppm (31.3 mg/kg/day) based on an increased incidence of clinical signs (females), decreased body weight and food consumption (females) and alterations in clinical pathology parameters (both sexes); NOAEL was 400 ppm (10 mg/kg/day).

The LOAEL for plasma cholinesterase inhibition was 125 ppm (3.1 mg/kg/day) for females; a NOAEL was not established. The LOAEL for plasma cholinesterase inhibition was 400 ppm (10 mg/kg/day) for males; the NOAEL was 125 ppm (3.1 mg/kg/day).

The LOAEL for RBC cholinesterase inhibition was 400 ppm (10 mg/kg/day) for males and females; the NOAEL was 125 ppm (3.1 mg/kg/day).

The LOAEL for brain cholinesterase inhibition was 125 ppm (3.1 mg/kg/day) for females; a NOAEL was not established. The LOAEL for brain cholinesterase inhibition was 400 ppm (10 mg/kg/day) for males; the NOAEL was 125 ppm (3.1 mg/kg/day).

In a five-week study (MRID 42022801), carbaryl (99.3% a.i.) was administered in the diet to six beagles/sex/group at doses of 0, 20, 45 or 125 ppm. Actual mg/kg/day doses for males were 0, 0.59, 1.43 and 3.83 mg/kg/day, respectively; doses for females were 0, 0.64, 1.54 and 4.11 mg/kg/day, respectively. The following parameters were measured: clinical observations, body weights, food consumption, ophthalmoscopic examinations, plasma and RBC cholinesterase (at days -11, -8 and -5 pretest and then days 14 and 32 of the study), brain cholinesterase (at termination) and gross necropsies. This study was conducted to complete the information needed to satisfy the chronic toxicity study requirement in nonrodent species.

There were no deaths or treatment-related clinical signs of toxicity. There were no treatment-related effects on body weights, food consumption or ophthalmoscopic examinations. In males, there was a statistically and biologically significant decrease in plasma cholinesterase for the 125 ppm (22%↓) group.

The LOAEL for systemic toxicity and for RBC and brain cholinesterase inhibition was >125 ppm (males: 3.83 mg/kg/day; females: 4.11 mg/kg/day); the NOAEL was ≥ 125 ppm.

The LOAEL for plasma cholinesterase inhibition for males was 125 ppm; the NOAEL was 45 ppm (1.43 mg/kg/day). The LOAEL for cholinesterase inhibition for females was >125 ppm; the NOAEL was ≥ 125ppm.

Together, these studies are **acceptable** and **satisfy** the guideline requirements for a chronic toxicity study in a nonrodent species (83-1).

Chronic toxicity/carcinogenicity in rats

In a combined carcinogenicity/chronic toxicity study (MRID No. 42918801), 70 Sprague-Dawley Crl:CD®BR rats/sex/group were administered technical Carbaryl (99% a.i.) in the diet at dosages of either 0, 250, 1500 or 7500 ppm for 104 weeks (males: 0, 10.0, 60.2 and 349.5 mg/kg/day; females: 0, 12.6, 78.6 and 484.6 mg/kg/day). An additional 10 animals/sex/dose were administered the same doses and were sacrificed after 53 weeks. Another 10 animals/sex from the control and high dose group animals were sacrificed at week 57 after switching the diet of the high dose animals to control feed for weeks 53-57 of the study.

There was no treatment-related effect on survival. There was an increased incidence of clinical signs of toxicity, including hunched posture, thin appearance, chromodacryorrhea and urine stains in the 7500 ppm group males. There was an increased incidence of alopecia and urine strains in the 7500 ppm group females.

Statistically significant decreases in mean body weight were observed in the 7500 ppm males (24-35%) and females (24-45%) and the 1500 ppm females (4-12%). Mean body weight gain over the course of the study was decreased in the 7500 ppm males (53%) and females (69%). There was a 18% decrease in body weight gain in the 1500 ppm females for the week 0-104 period only. Food consumption in the 7500 ppm group males and females was decreased (4-16% in males; 11-21% in females) during the study. In the recovery group, rebound in food consumption and body weight gain was seen, but mean body weight was still decreased 23% for both the 7500 ppm males and females at week 57.

There was an increased incidence of unilateral and bilateral cataracts in the 7500 ppm males and females. A consistent decrease in WBC and lymphocyte count in the 7500 ppm males and females was seen. Alterations in clinical chemistry in the 7500 ppm males and females included significant increases in cholesterol and BUN and significant decreases in AST, ALT and CPK. Plasma cholinesterase was decreased in the 7500 ppm males (27-42%) and females (46-57%) at all of the testing intervals (weeks 27, 53, 79 and 105), however all of the changes were not statistically significant. RBC cholinesterase was decreased in the 7500 males (19-37%) and females (25-38%) and in the 1500 ppm males (10-23%) and females (12-26%) at most of the testing intervals. At weeks 53 and 105, brain cholinesterase was statistically significantly decreased in the 7500 ppm males (8-28%) and females (22-31%). In the recovery group, cholinesterase values had returned to normal levels by week 56.

There was a slightly increased incidence of erythrocytes in the urine of the 7500 ppm males and occult blood in the 7500 ppm males and females. An increased incidence of dark urine in the 1500 ppm females and in the 7500 ppm males and females was also found.

There were no treatment-related macroscopic findings at the week 53 and 57 necropsies. At the week 105 necropsy, the macroscopic findings at an increased incidence in the 7500 ppm males and females, which were also associated with microscopic changes, included pale areas in the lungs and liver and urinary bladder masses. A decreased absolute weight and an increased relative weight of the kidneys, lungs, spleen and liver were found in the 7500 ppm males and females. At the week 53 necropsy, there were slight increases in the incidence of microscopic changes in the kidney and liver of the 7500 ppm males and females. At the week 105 necropsy, there was a wide variety of changes in multiple organs of males and females in the 7500 ppm group. In the liver, there was an increased incidence in the following: hepatocytic hypertrophy in males and females; and eosinophilic foci and pigment in females. In the urinary bladder, there was an increased incidence of transitional cell hyperplasia, squamous metaplasia, high mitotic index and atypia in males and females. In the lung, there was an increased incidence of focal pneumonitis and foamy macrophages in males and females. In the kidney, there was an increased incidence of transitional cell hyperplasia in males. In the thyroid, there was an increased incidence of follicular cell hypertrophy in males and females. Degeneration of the sciatic nerve and skeletal muscle was observed at an increased incidence in males and females.

The study demonstrated that Carbaryl is carcinogenic in male and female rats at 7500 ppm

(refer to p.2 -12 for further details).

The systemic LOAEL was 1500 ppm (78.6 mg/kg/day) in females based on decreased body weight and body weight gain; the NOAEL was 250 ppm (12.6 mg/kg/day). The systemic LOAEL was 7500 ppm (349.5 mg/kg/day) in males based on an increased incidence of clinical signs of toxicity, decreases in body weight, body weight gain and food consumption, an increased incidence of cataracts, alterations in clinical pathology parameters, organ weight changes, and an increased incidence of nonneoplastic microscopic changes. The systemic NOAEL was 1500 ppm (60.2 mg/kg/day) in males.

The LOAEL for plasma cholinesterase inhibition was 7500 ppm in males (27-47% decrease) and females (46-57% decrease); the NOAEL was 1500 ppm.

The LOAEL for RBC cholinesterase inhibition was 1500 ppm in males (10-23% decrease) and females (12-26% decrease); the NOAEL was 250 ppm.

The LOAEL for brain cholinesterase inhibition was 7500 ppm in males (8-28% decrease) and females (22-31% decrease); the NOAEL was 1500 ppm.

This study is classified as **Acceptable** and **satisfies** the guidelines for a combined carcinogenicity/chronic toxicity feeding study in rats (83-5).

Carcinogenicity in Mice

In a carcinogenicity study (MRID No. 42786901), 80 CD-1® mice/sex/group were administered technical Carbaryl (99.3% a.i.) in the diet at dosages of either 0, 100, 1000 or 8000 ppm for 104 weeks (males: 0, 14.73, 145.99 and 1248.93 mg/kg/day; females: 0, 18.11, 180.86 and 1440.62 mg/kg/day, respectively.) Four males in the 8000 ppm group died during the first week of treatment; the cause of death was not determined. Survival rates were not affected by treatment.

Animals in the 8000 ppm group, especially the females, developed clinical signs of toxicity, including hunched posture, thin and languid appearance, squinted and opaque eyes, urine stains, redness to various body areas, rough hair coat, soft feces and low body temperature. Mean body weights were statistically significantly decreased for the 8000 ppm males and females for the majority of the study (males 9-13%; females 5-14%). Mean body weight gain for the 8000 ppm males and females was decreased throughout the study (males 23-38%; females 10-32%). Mean food consumption was statistically significantly decreased in the 8000 ppm females (7-10%). Hematology parameters, including RBC, hemoglobin and hematocrit, were statistically significantly decreased in the 8000 ppm females at week 53 and 8000 ppm group males at week 105. Total leukocyte count and counts of lymphocytes and eosinophils were significantly increased in the 8000 ppm group females at week 53. Platelet counts were significantly increased in this group at week 105.

RBC cholinesterase (ChE) was statistically significantly decreased in the 1000 ppm (23%↓) and 8000 ppm (30% ↓) group males at week 53. RBC ChE was decreased in the 8000 ppm group females (24% ↓) at week 105, although the change was not statistically significant. Brain ChE was statistically significantly decreased in the 1000 and 8000 ppm group males at both weeks 53 and 105 (13-18% ↓ for the 1000 ppm group; 40-57% ↓ for the 8000 ppm group) and in the 8000 ppm females (34-47% ↓). Brain ChE was also significantly decreased (13% ↓) in the 1000 ppm group females at week 53. However, the percentage decreases from the control level were less than 20% for the 1000 ppm group males and females at both weeks 53 and 105. Therefore, the biological significance of these findings is questionable. Plasma ChE values were not affected by treatment.

There were no treatment-related macroscopic effects at the week 53 sacrifice, however at the week 105 sacrifice the incidence of opaque eyes was increased in the 8000 ppm group (males: 1/37 controls vs. 4/30; females: 2/34 controls vs. 16/32). The most consistent organ weight changes at both necropsies were increased relative liver and kidney weights. On microscopic examination, there was an increased incidence of chronic progressive nephropathy in the 1000 ppm males and 8000 ppm males and females at the interim sacrifice. The severity of extramedullary hematopoiesis and pigment in the spleen in the 8000 ppm males and females was increased at the interim sacrifice. There was a dose-related increased incidence of intracytoplasmic protein-like droplets in the urinary bladder in the 1000 and 8000 ppm group males and females at the terminal and unscheduled sacrifices. The incidence of animals with cataracts was increased, but not dose-related, in the 8000 ppm group males and females.

The study demonstrated that Carbaryl is carcinogenic in mice at doses of 100 ppm (14.73 mg/kg/day) and higher in males and 8000 ppm (1440.62 mg/kg/day) in females (refer to p.12 - 22 for further details).

The systemic LOAEL was 1000 ppm (M: 145.99 mg/kg/day; F: 180.86 mg/kg/day) based on an increased incidence of intracytoplasmic droplets in the superficial epithelial cells of the urinary bladder in males and females and chronic progressive nephropathy in males. The systemic NOAEL was 100 ppm (M:14.73 mg/kg/day; F: 18.11 mg/kg/day).

The RBC cholinesterase inhibition LOAEL in males was 1000 ppm (23% ↓ at week 53); the NOAEL was 100 ppm. The RBC cholinesterase inhibition LOAEL in females was 8000 ppm (24%↓at week 105); the NOAEL was 1000 ppm.

The plasma cholinesterase inhibition LOAEL was >8000 ppm (M: 1248.93 mg/kg/day; F: 1440.62 mg/kg/day); the NOAEL was ≥ 8000 ppm.

The brain cholinesterase inhibition LOAEL for males and females was 8000 ppm (M: 40-57%↓; F: 34-47%↓); the NOAEL was 1000 ppm.

This study is classified as **Acceptable** and **satisfies** the guidelines for a carcinogenicity study in

mice (§83-2).

5. Mode of Action Studies

In a DNA binding study (MRID 43282201), [1-^{14}C]-naphthyl-N-methylcarbamate (14-C carbaryl) was tested for the ability to bind to liver DNA in male CD1 mice treated with a single radiolabelled dose of carbaryl (75 mg/kg) or in mice pretreated with 8000 ppm (approximately 1143 mg/kg/day) unlabelled carbaryl in the diet for two weeks followed by a single 75 mg/kg radiolabelled dose. Binding of radiolabel to chromatin protein isolated from the livers of mice treated with a single dose or in pretreated mice was similar (specific activities ranging from 340.3-537.0 dpm/mg). No radioactivity was detectable in DNA samples isolated from mice treated with radiolabelled carbaryl (Covalent Binding Index < 0.1). According to the report, this maximum binding ability of carbaryl is more than 5 orders of magnitude below the Covalent Binding Index of aflatoxin B_1, and more than 4000 times lower than the Covalent Binding Index for 2-acetylaminofluorene. This study demonstrated the interaction of carbaryl with chromatin protein, but no significant interaction with DNA in the liver of male CD1 mice treated with either a single 75 mg/kg dose or in mice pretreated with 8000 ppm (1143 mg/kg/day) carbaryl in the diet followed by a single 75 mg/kg radiolabelled dose. This study was not conducted to satisfy a specific guideline requirement, but fulfills the purpose for which it was conducted.

In a special study (MRID 45236602), sections of ten female liver and ten male kidney samples from control and 8000 ppm mice sacrificed after 52 weeks of carbaryl dietary exposure in the carcinogenicity study (Study HWA 656-138, MRID 42786901) were prepared with an immunohistochemical staining to identify proliferating cell nuclear antigen (PCNA) to assess cell cycling. The mean number of PCNA-positive cortical tubular cells in male mice was minimally higher in the carbaryl-treated (8000 ppm) group when compared to the control group; however, there was considerable variability in the results within the groups and a small amplititude of change between the treated and control groups. The mean number of PCNA-positive female hepatocytes was minimally higher in the carbaryl-treated (8000 ppm) group when compared to the control group. There was a high variability within the control and treated groups and all individual values within the carbaryl-treated group were within the range of values observed in the control group. The toxicological significance of these results is questionable.

The study is classified Unacceptable (non-guideline). This is a special study not submitted to fulfill a data requirement. The study was unacceptable because it did not include a positive control chemical that is known to stimulate cell proliferation.

In a mechanistic study (MRID 45365504) carbaryl (98.4% ai) was administered by gavage (5 ml) to 5 Sprague-Dawley rats/sex/group at doses of 0, 10 or 40 mg/kg/day for 14 days. The control group received the vehicle, 0.5% aqueous carboxymethylcellulose/0.1% Tween 80. A

satellite subgroup of 5 rats/sex/group received the same doses but were sacrificed after three days of treatment to check for hepatic cellular proliferation and liver histopathology. Animals were observed daily for mortality and clinical signs. Body weight was recorded on Days -1, 1, 7 and 14 and before necropsy; food consumption was measured weekly. At the interim sacrifice (day 4), the livers from the satellite subgroup animals were examined for histopathology and cellular proliferation. At study termination, hepatic cellular proliferation was assessed using immunostaining techniques and then microsomal preparations were used to determine cytochrome P-450 isoenzyme profiles.

There were no deaths during the study. Most animals in the 40 mg/kg/day group had clinical signs of toxicity indicative of cholinesterase inhibition. Males treated at 40 mg/kg/day had decreased body weight/body weight gain and food consumption. There were no histological changes in the livers of treated animals. There were no changes in total cytochrome P-450 content, benzoxyresorufin (BROD) and pentoxyresorufin (PROD) activities. A small increase in ethoxyresorufin (EROD) activity was observed in males treated at 40 mg/kg/day. T_4-UDP-glucuronidation (UGT) and T_3-UGT activities were increased in males treated at 40 mg/kg/day and females at 10 and 40 mg/kg/day; the findings were comparable to a phenobarbital-like inducer. There was an increase in cell cycling in males treated at 40 mg/kg/day at days 4 and 15 and all female groups at day 15 (not dose-related).

This study is classified acceptable/nonguideline. The study was not intended to fulfill a guideline requirement but as a special mechanistic study to define the mode of action of carbaryl's carcinogenicity.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

The CARC concluded that carbaryl was carcinogenic to male mice and induced tumors in male and female rats and female mice only at excessively toxic doses based on the following:

a. The reanalyses of rat tumor data showed that male rats had significant increasing trends and significant differences in the pair-wise comparisons of the 7500 ppm dose group with the controls for thyroid follicular cell adenomas and combined adenomas/carcinomas, as well as urinary bladder transitional cell papillomas, carcinomas, and combined papillomas/carcinomas, all at $p<0.01$. The increase in the incidence of combined thyroid follicular cell adenomas/carcinomas was driven by adenomas. At 7500 ppm, the incidences of thyroid follicular cell adenomas, urinary transitional cell papillomas and carcinomas exceeded the respective ranges for the historical controls (thyroid: follicular cell adenoma: 0% - 12%; urinary bladder: transitional cell papilloma: 0 % - 1.1% and transitional cell carcinoma: 0% - 1.4%). The female rats had a

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significant increasing trend ($p<0.01$) and a significant increase by pair-wise comparison of the 7500 ppm dose group with the controls for hepatocellular adenomas ($p<0.05$). The reread of tumor data by the PWG showed that the female rats had significant increasing trends for urinary bladder transitional cell papillomas, carcinomas and combined papillomas/carcinomas, all at $p<0.01$. There were significant differences in the pair-wise comparisons of the 7500 ppm dose group with the controls for urinary bladder transitional cell papillomas ($p<0.05$), carcinomas ($p<0.05$), and combined papillomas/carcinomas ($p<0.01$). The incidences of hepatocellular adenomas, urinary bladder transitional cell papillomas and urinary transitional cell carcinomas exceeded the respective ranges for the historical controls (hepatocellular adenomas; 0%-6.3%; urinary bladder transitional cell papilloma: 0 % - 1.4% and transitional cell carcinoma: 0% - 0%). The CARC noted that at the week 53 necropsy transitional epithelial hyperplasia, a preneoplastic stage, was observed in the urinary bladder of MDT males and HDT males and females. After the 4-week recovery period, this change was still present in HDT males and females. At the terminal necropsy, the transitional cell hyperplasia was observed in HDT males and females, along with an increased incidence of squamous cell metaplasia, high mitotic index and atypia. An increased incidence of thyroid follicular cell hypertrophy was observed in HDT males and females at the interim and terminal necropsies. It was still present in 3/9 HDT males vs 0/9 in controls after the 4 week recovery period. An increased incidence of hepatocellular hypertrophy was observed in MDT males and HDT males and females at the interim and terminal necropsies but not in the recovery animals. The CARC concluded that the HDT tested was excessive. The MDT was judged to be a below adequate dose for testing the carcinogenic potential of carbaryl. At this dose, there was no effect on body weight/body weight gain and only minor ChEI (less than 20% inhibition of plasma, RBC and brain in males and females at week 53, except for 26% inhibition of RBC in females; at week 105, only female RBC and brain were decreased by 22% and 16%, respectively). The CARC noted that the MDT male rats had transitional cell hyperplasia of the bladder, a preneoplastic lesion, at the week 53 necropsy. If the dose had been adequate, bladder tumors seen at the HDT may have occurred at the MDT.

b. The reanalyses of tumor data showed that male mice had significant increasing trends in kidney tubule cell adenomas ($p < 0.05$), carcinomas ($p < 0.05$) and combined adenomas/carcinomas ($p < 0.01$). In addition, a significant difference in the pair-wise comparison of the 8000 ppm dose group with the controls was noted for combined kidney tubule cell adenomas/ carcinomas. In mice, hemangiomas in the liver and spleen can progress to hemangiosarcomas (Pletcher, JM, personal communication). Therefore, the incidence of hemangiomas and hemangio-sarcomas at various sites was combined and analyzed. There were significant differences ($p < 0.05$) in the

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pair-wise comparison of the ≥100 ppm dose groups with the controls for hemangiosarcomas and in combined hemangiomas/ hemangiosarcomas at 1000 and 8000 ppm.

The female mice had a significant increasing trend in hepatocellular adenomas ($p< 0.01$), combined hepatocellular adenomas/carcinomas/hepatoblastomas ($p< 0.01$), hemangiosarcomas ($p< 0.01$), and combined hemangiomas/ hemangiosarcomas ($p< 0.05$). There were also significant differences in the pair-wise comparisons of the 8000 ppm dose group with the controls for hepatocellular adenomas ($p < 0.05$), combined hepatocellular adenomas/carcinomas/hepatoblastomas ($p <0.01$), and hemangiosarcomas ($p < 0.05$). Appropriate historical control data for various types of tumors were not available for comparison (CPRC, 1994). However, based on recently submitted historical control data on vascular tumors in the liver and spleen (sites for most hemangiomas/hemangiosarcomas), the incidence of hemangiosarcomas exceeded the range for the historical controls in both male and female mice (liver: 0%-8%; spleen: 0%- 4.2% for both sexes).

The CARC considered the dosing at the highest dose in male and female mice to be excessive because the decrease in body weight gain, clinical signs and cholinesterase inhibition, and histopathological changes in various organs were indicative of excessive toxicity. **The CARC concluded that the malignant vascular tumors in male mice also occurred at doses which were not excessive. In addition, the findings in female mice were supportive of vascular tumors in male mice.**

2. Mutagenicity

The DNA reactivity of carbaryl is manifested as chromosomal aberrations in cultured mammalian cells. These mutagenic effects are confined to *in vitro* studies with no evidence of genotoxicity in the whole animal. More recent studies with cultured cells have demonstrated effects on microtubule assembly, karyokinesis and cytokinesis as well as stress genes associated with oxidative damage.

3. Mode of Action Studies

It is the registrant's position that alterations in the metabolism, distribution and excretion pattern occur at high doses of carbaryl. The CARC confirmed the Metabolism Subgroup's conclusion that there was an apparent shift in the metabolic pathway from sulfation to glucuronidation at the 7500 ppm dose in rats. However, there was no clear evidence of new metabolites being formed or a shift in the level of metabolites. In addition, these metabolism studies were

conducted at high doses in rats, whereas hemangiosarcomas and combined hemangiomas/hemangiosarcomas were increased at all doses and at 1000 and 8000 ppm doses, respectively, in mice.

The DNA binding study demonstrated no subsequent interaction with DNA in the liver of male CD-1 mice; however, there was limited sensitivity with the method used in the study. One special study conducted to identify proliferating cell nuclear antigen (PCNA) to assess cell cycling in liver and kidneys of mice was unacceptable because it did not include a routine control chemical known to stimulate cell proliferation. In another special study, rats dosed by gavage for 14 days had increases in hepatic enzymes comparable to a phenobarbital-type induction and an increase in cell cycling.

The CARC concluded that the mechanistic studies were inadequate evidence to divert from the default linear low-dose extrapolation approach for risk assessment.

V. CLASSIFICATION OF CARCINOGENICITY

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the majority of the CARC voted to classify carbaryl as **"Likely to be carcinogenic to humans"** based on the following weight-of-the-evidence considerations:

1. Carbaryl induced a statistically significant increase in urinary bladder tumors in male and female rats, thyroid tumors in male rats and liver tumors in female rats. These tumors were induced at an excessively toxic dose (7500 ppm) and, therefore, were not relevant for human cancer risk assessment. However, there was evidence of preneoplastic lesions in the bladder in males at 1500 ppm, a dose which was not adequate for testing the carcinogenic potential of carbaryl. In mice, a treatment-related increase in malignant vascular tumors (hemangiosarcomas) was noted in males at all doses, both excessive and not excessive, whereas in females, this same tumor type was seen only at excessive doses.

2. Carbaryl is clastogenic in *in vitro* studies but its genotoxicity has not been demonstrated in the whole animal.

Three CARC members voted to classify carbaryl as "Suggestive Evidence of Carcinogenicity but Not Sufficient to Assess Human Carcinogenic Potential".

VI. QUANTIFICATION OF CARCINOGENICITY

The Committee recommended a low dose linear extrapolation approach using all dose levels for the quantification of human cancer risk based on the most potent vascular tumors in mice. This approach is supported by the lack of confirmation of a mode of action of carbaryl.

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