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

TXR 010961

MAY 12 1994

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Carbaryl (1-Naphthyl N-methylcarbamate)

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

FROM: Ray Landolt 5/6/94
Review Section I
Toxicology Branch II
Health Effects Division (7509C)
and
Esther Rinde, Ph.D. E. Rinde
Manager, Carcinogenicity Peer Review Committee
Science Analysis Branch
Health Effects Division (7509C)

TO: Dennis Edwards
Product Manager #19
Insecticide Branch
Registration Division (7505C)
and
Judy Loranger
Special Review and Reregistration Division (7508W)

THROUGH: Penelope Fenner-Crisp, Ph.D. Penelope A. Fenner-Crisp 5/10/94
Director, Health Effects Division (7509C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on October 27, and December 8, 1993 to discuss and evaluate the weight-of-the-evidence on carbaryl with particular reference to its carcinogenic potential.

Carbaryl was found to induce tumors at multiple organ sites in two rodent species (mouse and rat) at doses considered to be excessively toxic for carcinogenicity testing. Only hemangiosarcomas (malignant vascular tumors) in the CD-1 male mouse occurred at a lower dose which was considered sufficient and not excessive. The Committee agreed that carbaryl should be classified as a Group C - possible human carcinogen. There was much discussion regarding the method of quantitation with the use of a low dose extrapolation (Q_1^*) approach and a margin of exposure (MOE) approach for quantification of human cancer risk; the CPRC agreed that both approaches be presented for carbaryl at this time. In addition, an RfD approach would be provided to assess the most sensitive non-cancer health endpoint for comparison to the linear and MOE approaches. The CPRC agreed to request additional metabolism studies which could i) direct the selection of the more appropriate quantitative approach (Q_1^* vs. MOE) and ii) provide insight into the significance of the tumors seen only at excessively toxic doses. Additional genotoxicity studies were also requested by the CPRC which would also provide insight for the more appropriate quantitative approach.

A. Individuals in Attendance at one or both meetings:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Penelope Fenner-Crisp

Reto Engler

William Burnam

Karl Baetcke

Marcia Van Gemert

Kerry Dearfield

Elizabeth Doyle

Richard Hill

Hugh Pettigrew

Esther Rinde

Yin Tak Woo

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Ray Landolt[1]

Mike Ioannou

Lori Brunsman

Lucas Brennecke[2]
(PAI/Clement)

3. Other Attendees:

Michael Beringer, Virginia Dobozy, Timothy McMahon, Karen Whitby (HED)
Diane Mandell (Clement)

[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Signature indicates concurrence with pathology report.

B. Material Reviewed:

The material available for review consisted of DERs and other data summaries prepared by Ray Landolt, and statistical analyses prepared by Lori Brunsman. Also cited are Registration Standards of 1984 and 1988, Toxicology Chapter of the Registration Standard of August 10, 1983 (DER No. 003328) and February 22, 1988 (DER No. 006608), the Agency's 1977 Carcinogenic Assessment Group Report on Carbaryl, the Agency's 1981 Reproductive Effects Assessment Group Report, and the 1976 Neil Chernoff memo (Environmental Research Center, RTP, NC). The material reviewed is attached to the file copy of this report. Studies were submitted by Union Carbide and Rhone Poulenc.

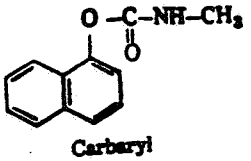
C. Background Information

Carbaryl with the trade name Sevin® was registered by Union Carbide in 1958. Rhone Poulenc Ag Company and Makhteshim Agan Corporation are the two producers registered in the U.S. Tolerances are established for residues of 1-naphthyl N-methyl carbamate in or on a wide range of raw agricultural commodities listed in 40 CFR 180.169. The insecticidal uses of Carbaryl are registered for terrestrial food and nonfood, aquatic food and nonfood, greenhouse, forestry, and domestic indoor and outdoor use. The primary usage of this broad spectrum carbamate insecticide is agricultural; however, indoor and outdoor homeowner, plus livestock and poultry uses account for over 50% of the usage (Registration Standard September 30, 1988).

A notice of Determination Not to Initiate A Rebuttable Presumption Against Registration (RPAR) was published in Federal Register December 12, 1980. Carbaryl was under consideration for the RPAR process in 1967 primarily because two laboratory studies conducted in the late 1960's indicated that Carbaryl induced teratogenicity when administered in low doses to pregnant dogs. In addition to teratogenicity, the Agency was concerned that the use of Carbaryl had potential carcinogenicity, mutagenicity, neurotoxicity and viral enhancement. The Agency concluded, at that time, that the overall weight of the evidence did not indicate that risk criteria warranting a RPAR had been met or exceeded.

The Tox. Chem No. is 160. The P.C. Code of Carbaryl is 056801. The Chemical Abstract Registry Number (CAS No.) of Carbaryl is 63-25-2.

The structure of Carbaryl is presented below:



Carbaryl

D. Evaluation of Carcinogenicity Data

To determine the carcinogenic potential of carbaryl in experimental animals, several studies were conducted in mice and rats prior to 1970. The studies, when reviewed individually, were considered deficient and of questionable value for drawing conclusions as to the carcinogenic potential of carbaryl. The dosing in these studies were much below the top dose levels used in the currently reviewed studies (see below), usually in the range of about 100 mg/kg or lower. These studies were evaluated by the Agency's Carcinogenicity Assessment Group (CAG) on September 28, 1977 with the conclusion that when these studies are considered collectively there is "no significant increase in tumor incidence in the treated groups as compared to the controls." The Scientific Advisory Panel (SAP) was of the opinion (September 19, 1980) that "the current data are adequate to indicate that carbaryl is not carcinogenic". The Agency concluded, based on the available carcinogenicity studies on carbaryl, that a rebuttable presumption was not warranted at that time (Fed. Reg. 45 (No. 241), December 12, 1980). While these studies individually are considered deficient, they add some perspective to events that may occur at lower carbaryl dosing.

Subsequently, the carcinogenicity data base on carbaryl was evaluated by HED (DER No. 007191, December 17, 1988) in concert with the California Department of Food and Agriculture (DER No. 007190, March 7, 1989) with the conclusion that additional mouse and rat carcinogenicity studies should be performed. The two 1993 studies described below were conducted in response to the requirement levied in light of the Agencies' conclusions.

1. Mouse 2-Year Carcinogenicity Study

Reference: Oncogenicity Study with Carbaryl Technical in CD-1 Mice. Hazleton Washington, Inc., Report No. HWA 656-138, MRID No. 427869-01. Study dated May 20, 1993.

a. Experimental Design

Carbaryl 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 chemical misdose (9 males and 8 females).

b. Discussion of Tumor Data

Male mice had significant increasing trends in kidney tubule cell adenomas ($p < 0.05$), carcinomas ($p < 0.05$) and combined adenomas/carcinomas ($p < 0.01$), and hemangiomas ($p < 0.05$). There were also significant differences in the pair-wise comparisons of the 1000 ppm dose group with the controls for hemangiosarcomas and combined hemangiomas and/or hemangiosarcomas, and significant differences in the pair-wise comparison of the 8000 ppm dose group with controls for combined kidney tubule cell adenomas/carcinomas, and combined hemangiomas and/or hemangiosarcomas (all at $p < 0.05$).

Female mice had significant increasing trends in hepatocellular adenomas, combined hepatocellular adenomas/carcinomas, hemangiosarcomas, and combined hemangiomas and/or hemangiosarcomas, all at $p < 0.01$. There were also significant differences in the pair-wise comparisons of the 8000 ppm dose group with the controls for hepatocellular adenomas ($p < 0.01$), combined hepatocellular adenomas/carcinomas ($p < 0.01$), and hemangiosarcomas ($p < 0.05$).

The CPRC agreed that the statistically significant increase in hemangiosarcomas, and combined hemangiomas and/or hemangiosarcomas in male mice at the mid-dose level were related to carbaryl administration and relevant to human risk assessment. There was extensive discussion, however, surrounding the statistically significant tumor increase at the HDT and its relevance to human carcinogenicity. This dose was judged by the CPRC to be excessive for carcinogenicity testing (see section 1.d.).

The following statistical analyses were based upon the Exact trend test because of the small numbers of tumors observed in selected instances. The Fisher's Exact test was used for pair-wise comparisons. See Tables 1, 2, 3, and 4 for tumor analysis results.

Table 1. Carbaryl - Charles River CD-1 Mouse Study

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	0/66	0/69	3[a]/68
(%)	(0)	(0)	(0)	(4)
p =	0.016*	1.000	1.000	0.128
Carcinomas	0/66	0/66	0/69	3[b]/68
(%)	(0)	(0)	(0)	(4)
p =	0.016*	1.000	1.000	0.128
Combined	0/66	0/66	0/69	6/68
(%)	(0)	(0)	(0)	(9)
p =	0.000**	1.000	1.000	0.015*

[+]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 $p < 0.01$.

Table 2. Carbaryl - Charles River CD-1 Mouse Study

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

	Dose (ppm)			
	0	100	1000	8000
Hemangiomas	0/66	1/66	1/69	3[a]/68
(%)	(0)	(2)	(1)	(4)
p =	0.046**	0.500	0.511	0.128
Hemangiosarcomas	2/66	5/66	9[b]/69	7/68
(%)	(3)	(8)	(13)	(10)
p =	0.200	0.220	0.033*	0.090
Combined	2/66	6/66	10/69	10/68
(%)	(3)	(9)	(14)	(15)
p =	0.063	0.137	0.019*	0.017*

*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 72, dose 8000 ppm.

[b]First hemangiosarcoma observed at week 81, dose 1000 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 $p < 0.01$.

Table 3. Carbaryl - Charles River CD-1 Mouse Study

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

	Dose (ppm)			
	0	100	1000	8000
Adenomas	0/63	0/70	1/66	7[a]/61
(%)	(0)	(0)	(2)	(11)
p =	0.000**	1.000	0.512	0.006**
Carcinomas	1/63	1/70	1/66	3[b]/61
(%)	(2)	(1)	(2)	(5)
p =	0.098	0.725	0.740	0.297
Combined	1/63	1/70	2/66	10/61
(%)	(2)	(1)	(3)	(16)
p =	0.000**	0.725	0.518	0.004**

[+]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 104, dose 8000 ppm.

[b]First carcinoma observed at week 74, 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 $p < 0.01$.

Table 4. Carbaryl - Charles River CD-1 Mouse Study

Female Vascular Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)			
	0	100	1000	8000
Hemangiomas	1/63	0/70	1[a]/66	0/61
(%)	(2)	(0)	(2)	(0)
p =	0.384	0.474	0.740	0.508
Hemangiosarcomas	2/63	3/70	3/66	9[b]/61
(%)	(3)	(4)	(5)	(15)
p =	0.003‾	0.550	0.522	0.024*
Combined	3/63	3/70	4/66	9/61
(%)	(5)	(4)	(6)	(15)
p =	0.008‾	0.609	0.526	0.056

[+]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.

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 $p < 0.01$.

Historical control data provided by the registrant were only 78 weeks in duration (with the exception of 2 studies, which were 88 weeks); whereas the current study was 104 weeks. These data were considered to be inappropriate for comparison purposes.

c. Non-neoplastic lesions and other findings

Statistical evaluation of mortality indicates 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 cholinesterase (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 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.

2. Two-Year Chronic/Carcinogenicity in Rats[3]

Reference: Special Reports on Chronic Oral feeding of Carbaryl to Rats. Mellon Institute report No. 21-88, submitted by Union Carbide Corp. Study dated 1958. MRID No. 00080533.

a. Experimental Design

Five groups of 20 CF-N rats/sex/group were fed dietary levels of 0, 50, 100, 200 or 400 ppm for two years.

b. Discussion of Tumor Data

There was no evidence for carcinogenicity due to carbaryl administration in this study.

c. Non-neoplastic Lesions and Other Findings

After one year, cloudy swelling of the convoluted and loop tubules of the kidney was reported in males and females at the 400 ppm level. At terminal sacrifice cloudy swelling of the hepatic cords about the central vein was observed in males at the 400 ppm level. In addition, a decrease in body weight was reported for males at the 400 ppm level. Concern for the cataractogenic property of 2-naphthol, a possible contaminant in the manufacture of 1-naphthyl N-methylcarbamate, was raised from the literature citation by Fitzhugh (Arch. Ophthalmal. 41:572-82, 1949). No cataracts were observed in rats at any of the four dose levels when examined with a hand slit-lamp after 419 and 719 days of the study (cataracts were however noted in the mouse study and in the later rat study). Clinical chemistry and ChE activity were not determined.

[3]There was also another 2 year study in mice listed in the 1-liner, (Mellon Inst. 26-53, 1963), in which no increases in tumors were reported at doses up to 400 ppm. Neither this rat study nor the mouse study were discussed by the CPRC; these studies were evaluated by the CAG group.

3. Two-Year Chronic/Carcinogenicity Rat Study

Reference: Combined Chronic Toxicity and Oncogenicity Study with Carbaryl Technical in Sprague-Dawley Rats; Hazleton Washington, Inc., Report No. HWA 656-139, MRID No. 429188-01. Study dated September 7, 1993.

a. Experimental Design

Groups of 10 Crl:CD®BR rats/sex/dose for the 53-week sacrifice and 70 rats/sex/dose for the 104-week sacrifice were fed dietary levels of 0, 250, 1500 or 7500 ppm carbaryl. These doses were equivalent to 0, 10, 60 or 350 mg/kg/day, respectively, for male and 0, 13, 79 or 486 mg/kg/day, respectively, for female rats.

To determine the extent of recovery from the 53-week study, additional groups of 10 rats/sex were fed 0 or 7500 ppm for 53 weeks then placed on control diet for 4 weeks. These animals were sacrificed during week 57 for complete necropsy, organ weights, clinical chemistry and histopathology.

b. Discussion of Tumor Data

Male rats had significant increasing trends in thyroid follicular cell adenomas ($p < 0.01$), combined thyroid follicular cell adenomas/carcinomas ($p < 0.01$), and urinary bladder transitional cell carcinomas ($p < 0.01$), papillomas ($p < 0.01$) and combined carcinomas and/or papillomas ($p < 0.01$). There were also 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, and urinary bladder transitional cell carcinomas, papillomas and combined carcinomas and/or papillomas, all at $p < 0.01$. Testes tumors were statistically analyzed and were not found statistically significant for interstitial cell tumors.

These statistical analyses were based upon the Exact test for trend because of the small numbers of tumors observed in selected instances in male rats. The Fisher's Exact test was used for pair-wise comparisons. See Tables 5 and 6 for tumor analysis results for male rats.

Female rats had significant increasing trends in hepatocellular adenomas, and urinary bladder transitional cell carcinomas, papillomas and combined carcinomas and/or papillomas, all at $p < 0.01$. There were significant differences in the pair-wise comparisons of the 7500 ppm dose group with the controls for hepatocellular adenomas ($p < 0.05$), and urinary bladder transitional cell carcinomas ($p < 0.05$), papillomas ($p < 0.05$) and combined carcinomas and/or papillomas ($p < 0.01$).

The HDT in this study was considered by the CPRC to be excessive for carcinogenicity testing (see section 3.d.). There

was extensive discussion surrounding the statistically significant tumor increase at the HDT and its relevance to human carcinogenicity.

These statistical analyses were based upon Peto's prevalence test since there was a statistically significant negative trend for mortality in female rats with increasing doses of carbaryl. See Tables 7, 8, and 9 for tumor analysis results for female rats.

Table 5. Carbaryl - Charles River Sprague-Dawley Crl:CDR®BR Rat Study

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

	Dose (ppm)			
	0	250	1500	7500
Adenomas	0/66	2/66	0/68	8[a]/68
(%)	(0)	(3)	(0)	(12)
p =	0.000**	0.248	1.000	0.004**
Carcinomas	0/66	0/66	0/68	1[b]/68
(%)	(0)	(0)	(0)	(1)
p =	0.254	1.000	1.000	0.508
Combined	0/66	2/66	0/68	9/68
(%)	(0)	(3)	(0)	(13)
p =	0.000**	0.248	1.000	0.002**

[+]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 93, dose 7500 ppm.

[b]First carcinoma observed at week 105, 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 $p < 0.01$.

Table 6. Carbaryl - Charles River Sprague-Dawley Crl:CD®BR Rat Study

Male 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	0/66	0/66	0/68	12[b]/68
(%)	(0)	(0)	(0)	(18)
p =	0.000**	1.000	1.000	0.000**
Carcinomas	0/66	0/66	0/68	11[a]/68
(%)	(0)	(0)	(0)	(16)
p =	0.000**	1.000	1.000	0.000**
Combined	0/66	0/66	0/68	22/68
(%)	(0)	(0)	(0)	(32)
p =	0.000**	1.000	1.000	0.000**

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

[a]First carcinoma observed at week 97, dose 7500 ppm.

[b]First papilloma observed at week 97, 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 $p < 0.01$.

Table 7. Carbaryl - Charles River Sprague-Dawley Crl:CD®BR Rat Study

Female Hepatocellular Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	250	1500	7500
Adenomas	1[a]/54	0/56	3/61	7/65
(%)	(2)	(0)	(5)	(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 $p < 0.01$.

Table 8. Carbaryl - Charles River Sprague-Dawley Crl:CD®BR Rat Study

Female Urinary Bladder Transitional Cell Tumor Rates[†]
and Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	250	1500	7500
Papillomas	1[b]/86	0/79	0/77	7/86
(%)	(1)	(0)	(0)	(8)
p =	0.000‾	-	-	0.027*
Carcinomas	0/33	0/34	0/43	6[a]/51
(%)	(0)	(0)	(0)	(12)
p =	0.000‾	-	-	0.014*
Combined	1/86	0/79	0/77	13/86
(%)	(1)	(0)	(0)	(15)
p =	0.000‾	-	-	0.002‾

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

[a]First carcinoma observed at week 98, dose 7500 ppm.

[b]First papilloma observed at week 42, 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 $p < 0.01$.

Table 9. Carbaryl - Charles River Sprague-Dawley Crl:CD®BR Rat Study

Female Thyroid Follicular Cell Tumor Rates*
and Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	250	1500	7500
Adenomas and/or Carcinomas Combined	1[b]/22	0/27	0/28	1[a]/46
(%)	(5)	(0)	(0)	(2)
p =	0.408	-	-	0.705

*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 105, dose 7500 ppm.

[b]First carcinoma observed at week 105, 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 $p < 0.01$.

Table 10 summarizes the historical control tumor incidence (%) reported for Sprague Dawley rats at Hazleton Washington for March 1985 to May 1992. Historical control data were not provided for the incidence of liver tumors in males or thyroid tumors in females. Study data for other tumors in individual historical control studies provided by the Registrant is presented in Tables 11 through 13.

The incidence of hepatocellular adenoma (11%) in females exceeds the historical control values for this tumor. The single incidence of a kidney transitional cell carcinoma in one high dose male was within the historical control range, but considered treatment-related in the high dose male because of the "proliferative, changes present throughout the urothelium." The incidence of thyroid follicular cell adenomas (12%) and carcinomas (1%) in males was at the upper end of the historical control range for these tumors. The incidences of urinary bladder transitional cell papillomas in males (18%) and females (8%) and carcinomas in males (16%) and females (12%) exceeds the historical control values for these tumors.

Table 10. Summary Table - Historical Control Tumor Incidence in Sprague Dawley Rats[a]

Liver, Thyroid, Kidney, and Urinary Bladder Tumors in Males and Females.

	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		
Transitional cell papilloma	0 - 0	0 - 0
Transitional 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.

Table 11. Historical Controls: 104-Week Studies.
Incidence of Transitional Cell Papillomas and Carcinomas
of the Urinary Bladder in Sprague-Dawley Rats*

Study No.	Incidence (%)			
	Transitional Cell Papillomas		Transitional Cell Carcinomas	
	Males	Females	Males	Females
14DE	0/49 (0)	0/50 (0)	0/49 (0)	0/50 (0)
15DE	0/48 (0)	0/47 (0)	0/48 (0)	0/47 (0)
16DE	0/70 (0)	0/69 (0)	1/70 (1)	0/69 (0)
17DE	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)
19DE	0/43 (0)	0/49 (0)	0/43 (0)	0/49 (0)
20DE	0/50 (0)	0/46 (0)	0/50 (0)	0/46 (0)
21DE	1/89 (1)	0/88 (0)	0/89 (0)	0/88 (0)
22DE	0/50 (0)	0/49 (0)	0/50 (0)	0/49 (0)
23DE	0/47 (0)	0/47 (0)	0/47 (0)	0/47 (0)
28DE	0/49 (0)	0/50 (0)	0/49 (0)	0/50 (0)
32DE	0/67 (0)	1/69 (1)	0/67 (0)	0/69 (0)
33DE	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)
34DE	0/50 (0)	0/48 (0)	0/50 (0)	0/48 (0)
35DE	0/47 (0)	0/49 (0)	0/47 (0)	0/49 (0)
107DE	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)
108DE	0/48 (0)	0/50 (0)	0/48 (0)	0/50 (0)
109DE	0/57 (0)	0/60 (0)	0/57 (0)	0/60 (0)
110DE	0/15 (0)	0/13 (0)	0/15 (0)	0/13 (0)
111DE	0/59 (0)	0/60 (0)	0/59 (0)	0/60 (0)
200DE	0/49 (0)	0/49 (0)	0/49 (0)	0/49 (0)
201DE	0/54 (0)	0/51 (0)	0/54 (0)	0/51 (0)
202DE	0/59 (0)	0/59 (0)	0/59 (0)	0/59 (0)
203DE	0/50 (0)	-	0/50 (0)	-
204DE	0/49 (0)	0/48 (0)	0/49 (0)	0/48 (0)
205DE	0/60 (0)	0/59 (0)	0/60 (0)	0/59 (0)
211DE	0/52 (0)	0/55 (0)	0/52 (0)	0/55 (0)
213DE	0/50 (0)	0/46 (0)	0/50 (0)	0/46 (0)
Weighted average	(0.04)	(0.05)	(0.05)	(0.0)

*Historical data for transitional cell papillomas and carcinomas in the urinary bladder were obtained from 27 carcinogenicity studies with male and female Sprague-Dawley rats conducted at Hazleton Washington between 1985 and 1992. Studies were 104 weeks in duration. Data were provided by the registrant.

Table 12. Historical Controls: 104-Week Studies. Incidence of Follicular Cell Adenomas and Carcinomas of the Thyroid in Male Sprague-Dawley Rats[a]

Study No.	Incidence (%)	
	Adenoma	Carcinoma
14DE	6/50 (12)	0/50 (0)
15DE	3/48 (6)	0/48 (0)
16DE	2/70 (3)	2/70 (3)
17DE	2/50 (4)	1/50 (2)
19DE	1/47 (2)	0/47 (0)
20DE	2/50 (4)	3/50 (6)
21DE	1/88 (1)	2/88 (2)
22DE	0/49 (0)	0/49 (0)
23DE	4/50 (8)	2/50 (4)
28DE	2/49 (4)	2/49 (4)
32DE	3/65 (5)	0/65 (0)
33DE	2/49 (4)	0/49 (0)
34DE	1/50 (2)	0/50 (0)
35DE	2/48 (4)	1/48 (2)
107DE	0/50 (0)	0/50 (0)
108DE	4/48 (8)	0/48 (0)
109DE	1/59 (2)	0/59 (0)
110DE	0/15 (0)	1/15 (7)
111DE	4/60 (7)	4/60 (7)
200DE	3/50 (6)	4/50 (8)
201DE	0/53 (0)	1/53 (2)
202DE	2/59 (3)	2/59 (3)
203DE	2/50 (4)	2/50 (4)
204DE	1/49 (2)	0/49 (0)
205DE	1/60 (2)	0/60 (0)
211DE	1/55 (2)	0/55 (0)
213DE	2/50 (4)	0/50 (0)
Weighted average	(3.7)	(2.0)

[a]Historical data for follicular cell adenomas and carcinomas in the thyroid were obtained from 27 carcinogenicity studies with male Sprague-Dawley rats conducted at Hazleton Washington between 1985 and 1992. Studies were 104 weeks in duration. Data were provided by the registrant; data were not provided for females.

Table 13. Historical Controls: 104-Week Studies. Incidence of Hepatocellular Adenomas in the Liver in Female Sprague-Dawley Rats[a]

Study No.	Adenoma
14DE	1/50 (2)
15DE	3/48 (6)
16DE	3/69 (4)
17DE	1/50 (2)
19DE	0/50 (0)
20DE	2/48 (4)
21DE	0/89 (0)
22DE	1/50 (2)
23DE	0/49 (0)
28DE	0/50 (0)
32DE	3/69 (4)
33DE	1/50 (2)
34DE	3/49 (6)
35DE	1/49 (2)
107DE	1/50 (2)
108DE	0/50 (0)
109DE	1/60 (2)
110DE	0/13 (0)
111DE	0/60 (0)
200DE	0/50 (0)
201DE	0/53 (0)
202DE	0/60 (0)
203DE	-
204DE	0/50 (0)
205DE	0/59 (0)
211DE	0/55 (0)
213DE	0/46 (0)
Weighted average	(1.6)

[a]Historical data for hepatocellular adenomas in the liver were obtained from 27 carcinogenicity studies with female Sprague-Dawley rats conducted at Hazleton Washington between 1985 and 1992. Studies were 104 weeks in duration. Data for females were provided by the registrant; no data were provided for males.

c. Non-neoplastic Lesions and Other Findings

Statistical evaluation of mortality indicated no significant incremental changes with increasing doses of carbaryl in male rats. Female rats showed a statistically significant decreasing trend in mortality with increasing doses of carbaryl. Clinical signs of toxicity observed for the high dose male 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 decreased by the 13, 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. ChE activities (plasma, RBC and brain) of the recovery animals returned to control values when measured at week 56.

At week 53, absolute organ weights of lung, liver, spleen and kidneys were decreased and organ/body weight ratios were significantly increased in HDT male and female rats. 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 HDT for the liver, urinary bladder, lung, kidney, thyroid and sciatic nerve. An increased incidence of hepatocyte hypertrophy was observed in male and female rats accompanied by an increased incidence of eosinophilic foci of 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 Dosing for Assessment of Carcinogenic Potential

The high dose was considered excessive for carcinogenicity testing based on a significant ($p < 0.05$) decrease in body weight gains during week 13 for males and females by 40% and 52%, respectively, as compared to controls. Decreased food efficiency, alterations in hematology and clinical chemistry values were also reported in both sexes at the high dose level. By weeks 52-53 plasma, erythrocyte 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, erythrocyte 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, erythrocyte 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, erythrocyte and brain ChE activities in female rats fed the 1500 ppm level decreased significantly by 22% and 16%, respectively. The CPRC agreed that the HDT in this study was excessively high for carcinogenicity testing. The CPRC also agreed that the mid-dose level was below the level that would be considered adequate for carcinogenicity testing.

E. Additional Toxicological Data on Carbaryl

1. Metabolism

Reference: Metabolism of Carbaryl in the Rat, Study No. 25051, and Metabolism of Carbaryl in the Dog, Study No. 25050. MRID No. 254104. Submitted by Union Carbide. Dated May 11, 1978.

Metabolism of carbaryl has been investigated in numerous species including the rat, guinea pig, dog, pig, sheep, monkey and humans. These studies have not been subjected to current acceptance criteria for metabolism guideline data requirements (158.135) and may not conform to the Agency's standard for testing.

Although the metabolism of carbaryl has been studied in numerous mammalian species, including man, the Agency in the Carbaryl Registration standard of 1984 requested a metabolism study in rats and dog to clarify the claim that metabolism of carbaryl in the dog is unique and may account for the teratogenicity reported in two dog teratology studies. In response to the Registration Data Call-In, metabolism studies in the rat and dog were reviewed (DER 005112) May 15, 1986.

In these studies female rats, dosed orally with 1-naphthyl-^{14}C-carbaryl in corn oil at 2.5 mg/kg, eliminated 74% of the dose in the urine and 1.8% in the feces within 24 hours with 91.3% of the dose accounted for in urine and feces within 48 hours. For comparison, male and female beagle dogs dosed orally with 1-naphthyl-^{14}C-carbaryl in gelatin capsules at 2.5 mg/kg eliminated 30-35% of the dose in the urine and 30-43% in the feces within 24 hours with 32-43% of the dose accounted for in the urine and 33-45% in the feces by day 4. Based on the existing rat and dog metabolism studies the proposed metabolic pathway of carbaryl is qualitatively similar for these two species (DER 005112).

These studies were deficient for the rat and acceptable for the dog. Data reviewed in these studies have not clarified whether there are species differences or similarities which can be identified quantitatively due to the physical form and vehicle used

for oral administration of the test material. Rhone Poulenc, in its letter of November 12, 1991, committed to providing an acceptable rat metabolism study.

During the Committee's deliberations, there was some discussion of the metabolic pathways of carbaryl. Hydrolysis of carbaryl to naphthol is a major pathway. This pathway appears operative in rat and man. Carbaryl can also undergo enzymatic oxidative metabolism. Both hydrolytic and oxidative metabolites are excreted as glucuronides and sulphates. It was noted that many of the pathways observed produced epoxide intermediates which could be a source of reactive products during carbaryl metabolism. Furthermore, hydrolysis of carbaryl probably results in loss of antiChE activity, but oxidative metabolites may retain antiChE activity. The increasing cholinesterase inhibition by carbaryl as dose increased may be attributable to this if there is a shift from the hydrolytic pathway to more prominence of the oxidative pathways.

These possible changes in metabolic pathways and metabolite profiles coupled with the lack of tumors at lower doses (low dose of current studies and the collective examination of the earlier studies) led the CPRC to discuss the possibility of a threshold dose above which the animals begin to react to carbaryl exposure in an altered fashion. The appearance of multiple tumors at the excessively high dose was an aspect of this consideration. This would impact the choice of the most appropriate method of quantitation of the induction of tumors by carbaryl.

The CPRC agreed that additional examination of metabolism would be useful. The CPRC discussed the lack of information on the comparative metabolism of carbaryl at low- vs high-dose levels. Particularly useful would be data on the metabolism, metabolic pathways, metabolite profile, and tissue disposition of carbaryl at low- and high-dose levels, identification of doses of carbaryl at which metabolic saturation or pathway shift occurs, and possible epoxide formation with carbaryl administration. This information would be useful to help in the analysis of the carcinogenicity studies in the mouse (MRID No. 427869-01) and rat (MRID No. 429188-01) described earlier.

Therefore, the CPRC is requesting studies that analyze the dose-dependent metabolism of carbaryl (especially disposition of the compound). The CPRC is also requesting that a metabolism study be performed in which radiolabeled material is administered in order to measure carbaryl's ability to bind to DNA in target tissues.

2. Genotoxicity

Reference: Mutagenicity Test on Carbaryl (Technical) in the Ames Salmonella/Microsome Reverse Mutation Assay, MRID No. 413703-03; Mutagenicity Test on Carbaryl (Technical) in the CHO/HGPRT Forward Mutation Assay, MRID No. 414202-01; Mutagenicity Test on Carbaryl (Technical) in the in vitro Cytogenetic Assay (CHO), MRID No. 413703-04; Mutagenicity Test on Carbaryl (Technical) in the in vitro Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay, MRID No. 413703-01. All studies submitted by Rhone-Poulenc. Studies dated January 26, 1990.

The numerous mutagenicity studies conducted during the period of 1965 to 1978 were also reviewed as were the carcinogenicity and teratogenicity studies to determine whether a RPAR should be issued for carbaryl. The Agency reviewed these studies collectively and determined "that carbaryl is not a potent mutagen in the reported studies and probably acts as a weak mutagen" (Reproductive Effects Assessment Group Report No. EPA-600/6-81-001 January 1981). The mutagenicity data base on carbaryl was reviewed in concert with the California Department of Food and Agriculture March 7, 1989 (DER 007190) with a divergence of opinion on the adequacy of the mutagenicity data base on carbaryl. Subsequently, four mutagenicity replacement studies were submitted in response to a requirement by California Department of Food and Agriculture. These studies were reviewed by USEPA (DER 008115 and 008450). Three of the four were acceptable and satisfy the guideline data requirements. Carbaryl was not mutagenic in the Salmonella typhimurium assay or the unscheduled DNA synthesis (UDS) assay. This is consistent with several published studies.

Very large increases in structural aberrations (breaks, deletions, triradials, quadriradials) were observed at concentrations from 150-300 ug/ml in cultured Chinese hamster ovary (CHO) cells in the presence of exogenous metabolic activation. Other reports show clastogenic activity in cultured Chinese hamster fibroblasts (Mutat Res 48: 337-354, 1977) and Chinese hamster V79 cells (Mutat Res 119: 319-330, 1983) in the absence of metabolic activation. It is also of interest to note no indication of increased aneuploid/polyploid cells in the submitted study since there is evidence to suggest carbaryl can induce numerical aberrations (review Mutat Res 167: 149-169, 1986; In Vitro 15: 172-173, 1979).

The CPRC agreed that additional genotoxicity studies should be conducted with carbaryl. Specifically needed are in vivo cytogenetics studies in rodents (micronucleus assay with antibodies to provide insight on structural and/or numerical aberrations). This information would help clarify outstanding questions regarding the genotoxicity of carbaryl, and would help in the determination of the mode of carcinogenic action.

3. Subchronic and Chronic Toxicity

Reference: Subchronic Feeding Study in Rats. J. Agr. Food Chem; 9: 30-39, 1961 DER 003328.

Two groups of 5 rats/sex/group were fed 1500 and 2500 ppm for 96 days. A decrease in body weight gain in females and a significant increase in male liver weight were reported for the 2500 ppm level. At the 1500 and 2500 ppm levels there was a significant increase in kidney weight of females accompanied by cloudy swelling of the kidney tubules. There were no effects on plasma, erythrocyte and brain ChE activity (DER 003328).

Reference: One Year Oral Toxicity in Dogs. Mellon Institute Report No. 21-89, October 3, 1958. MRID No. 00080532.

Four groups of 3 or 4 adult Basenji Cocker hybrid dogs/level were administered oral doses (gelatin capsule) of carbaryl at 0, 0.45, 1.8, or 7.2 mg/kg five days a week for one year (comparable to 25, 100 or 400 ppm). A total of ten males and four females were tested, the high dose level consisting of 4 males, no females. No effects on body weight, food intake, mortality, hematology, clinical chemistry, plasma or erythrocyte ChE, or liver and kidney weight were reported. Histopathology of the kidneys revealed diffuse cloudy swelling of the proximal convoluted and loop tubules at the 7.2 mg/kg level. These findings "were considered transitory and not related to carbaryl, but due to biological variability which was within the normal range for these dogs" (DER 003328). The study does not satisfy the minimum data requirements for a non-rodent study and a replacement study was requested (DER 004546).

Reference: One-Year Oral Toxicity Study in Beagle Dogs. Hazleton Laboratories America, Inc. Report No. 400-715, March 18, 1987, MRID 401667-01.

Reference: Five Week Subchronic Toxicity Study in Dogs, Hazleton Laboratories America, Inc. Report No. 656-152, March 28, 1991 MRID 420228-01.

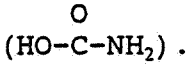
Four groups of 6 purebred beagle dogs/sex/group were fed dietary levels of carbaryl at 0, 125, 400, or 1250 ppm for one year (DER 006401). These doses were equivalent to 3.37, 11.23 and 33.83 mg/kg/day for males and 3.73, 12.17 and 34.43 mg/kg/day for females. In the one-year feeding study a systemic NOEL of 400 ppm was established. The LOEL was 1250 ppm with a significant ($p < 0.05$) decrease in body weight (50%), non-significant decrease in food consumption (23%) and a significant ($p < 0.05$) decrease in albumin (11%) values at this level. The question of pathologic changes in the kidneys raised in the original dog study were addressed by HED pathologist, Dr. Kasza. His evaluation of this second dog study concluded that "compound-related changes in the urinary system in the present one-year dog study could not be

established." A ChE NOEL was not determined in this study with a significant ($p < 0.05$) decrease in plasma (23%) and brain (20%) ChE activity in female dogs at the 125 ppm level (DER 007086).

To determine whether the marginal effects on ChE activity at the 125 ppm level were reproducible, a 5-week subchronic feeding study was conducted at 20, 45 and 125 ppm carbaryl. This 5-week dog feeding study was without affect on plasma, erythrocyte or brain ChE activity. However, when considered with the one-year dog feeding study a NOEL of 45 ppm and a LEL of 125 ppm were demonstrated with a significant decrease in plasma (23%) and brain (20%) ChE activity at the 125 ppm level (DER 009776).

4. Structure-Activity Relationships

Carbaryl is structurally related to nine other carbamate insecticides possessing the carbamic acid moiety:



As a N-methyl carbamate, it is expected to behave in a biologically similar manner to other structurally related carbamates. The limited information provided by the toxicology one-liners indicate the following carcinogenic concerns for these N-methyl carbamates.

Aldicarb - No carcinogenic potential in mice or rats.
Some mutagenic (clastogenic) activity reported in the literature.

Bendiocarb - No carcinogenic potential in mice or rats.
Positive for genotoxicity; increased chromosomal aberrations with activation, negative without S-9 activation.

Carbofuran - No carcinogenic potential in mice or rats.
Positive for genotoxicity in two separate Ames assays using Salmonella strain TA 1535 without activation. In two other Ames assays, a positive response was induced in strain TA 100 with and without activation, and in strains TA 98 and TA 100 with activation. In the mouse lymphoma assay a positive response was induced with and without activation.

Cloethocarb - No carcinogenic potential in mice or rats.
Not mutagenic in the test systems assayed.

Methiocarb - No carcinogenic potential in rats; mouse data are not available.
Not mutagenic in the test systems assayed.

Methomyl - No carcinogenic potential in mice or rats.
Not mutagenic in the test systems assayed.

Mexacarbate - No carcinogenic potential in mice or rats.
Positive in Chinese hamster ovary cells with activation.

Propoxur - Classified as Group B2 carcinogen based on bladder tumors in male and female rats at 5000 ppm. In addition, carcinoma of uterus was reported in female rats at 5000 ppm.
Not mutagenic in the test systems assayed.

Trimethacarb - No carcinogenic potential in mice or rats.
Positive for genotoxicity, with increase in chromosomal aberrations with and without activation.

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.

F. Weight of the Evidence Considerations

The Committee considered the following observations regarding the toxicology of carbaryl for a weight-of-the-evidence determination on its carcinogenic potential:

1. Male and female CD-1 mice were fed 0, 100, 1000, or 8000 ppm of carbaryl for 105 weeks. The highest dose tested (HDT) in the mouse study, 8000 ppm, was determined to be excessively high for carcinogenicity testing. This was based on decreases in hematology values and brain cholinesterase, clinical signs of toxicity, histopathological changes of the bladder, kidneys, and spleen and decreased body weight gains by week 13 in both sexes. Because of the excessive dosing in this study, the relevance to carcinogenicity in humans of the tumors occurring at the HDT was questioned by the CPRC.

In male mice, there were increases in hemangiosarcomas in the 1000 ppm dose group and combined hemangiomas/hemangiosarcomas in the 1000 and 8000 ppm dose groups, which were statistically significant ($p < 0.05$) by pair-wise comparison with control animals. In addition, there was a statistically significant positive trend ($p < 0.05$) for hemangiomas.

In male mice, there was also an increase in combined kidney tubule cell adenomas/carcinomas in the 8000 ppm dose group which was statistically significant ($p < 0.05$) by pair-wise comparison with the control group. This is considered a rare tumor type. In addition, there were also statistically significant positive trends (all at $p < 0.05$) in kidney tubule cell adenomas, carcinomas, and combined tumors.

In female mice, there was an increase in hemangiosarcomas in the 8000 ppm dose group which was statistically significant ($p < 0.05$) by pair-wise comparison with control animals. In addition, there were statistically significant positive trends ($p < 0.01$) for hemangiosarcomas and combined hemangiomas and/or hemangiosarcomas.[4]

In female mice, there were also increases in hepatocellular adenomas and combined adenomas/carcinomas in the 8000 ppm dose group which were statistically significant ($p < 0.01$) by pair-wise comparison with the control group. In addition, there were statistically significant positive trends ($p < 0.01$) for hepatocellular adenomas, and combined hepatocellular adenomas/carcinomas.

---

[4]When the data were analyzed excluding the high dose group, the incidences of hemangiosarcomas and combined hemangiomas/hemangiosarcomas in male mice were statistically significant by pair-wise comparison with controls and had statistically significant trends. All other tumor types in the mouse and rat were no longer significant by this analysis. [See Brunsman Memo 12/1/93, attached to file copy of this report.]

2. Male and female Sprague-Dawley rats were fed 0, 250, 1500, or 7500 ppm of carbaryl for 105 weeks. The highest dose tested (HDT), 7500 ppm, was determined to be excessively high for carcinogenicity testing. This was based on body weight losses during week 13 and decreased plasma, erythrocyte and brain cholinesterase activities, decreased and alterations in hematology and clinical chemistry in both sexes. Neoplastic histopathological findings were limited to the HDT for the thyroid, urinary bladder, and liver. Because of the excessive dosing in this study the relevance, to carcinogenicity in humans, of the tumors occurring at the HDT was questioned by the CPRC.

In male rats there were increases in thyroid follicular cell adenomas, and combined adenomas/carcinomas in the 7500 ppm dose group which were statistically significant ($p < 0.01$) by pair-wise comparison with control animals. The incidence of the thyroid adenomas was at the upper range of the testing laboratory's historical controls. In addition, there were statistically significant positive trends ($p < 0.01$) for thyroid follicular cell adenomas, and combined adenomas/carcinomas.

In male rats there were also increases in urinary bladder transitional cell carcinomas, papillomas, and combined carcinomas/papillomas in the 7500 ppm dose group which were statistically significant (all at $p < 0.01$) by pair-wise comparison with control animals. The incidence of the urinary bladder tumors exceeded the upper range of the testing laboratory's historical controls. In addition, there were statistically significant positive trends ($p < 0.01$) for urinary bladder transitional cell carcinomas, urinary papillomas, and combined carcinomas/papillomas.

In female rats there were increases in urinary bladder transitional cell carcinomas, papillomas, and combined carcinomas/papillomas in the 7500 ppm dose group which were statistically significant (all at $p < 0.01$) by pair-wise comparison with control animals. The incidence of the urinary bladder tumors exceeded the upper range of the testing laboratory's historical controls. In addition, there were statistically significant positive trends (all at $p < 0.01$) for urinary bladder transitional cell carcinomas, papillomas, and combined carcinomas/papillomas .

In female rats there was also an increase in hepatocellular adenomas in the 7500 ppm dose group which was statistically significant (at $p < 0.05$) by pair-wise comparison with control animals. The incidence of these tumors exceeded the upper range of the testing laboratory's historical controls. In addition, there was a statistically significant positive trend ($p < 0.01$) for hepatocellular adenomas.

3. Concerns were raised regarding the relevance for human risk assessment of the tumors seen at the HDT, based on possible perturbations in metabolic or other physiologic functions at excessive doses. In studies where dosing did not exceed 400 ppm, no increases in tumor incidences were reported. This is supported by the collective examination of the many earlier carcinogenicity studies (while individually not acceptable for several reasons) where the conclusion was that carbaryl at these lower doses did not present a carcinogenicity concern. Also, the incidences of tumors in the mouse and rat were no longer significant when the data were analyzed excluding the high dose group with the exception of the incidences of hemangiosarcomas and combined hemangiomas/ hemangiosarcomas in male mice (there was still pair-wise significance at the lower, non-excessive mid-level dose).

These concerns and the examination of the metabolism information (see Section E.1.) led to a discussion of the most appropriate method of quantitation, in particular whether a margin of exposure (MOE) approach would be appropriate for carbaryl. In other words, was there enough evidence to suggest that a threshold dose might be identified above which the animals were reacting to carbaryl exposure in an altered fashion? The Committee was split in this consideration, but did agree that additional studies would be useful to provide insight into the mode of carcinogenic action (see 5. below).

4. From submitted studies, carbaryl was not mutagenic in the Salmonella assay or in an unscheduled DNA synthesis (UDS) assay. However, carbaryl induced a very large increase in structural aberrations in cultured CHO cells in the presence of exogenous metabolic activation. The published literature supports the clastogenic activity and also suggests that carbaryl may induce numerical aberrations. This, coupled with the known metabolic profile at low doses which shows epoxide formation, suggests that carbaryl may generate a genotoxic species of concern for carcinogenic potential.

5. There are additional studies that the CPRC deems necessary to help direct the selection of the more appropriate quantitative approach (Q_1^* vs. MOE) and provide insight into the presence of the tumors seen only at excessively toxic doses. The CPRC has requested additional studies in the areas of metabolism and genotoxicity as follows:

a. Analysis of the dose-dependent metabolism of carbaryl (especially disposition of the compound) at low and high doses;

b. Administration of radiolabeled carbaryl to mice and rats with measurement of carbaryl's ability to bind to DNA in target tissues;

c. <u>In vivo</u> cytogenetics studies in rodents.

These data are necessary to shed light on possible mechanism(s) of induction of the blood vessel tumors and selection of the quantitation model to use. The metabolism studies would ascertain more definitively the possible role of increasing dose in altering the animals' responses. The binding study and cytogenetics study would more closely examine the possible role of a reactive metabolite (epoxide?) in causing the blood vessel tumors. These data would also be useful to provide insight to the appearance of the tumors seen at the excessive high doses in the animal carcinogenicity studies and if there is any relevance for human cancer risk. Once these data and all appropriate publicly available information are assembled and analyzed, a reevaluation of the possible differences in metabolite profile, in proportions of individual metabolites, in pharmacokinetics of carbaryl, and in DNA binding will be necessary to link any observed differences to some adverse effect, such as tumor induction.

6. Carcinogenicity in animals -- Carbaryl

After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to carbaryl resulted in an increased incidence of hemangiosarcomas (malignant vascular tumors) and combined hemangiosarcomas/hemangiomas in mice, combined kidney tubule cell adenomas/carcinomas in mice, combined thyroid follicular cell adenomas/carcinomas in rats, and urinary bladder transitional cell carcinomas and combined adenomas/carcinomas in rats. Previous negative studies conducted at much lower doses do not rebut these findings. Although there is some question about the relevance of tumors at the HDT for human risk assessment, the increased tumor incidences, in particular, the malignant hemangiosarcomas in the mouse, support the finding that carbaryl is an animal carcinogen. The relevance of the tumor data to an evaluation of carbaryl's potential for human carcinogenicity is discussed elsewhere in this report.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogenic Risk Assessment" (FR51: 33992-34003, 1986) for classifying the weight of evidence for carcinogenicity.

The Committee agreed that carbaryl should be classified as a Group C - possible human carcinogen based on the finding of a statistically significant increase in hemangiosarcomas (malignant vascular tumors) and combined hemangiomas/hemangiosarcomas in male mice at a dose which was sufficient, but not excessive. There was much discussion regarding the method of quantitation with the use of a low dose extrapolation (Q_1^*) approach and a margin of exposure (MOE) approach for quantification of human cancer risk; the CPRC agreed that both approaches be presented for carbaryl. In addition, an RfD approach would be provided to assess the most sensitive non-cancer health endpoint for comparison to the linear and MOE approaches.

The rationale to support a low dose extrapolation (Q_1^*) approach for carbaryl was based on the finding of a statistically significant increase in hemangiosarcomas (malignant vascular tumors) in male mice at a dose which was sufficient for assessing carcinogenic potential. This same tumor type was also seen in female mice, albeit at a dose considered to be excessively toxic. There is evidence that carbaryl has some genotoxic activity and the ability to generate a reactive metabolite (epoxide). It was noted that although metabolic pathways may be altered at doses considered to be excessively toxic, this has not been definitively demonstrated for carbaryl. It is also not known whether alteration of the metabolism of carbaryl at low versus high doses is dose-dependent. It was unclear what impact the other tumor types seen at the high excessive doses in the mouse and rat studies may have on human cancer risk. Overall, this evidence was considered by part of the CPRC to constitute rationale to support a low dose extrapolation (Q_1^*) approach for carbaryl. While the consensus of the Committee led to a Group C classification, there were some members who felt that this evidence was sufficient to suggest an even higher classification.

The rationale for a MOE approach supported by the other part of the CPRC was based on the consideration that the tumors seen at the excessive doses are not relevant for human cancer risk. Only the hemangiosarcomas in the male mouse were considered to be relevant for human risk assessment, since they also occurred at a dose which was <u>not</u> excessive and the increased incidence at this dose was statistically significant. The available metabolism data and lack of adverse effects at the lower doses suggested a threshold approach for carbaryl and support for a MOE approach.

All CPRC members agreed that metabolism studies were necessary to define any dose-dependent metabolism of carbaryl at low- versus high-doses, and to demonstrate whether altered metabolism (e.g. saturation of metabolic pathways) could produce metabolites not seen at lower doses. For this reason, the CPRC requested additional metabolism data. Additional genotoxicity studies were also requested.

The Committee agreed that both the low dose extrapolation (Q_1^*) and margin of exposure (MOE) approaches for quantification of human cancer risk should be presented for carbaryl:

1) The data set to use for the Q_1^* derivation should be the male mouse combined hemangioma/hemangiosarcoma response. The highest dose used should be excluded from the calculation because it was deemed excessive and should not be used in the Q_1^* derivation.

2) The MOE approach takes into account a weight-of-the-evidence approach which includes among many considerations the neoplastic, non-neoplastic and/or target organ effects that may be plausibly associated with the etiology and appearance of the tumor type. In the case of carbaryl, there were no non-neoplastic effects associated with the hemangiosarcomas from which to select an appropriate dose to use in the MOE calculation. Therefore, the most appropriate dose to use in the MOE calculation is the lowest dose used in the mouse carcinogenicity study where there was not a statistically significant increase in tumors.