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and
Toxic Substances

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MEMORANDUM

SUBJECT: CARBARYL - Review of Registrant's Submission on Cancer Issues

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Reregistration Branch I, Health Effects Division (7509C)

THRU: Whang Phang, Ph.D., Branch Senior Scientist 10/17/01
Reregistration Branch I, Health Effects Division (7509C)

TO: Betty Shackleford/Anthony Britten
Special Review and Reregistration Division (7508C)

Action Requested: The registrant submitted the following data to address the carcinogenicity potential of carbaryl: 1) historical control data on vascular tumors in CD-1 mice (MRID 45365501); 2) reexamination of the microscopic slides from the interim necropsies of the rat combined chronic toxicity/carcinogenicity study and the mouse carcinogenicity study (MRID 45365503); 3) evaluation of urinary bladder carcinogenicity of carbaryl in rats (MRID 45365502); and 4) 14-day mechanistic study in rats (MRID 45365504). The Special Review and Reregistration Division requested that RRB1 review these data.

Recommendation: The data have been reviewed and will be presented to the HED Cancer Assessment Review Committee at the upcoming meeting. The details of the HED evaluation of each report are presented below.

BACKGROUND

The carcinogenic potential of carbaryl was evaluated by the HED Carcinogenicity Peer Review Committee on October 27 and December 8, 1993 in a report dated May 12, 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 genotoxicity studies 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. Since the CPRC meeting, the registrant has submitted additional data to address these issues. One of these submissions (D271443) contained the registrant's position on cancer issues. This paper referred to reexamination of the interim necropsy slides, a 14-day mechanistic study, a paper on bladder tumors and additional historical control data on vascular tumors in mice. In the RRB1 review, it was noted that OPP had no record of receiving these data. The registrant has now submitted the data.

REVIEW

1) Citation: Klonne D.R. (1995). Carbaryl - Mouse Historical Control Data Position Pager. Rhone-Poulenc Agrochimie, Research Triangle Park, NC, Laboratory No.: none, November 1995. MRID 45365501. Unpublished.

In the 2-year mouse carcinogenicity study (conducted 1990-1992), there was a non-statistically significant increase in vascular tumors at 100 and 1000 ppm and a statistically significant increase at 8000 ppm in male mice. Historical control data from the conducting laboratory were only available for studies conducted for 18 months. In this submission, the registrant provides historical control data from other sources and concludes the following:

1) There appears to be an increase in the spontaneous vascular tumor incidence from 18 to 24 months of age in mice. There also appears to be an increase in the spontaneous vascular tumor incidence over the last 10 years in CD-1 mice.

2) Based on a weight-of-the-evidence, the vascular tumor incidence in the low dose, but not the middle dose, of the carbaryl study falls within the control range. As the high dose exceeded the maximum tolerated dose (MTD), it is not considered scientifically valid for these comparisons.

3) Based on statistical analyses and from comparison to various historical control databases, the low dose in the carbaryl study should be considered the NOAEL for carcinogenicity.

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The registrant states that previous data indicated that carbaryl does not appear to be acting through a genotoxic mechanism. Thus, carbaryl should not be regulated as a non-threshold carcinogen; additional data from studies currently being conducted will clarify this position.

The registrant has separated out the hemangiomas and hemangiosarcomas in the liver and spleen since the majority of tumors were found in these two organs. The registrant states that vascular tumors in other organs are rare and generally not listed and/or routinely histologically evaluated. The number of tumors in each organ is presented in Table 1.

Table 1: Incidence of Hepatic and Splenic Vascular Tumors in Male CD-1 Mice in 2-year carcinogenicity study[a]

	No. of Tumor-Bearing Animals (% incidence)					
	Liver		Spleen		Total	
Dose (ppm)	Hemangiomas	Hemangiosarcomas	Hemangiomas	Hemangiosarcomas	Liver	Spleen
0	0 (%)	0 (0%)	0	2 (2.5%)	0 (0%)	2 (2.5%)
100	1 (1.3%)	3 (3.8%)	0	2 (2.5%)	4 (5%)	2 (2.5%)
1000	0 (0%)	5 (6.3%)	0	4 (5%)	5 (6/3%)	4 (5%)
8000	1 (1.3%)	6 (7.5%)	0	2 (2.5%)	7 (8.8%)	2 (2.5%)

a Extracted from Text Table 2A, page 6, of MRID 45365501

In female mice, an increase in vascular tumors was observed only at 8000 ppm; the incidence at the low- and mid-doses were consistent with the concurrent controls. Since the 8000 ppm dose exceeded the MTD, further discussion in this paper only addresses tumors at 100 and 1000 ppm. It is noted that 1 hemangioma at 1000 ppm and 2 at 8000 ppm were missed by restricting tumors to the liver and spleen. At 8000 ppm, there were 7 hemangiosarcomas reported in the carbaryl study, whereas Table 2A of MRID 45365501 lists 6 of these tumors in the liver and 2 in the spleen for a total of 8. The reason for this discrepancy is not clear.

Data from Corning Hazleton Virginia (CHV)

The data from CHV were from 25 studies conducted in CD-1 mice between 1988-1993. The only study conducted for 104 weeks was the one with carbaryl. Table 2 presents these data along with the incidence in the carbaryl study. The registrant concludes that the incidence of hepatic hemangiomas for all carbaryl-treated groups falls within the historical control range. The incidence of hepatic hemangiosarcomas below the MTD did not exceed the historical control. The incidence of splenic hemangiomas for all carbaryl groups falls within the historical control range. The incidence of splenic hemangiosarcomas and total tumors for the 1000 ppm group slightly exceeded the historical control range.

Table 2: CHV historical control data on hepatic and splenic vascular tumors in male CD-1 mice[a]

		Hemangioma		Hemangiosarcoma		Total	
Organ	Source	Mean (%)	Range (%)	Mean (%)	Range (%)	Mean (%)	Range (%)
Liver (n=1433)	CHV data	0.3	0-2	1.3	0-6.7	1.6	0-8.4
	Carbaryl incidence (%)[b]	---	0,1.3,0	---	0,3.8,6.3	---	0, 5, 6.3
Spleen (n=1424)	CHV data	0.2	0-3.1	0.9	0-4.2	1.1	0-4.2
	Carbaryl incidence (%)[b]	---	0, 0, 0	---	2.5, 2.5, 5	---	2.5, 2.5, 5

a Extracted from Table 3, page 8 of MRID 45365501
b Percent tumor incidence for the 0, 100 and 1000 ppm treatments, respectively

Data from Corning Hazleton Wisconsin (CHW)

There are data from 11 studies conducted with CD-1 mice between 1986-1993 from CHW for study durations of 78, 91 and 104 weeks. The incidence of hepatic hemangiomas for all carbaryl-treated groups falls within this historical control range. The incidence of hepatic hemangiosarcomas at 1000 ppm exceeded the historical control range. For total tumors, both the 100 and 1000 ppm groups exceeded the historical control range. The incidence of splenic hemangiomas for all carbaryl groups falls within the historical control range; for hemangiosarcomas, both the 100 and 1000 ppm groups exceeded the historical control. The incidence of total tumors at 1000 ppm exceeded the historical control range. Table 3 presents these data along with the incidence in the carbaryl study.

Table 3: CHW historical control data on hepatic and splenic vascular tumors in male CD-1 mice[a]

		Hemangioma		Hemangiosarcoma		Total	
Organ	Source	Mean (%)	Range (%)	Mean (%)	Range (%)	Mean (%)	Range (%)
Liver (n=1433)	CHW data	0.2	0-1.7	0.8	0-4	1.0	0-4
	Carbaryl incidence (%)[b]	---	0,1.3,0	---	0,3.8,6.3	---	0, 5, 6.3
Spleen (n=1424)	CHW data	0.4	0-4	0.4	0-1.7	0.8	0-4
	Carbaryl incidence (%)[b]	---	0, 0, 0	---	2.5, 2.5, 5	---	2.5, 2.5, 5

a Extracted from Table 4, page 9 of MRID 45365501
b Percent tumor incidence for the 0, 100 and 1000 ppm treatments, respectively

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Data from Charles River Laboratories, 1981-1991

Data are provided from Charles River Laboratories (CRL) on the incidence of vascular tumors from 13 studies conducted from 1981-1991 for either 18, 21 or 24 months. The data were submitted to demonstrate that spontaneous vascular tumors increase as animals age from 18 to 24 months. The registrant indicates that there is a 2-fold increase in the % mean incidence of hepatic hemangiomas and hemangiosarcomas from the 18-month to 21-month interval but not from 21 to 24 months. The mean and range of splenic hemangiosarcomas does not change from 18 to 24 months. The data are presented in Table 4.

Table 4: Charles River historical control data on hepatic and splenic vascular tumors in male CD-1 mice, 1981-1991[a]

		18 Months		21 Months		24 Months	
Organ	Tumor Type	Mean (%)	Range (%)	Mean (%)	Range (%)	Mean (%)	Range (%)
Liver	Hemangiomas	0.39	0-2.5	0.81	0-3.33	0.96	0-4
		N=770		N=370		N=521	
	Hemangio-sarcomas	1.04	0-3.85	2.7	0-6	2.11	0-8
		N=770		N=370		N=521	
Spleen	Hemangiomas	NL		NL		NL	
	Hemangio-sarcomas	0.52	0-2.53	0.81	0-2	0.58	0-2.08
		N=767		N=369		N=519	

a Extracted from Table 5, page 10 of MRID 45365501
NL=not listed

Data from Charles River Laboratories, 1978-1984

The data from 1978-1984 are presented to demonstrate that there was a little increase in the incidence of hepatic and splenic vascular tumors from 18 to 24 months in earlier time periods (Table 5). When these data are compared to more recent CRL data, the incidences at 18 months are similar. However, when the data at 24 months are compared (1981-1999 vs 1978-1984), there is a tendency for the mean % incidence to be similar; however, the upper end of the range increases approximately 4-fold for liver vascular tumors and 1.5-fold for hemangiosarcomas in the spleen in the newer data (1.4, 2.8 and 1.4% vs 4, 8 and 2.08%).

Table 5: Charles River historical control data on hepatic and splenic vascular tumors in male CD-1 mice, 1978-1984[a]

		Study Duration			
		18 Months		21-24 Months	
Organ	Tumor Type	Mean (%)	Range (%)	Mean (%)	Range (%)
Liver	Hemangiomas	0.6	0-2	1.0	0-1.4
		N=499		N=482	
	Hemangiosarcomas	0.4	0-4	1.0	0-2.8
		N=499		N=482	
Spleen	Hemangiomas	0.2	0-0.7	NL	
		N=497			
	Hemangiosarcomas	0.6	0-2.1	0.6	0-1.4
		N=497		N=479	

a Extracted from Table 7, page 12 of MRID 45365501
NL=not listed

Data from Pharmaco LSR (PLSR)

These data are from approximately 27 studies conducted for 18 months and 17 studies conducted for 88-104 weeks with CD-1 mice between 1986-1993. The incidence (mean and upper end of range) of hepatic hemangiomas is much lower at 88-104 interval than at 78 weeks. But, the incidence of hepatic hemangiosarcomas increases 3-fold for the mean and 2-fold for the upper end range from 78 weeks to the 88-104- week interval. The mean incidence of splenic hemangiosarcomas increased 4-fold and the range 1.5-fold from 78 weeks to the 88-104 week interval. When comparing the incidence in the carbaryl study, all treated groups fall within these historical control data for the hepatic vascular tumors. Splenic hemangiomas fall within the historical control range but hemangiosarcomas and total tumors are exceeded at 1000 ppm.

The registrant concludes that these data are consistent with the CRL data and demonstrate that the incidence of spontaneous vascular tumors increases with time from 18 to 24 months. The incidence of tumors from the carbaryl study generally fall within the historical control data from PLSR, except for the 1000 ppm group for total vascular tumors and hemangiosarcomas of the spleen. The data are compared to the carbaryl incidence of splenic and hepatic tumors in Table 6.

Table 6: PLSR historical control data on hepatic and splenic vascular tumors in male CD-1 mice[a]

		Hemangioma		Hemangiosarcoma		Total	
		78 Weeks					
Organ	Source	Mean (%)	Range (%)	Mean (%)	Range (%)	Mean (%)	Range (%)
Liver (n=1444)	PLSR data	0.48	0-5.4	0.76	0-3.8	1.24	0-6.6
Spleen (n=1444)	PLSR data	---	---	0.21	0-2	---	---
		88-104 Weeks					
Liver (n=1017)	PLSR	0.1	0-1.7	2.56	0-7.7	2.66	0-7.7
	Carbaryl incidence (%)[b]	---	0, 1.3, 0	---	0, 3.8, 6.3	---	0, 5, 6.3
Spleen (n=914)	PLSR	0.11	0-1.7	0.77	0-3.3	0.88	0.33
	Carbaryl incidence (%)[b]	---	0, 0, 0	---	2.5, 2.5, 5	---	2.5, 2.5, 5

a Extracted from Table 8, page 13 of MRID 45365501
b Percent tumor incidence for the 0, 100 and 1000 ppm treatments, respectively

Data from Maita *et al*

Data from 11 studies of 104 weeks duration between 1982 and 1987 on the incidence of hepatic and splenic tumors were presented in a publication by Maita *et al.*[1] CD-1 mice in this study were from a Charles River facility in Japan. Comparing to the carbaryl study, the incidence for these tumors falls within the mean % historical control data, except the hemangiosarcomas in the liver and spleen exceed the values provided by Maita *et al.* The registrant states that the incidence of hemangiosarcomas is significantly lower than the values provided by both Charles River and Pharmaco LSR. This could be the result of genetic differences or may be a function of the age of the data and the tendency to have higher rates of spontaneous vascular tumors in these mice over the last 10 years. Since the Maita *et al.* data overlap with the older CRL data, it indicates that these data may not be appropriate to use for judging the incidence of vascular tumors in the carbaryl study. The data are compared to the carbaryl incidence of splenic and hepatic tumors in Table 7.

[1] Toxicologic Pathology 16(3); 340, 1988

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Table 7: Maita *et al* historical control data on hepatic and splenic vascular tumors in male CD-1 mice[a]

		Hemangioma	Hemangiosarcoma
Organ	Source	Mean (%)	Mean (%)
Liver (n=891)	CR data	1.5	0.45
	Carbaryl incidence (%)[b]	0, 1.3, 0	0, 3.8, 6.3
Spleen (n=891)	CR data	0.56	0.45
	Carbaryl incidence (%)[b]	0, 0, 0	2.5, 2.5, 5

a Extracted from Table 9, page 15 of MRID 45365501
b Percent tumor incidence for the 0, 100 and 1000 ppm treatments, respectively

Data from Chandra and Frith

Data presented by Chandra and Frith covered 11 studies of 104 weeks duration from 1983 to 1990. A total of 725 CD-1 mice from unspecified Charles River laboratories were in the database. The mean % incidence of hemangiomas and hemangiosarcomas for males was 0.8% and 0.6%, respectively. No organ was listed as the incidence was a composite of vascular tumors. Also, there were no incidence ranges provided. The registrant states that if one assumes the tumors were primarily in the liver and spleen, the incidence of tumors in this database is much lower than any of the other databases.

2) Citation: Debruyne E. and Irisarrri E. (1996). Carbaryl Technical - Chronic Toxicity Studies in the Rat (HWA Study N°656-139) and the Mouse (HWA Study N°656-138), Evaluation of Histological Studies. Rhone-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis Cedex, Laboratory No.: R&D/CRSA/Tox-HPA-4, March 19, 1996. MRID 45365503. Unpublished

Histological slides from the 52 week interim necropsy of the carcinogenicity studies were re-examined for unreported microscopic changes indicative of potential oncogenic effects at the end of the 104-week studies. In the original study reports, no changes were reported. The slides were examined independently by two Rhone-Poulenc pathologists and their findings reported as a consensus. A proportion of slides were examined by four external consultants. It is reported that their conclusions were in agreement with the opinion of the two reviewing pathologists.

Combined Chronic Toxicity/Carcinogenicity Study in Rats (HWA Study N°656-139)

In this study, male and female rats were dosed at 0, 250, 1500 or 7500 ppm for at least 104 weeks. The control and HDT groups consisted of 90 rats/sex/group, whereas the LDT and MDT groups had 80 rats/sex/group. Ten rats/sex/group were sacrificed after 52 weeks. After 52 weeks of treatment, another 10 rats/sex in the control and HDT group were designated as recovery animals; they received basal diet for another 4 weeks and then were sacrificed. All preserved tissues from the control and HDT groups and animals that died prior to study termination were examined microscopically at 53-57 weeks. Gross lesions, lungs, liver and kidneys were examined from all animals in the LDT and MDT groups. The same protocol

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was used for the terminal necropsies, except after the initial histopathological evaluation, urinary bladder, thyroid, sciatic nerve, skeletal muscle and female pancreas from the MDT and LDT groups were also examined. After 52 weeks, there was no indication that carbaryl was associated with carcinogenicity. After 104 weeks, the following changes were noted in the HDT males and females: urinary bladder - transitional cell hyperplasia, papilloma and carcinoma (males and females); kidney - transitional cell hyperplasia and a single carcinoma (males); liver - hepatocellular hypertrophy (males and females), hepatocellular adeomas (females); thyroid - follicular cell adenoma and carcinoma (males). The report states that there was no evidence of compound-related lesions in the LDT and MDT groups.

The following are the findings of the re-examination of the 53-57 week slides.

Urinary Bladder - There was an increased incidence of transitional epithelial hyperplasia in the urinary bladder of the MDT males and HDT males and females at the week 53 sacrifice. 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 uroethium. There were no signs of inflammatory infiltration. The incidence of the hyperplasia is presented in Table 8.

Table 8: Incidence of Transitional Epithelial Hyperplasia in Bladder of Rats After 53-57 Weeks of Carbaryl Administration[a]

	Dosages (ppm)			
	0	250	1500	7500
Males Week 53 Week 57	 0/9 0/9	 0/10 -	 3/10 -	 9/9 7/9
Females Week 53 Week 57	 1/9 1/10	 0/10 -	 0/9 -	 9/9 5/10

a Extracted from Text Table 1 of MRID 45365503

Kidney - There was an increased incidence of hyperplasia of the cuboidal epithelium lining the papillary surface of the renal pelvis in the 1500 and 7500 ppm males relative to the controls at the week 53 sacrifice. After the 4-week recovery period (week 57), no difference between treatment and controls was observed. Female rats were unaffected. The incidence of this finding is presented in Table 9.

Table 9: Incidence of Urothelial Hyperplasia of the Renal Pelvis in SD Rats After 53-57 Weeks of Carbaryl Administration[a]

	Dosages (ppm)			
	0	250	1500	7500
Males				
Week 53	0/9	0/10	3/10	7/9
Week 57	3/8	-	-	2/9
Females				
Week 53	0/10	1/10	2/10	0/10
Week 57	0/10	-	-	1/10

a Extracted from Text Table 2 of MRID 45365503

Liver - 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. The incidence of this finding is presented in Table 10.

Table 10: Incidence of Hepatic Hepatocellular Hypertrophy in SD Rats After 53-57 Weeks of Carbaryl Administration[a]

	Dosages (ppm)			
	0	250	1500	7500
Males				
Week 53	0/9	0/10	2/10	8/9
Week 57	0/9	-	-	1/9
Females				
Week 53	0/10	0/10	0/10	10/10
Week 57	0/10	-	-	1/10

a Extracted from Text Table 3 of MRID 45365503

Thyroid - Follicular cell hypertropy 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 controls, there was no dose-related effect. The report states that the incidence was comparable to controls after the recovery period, but the data show 3/9 HDT males affected vs 0/9 at week 57. The incidence of the finding in males is presented in Table 11.

Table 11: Incidence of Thyroid Follicular Cell Hypertrophy in Male SD Rats After 53-57 Weeks of Carbaryl Administration[a]

	Dosages (ppm)			
	0	250	1500	7500
Males				
Week 53	3/9	5/10	4/10	7/9
Week 57	0/9	-	-	3/9

a Extracted from Text Table 4 of MRID 45365503

Carcinogenicity Study in Mice (HWA Study N°656-138)

After 104 weeks of treatment, there was an increased incidence of the following: hemangiomas/hemangiosarcomas in all treated males and HDT females; renal tubular cell adenomas and carcinomas in HDT males; and hepatocellular adenomas and carcinomas in HDT females. The report states that no significant finding considered treatment-related were noted in the liver or kidney of CD-1 mice treated for 52 weeks at a dietary concentration of 7500 ppm.

3) Citation: Cohen S. M. (1995). Evaluation of the Urinary Bladder Carcinogenicity of Carbaryl in Rats. Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, Laboratory No.: none, November 2, 1995. MRID 45365502. Unpublished.

The number of animals with hyperplasia, papilloma and carcinoma of the urinary bladder and hyperplasia and carcinoma of the kidney pelvis from the rat combined chronic toxicity/carcinogenicity study cited in this paper is presented in Table 12.

Table 12: Incidence of Bladder and Renal Pelvis Changes in SD Rats Treated with Carbaryl[a]

	Urinary Bladder						Kidney			
Dose (ppm)	Hyperplasia		Papilloma		Carcinoma		Hyperplasia		Carcinoma	
	M	F	M	F	M	F	M	F	M	F
0	8	6	0	1	0	0	12	21	0	0
250	8	6	0	0	0	0	7	41	0	0
1500	10	6	0	0	0	0	9	28	0	0
7500	54	56	13*	7	11	6	30	21	1	0

a Extracted from text table on page 5 of MRID 45365502

* Includes the squamous cell papilloma noted for animal C09446

The paper states that there were no hyperplastic or neoplastic lesions in the bladder after 52 weeks of treatment. This conflicts with MRID 45365503, the re-examination of the slides from the week 53 and

57 sacrifices discussed above. Apparently, this paper was prepared prior to the re-examination.

No hyperplastic or neoplastic lesions were observed in the carcinogenicity study in CD-1 mice. In mice fed the highest dose, there were eosinophilic inclusions in the superficial umbrella transitional cells of the bladder epithelium. There was no evidence of necrosis, inflammation, hyperplasia or neoplasia in the mouse bladder.

In the rat study, hyperplastic and neoplastic lesions in the urinary bladder were seen only at the highest dose with a similar response in both sexes. There was also a significant increase in renal pelvic hyperplasia in the male at the highest dose along with one carcinoma. The paper states that the highest dose in both the rat and mouse studies was in excess of the maximum tolerated dose. Weight gain decreased more than 10% compared to controls, particularly in the rat. The author states that this raises the question of the legitimacy of results in the HDT male rats.

The paper states carbaryl has been extensively evaluated in various *in vitro* and *in vivo* assays for genotoxicity, including an *in vivo* assay in the rat. There was also no evidence of DNA binding. Based on these results and the overall structure of the chemical and its metabolites, the weight of evidence strongly suggests that carbaryl does not act via a genotoxic mechanism. The author states that since direct interaction with DNA is highly unlikely, increased cell proliferation in the target tissue is the most likely mehanism for the carcinogenicity. Although the actual mechanism by which carbaryl produces increased cell proliferation in the rat bladder has not been examined, there is information from the studies performed and in the literature to evaluate possible hypotheses. The most common cause of bladder carcinogenesis by non-genotoxic compounds is toxicity with consequent regenerative hyperplasia. This is usually associated with the formation of calculi in the bladder lumen; however, regenerative hyperplasia can be produced by toxic chemicals or their metabolites without the formation of calculi. Based on extensive hyperplasia in the bladder without associated necrosis, inflammation or regeneration, it is more likely that the epithelial proliferation is due to a mitogenic effects of carbaryl, or more likely, one or more of its metabolites. Propoxur, an aromatic carbamate like carbaryl, produced a low incidence of bladder cancer at a dose of (8000 ppm) similar to carbaryl in the rat bladder epithelium, but not in the mouse. There was also no calculus formation in the bladder with this chemical. The paper states that a proliferative effect was detected and it appeared to be a direct mitogenic effect rather than a toxic effect with regeneration.

Like propoxur, carbaryl can be metabolized by essentially three major pathways: 1) epoxide formation with subsequent metabolism to mono- and biphenolic metabolites, most of which are conjugated as glucuronides or sulfates; 2) hydrolysis of the carbamate; and 3) oxidation of the alkyl moiety. For carbaryl, there is no evidence that the hydrolysis product, 1-naphthol, has any effect on the rat bladder epithelium. In contrast, the mono- and diphenolic metabolites are similar to the metabolites of propoxur that are known to affect the rat bladder epithelium. The latter category includes the compounds butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The diphenolic compounds resemble catechol which has been observed to have an effect on rat bladder epithelium. The paper states that although there is no clear evidence for which metabolites of carbaryl or propoxur, BHA or BHT cause the proliferation in the rat bladder epithelium after administration of high doses in the diet, the

evidence suggests that the monophenolic metabolites are most likely responsible. NOTE: This appears to conflict with previous statements which implicate diphenolic compounds and may be an error.

Carbaryl is completely absorbed from the gastrointestinal tract when fed in the diet and is rapidly excreted so that 85-95% of the dose is excreted in the urine within 24 hours of administration. Due to the reservoir function of the bladder, the urine is present and in contact with epithelial cells for considerably longer periods of time.

The paper concludes that the evidence suggest that carbaryl produces tumors by increasing cell proliferation. This is accomplished most likely by a direct mitogenic effect of carbaryl, or more likely, by one or more of its metabolites. This could occur by an alteration of growth factor or cell interaction. There is unlikely to be a carcinogenic hazard to humans exposed to considerably lower doses than the high dose in the rat combined chronic toxicity/carcinogenicity study.

4) Berthe P (1997). Carbaryl 14-Day Toxicity Study in the Rat by Gavage. Rhone-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis Cedex, Study SA95515, January 9, 1997, MRID 45365504. Unpublished

In a special 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 cyclying 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.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 10/17/01
Reregistration Branch I, Health Effects Division (7509C)
Branch Senior Scientist: Whang Phang, PhD Whang Phang, Date 10/25/01
Reregistration Branch I, Health Effects Division (7509C)

TXR#0050256

DATA EVALUATION RECORD

STUDY TYPE: Special Mechanistic Study

DP BARCODE: DP Barcode: D274052; Submission: S595378
P.C. CODE: 056801

TEST MATERIAL (PURITY): Carbaryl (98.4% ai)

CITATION: Berthe, P (1997). Carbaryl 14-Day Toxicity Study in the Rat by Gavage. Rhone-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis Cedex, France. Laboratory report number SA 95515, January 9, 1997. MRID 45365504 Unpublished

SPONSOR: Rhone-Poulenc Agrochimie

EXECUTIVE SUMMARY:

In a special 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.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Carbaryl
   Description: white powder
   Batch #: 208115110
   Purity: 98.4% ai.

2. Vehicle Control: 0.5% carboxymethylcellulose/0.1% Tween 80

3. Test animals: Species: rat
   Strain:Sprague-Dawley
   Age and weight at study initiation: 9 weeks old; males: approximately 330g; females: approximately 230 g
   Source: Iffa-Credo, L'Arbresle, France
   Housing: individually in stainless steel wire cages
   Diet: Certified Rodent Pellet Diet A04C ad libitum
   Water: municipal water supply ad libitum
   Environmental conditions: Temperature: 20-24^{0}C
   Humidity: 40-70%
   Air changes: 10 to 15 per hour
   Photoperiod: 12-hour light/12-hour dark
   Acclimation period: 6 days

B. STUDY DESIGN:

1. In life dates - start: January 9, 1996 end: January 23/24, 1996

2. Animal assignment

Carbaryl was administered by gavage (5 ml) to 5 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.

3. Statistics - Standard statistical methods were used to analyze organ weights, organ/body weights, body weight, food consumption, cytochrome P-450 enzymatic activities, specific cytochrome P-450 enzymatic activities, T_4-UGT and T_3-UGT.

C. METHODS:

1. Observations:

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

2. Body weight

Animals were weighed once during the acclimation period, on the first day of dosing, at weekly intervals and before necropsy.

3. Food consumption

The weight of food supplied and that remaining were recorded weekly.

4. Post-mortem Examinations

Sacrifices and necropsies were performed on study days 4 and 15. The necropsies included examination of all major organs. Livers were weighed and microscopic slides prepared from sections of the organs.

5. Hepatotoxicity Testing

At the final necropsy, the remaining portions of the liver from all animals were homogenized for microsomal preparations. Cytochrome P-450 specific enzymes were assessed using the following methodology: Western blotting for qualitative investigation and enzymology for quantitative determination of P-450 activities.

a) Total cytochrome P-450 content

Total cytochrome P-450 content on two replicates of the microsomal preparations was determined by spectrophotometry using a reduced CO differential spectrum.

b) Specific cytochrome P-450 isoenzyme content

P-450 specific isoenzymes were examined by western blotting after electrophoresis of microsomal preparations. The antibodies tested, enzymatic activity and typical inducing agents are listed below.

Family	Enzymatic Activity	Typical Inducing Agents
CYP 1A1 1A2	ethoxyresorufin (EROD)	B-naphthoflavone
CYP 2B1/2 2B1 2B2	pentoxyresorufin (PROD)	phenobarbital
2E		isoniazid
CYP 3A	benoxyresorufin (BROD)	pregnenolone 16 alpha-carbonitrile phenobarbital
CYP 4A	lauric acid hydroxylation	clofibric acid

c) P-450 enzymatic activities

Specific cytochrome P-450 enzymatic activities were evaluated by spectrofluorimetry using the following substrates:

- benoxyresorufin
- ethoxyresorufin
- pentoxyresorufin

and by HPLC with fluorimetric detection. Ethoxyresorufin is a selective substrate for isoform 1A, the isoform 2B metabolizes preferentially the O-dealkylation for pentoxyresorufin and the benzoxyresorufin O-debenzylation is mainly metabolized by the isoform 3A.

d) T_4 and T_3 UDP-glucuronidation activities

Thyroxine UDP-glucuronyltransferease (T_4- UGT) and 3,3',5-triiodothyronine UDP-glucuronyltransferase (T_3 -UGT) activities were evaluated on the microsomal preparations.

e) Cell cycling assessment

An immunohistochemical staining was used to measure the proliferating cell nuclear antigen (PCNA) in order to assess cell cycling. A monoclonal antibody raised against the PCNA was applied to formalin-fixed, paraffin-embedded, deparaffinized liver sections. Measurements were performed at a magnification of x400 using an eye-piece reticule of 1 square millimeter

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divided into 100 squares. All the PCNA-positive hepatocytes in ten reticular surfaces were counted and classified according to their staining characteristics into G1, G2 or S phase of the cell cycle and reported as mean group values.

II. RESULTS

A. Observations :

1. Toxicity - Signs of cholinesterase inhibition, including reduced motor activity, tremors, staggering steps, increased salivation, piloerection, soft feces and polypnea, were observed in most animals treated at 40 mg/kg/day during 1 day of treatment. No clinical signs of toxicity were observed in the control or 10 mg/kg/day groups.

2. Mortality - None of the animals died during the study.

B. Body weight and body weight gain:

Body weight was significantly reduced at the end of the first and second weeks (8% and 7%, respectively) in males treated at 40 mg/kg/day. Body weight gain was reduced at the end of the first week but was comparable to controls at the end of the second week.

C. Food consumption

Males treated at 40 mg/kg/day had lower food consumption (21% decreased) at the end of the first week but values were comparable to controls at the end of the second week.

D. Post-mortem Examinations

1) Organ Weights - There were no organ weight changes after 3 or 14 days of treatment.

2) Gross Pathology - There were no gross pathology changes.

3) Microscopic Pathology - There were no microscopic changes after 3 or 14 days of treatment.

E. Hepatotoxicity Testing

1) Total cytochrome P-450 content

There were no significant differences in P-450 content at either dose of carbaryl.

2) Specific cytochrome P-450 isoenzyme content

The western blotting with anti-P450 antibodies showed that:

1) The level of isoforms 1A, 2B and 3A were not detected in any control or treated groups.

2)The levels of isoforms 2E and 4A in the treated groups were not increased over those in the controls.

In general, carbaryl treatment did not modify the level of expression of isoforms 1A, 2B, 3A, 2E and 4A.

3) P-450 enzymatic activities

The specific cytochrome P-450 activities were evaluated by spectrofluorimetry using substrates which were preferred by the individual isoforms. There was no effect of carbaryl on BROD and PROD activities or on lauric acid hydroxylation compared to control values. A statistically significant increase in EROD activity was observed in males treated at 40 mg/kg/day (5.5-fold increase). However, the increase was very weak compared to B-naphthoflavone (Table 1). It is noted that the control values for the reference compounds were quite different from the controls of carbaryl-treated groups. For example, in the mean data for BROD (pmol/min/mg protein) for the reference compounds (Table 10 of the study report), the mean male and female control values were 29.50 and 7.66 for males and females, respectively. When testing the carbaryl-treated animals, the mean male and female control values were 16.23 and 0.00 , respectively (unnumbered table on page 62 of the study report). The units of BROD are not provided in the second table but it assumed to be the same as Table 10.

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Table 1: Mean Ethoxyresorufin Levels in Rats Treated with Carbaryl and β-NF[a,b]

Dose (mg/kg/day)		Ethoxyresorufin			
		Carbaryl-treated		β-NF	
		female	male	female	male
0	N	5	5	5	5
	Mean	0.00	2.78	38.80	153.49
	Std	0.00	5.03	11.82	29.87
10	N	5	5		
	Mean	0.64	5.50		
	Std	1.43	3.98		
40	N	5	5		
	Mean	2.02	15.44*		
	Std	3.39	4.18		
75	N			5	5
	Mean			935.61	532.75
	Std			428.97	74.27

a Extracted from Table 11 (page 63) and unumbered table (page 64) of MRID 45365504
b Units were not provided on this table; it is assumed that they are pmol/min/mg protein which were the units provided for the reference compounds (Table 11)
* alpha = 0.05

4) T_4 and T_3 UDP-glucuronidation activities

T_4- and T_3- UGT activities (Table 2) were significantly increased at 40 mg/kg/day in males and 10 and 40 mg/kg/day in females (1.5 increase in T_4-UGT activity and 1.8-fold increase in T_3- UGT activity in both male and female rats). The increases were observed on activated microsomes only. The study author stated that the effects were similar to a phenobarbital-like inducer.

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Table 2: T_4-UGT and T_3-UGT values (pmol/min/mg protein) in rats treated with carbaryl[a]

	Dose Levels (mg/kg/day)					
	Males			Females		
	0	10	40	0	10	40
T_4-UGT						
Native	1.91±0.42	1.64 ±0.29	2.32±0.52	1.52±0.15	1.76±0.35	1.86±0.30
Activated	3.44±0.64	3.83±0.86	5.12±0.95*	1.70±0.16	2.35±0.48*	2.50±0.43*
T_3-UGT						
Native	2.34±0.27	2.18±1.19	3.47±1.20	1.59±0.63	2.25±0.18	2.37±0.59
Activated	3.99±0.24	4.81±2.83	7.69±2.20*	1.95±0.75	3.27±0.73*	3.50±1.07*

a Extracted from Tables 1 and 2 (pages 217-219) of MRID 45365504
* Significantly different from control using Dunnett test, $p<0.05$

5) Cell cycle assessment

On study day 4, there was a moderate increase in the mean total number of PCNA-positive cells in males treated at 40 mg/kg/day. An increase in phase G1 cells accounted for the increase. At the final sacrifice, there was a moderate increase in the PCNA-positive cells in males treated with 40 mg/kg/day. Both phases G1 and S were increased. Females treated at 10 and 40 mg/kg/day also showed an increase in PCNA-positive cells but the increases were not dose-related. The findings are presented in Table 3.

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Table 3: Mean (Standard Deviation) Values for PCNA Positive Cells[a]

	Cell Phase -			
	G1	S	G2	Total
Males				
Day 4				
control	0.60 (0.55)	10.80 (4.44)	0.00 (0.00)	11.40 (4.56)
10 mg/kg/day	0.00 (0.00)	2.80 (2.17)	0.00 (0.00)	2.80 (2.17)
40 mg/kg/day	12.40 (11.13)	11.80 (10.83)	0.20 (0.45)	24.40 (22.10)
Day 15				
control	0.00 (0.00)	3.46 (3.29)	0.00 (0.00)	3.46 (3.29)
10 mg/kg/day	0.20 (0.45)	0.40 (0.89)	0.00 (0.00)	0.60 (1.34)
40 mg/kg/day	7.20 (4.09)	9.00 (4.24)	0.20 (0.45)	16.40 (6.84)
Females				
Day 4				
control	0.40 (0.89)	4.20 (6.26)	0.00 (0.00)	4.60 (7.13)
10 mg/kg/day	9.20 (10.62)	10.40 (7.30)	0.40 (0.89)	20.00 (17.64)
40 mg/kg/day	1.40 (2.19)	0.80 (0.84)	0.00 (0.00)	2.20 (2.68)
Day 15				
control	0.00 (0.00)	1.00 (2.24)	0.00 (0.00)	1.00 (2.24)
10 mg/kg/day	8.60 (5.73)	11.60 (7.44)	1.60 (2.19)	21.80 (14.69)
40 mg/kg/day	5.60 (4.93)	10.40 (7.64)	0.20 (0.45)	16.20 (12.40)

a Extracted from Table 14 (pages 70-73) of MRID 45365504

III. DISCUSSION

A. Study Deficiencies

The report does not contain the individual animal data for enzymatic activity evaluation for the reference compounds (PB and β-NF). The enzymatic activity values for the control group of the reference compounds are different from those of the control group of the carbaryl-treated study. It appears that these assays were conducted at different times. Yet, the methods section of the report states that the determinations were conducted at the same time as the study samples. This difference needs to be explained.

B. Study Author's Conclusions

The study author concluded that, based on PCNA and UGT activity results, there was no

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NOAEL in the study.

C. Reviewer's Conclusions

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