012892

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

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carbaryl - Memorandum of Meeting of Cancer Assessment Review Committee (CARC) Metabolism Subgroup

PC Code: 056801

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer
Reregistration Branch I, Health Effects Division (7509C) Virginia A Dobozy 10/5/98

TO: Linda Propst/Kathryn Boyle
Special Review and Reregistration Division (7508W)

THRU: William Burnam, Chairman WB
Cancer Assessment Review Committee, Health Effects Division (7509C)

A group of Office of Pesticide Programs (OPP) scientists with expertise in metabolism (hereby referred to as Cancer Assessment Review Committee [CARC] Metabolism Subgroup) met on September 3, 1998, to review the metabolism data base for carbaryl. When the HED Cancer Peer Review Committee (CPRC) previously reviewed carbaryl on October 27 and December 8, 1993, the chemical was classified as a Group C - possible human carcinogen. Both a MOE and Q_1^* were selected for quantitation of cancer risk. The Committee requested additional metabolism studies to help: 1) direct the selection of the more appropriate quantitative approach for cancer risk; and 2) provide insight into the significance of the tumors seen only at excessively toxic doses. The CARC Metabolism Subgroup was convened to review the metabolism data submitted since 1994 and to determine if these studies help clarify the most appropriate method of quantitation for cancer risk assessment. It should be noted that revised draft cancer assessment guidelines, the 1996 *EPA Proposed Guidelines for Carcinogen Risk Assessment*, have been published since the CPRC meeting. The Guidelines describe linear and non-linear (MOE) approaches to dose extrapolation for risk estimation. A default assumption of linearity is appropriate when the evidence supports a mode of action of gene mutation due to DNA reactivity or supports another mode of action that is anticipated to be linear. The default assumption of linearity is also appropriate when evidence shows no DNA reactivity or other support for linearity, but neither is there sufficient evidence of a nonlinear mode of action to support a nonlinear procedure. A default of nonlinearity is appropriate when there is no evidence for linearity and sufficient evidence to support an assumption of nonlinearity and a nonlinear procedure.

The CARC Metabolism Subgroup concluded that, based on the draft Guidelines, **the data from the available metabolism studies are not adequate to support a nonlinear mode of action and therefore recommends that the default linear approach should be used for risk quantitation.** Attached is a copy of the Memorandum of the meeting.

cc: William Burnam (SAB), Byong-Han Chin (RRB1), Ghazi Dannan (TB2), Alberto Protzel (TB1), Timothy McMahon (Antimicrobial Division), Jess Rowland (RCAB), Rick Whiting (SAB), Cancer File (SAB)

CARBARYL - MEMORANDUM OF MEETING OF CARC METABOLISM SUBGROUP

On September 3, 1998, a group of Office of Pesticide Programs (OPP) scientists with expertise in metabolism (hereby referred to as the Cancer Assessment Review Committee [CARC] Metabolism Subgroup) met to discuss the metabolism data base (submitted since 1994) for carbaryl and to determine if these studies help clarify the most appropriate method of quantitation for cancer risk assessment. Carbaryl was reviewed by the Cancer Peer Review Committee on October 27 and December 8, 1993. At that time, the chemical was classified as a Group C - possible human carcinogen based on a statistically significant increase in hemangiosarcomas and combined hemangiomas/hemangiosarcomas in male mice at a dose which was sufficient but not excessive (1000 ppm). There were additional tumors at the highest dose, 8000 ppm, in mice and at 7500 ppm in rats but the Committee judged that these doses were excessive. Both a MOE and Q_1* were selected for quantitation of cancer risk. The Committee requested additional metabolism studies to help 1)direct the selection of the more appropriate quantitative approach for cancer risk; and 2) provide insight into the significance of the tumors seen only at excessively toxic doses. It should be noted that the 1996 *EPA Proposed Guidelines for Carcinogen Risk Assessment* describe linear and non-linear (MOE) approaches to dose extrapolation for risk estimation. A default assumption of linearity is appropriate when the evidence supports a mode of action of gene mutation due to DNA reactivity or supports another mode of action that is anticipated to be linear. The default assumption of linearity is also appropriate when evidence shows no DNA reactivity or other support for linearity, but neither is there sufficient evidence of a nonlinear mode of action to support a nonlinear procedure. A default of nonlinearity is appropriate when there is no evidence for linearity and sufficient evidence to support an assumption of nonlinearity and a nonlinear procedure.

I. Documents Reviewed

The following is a list of documents (with summaries) reviewed by the CARC Metabolism Subgroup:

A. May 12, 1994 Report of the HED Carcinogenicity Peer Review for Carbaryl

In the mouse carcinogenicity study (MRID 42786901), 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 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 $p<0.05$).

3

Female mice had significantly 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. The CPRC judged the 8000 ppm dose to be excessive based on the severity of the effects on body weight, cholinesterase inhibition, clinical signs and histopathological changes.

In the rat combined study (MRID 42918801), 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 tumors.

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 CPRC judged the 7500 ppm dose to be excessive based on the severity of the effects on body weight gains, clinical pathology and cholinesterase inhibition.

B. DER for MRID 43282201 - DNA Binding Study

The objective of this study was to test the ability of carbaryl to bind to liver DNA in male CD-1 mice. The high dose of 8000 ppm was administered for two weeks and followed by a single radiolabeled dose of 75 mg/kg. At this dose, carbaryl interacted with chromatin protein but not with DNA. However, the methods used do not permit a definitive conclusion about the DNA binding. A more detailed discussion is under II. Discussion of Strengths and Weaknesses of Metabolism Studies.

C. DER for MRID 43332101 - Metabolism study

This was a standard metabolism study in male and female Sprague-Dawley rats at doses of 1 mg/kg (single & repeated low oral radiolabeled doses; intravenous dose) and 50 mg/kg (single high oral radiolabeled dose). The study demonstrated that absorption was essentially complete. At 168 hrs post-dose, there were negligible percentages of the dose in any tissue; kidney and blood contained highest concentrations of residual radioactivity. Excretion was largely through urine (88-95% of radioactivity recovered). Conjugated and non-conjugated metabolites were identified and a metabolic scheme was proposed.

4

D. DER for MRID 43832601 - P450 Induction Study

The objective of this study was to test the potential of carbaryl to induce hepatic cytochrome P450 in male CD-1 mice at a dose of 8000 ppm. The study demonstrated that carbaryl is a weak "phenobarbital-type" inducer of liver metabolizing enzymes.

E. DER for MRID 44402501 - Carbaryl Metabolism in 15 Month-old Rats

The objective of this study was to investigate the mechanism that lead to tumors during the final year of the combined rat study. Carbaryl was administered at a single oral dose of 50 mg/kg or was administered in the diet at 250, 1500 or 7500 ppm for 83 days followed by 2 mg/kg/day carbaryl for 7 days. The study demonstrated that the sulfate conjugation pathway was saturable after 83 days at 7500 ppm as demonstrated by the apparent decrease in naphthyl sulfate (as a percentage of dose) and possible increase in naphthyl glucuronide (as a percentage of dose) at this dose as compared to the lower dose and control groups.

F. Registrant's Summary of the Carbaryl Toxicology Studies submitted October 15, 1996 (before the completion of MRID 44402501).

The following is a summary of the registrant's interpretations of the metabolism data.

1. DNA Binding Study - Concluded that carbaryl shows no genotoxic potential as demonstrated in the DNA binding *in vivo*.

2. Liver cytochrome P-450 inducer phenotyping in male CD-1 mice - Concluded that carbaryl is an enzyme inducer in mice with a pattern similar to barbiturates. In view of the magnitude of the dose, carbaryl is a low potency inducer in comparison to reference compounds.

3. Rat metabolism study - Concluded that carbaryl was almost completely absorbed and metabolized (≈90%) and that there was essentially no difference in the metabolism between sexes or in the low versus high dose. Likewise, there was no difference in the metabolism between the single versus multiple doses. The low dose was completely eliminated in 12 hours and the high dose in 24 hours.

Two metabolites, 5, 6-dihydro-5,6-dihydroxy carbaryl and 3,4-dihydro-3,4-dihydroxy carbaryl were identified to be approximately 8% and 1%, respectively, of the total dose and were found to be conjugated to glucuronide. These metabolites very likely formed from the metabolism of epoxide intermediates. Other metabolites which were likely formed from the epoxide intermediates were identified as 5-hydroxycarbaryl (13%) and 4-hydroxycarbaryl (6%) and conjugated carbaryl (3%). Thus, in young rats, carbaryl is completely absorbed and rapidly excreted. Approximately, 31% of the total dose appears to be metabolized through epoxide intermediates which are rapidly metabolized and excreted as any endogenous expoxides would be.

5

Epoxide intermediates have been proposed to be the proximate carcinogen for several classes of carcinogens. These reactive intermediates may be routinely handled by the body like other exogenous epoxides or could be available to react with cellular components. Thus, it is possible that excessively high doses of carbaryl could alter the normal metabolism, distribution, and/or excretion pattern in many different ways, e.g., saturate the normal metabolic pathways with a shift of carbaryl metabolism through the epoxide intermediates, deplete the available glutathione stores available for conjugation, increase the half-life of the epoxide intermediates, increase tissue concentrations of carbaryl and its metabolites due to diminished ability for excretion, etc. Additionally, saturation of many enzyme systems with large carbaryl substrate concentrations could allow accumulation of endogenous chemicals and by-products of normal metabolism that would otherwise be detoxified and excreted.

Geriatric changes in the last year of life in rodents compromise the hepatic and renal ability to detoxify and excrete metabolites. Based on the results of the 2-year rat study, the animals may have been able to more effectively metabolize and detoxify the high doses of carbaryl in the early part of life but unable to metabolize and detoxify such doses when normal degenerative changes occurred with old age.

II. Discussion of Strengths and Weaknesses of Metabolism Studies

The CARC Metabolism Subgroup discussed each of the metabolism studies in detail. The following strengths and weaknesses of the data were identified.

1. The two metabolism studies conducted in mice are of prime importance because the only tumors which were found at adequate, but not excessive, doses were the hemangiomas and hemangiosarcomas in mice at 1000 ppm. Concerning the DNA binding study, the subgroup concluded that the study did not conclusively demonstrate that carbaryl does not bind to DNA in the liver of male CD-1 mice. Due to the limited sensitivity of the method used in this study, it could not be determined if the radioactivity from the test material binds to DNA or not. Some of the radioactivity bound might have resulted from binding to protein and low levels of binding to DNA may have gone undetected. Examination of the interaction of carbaryl with specific nucleotides within the DNA would have provided better information on the potential DNA reactivity of carbaryl. In additon, the liver was not the target tissue in the mouse carcinogenicity study. Tumors were found in the blood vessels (hemangiomas/ hemangiosarcomas) at sufficient doses (1000 ppm) and kidney tubule cell tumors at excessive doses (8000 ppm).

2. The P450 study demonstrated that carbaryl is a weak "phenobarbital-type" inducer. There were no other studies in mice that explored the effect of increasing doses on carbaryl metabolism. Since tumors occurred at 1000 ppm, studies to establish a nonlinear approach for risk estimation should examine if there was a change in metabolism at this dose. However, it should be emphasized that an alteration in metabolism, by itself, is not sufficient evidence of a nonlinear mode of action.

3. The registrant made multiple postulations about alterations in the metabolism, distribution and excretion pattern of carbaryl at high doses, including the formation of reactive expoxides, depletion of glutathione, increase in the half-life of the epoxide intermediates and increased tissue concentrations of carbaryl. Concerning the rat studies, the subgroup concluded that there was an apparent shift in the metabolic pathway from sulfation to glucuronidation at the high dose (7500 ppm). However, there was no clear evidence of new metabolites being formed or a shift in the level of metabolites, except for those produced by sulfation or glucuronidation, or that older animals were unable to metabolize and detoxify high doses of carbaryl. In addition, there was no evidence of a correlation between the metabolic shift and increased toxicity or tumor formation.

III. Conclusions about Metabolism Data Base

Based on the 1996 *EPA Proposed Guidelines for Carcinogen Risk Assessment*, **the CARC Metabolism Subgroup concluded that the data from the available metabolism studies are not adequate to support a nonlinear mode of action and therefore recommends that the default linear approach should be used for risk quantitation.**

7

Carbaryl Metabolism Subgroup

William Burnam _____

Byong-Han Chin _____ 9/21/98

Ghazi Dannan _____ 9/22/98

Alberto Protzel _____ 9/17/98

Timothy McMahon _____ 9/22/98

Virginia Dobozy, Rapporteur _____ 9/22/98

8