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OFFICE OF  
PESTICIDES AND TOXIC  
SUBSTANCES

MEMORANDUM

SUBJECT: Carbaryl (1-naphthyl N-methylcarbamate)  
Mutagenicity Studies

Project No. 1-0950  
TOX Chem No. 160

FROM: Ray Landolt *RL 7/3/91*  
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TO: Dennis Edwards, Jr., PM 12  
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THRU: Mike Ioannou, Section Head  
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and  
Marcia van Gemert, Branch Chief  
Toxicology Branch II  
Health Effects Division (7509C) *J.M. Ioannou 7/3/91*  
*management 7/8/91*

Registrant: Rhone-Poulenc AG Company letter of January 26, 1990

Action Requested: The registrant has provided additional information for the two mutagenic studies (MRID No's 41370301 and 41370302) found not acceptable in the Toxicology review (DER 008115) of October 3, 1990.

Conclusion:

1. Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes MRID No's. 41370301 and 41810601 may be upgraded from unacceptable to acceptable and satisfies the mutagenic guideline data requirement 84-4.
2. Mammalian Cells in Culture Gene Mutation Assay in Chinese Hamster Ovary (CHO) Cells (MRID No's. 41370302 and 41810602) is not acceptable.
3. Four mutagenicity studies were reviewed in the Toxicology review (DER 008115) of October 3, 1990. Of these, the following three are acceptable and satisfy the guideline data requirement for mutagenicity testing.

84-2(a) Salmonella typhimurium/Mammalian Microsome Mutagenicity Assay (MRID 41370303).

84-2(b) Mammalian Cells in Culture Cytogenetic Assay in Chinese Hamster Ovary (CHO) Cells (MRID 41370304).

84-4 Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes (MRID 41370301).

Consideration given to the data submitted.

1. Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes (MIRD 41370301)

This study was not acceptable due to the presentation of average values without the actual grain counts or some indication of variability (standard deviations)

Response: The final report has been revised (MRID 41810601) to replace page 23, Table 1 and page 24, Table 2 with the requested information concerning the calculated means and standard deviation for the net nuclear grain counts.

The request for information on the stability of the test material was overlooked in this review. However, there is evidence in this study of a dose-related increase in cytotoxicity (% survival) in the treated hepatocytes (19 hrs. posttreatment). This study may be upgraded from unacceptable to acceptable.

Conclusion: Carbaryl is not genotoxic in this test system at 5.0 to 25.0 ug/mL and satisfies the guideline data requirement 84-2

2. Mammalian Cells in Culture Gene Mutation Assay in Chinese Hamster Ovary (CHO) Cells (MRID 41370302).

This study was not acceptable and does not support the conclusion that carbaryl is negative in this test system under the S9 metabolic activation and nonactivation conditions of this assay.

Response: The registrant has provided the following criteria that must be met for an assay to be considered positive:

1. "The test article must induce a statistically significant increase in the mutant frequency (95% level of significance using the Kastenbaum and Bowman test), and
2. The mutant frequency must also meet or exceed  $15 \times 10^{-6}$  in order to compensate for random fluctuations in the 0 to  $10 \times 10^{-6}$  background mutant frequency range that are typical for this assay.
3. A dose-related or toxicity-related increase in mutant frequency should be observed. Single dose increases must either be confirmed in a second trial or the number of mutant colonies must be more than twice the value needed to indicate a significant response.

Response: (continued)

4. The doses used for the evaluation should have a toxicity range between no toxicity and about 10% survival. (At concentrations less than 10% survival, the number of surviving cells is too small to obtain reliable results). Data is shown from doses with less than 10% survival but the results are not used in the analysis."

The registrant concluded that "in the study in question, when one considers the treatments with greater than 10% survival, none meet all three criteria. In fact, the only treatment that exceeds the first two criteria has a survival of 1.2% which is at a survival that is unacceptable in this assay. We have found that all the above criteria must be met in this assay for a reliable evaluation."

While the criteria submitted to determine the positive response in the CHO/HGPRT forward mutation assay are considered to be reasonable, the following deficiencies were not addressed:

1. The conflicting cytotoxicity data for the S9-activated assays provide no assurance that the final S9-activated mutation assay was conducted over an appropriate dose range;
2. Since the study author concluded that the new S9 batch was responsible for the increased cytotoxicity, the batch should have been replaced with a fully characterized batch;
3. There is concern that the increased cytotoxicity was accompanied by a decrease in assay sensitivity; and
4. Considering the adverse effects of carbaryl on cell cycling time, the expression period should have been increased to nine days.

Conclusion: The registrant's responses are not acceptable and have not addressed the deficiencies cited in this study. This study, Mammalian Cells in Culture Gene Mutation Assay in Chinese Hamster Ovary (CHO) Cells (MRID 41370302), remains unacceptable. However, the guideline data requirement for mutagenicity testing is satisfied and additional testing is not necessary.