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

OCT - 6 1994

MEMORANDUM

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

Subject: Carbaryl
84-2b Structural Chromosome Aberration Study

From: Ray Landolt 9/28/94
Review Section I
Toxicology Branch II
Health Effects Division (7509C)

P.C. Code 056801
Barcode D197680
Submission S455305
MRID 430393-01

To: Linda Propst, PM 73
Reregistration Branch
Special Review & Reregistration Division (7508W)

Thru: Mike Ioannou, Section Head
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Toxicology Branch II
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and
Marcia van Gemert, Branch Chief
Toxicology Branch II
Health Effects Division (7509C)

J M. Ioannou 9/29

mvanGemert 10/5/94

Registrant: Rhone-Poulenc Ag Company, letter of December 2, 1993

Action Requested: Review "Study to Evaluate the Chromosome Damaging Potential of Carbaryl Technical by its Effects on the Bone Marrow Cells of Treated Rats"

Executive Summary: In an in vivo cytogenetic assay (MRID 43039301), 3 groups of 5 Sprague-Dawley rats/sex/dose received a single oral dose of 30.0, 60.0 or 120 mg/kg carbaryl technical (99.7%) in 0.25% methylcellulose. The dose administered was based on a range-finding study with an LD_{50} of approximately 231 mg/kg. Carbaryl was negative for structural aberrations at doses ranging from 30 to 120 mg/kg. Clinical signs of toxicity were observed at the 120 mg/kg level.

This study is acceptable and satisfies the Guideline Data Requirement 84-2b for mutagenic testing.

Conclusion: Carcinogenicity Peer Review Committee (CPRC), May 12, 1994 requested an in vivo cytogenetic study in rodents (micronucleus assay with antibodies to provide insight on structural and/or numerical aberrations). This information would help clarify questions regarding the genotoxicity of carbaryl, and would help in the determination of the mode of carcinogenic action. This request by CPRC is an outstanding data gap for carbaryl.

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DATA EVALUATION REPORT

CARBARYL TECHNICAL

Study Type: Mutagenicity: _In Vivo_ Cytogenetic Assay with Rats

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Marisa B. Brett Date 2/25/94
Marisa B. Brett, B.A.

Independent Reviewer Nancy E. McCarroll Date 2/25/94
Nancy E. McCarroll, B.S.

QA Manager William L. McLellan Date 2/25/94
William L. McLellan, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 3-46
Clement Number: 174
Project Officer: Caroline Gordon

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CARBARYL TECHNICAL

GUIDELINE SERIES 84: MUTAGENICITY IN VIVO MAMMALIAN CYTOGENTICS

MUTAGENICITY STUDIES

EPA Reviewer: Ray Landolt
Review Section I,
Toxicology Branch II/HED (7509C)

Date: 9/20/94

EPA Mutagenicity Reviwer: Byron T. Backus, Ph.D.
Review Section II
Toxicology Branch II/HED(7509C)

Date: 9/21/94

DATA EVALUATION REPORT

STUDY TYPE: MUTAGENICITY: In Vivo cytogentic assay with rats

PC CODE: 056801

TOX CHEM. NUMBER: 160

MRID Number: 430393-01

TEST MATERIAL: Carbaryl technical

SYNONYMS: 1-Naphthyl methylcarbamate; $C_{12}H_{11}NO_2$, Sevin

SPONSOR: Rhone-Poulenc Ag Company, Lyon Cedex, France

TESTING FACILITY: Hazleton Microtest, Harrogate, North Yorkshire, England

TITLE OF REPORT: Technical study to Evaluate the Chromosome Damaging Potential of Carbaryl by Its Effects on the Bone Marrow Cells of Treated Rats.

AUTHOR McEnaney, S.

STUDY NUMBERS: Hazleton UK Study Number 198/64; Hazleton Microtest Study Number RPF 5/RBM

REPORT ISSUED: September 7, 1993

EXECUTIVE SUMMARY: In an in vivo cytogenetic assay, groups of five male and five female Sprague-Dawley rats were administered a single oral gavage dose of 30.0, 60.0, or 120 mg/kg carbaryl technical in 0.25% metylcellulose. Bone marrow cells were harvested 6, 24, and 48 hours postexposure. Doses selected were based on the findings of a preliminary range-finding study which identified an LD_{50} of approximately 231 mg/kg.

Systemic toxicity (i.e., lethargy and tremors) was reported for the majority of high-dose animals. The mitotic indices in the treatment of groups did not differ significantly from the vehicle controls. There was, futhermore, no significant increase in the frequency of cells with stauctural aberrations at doses ranging from 30.0-120 mg/kg at any harvest time. Results with the positive control confirmed the sensitivity of the test system to detect a clastogenic effect.

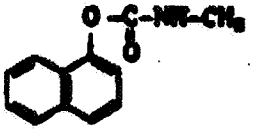
CARBARYL TECHNICAL IN VIVO MAMMALIAN CYTOGENETICS

This study is classified as Acceptable and satisfies the guideline requirements for genetic effects, Category II, Structural Chromosome Aberrations.

A. MATERIALS:

1. Test Material: Carbaryl technical

Description: White powder
Identification number: Batch number sevin; Lot 198
Purity: 99.7%; analysis performed by the sponsor
Receipt date: September 25, 1992
CAS number: 63-25-2
Structure:



Stability: Expiration date: August 17, 1993
Contaminants: None listed
Solvent used: 0.25% (w.v) methylcellulose (MC)
Other provided information: The test material was stored at room temperature in the dark. Dosing suspensions were prepared with homogenization no more than 2 and 3/4 hours prior to use. Analytical determinations on representative dosing suspensions were not performed.

2. Control Materials:

Vehicle/final concentration/route of administration: 0.25% MC was administered once by oral gavage at a dosing volume of 10 mL/kg.

Positive/final dose(s)/route of administration: Cyclophosphamide (CP) was prepared in purified water and administered once by oral gavage at a dose of 40 mg/kg in a volume of 10 mL/kg.

3. Test Compound:

Route of administration: Oral gavage.

Volume of test substance administered: 10 mL/kg; based on individual body weights

Dose levels used:

(a) Range-finding study: 75.42, 116.0, 178.5, 274.6, 422.5, 650.0, and 1000 mg/kg

(b) Cytogenetic assay: 30.0, 60.0, and 120 mg/kg

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CARBARYL TECHNICAL **IN VIVO MAMMALIAN CYTOGENETICS**

4. Test Animals:

(a) Species: Rat; Strain: Sprague-Dawley; Age (at dosing): 38-45 days (range-finding study); 44-51 days (cytogenetic study); Weight range at dosing: range-finding study: 168-194 g (males); 159-185 g (females); cytogenetic study: 220-262 g (males); 174-213 g (females); Sex: Males and females; Source: Charles River UK Ltd., Margate, UK

(b) No. animals used per dose:

Range-finding study: 3 males 3 females per treatment group

Cytogenetic assay: (primary dose groups)

- Treatment groups: 15 males 15 females
- Positive control: 5 males 5 females.
- Vehicle control: 15 males 15 females.

Note: A secondary group of 5 males and 5 females receiving the high dose was included for use in case of mortality in the primary high-dose group. None of these animals were used for the cytogenetic analysis.

(c) Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Acute Range-Finding Study: Three males and three females/group received single oral gavage administrations of the selected doses; mortality was monitored for 3 days.

2. Cytogenetic Assay:

(a) Treatment and sampling times:

(1) Test compound/vehicle control

Dosing: x once _____ twice (24 hours apart)
_____ other (describe):

Sampling (after last dose): x 6 hours _____ 12 hours
x 24 hours x 48 hours _____ 72
____ other (describe):

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(2) Positive control

Dosing: __x__ once _____ twice (24 hours apart)
_____ other (describe):

Sampling (after last dose): _____ 6 hours _____ 12 hours
__x__ 24 hours _____ 48 hours _____ 72
_____ other (describe):

Administration of spindle inhibitor:

Inhibitor used/dose: Colchicine/1 mg/kg

Administration time: Approximately one hour prior to sacrifice.

Route of administration __x__ i.p. _____ other (describe)

(b) Tissues and cells examined:

__x__ bone marrow _____ other (list):

No. of cells per animal per treatment group examined: 100.

No. of cells per animal per control group examined: 100.

The mitotic index (MI) was determined for each animal by counting the number of mitotic cells per 500 scored cells.

Note: 100 cells per animal per treatment group were examined only when 100 cells with 40-42 chromosomes were available.

(c) Details of cell harvest and slide preparation: Five males and five females in the treatment and vehicle control groups were sacrificed by CO_2 asphyxiation and cervical dislocation at 6, 24, and 48 hours postexposure. Animals in the positive control groups were sacrificed 24 hours posttreatment. Bone marrow was flushed from both femurs with phosphate buffered saline, centrifuged, resuspended in 0.075M KCl and incubated at 37°C for 30 minutes. Cells were fixed in methanol/glacial acetic acid (3:1), repeatedly centrifuged and refrigerated until use. For slide preparation, cells were pelleted, resuspended in a minimal amount of fresh fixative, dropped onto slides, air dried, and stained with 4% Giemsa in a pH 6.8 buffer.

Note: Slides were coded prior to analysis for the evaluation of chromosome aberrations, but not for the mitotic index analysis.

(d) Evaluation criteria:

• Assay validity: The assay was considered acceptable if: (1) the heterogeneity χ^2 test demonstrated acceptable within-group variability, (2) the proportion of cells from vehicle control animals with structural aberrations fell within the provided historical control range, (3) at least 8/10 animals and 800/1000 cells were available for analysis at each treatment time for each

dose group, and (4) the positive control (CP) induced a statistically significant ($p \leq 0.05$) increase in the number of cells with structural aberrations.

- Positive response: The assay was considered positive if a statistically significant ($p \leq 0.05$) dose-related increase in the number of structural aberrations was observed at more than one dose or harvest time, and if the frequency of cells with structural aberrations was beyond the historical control range.

(e) Statistical methods: The percentage of cells with structural aberrations (including and excluding gaps) was evaluated for significance ($p \leq 0.05$) in comparison to the vehicle controls using a 2 x 2 contingency test for χ^2.

C. REPORTED RESULTS:

1. Acute Dose-Finding Study: Groups of three male and three female rats were administered single oral gavage doses of 75.42, 116.0, 178.5, 274.6, 422.5, 650.0, or 1000 mg/kg carbaryl technical. No deaths were reported in groups treated with ≤116.0 mg/kg. Three days post-dosing, three males receiving 178.5 mg/kg died. At 274.6 mg/kg, one male and two females died; two males and three females died at 422.5 mg/kg. All animals treated with 650.0 mg/kg died, and with the exception of one male with a blocked gavage needle whose survival was considered "misleading," all animals succumbed to treatment with the high dose. Clinical signs were not reported. From these findings, the LD_{50} was estimated to be 231 mg/kg. Based on these results, the cytogenetic assay was conducted with 30.0, 60.0 and 120 mg/kg. The high dose was ≈52% of the LD_{50}.

2. Cytogenetic Assay: The report indicated that the majority of the high-dose group animals exhibited lethargy and tremors immediately after compound administration; further signs of toxicity, if any, were not described. Representative results from the bone marrow cytogenetic phase of the assay are presented in Table 1. The MIs for all treatment groups at all harvest times did not differ significantly from the concurrent vehicle controls. As shown, no significant increases in the percentage of cells with structural aberrations were scored in bone marrow cells harvested from male and female rats 6, 24, or 48 hours postexposure to 120 mg/kg carbaryl technical. Similar findings were observed in the lower treatment groups at all sacrifice intervals. By contrast, the positive control (40 mg/kg CP) elicited a marked clastogenic response in both male and female rats; the data combined for both sexes were significant ($p \leq 0.001$). Only 246 cells were analyzable from the positive control animals but this deviation from the protocol was not considered to have affected the overall study outcome. A slight but not significant increase in numerical chromosome aberrations was observed in the high-dose group (males and females combined) at the 48-hour sacrifice. However, this value was within the historical control range provided by the study author.

TABLE 1. Representative Results of the Rat Bone Marrow Cytogenetic Assay with

Substance	Dose/kg	Exposure Time[a]	No. of Animals Analyzed	% Mitotic Index	No. of Metaphases Analyzed	% Cells with Structural Aberrations[b,c]	Total No. o Structural Aberrations
Vehicle Control							
0.25% Methylcellulose	10 mL	6 h	5 M	0.8	500	0.0	0
		6 h	5 F	1.8	500	0.0 (0.0)[e]	0
		24 h	5 M	1.2	353	0.3	1
		24 h	5 F	1.8	500	0.6 (0.5)	3
		48 h	5 M	1.1	500	0.0	0
		48 h	5 F	0.9	500	0.2 (0.1)	1
Positive Control							
Cyclophosphamide	40 mg	24 h	5 M	--	142[f]	55.6	168
		24 h	5 F	--	184	55.8 (55.7)[e]	95
Test Material							
Carbaryl technical	.120 mg[g]	6 h	5 M	1.2	500	0.2	2
		6 h	5 F	1.2	500	0.0 (0.1)	0
		24 h	5 M	1.7	446	0.7	4
		24 h	5 F	2.2	500	0.8 (0.7)	4
		48 h	5 M	1.9	498	0.2	1
		48 h	5 F	1.2	500	0.4 (0.3)	2

[a] Time of cell harvest after compound exposure
[b] Excluding gaps
[c] Calculated by our reviewers
[d] Abbreviations used:

TE = Chromatid exchange
SE = Chromosome exchange
OT = Other (i.e., pulverized cell and/or cell with >7 aberrations)
TD = Chromatid deletion
SD = Chromosome deletion

[e] Values in () are the combined results for males and females; statistical evaluations were performed only on combined
[f] Only a total of 246 cells were available for analysis from cyclophosphamide-treated animals; this protocol deviation
altered the study outcome.
[g] Results for the low- or mid-dose groups (30.0 or 60.0 mg/kg, respectively) at all sacrifice times did not suggest a c

*Significantly higher than the vehicle control group ($p \leq 0.001$) in a 2 x 2 contingency test for χ^2.

Note: Data were extracted from the study report, pp.23-26, 38-44, and 52.

Based on the overall results, the study author concluded that carbaryl technical was not clastogenic in this in vivo rat bone marrow cytogenetic assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that this cytogenetic study was properly conducted and that the study author's interpretation of the data was correct. Although clinical signs should have been more thoroughly assessed in both the initial range-finding assay and the main cytogenetic assay, carbaryl technical was evaluated to a level (120 mg/kg) that caused systemic toxicity but failed to induce a clastogenic response in a well-controlled study. In addition, the sensitivity of the test system to detect clastogenesis was clearly demonstrated by the significant ($p \leq 0.001$) increases in structural chromosome aberrations obtained in both male and female rats exposed to the positive control (40 mg/kg CP).

We conclude, therefore, that the study provided acceptable evidence that carbaryl technical was not clastogenic in this whole animal assay.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement was signed and dated September 7, 1993).