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

MAY 31 1989

007229

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg. No. 100-524 (Diazinon Technical): Mutagenicity Studies and Studies with 2-PAM (2-Pyridine Aldoxime Methiodide) and Atropine as Antidotes to Diazinon Poisoning

FROM: Krystyna K. Locke, Toxicologist
Section I, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Krystyna K. Locke 5/11/89

TO: George T. LaRocca, PM Team (15)
Insecticide-Rodenticide Branch
Registration Division (H7505C)

5/24/89

THRU: Edwin R. Budd, Section Head
Section I, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

EB 5/25/89

TB Project Nos.: 9-0274, 9-0276 and 9-0278

Tox. Chem. No.: 342

In compliance with EPA's Data Call In, Ciba-Geigy Corporation has submitted the following studies on mutagenicity of Diazinon and antidotes to Diazinon poisoning:

Volume 2. Toxicological Assessment of Diazinon (compilation of published and unpublished mutagenicity studies with Diazinon; C. Breckenridge; Ciba-Geigy Corporation, Greensboro, NC; May 31, 1988.
Accession/MRID No.: 406957-01
Record No.: 234064

Volume 3. Toxicological Assessment of Diazinon (compilation of published studies on antidotes to Diazinon poisoning; C. Breckenridge; Ciba-Geigy Corporation, Greensboro, NC; May 31, 1988.
Accession/MRID No.: 406957-01
Record No.: 234064

Volume 4. Nucleus Anomaly Test in Somatic Interphase Nuclei of Chinese Hamster with Diazinon Technical; G. Hool; Ciba-Geigy Limited, Basle, Switzerland; No. 801503; November, 5, 1981.
Accession/MRID No.: 406608-01
Record No.: 234057

Volume 5. L517Y/TK +/- Mouse Lymphoma Mutagenicity Test; P. Dollenmeier and D. Muller; Ciba-Geigy Limited, Basle, Switzerland; No. 840396; July 31, 1986.
Accession/MRID No.: 406608-02
Record No.: 234058

Volume 6. Sister Chromatid Exchange Study in Chinese Hamster; G. Hool; Ciba-Geigy Limited, Basle, Switzerland; No. 801504; October 13, 1981.
Accession/MRID No.: 406608-03
Record No.: 234059

Volume 7. Sister Chromatid Exchange Test on Human Lymphocytes Cell Line CCL 156 in Vitro; F. Strasser; Ciba-Geigy Limited, Basle, Switzerland; No. 871697; May 16, 1988.
Accession/MRID No.: 406608-04
Record No.: 234059

Volume 8. Micronucleus Test on Diazinon in the Mouse; Carla Ceresa; Ciba-Geigy Limited, Basle, Switzerland; No. 871696; May 24, 1988.
Accession/MRID No.: 406608-05
Record No.: 234057

Five mutagenicity studies reported in Volumes 4-8 have also been included in Volume 2. Toxicology Branch/IRS has completed an evaluation of the mouse lymphoma study (Volume 5) in March,1988; this review was submitted to Registration Division (G.T. LaRocca) under separate cover (Memorandum dated March, 1989). Technical Diazinon was not mutagenic in that study, but the study was classified as Provisionally Acceptable pending submission of sample calculations. If the requested data are satisfactory, this study will be upgraded to Acceptable. The evaluations of the remaining studies (Volumes 4, 6, 7 and 8) are currently being submitted (Attachment I). Each of these four studies was classified by Toxicology Branch/IRS as Unacceptable, mostly because of deficiencies in experimental procedures used.

Volume 2 consists mostly of mutagenicity studies (and their abstracts) with Diazinon, published in the open literature. Toxicology Branch/IRS performed a cursory review of these studies, but not an in-depth review. Using studies compiled in Volume 2, Ciba-Geigy (C. Breckenridge, Ph.D.) prepared a mutagenicity profile for Diazinon (Attachment II). Of the 36 studies referenced in that profile, Diazinon was negative for mutagenicity in 23 studies, positive in 6, inconclusive in 6, and could not be evaluated (insufficient data) in 1. However, it should be emphasized that studies published in the open literature generally do not meet EPA guidelines and that Toxicology Branch/IRS disagreed with the results/conclusions of at least 9 studies which were included in that profile. Five of

these studies (recently evaluated) were discussed above. The remaining 4 studies were evaluated several years ago and also classified as Unacceptable or Inconclusive. Nevertheless, Ciba-Geigy regarded these studies as fully acceptable in their mutagenicity profile (see Attachment II, Comments, for details).

Volume 3 contains only studies published in the open literature and concerned mostly with atropine and 2-PAM as antidotes to Diazinon poisoning. Toxicology Branch/IRS performed a cursory review of these studies, but not an in-depth review. Of the 24 studies on Diazinon poisoning included in Volume 3, 8 studies (Nos. 47-54, Attachment III) involve experimental exposure of domestic animals (rats, rabbits, guinea pigs, cats, dogs, sheep, cattle, geese, ducks and turkeys) to Diazinon and subsequent treatment with one or both antidotes. The remaining 16 studies are actually case studies of unintentional or intentional human poisoning with Diazinon and subsequent treatment with one or both antidotes. Ciba-Geigy (C. Breckenridge) has summarized these human case studies, showing estimated doses of ingested Diazinon, treatment schedules with antidotes and outcomes of treatments (study Nos. 55-70, TABLE 4, Attachment IV).

In conclusion:

1. Because the recently submitted mutagenicity studies are either unacceptable (4 studies: structural chromosome aberrations in mouse and Chinese hamster bone marrow, and sister chromatid exchanges in Chinese hamster bone marrow and human lymphocytes in vitro) or provisionally acceptable (1 study: gene mutation in mouse lymphoma cells), the requirement for mutagenicity testing under 84-2, for Diazinon Technical, still remains unfulfilled.

2. Based on an evaluation (by Ciba-Geigy) of the open literature concerned with antidotes to Diazinon poisoning, a combination of atropine and 2-PAM was the most effective method for managing human and domestic animal poisoning with Diazinon.

Attachment I

Recently Evaluated Mutagenicity Studies with Diazinon Technical

EPA No.: 68D80056
DYNAMAC No.: 166-A
TASK No.: 1-66A
May 8, 1989

DATA EVALUATION RECORD

DIAZINON

Mutagenicity--Nucleus Anomaly Test in Chinese Hamsters

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 5/8/89

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EPA No.: 68D80056
DYNAMAC No.: 166-A
Task No.: 1-66A
May 8, 1989

DATA EVALUATION RECORD

DIAZINON

Mutagenicity--Nucleus Anomaly Test in Chinese Hamsters

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation
Signature: Nancy E. McCarroll
Date: 5-8-89

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation
Signature: /for I. Cecil Felkner
Date: 5-8-89

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation
Signature: Roman J. Pienta
Date: 5-8-89

Krystyna Locke, Ph.D.
EPA Reviewer, Section I
Toxicology Branch I (H-7509C)
Signature: Krystyna Locke
Date: 5/9/89

Edwin Budd
EPA Section Head, Section I
Toxicology Branch I (H-7509C)
Signature: Acting
Date: 5/5/89

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity--Nucleus anomaly test in Chinese hamsters.

ACCESSION/MRID NUMBER: 406608-01.

TEST MATERIAL: G 24 480.

SYNONYM(S): Diazinon.

STUDY NUMBER(S): 801503.

SPONSOR: CIBA-GEIGY Corp., Greensboro, NC.

TESTING FACILITY: CIBA-GEIGY Ltd., Basle, Switzerland.

TITLE OF REPORT: Diazinon--Structural Chromosome Aberration Test--Nucleus Anomaly Test in Somatic Interphase Nuclei.

AUTHOR(S): Hool, G.

REPORT ISSUED: November 5, 1981.

CONCLUSIONS - EXECUTIVE SUMMARY: Male and female Chinese hamsters (six/sex) were administered single oral doses of 6.5, 13.0, or 26.0 mg/kg G 24 480 for 2 consecutive days. In the high-dose group, one male and one female died following the second administration of the test material; one control group female died during the same dosing interval. Based on the analysis of bone marrow cells from six animals/sex/group in the mid-dose group and three animals/sex/group in the high- and low-dose groups, there were no significant increases in the frequency of nuclear anomalies (micronuclei). The results, however, do not provide clear evidence of overt toxicity to the animals or cytotoxicity to the target organ; the data are, therefore, considered insufficient to establish that the maximum tolerated dose was administered. Additionally, less than the recommended number of animals per group (8 to 10 animals equally divided between sexes -- see Section E, Reviewers' Discussion and Interpretation of Study Results) were analyzed for micronuclei induction in the low- and high-dose groups and the statement on good laboratory practices was issued more than 6 years after the study was completed. *

Classification: The study is unacceptable.

A. MATERIALS:

1. Test Material: G 24 480 from batch No. EN 30554 was not described. The test material was prepared in polyethylene glycol 400 to achieve doses of 6.5, 13, and 26 mg/kg and was administered by gavage once daily for 2 consecutive days. Dose selection was based on the results of an earlier study, indicating that the LD_{50} in Chinese hamsters for both sexes was 76 (44-125) mg/kg.

2. Test Animals: Male and female Chinese hamsters (Cricetulus griseus) were used in this study; the source was not specified. The males weighed between 20 and 28 g and the females weighed between 20 and 25 g; the age of the hamsters at the onset of the study was not reported.

[1] CIBA-GEIGY Lab Report GU 2.1, dated December 1, 1980.

* GLP Statement not required for studies started before 15/29/83 40 CFR Sec 160.1 AGS 3/24/89

Animal Maintenance: Acclimation to laboratory conditions and animal housing were not reported. The animals were maintained in an air-conditioned room that was controlled for temperature (22-24°C), humidity (40-48%), and light (12 hours). Standard diet, NAFAG No. 924 (source not specified), and tapwater were provided ad libitum. The report did not state whether the animals were randomized; animals were identified according to cage assignment.

3. Nuclear Anomaly Test:

   a. Test Animals and Compound Administration: Twelve hamsters (six males and six females) per group were administered the selected concentrations of the test material, vehicle (polyethylene glycol 400), or the positive control, 128 mg/kg cyclophosphamide (CP), in two single applications separated by a 24-hour interval. Dosing solutions were prepared to yield volumes of 20 mL/kg.

   b. Animal Sacrifice/Bone Marrow Harvest: Twenty-four hours after the second application of the test material, vehicle, or positive control, the animals were sacrificed by cervical dislocation. Bone marrow cells were harvested from both femurs by aspiration into 0.5-μL rat serum. Aspirates were mixed, dropped onto slides, and air dried. Prepared slides were stained in undiluted and diluted May-Grunwald (1:1 in H_2O), counterstained in 40% Giemsa, and mounted.

   c. Slide Analyses: Coded slides from three males and three females per group were analyzed. One thousand bone marrow cells per animal were scored for the following nuclear anomalies: single Jolly bodies, fragments of nuclei in erythrocytes, micronuclei in erythroblasts, micronuclei in leukopoietic cells, and polyploid cells.

4. Evaluation Criteria: No criteria for a positive response, the validity of the assay, or the biological significance of the findings were present.

5. Statistical Analysis: The data were analyzed for significance using the X^2 test at $p < 0.05$.

B. Protocol: A protocol was not provided.

C. REPORTED RESULTS:

Nuclear Anomaly Test: One control group female and two high-dose animals (one male and one female) died after the second day of compound administration. All other animals survived until sacrifice and no other toxic signs were reported. No appreciable increase in the percentage of cells with nuclear anomalies was observed in males or females exposed to 6.5 and 26.0 mg/kg of the test material. The report stated that two mid-dose females had elevated nuclear anomaly rates; therefore, slides prepared for all animals in the mid-dose group (six males and six females) were analyzed. Although the total percent cells with nuclear anomalies (0.2%) was slightly increased, compared to the control group value (0.12%), the increase was not significant. By contrast, significant increases ($p < 0.05$) in the total percent of cells with nuclear anomalies were noted in the positive control (CP) group.

Representative data combined for both sexes are presented in Table 1.

D. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

1. The author concluded, "Under the conditions of this experiment, no evidence of mutagenic effects was obtained in Chinese hamsters treated with G 24 480."

2. A statement of compliance with good laboratory practices was signed and dated June 1, 1988; however, the study was completed on November 5, 1981.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The study author provided additional information (dated January 4, 1986) to justify the use of the nucleus anomaly test as an alternative to the micronucleus assay. However, the suitability of the nucleus anomaly test as an appropriate test system for the evaluation of genotoxic effects in bone marrow cells is not in question. We assess that the assay as performed does not provide acceptable evidence of a negative response for the following reasons:

1. Although two deaths occurred following the second administration of the high dose (26 mg/kg), the relevance of this finding is diminished by the death of a control group animal during the same dosing interval. Similarly, no other evidence of clinical toxicity nor any indication of target cell cytotoxicity was noted. The data are, therefore, insufficient to establish that the maximum tolerated dose of G 24 480 was assayed.

TABLE 1. Representative Results of the Nucleus Anomaly Test (Micronucleus Assay) in Chinese Hamsters with G 24 480

Substance	Dose (mg/kg)	Animals[a] Exposed per Group	No. of Animals[b] Analyzed per Group	No. of Cells Analyzed per Group	Percent Cells with Nuclear Anomalies[c]					
					Jolly Bodies	Nuclear Fragments in Erythrocytes	Micronuclei in Erythroblasts	Micronuclei in Leukopoietic Cells	Polyploid Cells	Total
Vehicle Control										
Polyethylene glycol 400	--	12	6	6000	0.1	0.00	0.00	0.03	0.00	0.12
Positive Control										
Cyclophosphamide	128	12	6	6000	8.8	0.98	2.55	0.47	0.02	12.85*
Test Material										
G 24 480										
	6.5	12	6	6000	0.1	0.00	0.00	0.02	0.00	0.10
	13.0	12	12	13000[d]	0.2	0.02	0.00	0.01	0.00	0.20
	26.0	12	6	6000	0.1	0.00	0.00	0.00	0.00	0.12

[a]Six males and six females.

[b]The slides from three males and three females were analyzed for vehicle and positive control groups and low- and high-dose groups; slides from all animals in the mid-dose group were analyzed.

[c]Average values calculated by our reviewers.

[d]Two thousand cells were evaluated for a single animal.

*Significantly different from control value ($p<0.05$) by X^2 test.

2. The number of animals evaluated for micronuclei induction in high- and low-dose groups (3 males and 3 females/group) does not conform with EPA [Federal Register, Vol. 50, No. 188, Subpart F 798.5395(d)(iii)] or OECD (Section 71) guidelines (10 animals/group; 5 males/5 females), or with the Gene-Tox recommendation[2] of at least 8 animals/group.

We conclude, therefore, that the study is unacceptable.

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 7 and 8.

[2]Heddle, J. A., Hite, M., Kirkhart, B., Mavournin, K., MacGregor, J. T., Newell, G. W., and Salamone, M. F. The induction of micronuclei as a measure of genotoxicity. A Report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123(1983): 61-118.