10-9-92

DOC 930148

FINAL

DATA EVALUATION REPORT

D-NC 302 (ASSURE)

Study Type: Mutagenicity: Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes

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 Nancy E. McCarroll Date 10/9/92
Nancy E. McCarroll, B.S.

Independent Reviewer Lynne T. Haber Date 10/9/92
Lynne T. Haber, Ph.D.

QA/QC Manager Sharon Segal Date 10/9/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-118
Clement Number: 93-96
Project Officer: James Scott

GUIDELINE §84: MUTAGENICITY
UDS

EPA Reviewer: Whang Phang, Ph.D.
EPA Review Section (III),
Toxicology Branch II/HED (H-7509C)
EPA Secondary Reviewer: Byron Backus, Ph.D.
EPA Review Section (II),
Toxicology Branch II/HED (H-7509C)

Signature: Whang Phang
Date: 10/23/92
Signature: Byron T. Backus
Date: 10-14-92

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro unscheduled DNA synthesis assay in primary rat hepatocytes.

EPA IDENTIFICATION NUMBERS.:

Tox Chem. Number:

MRID Number: 419366-02

TEST MATERIAL: D-NC 302 technical

SYNONYM: Assure

SPONSOR: Nissan Chemical Industries, Ltd., Tokyo, Japan

STUDY NUMBER: 19

TESTING FACILITY: IIT Research Institute, Chicago, IL

TITLE OF REPORT: DNA Repair Assay in Primary Rat Hepatocyte Cultures on D-NC 302

AUTHOR: Ketels, K.V.

REPORT ISSUED: Amended final report: June 1991

CONCLUSIONS-EXECUTIVE SUMMARY: Concentrations ranging from 10 to 5000 µg/mL D-NC 320 did not induce unscheduled DNA synthesis (UDS) in primary rat hepatocytes. Doses ≥100 µg/mL were insoluble and levels ≤100 µg/mL (50 and 10 µg/mL) were presumed to be soluble; 5000 µg/mL was severely cytotoxic. It was concluded, therefore, that D-NC 302 was tested over an appropriate range of concentrations and failed to induce a genotoxic response.

STUDY CLASSIFICATION: Currently unacceptable. The study does not fully satisfy Guideline requirements (§84-4) for genetic effects, Category III, Other Mutagenic Mechanisms but can be upgraded if individual grain count data (including gross nuclear and cytoplasmic background counts) are provided. Additionally, information regarding the lowest insoluble and highest soluble levels that were tested in the UDS assay should be submitted.

2

A. MATERIALS:

1. Test Material: D-NC 302 technical

Description: Fine, light-brown powder
Identification number: Lot number: 302 DT 8501
Purity: 98.1%
Receipt date: May 23, 1986
Stability: Not reported
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provided information: The test material was stored in the dark at 4°C. Prior to use, test material solutions were prepared with the aid of a vortex mixer.

2. Indicator Cells: Primary rat hepatocytes were obtained by the in situ perfusion of the liver of a male Fischer 344 rat obtained from Harlan-Sprague-Dawley.

3. Control Substances: WME (see below) was used as the cell culture control; 1% DMSO was used as the solvent control; biphenyl (Bp) at 100 nM/mL (~15 µg/mL) was used as the negative control; and 2-acetylaminofluorene (2-AAF) at 200 nM/mL (~0.05 µg/mL) was used as the positive control.

4. Medium: WME: Williams Medium E with 20 mM glutamine and antibiotics; WME+: WME with 10% serum.

5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: 100, 500, 1000, 5000, 10,000 and 50,000 µg/mL.

(b) UDS assay: 10, 50, 100, 500, 1000, and 5,000 µg/mL; cells exposed to levels ≤1000 µg/mL were scored for UDS.

B. STUDY DESIGN:

1. Cell Preparation:

(a) Perfusion techniques: The rat was anesthetized with nembutal. The liver was perfused with 0.5 mM EGTA, and 100 units/mL collagenase, excised, and transferred to a petri dish; hepatocytes were dispersed and collected.

(b) Hepatocyte harvest/culture preparation: Recovered cells were counted, checked for viability and seeded at a density of ~$1x10^6$ cells, either into tissue culture flasks for the cytotoxicity assay, or onto coverslips in multi-well tissue culture dishes for the UDS assay. Cultures were placed in an incubator for a 2-hour attachment period, washed and fed WME prior to use.

2. Preliminary Cytotoxicity Assay: Triplicate hepatocyte cultures were exposed to six doses of the test compound, ranging from 100 to 50,000 µg/mL or the cell culture control (WME) for 18-20 hours. Viability was determined by trypan blue exclusion and cell count.

3. UDS Assay:

   (a) Treatment/Slide Preparation: Three prepared hepatocyte cultures were exposed for 18-20 hours to six selected doses of the test material, the untreated control (WME), the solvent control (DMSO), the negative control (Bp) or the positive control (2-AAF). Treatment medium contained 10 µCi/mL [^{3}H]thymidine. Treated hepatocytes attached to coverslips were washed, swollen with 1% sodium citrate, fixed in ethanol:glacial acetic acid (3:1), dried, mounted and coded.

   (b) Preparation of Autoradiographs/Grain Development: Slides were dipped into Kodak NTB emulsion, dried and stored at 4°C in light-tight boxes for 10 days. Slides were developed in Kodak D-19, stained with Harris' alum hematoxylin and eosin and counted.

   (c) Grain Counting: The nuclear grains of 150 randomly selected normal cells (50/slide) from each test, untreated, solvent, negative, or positive control group were scored for the incorporation of tritiated thymidine into DNA. Net nuclear grain counts were determined by subtracting the highest cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus from the nuclear grain count of each cell. Means and standard deviations were calculated for each set of slides.

4. Evaluation Criteria:

   (a) Assay Validity: For the assay to be considered valid, the untreated, solvent, and negative control cultures must have net nuclear grain counts <5 grains/nucleus and the positive control must induce a net nuclear grain count that is >5 grains/nucleus.

   (b) Positive Response: The assay was considered positive if the test material induced a dose-related increase in mean net nuclear grains that was >5 grains/nucleus at two doses, and at least one dose produced a significant response ($p \leq 0.001$).

   NOTE: The data were not evaluated for statistical significance.

5. Protocol: See Appendix A

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: The six doses (100-50,000 µg/mL) evaluated in the preliminary cytotoxicity test were insoluble in culture medium. Survival was reduced to ≤25.8% at the three highest doses (5000, 10,000, and 50,000 µg/mL); however, the response was not

dose related presumably because of the test material precipitation. At lower dose levels, survival proceeded in a more conventional dose-related manner and ranged from 32.9% at 1000 µg/mL to 64.2% at the lowest assayed concentration (100 µg/mL). Based on these findings, doses selected for the UDS were 10, 50, 100, 500, 1000, and 5000 µg/mL.

2. UDS Assay: Representative results from the UDS assay are presented in Table 1. No comment was made regarding test material insolubility. We assumed, therefore, that compound precipitation occurred at least down to the 100-µg/mL dose level. We further assume, based on information provided in the accompanying report of the mouse lymphoma assay (see DER 93-95), that 10-µg/mL dose level was probably soluble. As shown in Table 1, the highest assayed dose (5000 µg/mL) was cytotoxic and cells were not scored. Net nuclear grain counts for the remaining levels did not suggest a genotoxic response. By contrast, the positive control (200 nM/mL 2-AAF) induced a marked increase in UDS activity. The study author concluded, therefore, that D-NC 302 was negative in the primary rat hepatocyte assay.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that D-NC 302 was assayed over a concentration range that presumably included at least one soluble dose level (10 µg/mL) and several insoluble dose levels (100-5000 µg/mL) but failed to induce a genotoxic response. The demonstration of a cytotoxic effect at 5000 µg/mL further indicates that test material insolubility did not interfere with the ability of D-NC 302 to penetrate cellular membranes. Additionally, both the negative (100 nM Bp) and positive (200 nM 2-AAF) controls produced the expected results. We conclude, therefore, that D-NC 302 was evaluated over an appropriate range of concentrations and was found to be nongenotoxic in this test system.

The study is, however, unacceptable but can be upgraded if individual grain count data (including gross nuclear and background cytoplasmic grain counts) are provided. Additionally, information regarding the lowest insoluble and the highest soluble levels should be furnished.

E. QUALITY ASSURANCE MEASURES: Was the test performed under GLPs? Yes. (A quality assurance statement and a list of changes in the report were signed and dated June 11, 1991.)

F. CBI APPENDIX: Appendix A, Protocol, CBI pp. 19-25; Appendix B, Materials and Methods, CBI pp. 9-11.

CORE CLASSIFICATION: Unacceptable. The study does not satisfy Guideline requirements (§84.4) for genetic effects Category III, Other Mutagenic Mechanisms.

TABLE 1. Representative Results of the Unscheduled DNA Synthesis Rat Hepatocyte Assay with D-NC 302

Treatment	Dose	Number of Cells Scored	Average Net Nuclear Grains[a]
Cell Control			
Untreated culture	--	150	0.35
Solvent Control			
Dimethyl sulfoxide	1%	150	0.53
Negative Control			
Biphenyl	100 nM/mL	150	0.46
Positive Control			
2-Acetylaminofluorene	200 nM/mL	150	24.87
Test Material			
D-NC 302	10 µg/mL[b]	100	0.25
	1000 µg/mL[c]	100	0.68

[a]Individual means and standard deviations from the counts of 50 nuclei/slide were presented.

[b]Lowest assayed level; it was assumed that this concentration was soluble. Results for intermediate doses (50, 100, and 500 µg/mL) did not suggest a genotoxic effect.

[c]Highest dose scored for UDS; the highest assayed concentration (5000 µg/mL) was cytotoxic.

APPENDIX B

MATERIALS AND METHODS
CBI pp. 9-11

ASSURE 128711

Page_____ is not included in this copy.

Pages 8 through 19 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.