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

009404

APR 3 1992

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Cryolite (sodium fluoaluminate) --- Tox Data Submitted under MRID 418384-01, -02, and -03.

Case No. 819150/ ID# 075101

Chemical: 264(075101)
RD Record: S-399629
HED Project: 1-2236

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch - I
Health Effects Division (H7509C)

03-11-92

TO: Larry Schnaubelt/Brigid Lowry, PM 72
Reregistration Branch
Special Review and Reregistration Division (H7508W)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I
Health Effects Division (H7509C)

3/30/92

Registrant Atochem North America, Philadelphia, PA.

Request Review and evaluate the following three (3) mutagenicity studies, submitted to support the re-registration of cryolite; all performed by Pharmakon Research International (PH), Waverley, PA:

(1) Ames/Salmonella Plate Incorporation Assay on Kryocide (Ames), Study No. PH 301-ANA-001-90, Final Report dated March 19, 1991 (EPA MRID NO. 418384-01).

(2) In Vitro Chromosone Aberration Analysis of Kryocide in Human Lymphocytes (HLC) Study No. PH 324 ANA-001-90, Final Report dated March 18, 1991 (EPA MRID NO. 418384-02).

(3) Rat Hepatocyte Primary Culture /DNA Repair Test on Kryocide (HPC/UDS), Study No. PH 311-ANA-001-90, Final Report dated March 18, 1991 (GPA MRID NO. 418384-03)

TB CONCLUSIONS: The studies have been judged for regulatory purposes as follows (detailed reviews are attached to this memo):

Study (MRID)	REPORT	TB Evaluation
(1) Ames (418384-01)	Negative for inducing reversion in Salmonella strains, exposed w/without activation up to 10,000 ug/plate.	Acceptable
(2) HLC (418384-02)	Negative for inducing structural chromosome aberrations in human lymphocyte cultures exposed, w/without activations, up to the limit dose, 1000 ug/ml.	Acceptable
(3) HPC/UDS (418384-03)	Negative for inducing DNA repair in rat hepatocytes treated up to toxic doses (50 ug/ml)	Acceptable

(ATTACHMENT DER's)

Reviewed by: Irving Mauer, Ph.D., Geneticist,
Toxicology Branch I, (IRS)/HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I, (IRS)/HED (H7509C)

3-11-92

3/30/92

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DATA EVALUATION REPORT

I. SUMMARY

Study Type: (84-2) Mutagenicity - Gene mutation in bacteria (Ames Test)

MRID No.: 418384-01
PC No.: 075101
RD Record No.: S-399629
EPA ID No.: 075101
Tox Chem No.: 264
Project No.: 1-2236

Chemical: Cryolite [sodium fluoaluminate]

Synonyms: KRYOCIDE

Sponsor: Atochem North America
Philadelphia, PA

Testing Facility: Pharmakon Research International (PH), Waverly, MD

Title of Report: Ames/Salmonella Plate Incorporation Assay on Kryocide

Author: L. F. Stankowski

Study No.: PH 301-ANA-00I-90

Report Issued: March 19, 1991

TB Conclusions: Negative for inducing reverse gene mutation at the his locus in Salmonella typhimurium TA (Ames) strains, exposed with/without activation, to doses up to 10,000 ug/plate.

TB-I Evaluation: Acceptable

II. DETAILED REVIEW:

A. Test Material: Cryolite

Description: White powder
Batch (Lot): 86-12
Purity (%): (Not stated)
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism: Bacterial Cultures

Species: Salmonella typhimurium LT2
Strain: TA 1535, TA 1537, %A 1538, TA 98, TA 100 (all his-)
Source: Bruce N. Ames, Berkeley (UCal)

C. Study Design (Protocol):

This study was designed to determine the (reverse) mutagenic potential of cryolite when administered in vitro to histidine-requiring (his-) cultures (Ames TA-battery) of Salmonella typhimurium LT2, according to established procedures (referenced).

Statements of both Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were provided.

D. Procedures/Methods of Analysis:

Following a preliminary cytotoxicity screen (TA 100 and TA 1538 exposed at doses up to 5000 ug/plate), triplicate cultures of all five strains were exposed to test article for 48 hrs. both in the absence and presence of mammalian metabolic activation provided by the S9 (microsomal) fraction of liver homogenates from male S-D rats pretreated with Aroclor 1254, plus NADP(H)-generating co-factors. Strain specific mutagens[1] served as positive controls.

After the two-days incubation, revertant (his+) colonies were scored on all plates (using an Artek electronic colony counter, interfaced with an IBM PC/AT computer) and group revertant mean frequencies automatically provided by PC software, using a program developed by

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[1] Without activation: Sodium azide (10 ug/plate) for TA 100; 9-Amine acridine (150 ug/plate) for TA 1537; 2-Nitrofluorene (5 ug/plate) for TA 1538, TA 98.

With activation: 2-Anthramine (2.5 ug/plate) for all five tester strains.

Snee and Irr (Mutation Res. 85: 77-93, 1981).

The entire assay was repeated once.

E. Results: In preliminary dose-selection testing, Kryocide was not toxic up to the dose limit, 5000 ug/plate; hence the test article was tested in the main assays up to 10,000 ug/plate. At no dose in either assay were revertant frequencies in any test cultures statistically different from DMSO controls (summary tables attached here). In contrast, cultures exposed to reference mutagens responded positively with highly significant revertant frequencies.

Therefore, the author concluded that Kryocide was negative for inducing genemutation in Ames-testing.

F. TB-I Evaluation: Acceptable

Attachments (Data Tables)

Disk 1/Ames/Salmonella/Mauer/aw
RETYPED:Mauer/mcs/12/30/91:Memory-Cryolite.mcs-02/03/92
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CRYO-DER.IM/lca

Cryolite

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____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

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Reviewed by: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I, HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I, HED (H7509C)

03-19-92

3/30/92

DATA EVALUATION RECORD

MRID Number No.: 418384-02
PC No.: 075101
RD Record No.: S-399629
EPA ID No.: 075101
Tox. Chem. No.: 264
Project No.: 1-2236

I. SUMMARY

STUDY TYPE: Mutagenicity --- Chromosome damage in vitro (HLC/CA)

CHEMICAL: Cryocide

SYNONYMNS: KRYOCIDE®

SPONSOR: Atochem, Philadelphia, PA

TESTING FACILITY: Pharmakon Research International (PH) Waverly,PA

TITLE OF REPORT: in vitro Chromosome Aberration Analysis of Kryocide in Human Lymphocytes

AUTHOR(S):j. J. R. San Sebastian

STUDY NUMBER: PH 324-ANA-001-90

DATE ISSUED: March 18, 1991

CONCLUSIONS: Negative for inducing chromosome aberrations during the mitotic cycle of human lymphocytes in vitro up to 1000 ug/ml, w/w out activation

TB-I EVALUATION: ACCEPTABLE

II. DETAILED REVIEW

A. TEST MATERIAL Kryocide technical

Description: White powder
Batches (Lots): 86-12
Purity (%): (not stated)
Solvent/carrier/diluent: HPLC-grade water

B. TEST ORGANISM: Primary lymphocyte cultures

Species: Human blood donor (stated to be "healthy")

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the clastogenic (chromosome damaging) potential of the test article when administered in vitro to lymphocytes cultures from a volunteer blood donor, according to published (referenced) procedures.

Statements of Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were provided

D. PROCEDURES/METHODS OF ANALYSIS: Following cytotoxicity (dose selection) determinations (using cell proliferation kinetics and mitotic index in cultures treated up to 1000 ug/ml test article), duplicate phytohemagglutinin (PHA)-stimulated cultures of lymphocytes were exposed: (1) 4 hr after such PHA to 2 hours treatment, and harvested 48 hr later, in order to sample any effects during the G0/G1 mitotic phase; (2) 41 hr. after the PHA to 4 hours treatment, in order to sample effects during the S-phase; and (3) 69 hr after the PHA to 3 hours treatment in order to detect any effects during the G2 phase. Test cultures were incubated either in the absence or presence of a metabolic activation mixture consisting of rat hepatocyte microsomes (S-9) plus NADP(H)-generating co-factors. In addition to solvent (water) controls, other cultures were treated 41 hours after PHA stimulation, and harvested at 73 hours (ie., sampling the S-phase only) with the clastogens mitomycin-C (MMC) (0.5 ug/ml) and cyclophosphamide (CP, 40 ug/ml) to serve as positive controls for, respectively, the non-activation and activation series.

Two to three hours before harvest, all cell cultures were exposed to the metaphase-arresting alkaloid, colcemid, then prepared for microscopic chromosome analysis by conventional cytological techniques. Carnoy (3:1)-fixed slide preparations were stained with Giemsa, air-dried and permanently mounted.

A total of 100 metaphases per data point (50 per slide per test dose) were scored for the usual array of structural chromosome aberrations, and proportion data analyzed by Chi-square and t-testing.

E. RESULTS:

Cytotoxicity testing revealed that Kryocide was non-toxic (in terms of cell cycle kinetics and mitotic indices) up to the maximum dose tested, 1000 ug/ml, although a small degree of precipitation was evident at the highest concentrations in activated cultures (Report Tables 1, 2). Hence, the doses selected for the cytogenetic assay were 100,500 and 1000 ug/ml with/without S-9 mix for the three phases of the mitotic cycle: G0/G1, S, and G2.

At none of the dose levels during any phase of the mitotic cycle, however, did the test article induce statistically significant increases in aberration frequencies (Report Tables 4,5,6), nor in proportions of aberrant metaphases (Tables 7, 8, 9). By contrast, both clastogens produced the anticipated positive increases.

Hence, the investigator concluded that Kryocide technical was negative for chromosome aberrations up to the limit dose tested, 1000 ug/ul.

F. TB EVALUATION: ACCEPTABLE

ATTACHMENT (Data Tables)

Disk 3/Drive A/Kryocide/Mauer/aw/3-17-92.

Cryolite

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Toxicology Branch-I, HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I, HED (H7509C)

I. Mauer 12/20/91

Karl P. Baetcke 3/30/92

DATA EVALUATION RECORD

MRID NUMBER No.: 418384-03
RD Record No.: S-399629
EPA ID No.: 075101
Tox Chem. No.: 264
Project No.: 1-2236

I. SUMMARY

STUDY TYPE: MUTAGENICITY---Other genotoxic effects: DNA damage/repair in vitro (HPC/UDS)

CHEMICAL: Cryolite [sodium fluoaluminate]

SYNONYMNS: Kryocide®

SPONSOR: Atochem North America, Philadelphia, PA

TESTING FACILITY: Pharmakon Research International (PH), Waverly, PA

TITLE OF REPORT: Rat Hepatocyte Primary Culture/DNA Repair Test on Kryocide

AUTHOR(S): J.R. SanSebastian

STUDY NUMBER: PH 311-ANA-001-90

DATE ISSUED: March 18, 1991

CONCLUSIONS: Negative for inducing unscheduled DNA "repair" synthesis (UDS) in primary rat hepatocytes (HPC) exposed up to toxic doses (50 ug/ml).

TB-I EVALUATION: Acceptable

II. DETAILED REVIEW

A. TEST MATERIAL: Cryolite

Description: White powder
Batches (Lots): 86-12
Purity (%): [Not stated]
Solvent/carrier/diluent: Deionized water (DW)

B. TEST ORGANISM: Primary hepatocyte cultures

Species: Rat
Strain: Fischer 344 (F-344)
Age: "Young adult"
Weights - males (only): 152 g
Source: Taconic Farms, NY

C. STUDY DESIGN (PROTOCOL): This study was designed to determine the genotoxic (DNA damage -repair) potential of cryolite when administered in vitro to hepatocytes cultured from Fischer 344 rats, according to referenced procedures, presented as Appendix II of the Final Report.

Statements of both Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were provided.

D. PROCEDURES/METHODS OF ANALYSIS: Hepatocytes were removed from the liver of a male F-344 rat according to established in situ techniques, allowed to attach to coverslips under tissue culture medium in multiwell culture dishes, and exposed for 18-20 hours to water (solvent) or 10 graded concentrations of test article (1 thru 1000 ug/ml), concurrently with a fixed concentration of tritiated thymidine (10 u Ci/ml 3H-TdR, spec. act. 50-80 Ci/mM). Following treatment, the coverslip cultures were immersed in hypotonic saline (1% sodium citrate), then fixed in Carnoy's (3:1:: methanol: acetic acid), dried and mounted cell-side up onto standard glass microscope slides. The slide cultures were then treated to standard autoradiographic methodology (in a darkroom) by dipping in Kodak NTB-2 liquid photographic emulsion, drying overnight, and storage at 4^0C in light-tight slide boxes.

After seven-days exposure, the autoradiographs were developed in D19, fixed, washed, dried and stained in Harris alum-hematoxylin followed by eosin, and finally coverslipped. 2-Acetoamidofluorene (2AAF) served as positive control.

Unscheduled DNA "repair" synthesis (an indication of recovery from prior DNA damage) was measured by net increases in nuclear silver grains, quantified by determining nuclear and cytoplasmic labeling with an Artek 880 automated grain counter, attached to a microscope/video camera interfaced with an Apple II computer

programmed for such data aquisition. A total of 150 hepatocytes per dose were scored for such UDS determination; raw grain values were corrected for area/grain ratio.

E. RESULTS: Of the 10 doses of test article initially applied to the hepatocyte cultures, levels of 100 ug/ml and above were too toxic for scoring. Hence, the next lower dose, 50 ug/ml was selected as the highest dose for UDS evaluation, even though some cytotoxicity (detectable abnormal cell morphology) was evident. In none of the treated test cultures analyzed were net grain counts significantly different from the water/solvent value (Report Table 1, attached to this DER). By contrast, over 96% of 2-AAF treated hepatocyte responded positively.

The author concluded that Kryocide was negative for inducing UDS-DNA repair in primary rat hepatocyte cultures.

F. TB Evaluation: Acceptable

Attachment (Data Table)

EPA:Memory-PI-2236:Disk:Tox:IMauer:mcs:10/18/92:305-6193

TABLE 1

PH 311-ANA-001-90

Autoradiographic Analysis of DNA Repair in the Rat Hepatocyte Primary Culture/DNA Repair Test

Treatment	Concentration	Net Nuclear Grains Per 150 Hepatocytes x ± s.d.	Percent of Cells in Repair[a]
2AAF	1 x 10^{-7}M	19.5 ± 9.1*	96.7
dH_2O	1% (v/v)	-6.6 ± 5.6	1.3
Kryocide	1 µg/ml	-12.9 ± 6.9	2.0
Kryocide	5 µg/ml	-7.8 ± 5.7	2.0
Kryocide	10 µg/ml	-7.5 ± 7.2	4.7
Kryocide	50 µg/ml	-9.1 ± 7.0	0.7

*Positive finding. Mean net nuclear grain count ≥5 than the vehicle control.
[a]The percentage of cells that have net nuclear grain count ≥5.