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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: KRENITE - Miscellaneous Mutagenicity data submitted May 29, 1985, under Accession #256247.

EPA Registration No. 352-376 Caswell No. 465G

FROM: Irving Mauer, Ph.D., Geneticist
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10-02-85

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JEH 10/3/85

10/30/85

Registrant: E.I. du Pont de Nemours & Company

Action Requested:

Review and evaluate the following mutagenicity studies, originally submitted January 25, 1985, but not then evaluated because of missing report pages:

A. Unscheduled DNA Synthesis/Rat Hepatocytes in vitro HLR 680-82. (UDS/HPC)

B. In Vitro Assay for Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells HLR 683-82. (CHO/CA)

TB Evaluation/Conclusions:

Data Reviews for these studies are attached to this memo.

Study	Type	Reported Results	Evaluation
A	UDS/HPC	Negative	Acceptable
B	CHO/CA	Positive	Acceptable

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TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Krenite (ammonium salt of fosamine)

Caswell: 465G
EPA Tox Chem No.: N/A

Study Type: Mutagenicity: DNA damage/repair in rat hepatocytes (HPC/UDS)

Citation: Unscheduled DNA Synthesis/Rat Hepatocytes In Vitro

Accession No./MRID No.: 256247/na (EPA Registration No. 352-376)

Sponsor/Testing Lab.: Dupont/Haskell Labs

Study No./Date: Report No. HL 680-82 (MR No. 4487-001)

Test Material: H-14,506 (Krenite INR-1108), Lot. No. S60405-86, a clear, yellow liquid containing 41.5 percent ai [phosphonic acid, (amino carbonyl)-, monomethyl ester, ammonium salt].

Procedures:

Freshly isolated hepatocytes from 8-week male CD/SD rats were treated in culture with tritiated thymidine (5 µCi/ml) and 8 halflog concentrations of test material ranging from 1×10^{-5} mM to 10 mM (duplicates for each test concentration in two separate trials), according to standardized (referenced) procedures. Cells, attached to coverslips, were fixed and prepared for autoradiographic analysis. A solvent control and a positive control (the known mutagen, dimethylbenzanthracene, DMBA) were included in each trial.

Results:

At no concentration of test material in either assay was there a net increase of 5 silver grains or more per nucleus (after correction for background i.e., cytoplasmic, labeling), indicative

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of a positive response for induction of unscheduled DNA (repair) synthesis. DMBA induced a significant UDS response in both trials.

Conclusions:

The authors concluded that Krenite did not induce UDS in rat hepatocytes, under the conditions described.

TB Evaluation/Core:

Acceptable. Krenite is negative for UDS induction in rat hepatocytes.

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TOXICOLOGY BRANCH: DATA REVIEW

Chemical: Krenite (ammonium salt of fosamine)

Caswell: 465G
EPA Tox Chem No.: N/A

Study Type: Mutagenicity: Chromosome aberrations in vitro (CHO cells)

Citation: In Vitro Assay for Chromosome Aberrations in Chinese Hamster Ovary (CHO) Cells

Accession No./MRID No.: 256247/na (EPA Registration No. 352-376)

Sponsor/Testing Lab.: Dupont/Haskell Labs

Study No./Date: Report No. HL 683-82 (MR No. 4487-001)

Test Material: H-14,506 (Krenite lNR-1108), Lot. No. S60406-86, a clear, yellow liquid containing 41.5 percent ai [ammonium ethyl (amino carbonyl)-phosphonate]

Procedures:

Duplicate cultures of Chinese hamster ovary cells (CHO-K_1, BH-4 clone) were exposed to four concentrations of test material (ranging from 0.3 to 6.0 µL/ml) in repeat trials, both in the absence (10-hr exposure) and presence (2-hr exposure) of a mammalian metabolic activation system provided by Aroclor 1254-stimulated hepatic microsomes from young male CD rats (S-9 plus appropriate co-factors). After removal to fresh medium for an additional 8 hrs (including exposure to the mitotic arresting agent, Colcemid), cells were prepared for cytogenetic analysis, according to standard procedures. Both solvent (phosphate-buffered saline, PBS) and positive controls (respectively, ethylmethanesulfonate, EMS, for nonactivated, and cyclophosphamide, CP, for activated assays) were included in each trial.

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Results:

Preliminary cytotoxicity testing indicated relative cell survivals in nonactivated cultures after 6 hrs exposure to test material of 60 percent at 33.3 µL/ml, and 2.1/2.7 percent (duplicates) at 50 µL/ml; in activated cultures cell survival was 75 percent at 50 µL/ml after 6 hrs exposure, decreasing to 55 percent after 24-hr exposure. Therefore, concentrations of 33.3 µL/ml and 60 µL/ml were selected for nonactivated and activated assays, respectively. Statistically significant, dose-related increases in structural chromosome aberrations were observed at 16.7 and 33.3 µL/ml Krenite in nonactivated cultures, as well as at 15, 20, and 60 µL/ml in S-9 supplemented cultures. The authors concluded that, under the conditions of this assay, Krenite was clastogenic (= chromosome breaker) both with (3 doses) and without (2 doses) metabolic activation (equivalent to final Krenite concentrations by volume in medium of, respectively: 1.4%, 2.8%, 5.7%; and 1.6%, 3.2%).

TB Evaluation:

Acceptable. Krenite is a clastogen in CHO cells cultured in vitro.

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