



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

CASWELL FILE

007050

FEB 27 1989

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Hydroxyatrazine - Review and Evaluation of Mutagenicity
Study Submitted Under EPA MRID No. 40888101
EPA ID 100-529

TOX Chem No.: 486K (063)
TB Project No.: 9-0416A
RD Record No.: 235283

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Registrant: Ciba-Geigy, Greensboro, NC.

Request

Review and evaluate the following mutagenicity study
(cf. Atrazine Registration Standard):

Hydroxyatrazine: Tests for Other Genotoxic
Effects - Autoradiographic DNA Repair Test on
Human Fibroblasts, conducted at Ciba-Geigy's
Genetic Toxicology Laboratories, Basle (Suisse),
Study No. 871375, Final Report dated January 11,
1988 (EPA MRID No. 40888101).

TB Conclusion

Reportedly negative in human fibroblast cells treated
with hydroxyatrazine up to the limits of solubility (increasing
precipitation from 500 ug/mL) and severe cytotoxicity (1500 ug/
mL). The study is acceptable and the negative results
considered valid, but only in the absence of metabolic
activation. The substance was not tested with activation.

A detailed review of this study is attached to this memo..

Reviewed By: Irving Mauer, Ph.D., Geneticist
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DATA EVALUATION REPORT

I. SUMMARY

TB Project: 9-0416A
Caswell No.: 486K (063)
MRID No.: 40888101

Study Type: Mutagenicity - DNA Damage Repair in Human Cells (UDS in CRL 1521)

Chemical: Hydroxyatrazine (metabolite of atrazine, 063)

Sponsor: Ciba-Geigy, Greensboro, NC

Testing Facility: Ciba-Geigy, Basle (Switzerland)

Title of Report: Hydroxyatrazine: Tests for Other Genotoxic Effects, Autoradiographic DNA Repair Tests on Human Fibroblasts.

Author: A. Meyer

Study Number: 871375

Date of Issue: January 11, 1988

TB Conclusions:

Reportedly negative for induction of UDS repair in human cells treated with test substance up to limits of solubility (500 μ g/mL) and cytotoxicity (1500 μ g/mL).

Classification (Core-Grade):

ACCEPTABLE for nonactivation conditions only. Not tested with metabolic activation.

II. DETAILED REVIEW

A. Test Material: G 34 048 technical (hydroxyatrazine)

Description: (Not stated)
Batch (Lot): FL 870869
Purity (%): 96-99
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organisms: Mammalian cell cultures (established cell line)

Species: Human fibroblasts
Strain: CRL 1521
Source: American Type Culture Collection,
Rockville, MD

C. Study Design (Protocol):

A formal protocol was not presented, but the author stated that the assay was conducted according to OECD Guideline No. 482 (1987).

A Quality Assurance statement attesting to data and report audits, as well as a testament of compliance with EPA GLPs were included.

D. Procedure/Methods of Analysis:

Following preliminary toxicity testing, coverslip cultures of early passage CRL 1521 cells were exposed to six concentrations of the test substance, or to the vehicle (DMSO), or to the mutagen, 4-nitroquinoline-N-oxide (NQO, 5 μ M). At the same time, tritiated thymidine ($6\text{-}^3\text{H-TdR}$, sp.ac. 21 Ci/mmol) was added. After 5 hours incubation, the coverslip preparations were washed, fixed in Carnoy's fluid (ethanol:acetic acid, 3:1 v/v), mounted on microscope slides, and prepared for autoradiography by standard procedures. After 6 days exposure at 4 °C under light-tight conditions, the preparations were stained (H&E), and coded.

Silver grains over 150 nuclei per treatment were counted electronically on 3 slides (50 cells per slide), and the mean values of each group calculated. Background counts in cell-free areas, but not over cytoplasmic areas, were also recorded.

According to the laboratory's criteria, a substance is considered positive if one of the following is met:

- "- The mean number of silver grains per nucleus in relation to the vehicle control is more than doubled at any concentration.
- "- The mean number of silver grains per nucleus in relation to the vehicle control shows a concentration-dependent increase and at least at one concentration a statistically significant increase in comparison with the vehicle control is demonstrated. Statistical analysis will be carried out as follows: the values of silver grains per nucleus will be compared using Duncan's multiple range test (Ref. 4). Statistical significance will be judged to be achieved if the probability is less than 0.01.
- "- The percentage of nuclei with more than five silver grains per nucleus at any concentration is 10% or more" (quoted from the Final Report).

Results:

In the preliminary cytotoxicity test, precipitation was observed at concentrations of 500 $\mu\text{g/mL}$ test material and higher, and severe toxicity (< 25% of the cells viable, and in poor condition) occurred above 1500 $\mu\text{g/mL}$ (Report Table 1, attached). Hence, six concentrations of test substance were selected for the UDS assay, with 1500 $\mu\text{g/mL}$ as the HDT.

In the two experiments (initial and confirmatory), neither the mean number of silver grains nor the distribution of counts in test slides differed from solvent (or medium) control (Report Tables 2 and 4, attached). In contrast, the positive control, NQO, induced marked increases in grain counts in both experiments, 30 to 50 times solvent values, and virtually all cells had more than five nuclear grains.

The author concluded that the test substance did not produce DNA damage in human fibroblasts.

TB Evaluation:

The study was conducted adequately, and the negative results generated in repeat experiments can be considered valid.

However, this study can only be judged ACCEPTABLE in the absence of metabolic activation. No assay was performed with any of the several means of mammalian metabolic activation (addition of exogenous S9; cellular co-cultivation; inter alia), to achieve a comprehensive evaluation of this chemical in this in vitro system.*

Attachments

*CRL 1521 human fibroblast cells have little to no endogenous capacity to process xenobiotics.

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Pages 7 through 9 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.
- ☒ FIFRA registration data.
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