3/22/95

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

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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: BENZYL BENZOATE---Tox Data Submitted Under MRID 434133-02

ID #: 009501.

(PC CODE)
Chemical: 009501 (082)
RD Record: S477565
HED Project: D209717
Case No.: 4013

FROM: Irving Mauer, PH.D., Geneticist
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03-01-95

TO: Kathryn Davis/S. Bacchus, PM # 52
Special Review and Reregistration Division (7508W)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I
Health Effect Division (7509C)

3/3/95

Registrant: Center Laboratories, Port Washington, NY

Request: Review and evaluate the following mutagenicity assay:

(84-4) Unscheduled DNA Synthesis in Primary Hepatocytes of Male Rats in vitro w/Benzyl Benzoate, performed by CCR Cytotest Cell Research GMBH/KG, Rossdorf (W. Germany), Final Report No. 444100, dated August 12, 1994 (MRID 43413302).

TB CONCLUSION: The study is ACCEPTABLE in satisfying GDLN 84-4 of the mutagenicity test battery, and demonstrates that benzyl benzoate does not induce (is negative for) unscheduled DNA synthesis (UDS) in primary rat hepatocyte cultures exposed up to precipitating concentrations, 130 ug/ml and above.

ATTACHMENT: DER

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BENZYL BENZOATE (84-4)

EPA Reviewer: Irving Mauer, PH.D. Date: 02-17-95
Immediate Office, Toxicology Branch-I (7509C)
EPA Branch Chief: Karl P. Baetcke, PH.D. Date: 3/3/95
Toxicology Branch-I (7509C)

DATA EVALUATION RECORD

MRID No.: 434133-02
PC No.: 009501
RD Record No.: S477565
EPA ID No.: 009501
Tox Chem. No.: 082
Project No.: D209717

I. SUMMARY

STUDY TYPE: (84-4) Mutagenicity---other genotoxicity (UDS/HPC)--rat

CHEMICAL: Benzyl benzoate

SPONSOR: Center Laboratories (successor to: Allergopharma Joachim Ganze KG, Hamburg (RFG)

TESTING FACILITY: CCR Cytotest Cell Research GMBH, KG, Rossdorf (W. Germany), contracted by RCC, Research and Consulting Company, Itingen (Switzerland)

TITLE OF REPORT: Unscheduled DNA Synthesis in Primary Hepatocytes of Male Rats *in vitro* with Benzyl Benzoate.

AUTHOR: R. Fautz

STUDY NUMBER: CCR 444100

DATE ISSUED: August 12, 1994

EXECUTIVE SUMMARY: Negative for inducing unscheduled DNA synthesis (UDS) in primary rat hepatocyte cultures exposed up to a precipitating concentration, 130 ug/ml.

TB-I EVALUATION: ACCEPTABLE

II. DETAILED REVIEW

A. TEST MATERIAL: Benzyl benzoate

Description:	Clear, colorless liquid
Batches (Lots):	CN 3063303
Purity (%):	99
Solvent/carrier/diluent:	Dimethylsulfoxide (DMSO)

B. TEST ORGANISM: Rodent

Species:	Rat
Strain:	Wistar/WU
Age:	6-9 weeks
Weights - males only:	236-272 g
Source:	Charles River

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the genotoxic potential of the test article when administered in vitro to primary cultures of rat hepatocytes, and determining DNA repair (unscheduled DNA synthesis) by radiographic enumeration of silver grains, according to established (published) procedures and FIFRA/OECD Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: Following preliminary cytotoxicity testing, hepatocytes were isolated from Wistar male rats, and established for two hours on coverslips in complete Williams Medium. Triplicate cultures were then exposed for 18 hours to five graded concentrations of test article, together with a constant amount(5 u Ci/ml) of tritiated thymidine (spec. gr., 20 Ci/mmol, New England Nuclear, Dreicich, W. Germany). In addition to DMSO solvent controls, additional cultures were treated with the mutagen, 2-acetylaminofluorene (2AAF, 2.23 ug/ml), to serve as positive control. The entire assay was repeated.

After the treatment periods, all coverslip cultures were mounted on standard microscope slides, cell-side out, coated under darkroom conditions with photographic emulsion (Kodak NTB2), then stored in light-tight microslide boxes under refrigeration. After seven

days' storage slide preparations were developed (D-19) and fixed, and finally stained with hematoxylin and eosin (H&E).

At least 50 cells per coded slides and two slides per dose, were scored microscopically (under oil immersion optics) for silver grains over each nucleus (by Artek 880 or 982 Grain Counter), normalized (subtracted) by the mean of three adjacent nuclear-size cytoplasmic areas, to provide net nuclear silver grains, NNG. Heavily-labelled cells believed to be in replicative-(S-) phase were excluded from these counts.

Standard (conventionally accepted) criteria for assay validity, as well as for characterizing genotoxic response, were included in the Final Report. Statistical significance of data from test compared to solvent control series could be (if necessary) evaluated by the non-parametric Mann-Whitney "U" Test[1].

E. RESULTS: In the preliminary cytotoxicity assay, slight precipitation of the test article was noted at 86.7 ug/ml, which increased at 130 ug/ml and above. Cytotoxicity, however (as manifested by altered cell morphology and/or reduced number of adherent cells, in addition to the capability to take up neutral red) was not evident at doses up to the HDT (Report Table 1). Based on these results, the following eight test article doses were selected for both main (UDS) assays: 1.03, 2.05, 4.10, 8.13, 16.25, 32.50, 65.0 and 130 ug/ml.

In Experiment I, toxicity was observed in high dosed cultures (130 ug/ml), by reduction of neutral red uptake to 50% of the solvent value (Summary Table of Toxicity Data, attached to this DER), an effect only marginally evident in Experiment II at the same dosage. However, in neither trial did the test article at any concentration cause increases over solvent in NNG counts (such values were consistently negative, indicating no effect), nor any shift in their distribution, contrasted to the reproducible, significant increases in cultures treated with the reference mutagen (Experiments I and II: Summary of Results, also attached here).

[1]However, as stated by the performing laboratory:
"A statistical evaluation of the results was not necessary to perform, since the number of nuclear grain counts of the groups treated with the test article was in the range of the controls and net grain values obtained were consistently negative."

Therefore the investigator concluded benzyl benzoate was negative for inducing UDS in this cell system.

F. TB EVALUATION: ACCEPTABLE, in demonstrating UDS was not induced by benzyl benzoate in primary rat hepatocytes treated up to a precipitating concentration, 130 ug/ml.

ATTACHMENTS: Summary Data Tables

BENZYL BENZOATE (84-4)

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BENZYL BENZOATE

Page_____ is not included in this copy.

Pages 8 through 12 are not included.

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