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)

9-4-91
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DATA EVALUATION REPORT 008552

I. SUMMARY

Study Type: Mutagenicity - Chromosome Damage in vitro (CHO)

ID No.: 2749/2792
MRID No.: 418467-01
Caswell No.: 510A
Project No.: 1-1169

Chemical: Chlorpropham

Synonyms: CIPC

Study Number: (HLA) 12276-0-437

Sponsor: Chlorpropham Task Force
Liberty, Missouri

Testing Facility: Hazleton Laboratories America, Inc. (HLA)
Kensington, MD

Title of Report: Mutagenicity Test on Chlorpropham in an in vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells

Author: H. Murli

Study No.: (HLA) 12276-0-437
Report Issued: April 3, 1991

TB Conclusions: Reported to be negative for clastogenicity in non-activated Chinese hamster ovary (CHO) cells exposed in a single trial to relatively non-toxic doses up to 120 ug/ml, but positive at activated doses of 120 to 140 ug/ml, stated to be "severely toxic"

Classification: Presumptively positive under activation conditions, but UNACCEPTABLE as a comprehensive evaluation of the test article, since incompletely conducted (under non-activation conditions) and/or deficient (under activation).

1 2

II. DETAILED REVIEW:

A. Test Material: Chlorpropham

Description: Tan crystalline solid at room temperature, forming a clear colorless, viscous liquid when warmed in DMSO.
Batch (Lot): Stated as "Aliquot No. 41"
Purity (%): (Not stated)
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism Established mammalian cell strain

Species: Chinese hamster (ovary)
Strain: CHO-WBL
Source: Dr. S. Wolff, UCal, San Francisco (CA)

C. Study Design (Protocol):

This study was designed to assess the clastogenic (chromosome-breaking) potential of chlorpropham when administered in vitro to cultures of Chinese hamster ovary (CHO), according to established (referenced) procedures.

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 range-finding assays using bromodeoxyuridine (BrdUrd) to monitor cell cycle kinetics (as a measure of cytotoxicity), duplicate cultures of CHO cells were exposed to test material for either 7.75 or 17.5 hr in the absence of mammalian metabolic activation, or for 2 hr in its presence[1] (followed by re-incubation in fresh untreated medium for 5.25, 15.25 or 21.25 hr), then treated for 2.5 hr with the mitotic poison, colcemid, in order to accumulate cells in metaphase for chromosome analysis. In addition to solvent controls, other cell cultures were exposed to the clastogens, mitomycin-C (MMC), or cyclosphosphamide (CP), to serve as positive controls for the non-activation and activation series, respectively.

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[1] Liver microsomal fraction (S9) from male Sprague-Dawley rats pre-treated with Aroclor 1254, plus NADP(H)-generating co-factors.

2 73

Metaphase cells were then collected by mitotic shake-off, exposed to hypotonic saline (0.075 M KCl), fixed in Carnoy's, spread on standard glass microscope slides, and air-dried. Coded Giemsa- stained slide preparations (100 cells per slide) were scored for the standard array of structural chromosome aberrations[2], according to the following data classifications:

(1) Overall chromosome aberration frequencies (CA)
(2) Percent of cells with at least one aberration
(3) Percentage of cells with more than one aberration
(4) Evidence for a dose-response.

These data were statistically analyzed by Fisher's Exact Test, adjusted for multiple comparisons, and with significance established at $p < 0.01$. At least two independent trials were run under S9 activation, but only one complete assay in the absence of activation.

E. Results: In preliminary testing, solubility of the test article in tissue culture medium (i.e., giving clear solutions with no oily globules) was limited to 1550 ug/ml and below. Severe cytotoxicity (defined as a dead cell monolayer with no dividing cells) occurred at test article doses of 149 ug/ml and above with or without activation, but cell cycle delay was evident only in non-activated (-S9) cultures exposed to doses of 14.9 and 49.7 ug/ml (Report Table 1). Therefore, concentrations of test article selected for the main aberration assays in the absence of S9 were 10, 15 and 20 ug/ml for the 10-hr harvest, and 20, 40.1, 60.1, 80.2, 120 and 160 ug/ml for the 20-hr harvests; in the presence of S9, cultures to be harvested at 10 hr were exposed to 10, 20, 40, 60 and 80 ug/ml chlorpropham, whereas 20 hr cultures were treated to 80.2, 120 and 160 ug/ml.

Although no toxicity was observed in any non-activated (-S9) test cultures of the 10-hr (cell-cycle delayed) assay, these cultures were not scored for chromosome aberrations, since sufficient data were stated to be available from the 20-hr assay (Report Tables 2 and 3 attached to this DER). In this latter assay, increasing toxicity was manifest at 80 ug/ml and higher (and lethality at the HDT, 160 ug/ml), but at none of the four analyzable concentrations was any induced chromosome damage recorded.

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[2] Although chromosome ('tid and iso-) gaps were recorded, they were not analyzed as aberrations.

3 24

Activated (+S9) cultures of the single 10-hr assay showed only slight cytotoxicity at the HDT (80 ug/ml), but no significantly increased chromosomal damage (Report Tables 4 and 5). On the other hand, although no toxicity was observed in the initial 20-hr assay, significantly increased aberrations were evident at the highest analyzable dose, 120 ug/ml (Report Tables 6 and 7). Hence the latter +S9/20-hr experiment was repeated at concentrations ranging from 80 to 140 ug/ml.

In the repeat 20-hr harvest assay, the HDT proved to be cytotoxic in one duplicate set of cultures and, as well, manifested a significantly increased level of aberrations (5% vs. 0.5% in combined controls), whereas the second set at 140 ug/ml was only cytotoxic, but the increased level of chromosome damage (3.5%) did not achieve the 1% level of significance (Tables 8, 9). It was noted by the investigator that in both 20-hr activated trials, variable chromosomal responses were found between duplicate cultures exposed to the same concentrations (Report Tables 7 and 9).

Because of this lack of cultural concurrence (possibly attributable to variable cell cycle delay between replicate cultures exposed to the same test doses), further activated trials were conducted at the critical dose, 120 ug/ml, in addition to harvest periods extended to 26 hr as well as repeat 20-hr assays. In both sets of these timed assays, pooled data indicated significantly increased chromosomal damage (Report Tables 10, 12), but variable responses between replicate cultures (Report Tables 11, 13), thus apparently confirming the author's suggestion of variable cell cycle delay between replicates. For comparison, lab background data from an unstated number of previous assays have been summarized also in the Report (Table 14, also attached here).

Finally, both clastogens induced the expected positive responses, 25 to 75-fold higher than solvent/background controls.

The author concluded that chlorpropham was negative for the induction of chromosomal aberrations in non-activated cultures of Chinese hamster ovary cells, but "weakly clastogenic" under activation (+S9) conditions, but only at toxic concentrations of 120 to 140 ug/ml stated to cause "severe cell cycle delay."

F. TB-I Evaluation: This study cannot be properly evaluated with respect to the clastogenic potential of chlorpropham, since it is incomplete in several respects:

4 75

(i) Only a single trial under non-activation conditions was conducted and further, data from only one timed portion reported, namely, from the 20-hr harvest. Hence, the initial reported negative response was not confirmed, and thus the non-activated series incompletely conducted and/or reported.

(ii) Whereas it is entirely possible that differences in cell cycle kinetics between replicate cultures contributed to overall (summary) significantly positive responses at the higher dose levels tested (120 and 140 ug/ml), better support for this suggestion could have been afforded by more appropriate dose scheduling, which should have included tighter scaling, such as 90, 100, 110, 130 ug/ml), rather than slavish repetition at the same dosages.

Finally "severe" mitotic delay was just not demonstrated at chromosomally active concentrations, or at least, not entirely documented in this Report.

Attachments (Report Tables 1-14)

5 76

CHLO-DER.IM/lca

Chlorpropham

Page _____ is not included in this copy.

Pages 7 through 20 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of product 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.

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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.

CHLORPROPHAM TASK FORCE

P.O. BOX 336
LIBERTY, MO 64068

PHONE: (816) 781-1763 TELEFAX: (816) 781-1763

1. **NAME AND ADDRESS OF SUBMITTER:**

CHLORPROPHAM TASK FORCE
c/o John Wise & Associates, Ltd
P.O. Box 336
Liberty, MO 64068
Consortium No: 64592

418467-00

Submitted on Behalf Of the Following Members:

Aceto Agricultural Chemicals Corporation: EPA No. 2749
Atochem North America, Inc.: EPA No. 2792

In Support of the Following Registrations:
All registrations for the above companies containing the active ingredient Chlorpropham (018301).

* Chlorpropham Task Force will act as sole agent for all submitters.

2. **REGULATORY ACTION IN SUPPORT OF WHICH THIS PACKAGE IS SUBMITTED:**

GUIDANCE FOR THE REREGISTRATION OF PESTICIDE PRODUCTS CONTAINING CHLORPROPHAM (OPP NUMBER 018301) AS THE ACTIVE INGREDIENT, CASE NUMBER 0271, ISSUED DECEMBER, 1987.

3. **TRANSMITTAL DATE:** April 11, 1991

4. **LIST OF SUBMITTED STUDIES:**

41846701 Vol 1 of 1

Mutagenicity Test On Chlorpropham In An In Vitro Cytogenetic Assay Measuring Chromosomal Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells
40 CFR 158.340
FIFRA Guideline No. 84-2

COMPANY OFFICIAL: John M. Wise, Chairman _____
Name Signature

COMPANY NAME: Chlorpropham Task Force

COMPANY CONTACT: John M. Wise (816) 781-1763
Name Phone No.