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9-4-91

9/4/91

DATA EVALUATION REPORT

008552

I. SUMMARY

Study Type: Mutagenicity - In Vitro Cell Transformation (SHE)

ID No.: 2749/2792
MRID No.: 418455-01
Caswell No.: 510A
Project No.: 1-1169

Chemical: Chlorpropham

Synonyms: CIPC

Study Number: (HLA) 12276-0-485R

Sponsor: Chlorpropham Task Force
Liberty, Missouri

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

Title of Report: In Vitro Transformation Assay of Chlorpropham Using Syrian Hamster Cells

Author: J. A. Poiley

Study No.: (HLA) 12276-0-485R

Report Issued: March 29, 1991

TB Conclusions: Positive for inducing stable morphological transformation in Syrian golden hamster embryo (SHE) cells exposed by two different treatment regimens.

Classification: ACCEPTABLE

1 ~~2~~ 3

II. DETAILED REVIEW:

A. Test Material: Chlorpropham

Description: (Not stated)
Batch (Lot): Stated as "Aliquot No. 41"
Purity (%): (Not stated)
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism: Established mammalian cell line

Species: Syrian hamster (embryo)
Strain: SHE(HLA)
Source: Maintained at HLA (Molecular and Cellular Services Division), Kensington, MD

C. Study Design (Protocol):

This study was designed to assess the transforming potential of chlorpropham when administered in vitro to cultures of Syrian hamster embryo (SHE) cells, according to 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 preliminary cytotoxicity testing (Report Table 1), cultures (20 dishes/treatment) of SHE cells were exposed for either 7 days (continuous exposure treatment regimen), or for only 24 hr (stability/re-feeding treatment regimen), to 6 or 8 concentrations of test article, harvested after the one week's incubation and prepared for microscopic examination after fixation (methanol) and staining (Giemsa). In addition to solvent (0.2% DMSO) controls, other cultures were exposed to benzo(a)pyrene (BaP), serving as positive control.[1] Total colony number and the number of colonies with altered ("transformed") morphology per dish were recorded (raw data recorded as Report Tables 5 through 10); plating efficiencies and morphological transformants were calculated for summary tabulation (Report Tables 2 to 4), well as graphically represented (Report Figs. 1, 2 and 3). Transformation data were analyzed by Fisher's Exact Test on pooled data from two (independent) trials.

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[1] Since SHE cells appear to retain a broad range of metabolic activities, additional (exogenous) metabolic activation was not deemed necessary.

2 8/4

E. Results: In continuous (7-day) preliminary cytotoxicity testing, the test article was lethal at 55 ug/ml and above, with dose related relative (to DMSO controls) toxicities ranging from 0% at 5 ug/ml to 80% at 45 ug/ml (Report Table 1). With the 24-hr refeed regimen, however, concentrations up to 145 ug/ml (producing 74% relative toxicity) could be tolerated (Table 1A). Hence, 6 concentrations ranging from 5 to 30 ug/ml were selected for the continuous (7-day exposure) regimen, and 5 concentrations (85 to 115 ug/ml) for the 24-hr exposure (which included a 7-day refeeding regimen).

One weeks' continuous exposure to chlorpropham induced a significant increase in the frequency of transformants at 4 of the 6 dose levels tested in both assays (Report Table 2, 4 attached here). In both the initial and repeat 24-hr exposure/refeed assays, significant dose-dependent increases in stable morphological transformation was also achieved (Report Tables 3,4). The BaP positive control performed as expected.

The author concluded that chlorpropham was positive for inducing morphological transformation of SHE cells.

F. TB-I Evaluation: ACCEPTABLE

Attachments (Summary Data Tables)

4 96

TABLE 2

7 DAY CONTINUOUS EXPOSURE

SUMMARY OF SYRIAN HAMSTER EMBRYO CELL
TRANSFORMATION ASSAYS OF
CHLORPROPHAM

Dose			Total Colonies Scored	MT[1]/MT Freq[2]	MA[3]/MA Freq[4]	Average P.E.[5] ± S.E.%[6]	Relative P.E.%[7]	MT P value 1 Tail Fishers[8]
	DMSO	(0.2%)	1233	3 /0.243	58 /4.704	48 ± 1.4	100	
	MEDIUM		1101	5 /0.454	30 /2.725	45 ± 1.5	-	
B(a)P	1.25	µg/ml	1268	29 /2.287	76 /5.994	48 ± 1.3	100	0.0000[9]
	2.5	µg/ml	1214	23 /1.895	67 /5.519	48 ± 1.3	101	0.0000[9]
Dose:	5.0	µg/ml	1290	8 /0.620	66 /5.116	46 ± 1.2	97	0.1280
	10.0	µg/ml	1439	13 /0.903	70 /4.864	51 ± 1.0	108	0.0225[9]
	15.0	µg/ml	1358	11 /0.810	85 /6.259	49 ± 1.2	102	0.0651
	20.0	µg/ml	1203	11 /0.914	75 /6.234	44 ± 1.4	93	0.0286[9]
	25.0	µg/ml	1259	10 /0.794	85 /6.751	45 ± 1.1	94	0.0122[9]
	30.0	µg/ml	1041	13 /1.249	73 /7.012	37 ± 1.1	78	0.0041[9]

TABLE 3

24 HOUR EXPOSURE
SUMMARY OF SYRIAN HAMSTER EMBRYO
TRANSFORMATION ASSAYS OF
CHLORPROPHAM

	Dose	Total Colonies Scored	MT[1]/ MT Freq.[2]	MA[3]/ MA Freq.[4]	Average P.E.[5] ± S.E.[6]	Relative P.E.[7] (%)	MT P Value 1 Tail Fisher's[8]
	MEDIUM	1410	4 /0.284	23 /1.631	48 ± 1.8		
	DMSO (0.2%)	1543	3 /0.194	21 /1.361	52 ± 1.8	100	
B(a)P	1.25 µg/ml	1422	15 /1.055	71 /4.993	48 ± 1.2	92	0.0022[9]
	2.5 µg/ml	1562	12 /0.768	60 /3.841	53 ± 1.3	101	0.0184[9]
Dose:	40 µg/ml	1416	3 /0.212	49 /3.460	48 ± 1.7	92	0.6153
	50 µg/ml	1352	4 /0.296	67 /4.956	46 ± 1.6	88	0.4289
	60 µg/ml	1278	8 /0.626	68 /5.321	43 ± 1.7	83	0.0631
	70 µg/ml	1249	10 /0.801	70 /5.604	42 ± 2.1	81	0.0192[9]
	85 µg/ml	1221	10 /0.819	83 /6.798	41 ± 2.1	79	0.0173[9]
	100 µg/ml	554	20 /3.610	85 /15.343	19 ± 1.5	36	0.0000[9]

TABLE 4

7 DAY CONTINUOUS EXPOSURE
SUMMARY OF SYRIAN HAMSTER EMBRYO
TRANSFORMATION ASSAYS OF
CHLORPROPHAM

	Dose		Total Colonies Scored	MT[1]/ MT Freq.[2]	MA[3]/ MA Freq.[4]	Average P.E.[5] ± S.E.[6]	Relative P.E.[7] (%)	MT P Value 1 Tail Fisher's[8]
	DMSO	(0.2%)	1541	2 /0.130	18 /1.168	52 ± 2.0	100	
B(a)P	1.25	µg/ml	1538	16 /1.040	57 /3.706	52 ± 1.4	100	0.0006[9]
	2.5	µg/ml	1399	14 /1.001	64 /4.575	47 ± 1.3	91	0.0011[9]
Dose:	20	µg/ml	1413	8 /0.566	60 /4.246	48 ± 1.8	92	0.0408[9]
	30	µg/ml	1235	12 /0.972	87 /7.045	42 ± 2.2	80	0.0019[9]

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27

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TABLES 2, 3 and 4 (cont)

SUMMARY OF SYRIAN HAMSTER EMBRYO CELL TRANSFORMATION ASSAYS OF CHLORPROPHAM

[1]MT - Combined total no. of morphologically transformed colonies

[2]MT Freq - $\frac{\text{MT}}{\text{Total colonies scored}} \times 100$

[3]MA - Combined total no. of morphologically altered colonies

[4]MA Freq - $\frac{\text{MA}}{\text{Total colonies scored}} \times 100$

[5]Average PE - Average PE of combined trials

[6]Standard Error (SE) - $\frac{\text{Standard deviation of combined average PE}}{\sqrt{\text{combined total no. of dishes counted}}}$

[7]Relative PE - $\frac{\text{Average PE}}{\text{Average PE of solvent control}} \times 100$

[8]MT P value - Probability of significance of treatment related effects using Fishers Exact Test

[9]Significant treatment related increase compared to solvent control at $P<0.05$ using a

510A
CASWELL FILE

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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: ID# 018301. Chlorpropham, technical. Evaluation of Five Acute Toxicity Studies submitted by by Chlorpropham Task Force to support reregistration of Chlorpropham.

Shaughnessey No.: 018301
Tox Chem. No.: 510A
Project No.: 1-0640
Submission No.: S390681

TO: Lois Rossi, Team Manager, PM Team 74
Karen Farmer, PM 74 Team Reviewer
Special Review and Reregistration Division (H7508W)

FROM: Linnea J. Hansen, Ph.D.
Section IV, Tox. Branch I (H7509C)
Health Effects Division

THRU: Marion P. Copley, D.V.M., D.A.B.T., Section Head
Section IV, Tox. Branch I (H7509C)
Health Effects Division
6/26/91

CONCLUSIONS:

The acute toxicity studies submitted by the Chlorpropham Task Force for reregistration of Chlorpropham satisfy the guideline requirements and are classified as Acceptable for regulatory purposes. Results of the studies are summarized below:

Acute Oral Toxicity (81-1), Rat	LD_{50} = 4.2 kg (both sexes) Toxicity Category III
Acute Dermal Toxicity* (81-2), Rabbit	LD_{50} > 2000 mg/kg (both sexes) Toxicity Category III
Primary Ocular Irritation (81-4), Rabbit	Mild irritation, resolved by 72 hr. Toxicity Category III
Primary Dermal Irritation (81-5), Rabbit	PIS 0.1 at 24 and 48 hr (mild irritation. Toxicity Category IV

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9 10

Dermal Sensitization Potential (81-6), G. pig	Not a sensitizer

*Study classified as acceptable despite deficiencies because results are supported by previously submitted acceptable study (MRID# 410137-01)

ACTION REQUESTED:

The Chlorpropham Task Force submitted five acute toxicity studies on April 2 and May 2, 1990 for review to support reregistration of Chlorpropham (CIPC). An acute oral toxicity (81-1), dermal toxicity (81-2), ocular toxicity (81-4), dermal irritation (81-5) and dermal sensitization study (81-6) were submitted. An acute inhalation study (81-3) is not required for reregistration of this compound.

HANSEN/PC-1/CIPC.MEM/0001\CHLORPROPHAM/PROJ 1-0640/ACUTES/6/25/91