GUIDELINE SERIES 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE CYTOGENETICS

EPA Reviewer: Irving Mauer, Ph.D. Signature:
Geneticist, Toxicology Branch (I)/HED Date:
EPA Branch Chief: Karl Baetcke, Ph.D. Signature:
Toxicology Branch (I)/HED Date: 11/5/91

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mammalian cells in culture cytogenetic assay in human lymphocytes

EPA IDENTIFICATION Numbers:

Tox Chem. Number:

MRID Number: 415614-07

TEST MATERIAL: Dichlormid

SYNONYMS: Stauffer R-25788 technical

SPONSOR: ICI Americas Inc., Wilmington, DE

STUDY NUMBER: SV0311; Report No. CTL/P/2470

TESTING FACILITY: ICI Central Toxicology Laboratory, Macclesfield, Cheshire, UK

TITLE OF REPORT: Dichlormid: An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes

AUTHOR: Howard, C.A.

REPORT ISSUED: June 7, 1989

CONCLUSIONS-EXECUTIVE SUMMARY: Human lymphocytes, obtained from one male and one female donor were evaluated for clastogenic effects following exposure to three nonactivated and three S9-activated doses of dichlormid (75, 500, and 750 µg/mL). Results indicated that the high dose with and without S9-activation induced a cytotoxic effect; however, no significant increase in structural aberrations was seen at any concentration. It was, therefore, concluded that dichlormid was tested over an appropriate range of concentrations with no evidence of a clastogenic response in a well-controlled assay. The study satisfies Guideline requirements for genetic effects Category II, Structural Chromosome Aberrations.

STUDY CLASSIFICATION: The study is acceptable.

A. MATERIALS:

1. Test Material: Dichlormid (R-25788)

Description: Amber liquid
Identification No.: SC2/88
Purity: 97.2%
Receipt date: Not reported
Stability: Unspecified for this study; however, R-25788 was listed as stable at <100°C in an additional report submitted by the sponsor (see Data Evaluation Record 91-8).
Contaminants: None listed
Solvent used: Dimethyl sulfoxide (DMSO)
Other provide information: No information on test material storage conditions or the frequency of dose solution preparation were provided.

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO/1µg/mL

Positive: Nonactivation (concentrations, solvent): Mitomycin C (Mit C) was prepared in 0.85% physiological saline to yield a final concentration of 0.5 µg/mL.

Activation (concentrations, solvent): Cyclophosphamide (CP) was prepared in 0.85% physiological saline to yield final concentrations of 50 and 100 µg/mL. Only cultures treated with 100 µg/mL were scored.

3. Activation: S9 derived from AlpK:APfSD male rats

x Aroclor 1254	x induced	x rat	x liver
_____ phenobarbital	_____ noninduced	_____ mouse	_____ lung
_____ none		_____ hamster	_____ other
_____ other		_____ other	

The rat S9 liver homogenate was prepared by the performing laboratory.

S9 mix composition:

Component	Final Concentration in S9 Mix
Na_2HPO_4	75 mM
KCl	25 mM
NADP	3 mM
Glucose 6-Phosphate	4 mM
$MgCl_2$	6 mM
S9	50%

Note: 200 µl of the S9 mix were added to 10 mL of culture medium

4. Test Compound Concentration Used:

   (a) Preliminary cytotoxicity assay: A preliminary cytotoxicity assay was not performed.

   (b) Cytogenetic assay:

      (1) Nonactivated conditions: A concentration range of 2.5 to 1200 µg/mL was initially used; cultures exposed to 75, 500, and 750 µg/mL were scored for structural aberrations.

      (2) S9-activated conditions: As above.

5. Test Cells: Human lymphocytes were obtained from the blood of two healthy subjects (one male and one female) with an established history of low chromosome damage. Lymphocyte cultures were initiated and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 0.1 mg/mL phytohemagglutinin, and antibiotics.

   Properly maintained? Yes.

   Cell line or strain periodically checked for mycoplasma contamination? Not applicable.

   Cell line or strain periodically check for karyotype stability? Not applicable.

B. TEST PERFORMANCE:

1. Cell Treatments:

   (a) Cells exposed to test compound for:
   2 hours and 40 minutes-3 hours and 55 minutes (nonactivated)
   2 hours and 40 minutes-3 hours and 55 minutes (activated)

   (b) Cells exposed to positive controls for:
   2 hours and 40 minutes-3 hours and 55 minutes (nonactivated)
   2 hours and 40 minutes-3 hours and 55 minutes (activated)

   (c) Cells exposed to negative and/or solvent controls for:
   2 hours and 40 minutes-3 hours and 55 minutes (nonactivated)
   2 hours and 40 minutes-3 hours and 55 minutes (activated)

2. Cytogenetic assay:

   (a) Treatment: At ≈44 hours after initiation, replicate cultures (two/sex), were exposed to the selected test material doses, the solvent control (DMSO), or the positive controls (Mit C or CP) in both the presence and absence of S9-activation. At the end of treatment, cells were centrifuged, refed culture medium, and reincubated. Colchicine (final concentration, 1[illegible] µg/mL) was

added 2 hours before the cultures were harvested (72 hours postinitiation). Metaphase cells were collected, swollen in 0.075M KCl, and fixed in glacial acetic acid: methanol (1:3). Slides were stained with 10% Giemsa and coded.

(b) Metaphase analysis: Two hundred metaphase cells in treatment and solvent control groups (100 cells/culture/donor) were scored for chromosome aberrations; 25 cells/donor from one of the two replicate cultures for each positive control were also scored for aberrations. The mitotic index (MI) was determined for each treatment group.

(c) Statistical methods: The percentage of cells with chromosome aberrations (excluding gaps) was evaluated using the Fisher's exact test.

(d) Evaluation criteria: No criteria were provided to establish the validity of the assay or the biological significance of the results.

2. Protocol: See Appendix B.

C. REPORTED RESULTS: The highest nonactivated and S9-activated dose (750 µg/mL) was selected for the evaluation of chromosomal aberration based on a ≥50% decrease in the MI, compared to solvent control value. As the results presented in Table 1 show, MIs for 750 µg/mL-S9 were ~70% lower than the solvent control; with S9-activation, MIs for the high-dose cultures were ~50 to 60% lower than the solvent control. However, dichlormid at 75, 500, or 750 µg/mL +/-S9 did not induce a clastogenic response in replicate cultures from the two donors. By contrast, significant ($p<0.01$) chromosome damage was seen in cultures exposed to the nonactivated (0.5 µg/mL Mit C) and the S9-activated (100 µg/mL CP) positive controls. The study author, therefore, concluded that dichlormid was not clastogenic in cultured human lymphocytes.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study author's interpretation of the data was correct. Dichlormid was assayed to a cytotoxic level with no indication of a clastogenic effect either in the absence or presence of S9-activation. Additionally, the sensitivity of the test system to detect a clastogenic effect was adequately demonstrated by the significant results obtained in both donor cell cultures exposed to the nonactivated and S9-activated positive controls. The study, therefore, provides acceptable evidence of a negative response in this test system.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated May 22, 1989.)

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 11-13; Appendix B, Protocol, CBI pp. 19-24.

TABLE 1. Representative Results from the Human Lymphocyte In Vitro Cytogenetic Assay with Dichlormid

Substance	Dose/mL	S9-Activation	No. of Cells Scored	Mitotic Index (%)	Total No. Of Aberrations[a]	No of Cells with Aberrations[a]	Percent Cells with Aberrations[a]	Biologically Significant Aberrations[b] (No./Type)
Solvent Control								
Dimethyl Sulfoxide	1 µl	-	200[c]	15.0	0	0.00	0.00	--
	1 µl	-	200[d]	14.5	0	0.00	0.00	--
	1 µl	+	200[c]	15.0	0	0.00	0.00	--
	1 µl	+	200[d]	12.5	0	0.00	0.00	--
Positive Control								
Mitomycin C	0.5 µg	-	25[c]	5.0[e]	8	0.32	28.00[f]	2 B; 4 F-M; 2 Ot
	0.5 µg	-	25[d]	5.0[e]	5	0.20	20.00[f]	1 B; 2 F-M; 1 I; 1 Ot
Cyclophosphamide	100 µg	+	25[c]	4.0[e]	17	0.68	52.00[f]	9 B; 6 F-M; 2 Ot
	100 µg	+	25[d]	2.0[e]	9	0.36	36.00[f]	4 B; 4 F-M; 1 Ot
Test Material								
Dichlormid	750 µg[g]	-	200[c]	5.0[e]	0	0.00	0.00	--
	750 µg[g]	-	200[d]	4.5	0	0.00	0.00	--
	750 µg[g]	+	200[c]	7.5	0	0.00	0.00	--
	750 µg[g]	+	200[d]	5.0	0	0.00	0.00	--

[a]Gaps excluded.

[b]Abbreviations used:

B = Break I = Interchanges
F-M = Fragments and minutes Ot = other (not specified).

[c]Lymphocytes obtained from donor 1.

[d]Lymphocytes obtained from donor 2.

[e]Mitotic index was determined from a single culture.

[f]Significantly higher ($p<0.01$) than the solvent control by Fisher's exact test.

[g]Results for lower doses (75 and 500 µg/mL +/-S9) did not suggest a clastogenic effect.