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

JAN 10 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Polyhexamethylene biguanide (PHMB, Baquacil): Review of a Mutagenicity Study Submitted by the Registrant.

P.C. Code: 111801
Submission: S458169
DP Barcode: D210731
MRID # 416870-05

FROM: Timothy F. McMahon, Ph.D. 1/4/95
Pharmacologist, Review Section I
Toxicology Branch II, HED (7509C)

TO: Kathryn Scanlon / PM 53
Special Review and Reregistration Division (7508W)

THRU: Yiannakis M. Ioannou, Ph.D. 1/10/95
Section Head, Review Section I
Toxicology Branch II, HED (7509C)

and

Marcia Van Gemert, Ph.D. 1/10/95
Chief, Toxicology Branch II, HED (7509C)

Registrant: Zeneca, Inc.

Action Requested: Review of a mutagenicity study submitted by the registrant (in vitro cytogenetics assay).

Summary:

The clastogenic effects of Vantocil IB (19.6% active ingredient) were evaluated in human lymphocytes obtained from one male and one female donor (MRID # 416870-05). Lymphocytes were exposed to three concentrations of test chemical in the absence of metabolic activation (5, 25, and 50 µg/ml) and in the presence of rat liver S-9 (25, 100, and 187.5 µg/ml for donor 1; 25, 100, and 250 µg/ml for donor 2). Results indicated that the high dose(s) with and without metabolic activation induced a cytotoxic effect in cultures derived from both donors; however, no significant increase in structural aberrations was seen at any concentration. It was concluded that Vantocil IB was tested over an appropriate range of concentrations with no evidence of a clastogenic response in a well-controlled assay.

This study is classified as an **acceptable** study. It satisfies the guideline requirement for an in vitro cytogenetics study.

FINAL

DATA EVALUATION REPORT

PHMB

Study Type: Mutagenicity: Mammalian Cells in Culture Cytogenetic Assay in Human Lymphocytes

Prepared for:

Health Effects Division
Office of Pesticide Programs
Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Nancy E. McCarroll, B.S. Date 5/3/93

Independent Reviewer Kristin Jacobson, MSPH Date 5/3/93

QA/QC Manager Sharon Segal, Ph.D. Date 5/3/93

Contract Number: 68D10075
Work Assignment Number: 2-21
Clement Number: 73
Project Officer: Caroline Gordon

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GUIDELINE SERIES 84: MUTAGENICITY
MAMMALIAN CELLS IN CULTURE CYTOGENETICS

EPA Reviewer and Section
Head: Elizabeth Doyle, Ph.D.
Review Section IV,
Toxicology Branch II/HED

Signature: R. McLaughlin for E
Date: 12/20/93

DATA EVALUATION REPORT

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

EPA IDENTIFICATION Numbers:

Tox Chem. Number: 676

PC Code: 111801

MRID Number: 416870-05

TEST MATERIAL: Vantocil IB

SYNONYM: Polyhexamethylene biguanide; PHMB; baquacil

SPONSOR: ICI Americas Inc., Wilmington, DE

STUDY NUMBER: Report No. CTL/P/2582

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

TITLE OF REPORT: 'Vantocil' IB: An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes

AUTHOR: C.A. Howard

REPORT ISSUED: July 6, 1989

CONCLUSIONS-EXECUTIVE SUMMARY: Human lymphocytes, obtained from one male and one female donor, were evaluated for clastogenic effects following exposure to three nonactivated (5, 25, and 50 µg/mL) and three S9-activated doses (25, 100, and 187.5 µg/mL--donor 1; 25, 100, and 250 µg/mL--donor 2) of Vantocil IB. Results indicated that the high dose(s) with and without S9-activation induced a cytotoxic effect in cultures derived from both donors; however, no significant increase in structural aberrations was seen at any concentration. It was, therefore, concluded that Vantocil IB was tested over an appropriate range of concentrations with no evidence of a clastogenic response in a well-controlled assay.

STUDY CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-2) for genetic effects Category II, Structural Chromosome Aberrations.

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A. MATERIALS:

1. Test Material: Vantocil IB

Description: Colorless liquid
Identification No.: Reference numbers: BX2125; SC 17/88; Y00156/001/010
Purity: 19.6% active ingredient (a.i.) in water
Receipt date: Not reported
Stability: Unspecified
Contaminants: None listed
Solvent used: 0.85% physiological saline (saline)
Other provided information: No information on test material storage conditions or the frequency of dose solution preparation were provided. Analytical determinations were not performed to verify actual concentrations used in the study; solutions were not adjusted to 100% a.i.

2. Control Materials:

Negative: None

Solvent/final concentration: Saline 10 µL/mL

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

Activation (concentrations, solvent): Cyclophosphamide (CP) was prepared in 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.

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4. Test Compound Concentration Used:

   (a) Preliminary cytotoxicity assay: 50, 100, 500, 750, 1000, 2500, 3750, 5000, and 7500 µg/mL were evaluated in the cytotoxicity test.

   (b) Cytogenetic assay: A concentration range of 5 to 500 µg/mL +/-S9 was initially used; cultures exposed to 5, 25, or 50 µg/mL -S9 (both donors) and 25, 100, and 187.5 µg/mL +S9 (donor 1) or 25, 100, and 250 µg/mL +S9 (donor 2) were scored for structural aberrations.

5. Test Cells: Human lymphocytes were obtained from the blood of two healthy subjects (one male and one female) with established histories of low incidences of 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 checked for karyotype stability? Not applicable.

B. TEST PERFORMANCE:

1. Cell Treatments:

   Cells exposed to test compound, solvent or positive controls for:
   2 hours and 20 minutes-3 hours and 40 minutes (nonactivated)
   2 hours and 20 minutes-3 hours and 40 minutes (activated)

2. Preliminary Cytotoxicity/Cytogenetic Assays: Similar procedures were used for the preliminary assessment of cytotoxicity and for the cytogenetic assay.

   (a) Treatment: At ≈44 hours after initiation, replicate cultures (two/sex), were exposed to the selected test material doses, the solvent control (saline), 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, 10 µg/mL) was added 2 hours before the cultures were harvested at 72 hours postinitiation. Metaphase cells were collected, swollen in 0.075M KCl, and fixed in glacial acetic acid:methanol (1:3). Slides were prepared, 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; ~100 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:

1. Preliminary Cytotoxicity Test: No data were provided from the preliminary cytotoxicity test. The report indicated that the maximum dose(s) selected for the cytogenetic assays were determined "by a reduction in mitosis of approximately 50-80%".

2. Cytogenetic Assays:

(a) Nonactivated conditions: The three doses selected for chromosome analysis in the nonactivated phase of testing were 5, 25, and 50 µg/mL. As shown in Table 1, MIs for lymphocyte cultures from both donors were reduced ~≥50% compared to the solvent control. Data from individual donors further indicated that the three nonactivated doses were not clastogenic.

(b) S9-activated conditions: In the presence of S9 activation, there was no appreciable increase in the frequency of chromosomes with abnormal morphology in cultures exposed to 25, 100, or 187.5 µg/mL Vantocil IB (donor 1) or 25, 100, or 250 µg/mL of the test material (donor 2). Additionally, the high doses selected for the analysis (185.7 µg/mL--donor 1 and 250 µg/mL--donor 2) caused an ~≥50% reduction in the MI (Table 1).

By contrast to the lack of clastogenic response in the treatment groups, the nonactivated (0.5 µg/mL Mit C) and S9-activated (100 µg/mL CP) positive controls induced significant ($p \leq 0.01$) yields of cells with structural chromosome aberrations. The study author, therefore, concluded that Vantocil IB was not clastogenic in cultured human lymphocytes.

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

Substance	Dose/mL	S9-Activation	No. of Cells Scored	Mitotic Index (%)	Total No. Of Aberrations[a]	No of Cells with Aberrations[a,b]	Percent Cells with Aberrations[a]	Biologically Significant Aberrations[c] (No./Type)
Solvent Control								
0.85% Physiological saline	10 µl	-	200[d]	8.75	0	0	0.0	
	10 µl	-	200[e]	7.00	2	2	1.0	2F-M
	10 µl	+	200[d]	12.30	0	0	0.0	--
	10 µl	+	200[e]	8.90	1	1	0.5	1F-M
Positive Control								
Mitomycin C	0.5 µg	-	100[d]	2.70[f]	22	20	20.0*	13B; 3F-M; 6I
	0.5 µg	-	59[e]	2.40[f]	19	15	25.4*	14B; 3F-M; 2I
Cyclophosphamide	100 µg	+	100[d]	4.30[f]	30	26	26.0*	22B; 1F-M; 7I
	100 µg	+	100[e]	4.10[f]	16	14	14.0*	14B; 1F-M; 1I
Test Material								
Vantocil IB	50.0 µg[g]	-	200[d]	2.15	0	0	0.0	--
	50.0 µg[g]	-	145[e]	3.65	1	1	0.5	1BK
	187.5 µg[g]	+	200[d]	5.45	1	1	0.5	1BK
	250.0 µg[g]	+	111[e]	4.80	1	1	0.9	1F-M

[a]Gaps excluded.
[b]Calculated by our reviewers.

[c]Abbreviations used:

B = Break I = Interchanges
F-M = Fragments and minutes

[d]Lymphocytes obtained from donor 1.
[e]Lymphocytes obtained from donor 2.
[f]Mitotic index was determined from a single culture.
[g]Results for lower doses (5 and 25 µg/mL -S9--both donors, and 25 and 100 µg/mL +S9--both donors) did not suggest a clastogenic effect.

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

Note: Data were extracted from the study report; see CBI pp. 17, 18, and 26-29.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study author's interpretation of the data was correct. Vantocil IB was assayed to cytotoxic levels 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 individual 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; however, the date was not legible.)

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 11-13; Appendix B, Protocol, CBI pp. 19-25.

CORE CLASSIFICATION: Acceptable. The study satisfies Guideline requirements (§84-2) for genetic effects, Category II, Structural Chromosome Aberrations.

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