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

NOV 12 1997

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Vantocil IB® (Baquacil): Review of Mutagenicity Data.

EPA Identification Numbers:

DP Barcode: D240678 ~~D220840~~ P.C. Code: 111801
Submissions: S496691
MRIDs: 41404501; 42149905; 41404502; 42149903; 41096901;
41404503; 41687006; 42149906; 41687004.

TO: Connie Welch / Portia Jenkins
PM Team # 32
Registration Branch II, Antimicrobial Division

FROM: Timothy F. McMahon, Ph.D. 11-12-97
Pharmacologist, RASSB
Antimicrobial Division (7510W)

THRU: Winston Dang, Ph.D.
Leader, Team One
Antimicrobials Division (7510W)

and

Norm Cook 11-12-97
Chief, RASSB
Antimicrobials Division (7510W)

Action Requested: Review of mutagenicity data submitted by ICI Americas, Inc (now known as Zeneca) for the active ingredient Polyhexamethylene biguanide hydrochloride (Vantocil IB®).

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Data Summary:

The registrant, as part of the reregistration process for the active ingredient in Vantocil IB® (Polyhexamethylene biguanide hydrochloride, submitted mutagenicity data to OPP for review. The executive summaries of these reviews are shown below. Each review contains a conclusion as to the mutagenic potential observed in the study.

1) CITATION:Callander, R.D (1989): Vantocil IB: An Evaluation in the Salmonella Mutation Assay. ICI Central Toxicology Laboratory: Alderley Park, Macclesfield, Cheshire, UK. Report No. CTL/P/2406. Study Completion Date: June 7, 1989. Unpublished. MRID # 41687004.

EXECUTIVE SUMMARY: In two independently performed microbial gene mutation assays (MRID No. 41687004), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 were exposed to 0.32-500 µg/plate Vantocil IB (19.6% a.i.) in the absence or presence of S9 activation. Additional testing was carried out using comparable doses with and without S9 in TA1537 and TA98. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

The utility of testing a bactericidal agent in a microbial mutation assay is questionable. Nevertheless, cytotoxicity was observed for the majority of strains at ≥ 200 µg/plate +/- S9. All strains responded in the expected manner to the nonactivated and S9-activated positive controls. There was, however, no evidnece that Vantocil IB induced a mutagenic response in any strain at any nonactivated or S9-activated dose.

The study is classified as **Acceptable** and satisfies the guideline requirement for a microbial gene mutation assay (§84-2).

2) CITATION: Hastwell, R.M. and McGregor, D.B.(1979): Testing for Mutagenic Activity in Salmonella typhimurium Compound YO156/001/001. Inveresk Research International, Edinburgh, Scotland; Report No. CTL/C/1720. Study Completion Date: June 1979. Unpublished. MRID # 41687006/42149906.

EXECUTIVE SUMMARY: In a microbial gene mutation assay, Salmonella typhimurium strains TA1535, TA1537, TA 1538, TA98, and TA100 were exposed to 3.3, 10, 33.3, 100 or 333.3 µg/plate Compound Y0156/001/001 (20% a.i. in water) in the absence or presence of S9 activation. Additional testing was conducted with strains TA1538 and TA98 exposed to nonactivated doses of 25, 50, 100, 150, and 200 µg/plate. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in phosphate buffer.

Cytotoxicity was observed for the majority of strains at 333.3 µg/plate +/- S9. There was inconclusive evidence that the nonactivated test material induced a weak mutagenic response in strain TA1538; however, a parallel effect on the derivative strain TA98 was not seen. It should be noted that the test substance is a bactericidal agent, hence, this assay system is not appropriate. Additionally, numerous study deficiencies (e.g. no methodology, no direct acting positive controls, marginal responses with the S9-activated positive control) precluded a full evaluation and render the study **unacceptable** for regulatory purposes. This study **does not satisfy** the guideline requirement for a microbial gene mutation assay (§84-2).

3) CITATION: Randall, V. and Beck, S.L. (1989): "Vantocil" IB: An evaluation in the Mouse Micronucleus Test (Original Report, MRID No. 41096901); "Vantocil" IB : An Evaluation in the Mouse Micronucleus Test (Addendum, MRID No. 41404503). ICI Central Toxicology Laboratory, Alderley Park, Nr. Macclesfield, Cheshire, UK; Study No. CTL/P/2436. Study completion date: April 14, 1989. Unpublished.

EXECUTIVE SUMMARY: In a mouse micronucleus assay (MRID No. 41096901/41404503), groups of five male and five female C57BL/6JfCD-1/Alpk mice received single oral gavage administrations of 250 or 400 mg/kg Vantocil IB (19.6% a.i.) prepared in deionized water. Mice in the high-dose group were sacrificed at 24, 48 and 72 hours postadministration and harvested bone marrow cells were examined for the incidence of micronucleated polychromatic erythrocytes (MPEs). Low-dose animals were sacrificed at 24 hours.

Two animals receiving 400 mg/kg died prior to the scheduled sacrifice. There was also clear evidence of target cell cytotoxicity in the high-dose males and females at all sacrifice intervals. The positive control induced the expected high yield of MPEs in males and females. **Vantocil IB did not, however, induce a clastogenic or aneugenic effect in either sex at any dose or sacrifice time.**
The study is classified as Acceptable and satisfies the requirements for FIFRA Test Guideline 84-2 for a micronucleus assay.

4) CITATION: Trueman, R.W. (1989) Vantocil IB: An Assessment for the Induction of Unscheduled DNA Synthesis in Rat Hepatocytes In Vivo; ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. CTL/P/2603; Study Completion Date: August 29, 1989. (Unpublished) MRID NUMBER: 41404502/42149903.

EXECUTIVE SUMMARY: In two independently performed in vivo/in vitro unscheduled DNA synthesis (UDS) assays (MRID No. 41404502/42149903), groups of two to three male rats were administered single oral gavage doses of 750 or 1500 mg/kg Vantocil IB (19.6%) prepared in deionized water. Animals were sacrificed at 4 and 12 hours posttreatment and recovered hepatocytes were scored for UDS.

Clinical toxicity (i.e., excessive salivation and subdued nature) was observed at 1500 mg/kg; higher levels were lethal. No cytotoxicity for the target organ was seen at either level. The positive control induced the expected high yield of hepatocytes with net nuclear grains. **There was, however, no evidence that the Vantocil IB induced a genotoxic response at either dose or sacrifice time.**

This study is classified as Acceptable and satisfies the guideline requirement for a UDS assay (84-4).

5) CITATION: Howard, C.A. (1989) Vantocil IB: An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes; ICI Central Toxicology Laboratory, Alderley Park, Nr. Macclesfield, Cheshire, UK; Report No. CTL/P/2582; Study Completion Date: July 26, 1989. (Unpublished) MRID NUMBER: 41404501/42149905

EXECUTIVE SUMMARY: In an in vitro mammalian cell cytogenetic assays (MRID No: 41404501/42149905), human lymphocytes derived from male and female donors were exposed to Vantocil IB (19.6% a.i.in water) doses of 5, 25 or 50 µg/mL without S9 activation (both donors) and levels of 25, 100 or 187.5 µg/mL (male donor) or 25, 100 or 250 µg/mL (female donor) with S9 activation for approximately 2.5-3.5 hours. The S9 liver homogenate was derived from Aroclor 1254 induced Sprague-Dawley rat livers and the test material was delivered to the test system in physiological saline.

A 50% reduction in the mitotic index occurred at 50 _g/mL -S9 (both donors) and at 100 µg/mL +S9 (male donor) or at 250 µg/mL +S9 (female donor). The positive controls induced the expected high yield of chromosome aberrations in the lymphocytes derived from the male and female donors. **There was, however, no evidence that Vantocil IB induced a clastogenic effect.**

This study is classified as Acceptable and satisfies the guideline requirement for an in vitro cytogenetic assay.

Conclusions: The above studies on mutagenicity of Vantocil IB® containing the active ingredient polyhexamethylene biguanide at a concentration of 19.6% (considered the technical material) were submitted in order to fulfill the mutagenicity data requirements for reregistration of this chemical. The above studies fulfill the mutagenicity data requirements for this chemical and show no significant mutagenic effects of the test material in any of the assays employed.

VANTOCIL IB **SALMONELLA (84-2)**

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED (7509C)
EPA Reviewer: Tim McMahon, Ph.D.
Review Section I,
Toxicology Branch II/HED (7509C)

Signature: Nancy L. McCarroll
Date: 10/21/97

Signature: [signature]
Date: 10/24/97

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay; OPPTS 870.5265 [§84-2]

DP BARCODE: D220840 SUBMISSION NO.: S496691

PC CODE: 111801 TOX. CHEM. NO.: MRID NO: 41687006/42149906[1]

TEST MATERIAL (PURITY)/BATCH NO.: Compound Y0156/001/002 (20% a.i.in water)/ADGM 2253/77

SYNONYM(S): Vantocil IB; Polyhexamethylene biguanide hydrochloride

CITATION: Hastwell, R.M. and McGregor, D.B. (1979) Testing for Mutagenic Activity in Salmonella typhimurium Compound Y0156/001/002; Inveresk Research International, Edinburgh, Scotland; Report No. CTL/C/1720; Study Completion Date: June 1979. (Unpublished) MRID NUMBER: 41687006/42149906

SPONSOR: ICI Americas Inc., Wilmington, DE

EXECUTIVE SUMMARY: In a microbial gene mutation assay (MRID No. 41687006/42149906), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 were exposed to 3.3, 10, 33.3, 100 or 333.3 µg/plate Compound Y 0156/001/001 (20% a.i.in water) in the absence or presence of S9 activation. Additional testing was conducted with strains TA1538 and TA98 exposed to nonactivated doses of 25, 50, 100, 150 and 200 µg/plate. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in phosphate buffer.

Cytotoxicity was observed for the majority of strains at 333.3 µg/plate +/-S9. **There was inconclusive evidence that the nonactivated test material induced a weak mutagenic response in strain TA1538; however, a parallel effect on the derivative strain TA98 was not seen. It should be noted that the test substance is a bactericidal agent, hence, this assay system is not appropriate. Additionally, numerous study deficiencies (e.g., no methodology, no direct acting positive controls, marginal responses with the S9-activated positive control) precluded a full evaluation and render the study Unacceptable for regulatory purposes.** The study does not satisfy the guideline requirement for a microbial gene mutation assay (84-2).

[1]A Phase 3 Reformat of the study was also submitted and was assigned MRID No. 42149906.

COMPLIANCE: The study was completed prior to implementation of GLP standards; therefore, neither a GLP nor a Quality Assurance statement was present. A Data Confidentiality statements was, however, provided.

VANTOCIL IB **IN VIVO UDS (84-2)**

EPA Reviewer: Nancy McCarroll
Toxicology Branch 1/HED (7509C)

Signature: Nancy L. McCarroll
Date: 10/4/97

EPA Reviewer: Tim McMahon, Ph.D.
RASSB/AD (7510W)

Signature:
Date: 10/24/97

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vivo/in vitro unscheduled DNA synthesis assay in primary rat hepatocytes assay; OPPTS 870.5550 [§84-2]

DP BARCODE: D220840 SUBMISSION NO.: S496691
PC CODE: 111801 TOX. CHEM. NO.: MRID NO: 41404502/42149903[1]

TEST MATERIAL (PURITY): Vantocil IB (19.6% a.i.in water)

SYNONYM(S): Polyhexamethylene biguanide hydrochloride

CITATION: Trueman, R.W. (1989) Vantocil IB: An Assessment for the Induction of Unscheduled DNA Synthesis in Rat Hepatocytes In Vivo; ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK; Study No. CTL/P/2603; Study Completion Date: August 29, 1989. (Unpublished) MRID NUMBER: 41404502/42149903

SPONSOR: ICI Americas Inc., Wilmington, DE

EXECUTIVE SUMMARY: In two independently performed in vivo/in vitro unscheduled DNA synthesis (UDS) assays (MRID No. 41404502/42149903), groups of two to three male rats were administered single oral gavage doses of 750 or 1500 mg/kg Vantocil IB (19.6%) prepared in deionized water. Animals were sacrificed at 4 and 12 hours posttreatment and recovered hepatocytes were scored for UDS.

Clinical toxicity (i.e., excessive salivation and subdued nature) was observed at 1500 mg/kg; higher levels were lethal. No cytotoxicity for the target organ was seen at either level. The positive control induced the expected high yield of hepatocytes with net nuclear grains. **There was, however, no evidence that the Vantocil IB induced a genotoxic response at either dose or sacrifice time.**

This study is classified as Acceptable and satisfies the guideline requirement for a UDS assay (84-4).

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

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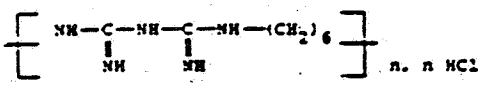
[1]A Phase 3 Reformat of the study was also submitted and was assigned MRID No. 42149903.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Vantocil IB

Description: Clear liquid
Lot/batch number: Sample reference No. BX2125
Purity: 19.6% a.i. in water
Receipt date: September 1988
Stability: Considered to be stable for at least 2 years.
CAS number: Not listed
Structure:



Vehicle used: Deionized water

Other provided information: The test material was stored in a sealed container at room temperature. The frequency of dosing solution preparation was not reported and achieved concentrations of test material were not verified. Stability of the test material has been verified in previous work conducted by the registrant (HED Document No. 010677). The technical grade of the active ingredient for Vantocil IB is considered to be 20%, based on previous discussions with the registrant.

2. Control Substances:

Vehicle control/concentration/route of administration: Deionized water was administered by oral gavage at 10 mL/kg.

Positive controls/concentration/route of administration: 6-p-Dimethyl-aminophenylazobenzthiazole (6BT) was prepared in corn oil and administered by oral gavage at a final dose of 40 mg/kg (4-and 12-hour sacrifices).

3. Medium: WME: Williams' Medium E containing 4 mM L-glutamine and antibiotics; WME+: Williams' Medium E as above supplemented with 10% fetal bovine serum (FBS).

4. Test Compound:

Route of administration: Once by oral gavage (dosing volume = 10 mL/kg).

Dose levels:

Acute Oral Toxicity Test: 700, 1000, 1500, 2000, 2500, 3000, 3500 and 5000 mg/kg (5 males/group).

UDS Assay: 375, 750 and 1500 mg/kg (2-3 males/group/sacrifice time)

Note: Two independent trials of the UDS assay were performed.

5. Test Animals:

(a) Species: Rat; Strain: Alderley Park (Alpk:APfSD); Age (at dosing): Young adults; Sex: Males; Weight range (at dosing): 200-298 g; Source: ICI Pharmaceuticals Barriered Animal Breeding Unit.

(b) Number of animals/dose:

Preliminary toxicity test: 5 males

UDS assay:

- Treatment groups: 2-3 males per trial per sacrifice time
- Positive controls: 1 male per trial per sacrifice time
- Vehicle control: 1 male per trial per sacrifice time

(c) Properly maintained? Yes.

B. TEST PERFORMANCE

1. UDS Assay:

(a) Perfusion techniques/hepatocyte harvest: At 4 and 12 hours postdosing, animals in the appropriate test material, vehicle or positive control groups were anesthetized with Fluothane and livers were perfused with buffer solutions and a collagenase solution. Livers were removed and finely chopped; the resulting crude homogenate was diluted with WME+, filtered and centrifuged. Pellets were resuspended in WEM+ and cell densities were adjusted to 1.5×10^5 viable cells/mL. Prepared hepatocytes in 3-mL volumes were plated onto coverslips placed in multi-well culture dishes. Three coverslips were made per suspension. Cultures were allowed to attach at 37_C with 5% CO_2 for 1.5-2 hours. Unattached cells were removed. Viable cells were incubated in fresh WME containing ^{3}H-thymidine (1 _Ci/mL) for 4 hours, washed and reincubated overnight in WME containing unlabeled thymidine.

(b) Slide preparation: Hepatocytes attached to coverslips were washed, fixed in glacial acetic acid:absolute alcohol (1:3), washed, dried and mounted.

(c) Preparation of autoradiographies/grain development: Slides were coated with Ilford K2 emulsion, exposed at 4_C in the dark for 14 days, developed in Kodak D19, stained with Meyers Haemalum and eosin Y phloxine and coded.

(d) Grain counting: When possible, the grains of at least 100 morphologically normal cells (50 cells/slide/animal) were counted. To determine the net nuclear grains (N-C), grains were counted in a nuclear-sized area within the most heavily labeled cytoplasmic area adjacent to each nucleus. The cytoplasmic grain count (C), was then subtracted from the nuclear grain count (N) of that cell. The percentage of cells in repair (i.e., cells with at least five N-C) was also calculated. Mean gross nuclear counts, mean cytoplasmic grain counts as well as the mean and standard deviations for N-C were calculated.

(e) Statistical methods: The data were not evaluated for statistical significance.

2. Evaluation Criteria: The assay was considered positive if the mean N-C count for any treatment group was _5 net nuclear grains with _20% of the cells in repair and the response was reproducible.

C. REPORTED RESULTS:

1. Acute Toxicity Test: Doses of 700-5000 mg/kg were evaluated in the acute toxicity study using five male rats per treatment group. Reported results indicated that 4 of 5 rats receiving _3000 mg/kg died within 6 hours of treatment. Additional deaths recorded within this time period included 1 of 5 males at 2000 mg/kg and 2 of 5 males at 2500 mg/kg. No other clinical signs were observed. Based on these findings, doses of 375, 700 and 1500 mg/kg were selected for further study.

2. UDS Assay:

Animal observations: Excessive salivation and a subdued nature were reported for rats exposed to 1500 mg/kg.

Hepatocyte analysis: There was no evidence of cytotoxicity in the recovered hepatocytes at any dose; consequently, animals exposed to the low dose (375 mg/kg) were not analyzed for UDS. No appreciable increases in N-C or the percentage of cells in repair were noted in the hepatocytes harvested at either sacrifice time from the male rats administered 750 or 1500 mg/kg Vantocil IB. Similar results were obtained in the independently performed repeat trial. By contrast to the negative findings with the test material, treatment of single animals in each trial with the positive control (6BT at 40 mg/kg) resulted in marked increases in N-C as well as the percentage of cells in repair. Since a genotoxic effect was not uncovered, the data from both trials were combined and are summarized in Table 1.

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Based on these findings, the study author concluded that Vantocil IB did not induce DNA repair in the hepatocytes of rats treated in vivo.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the study was properly conducted and that the study author correctly interpreted the data. Vantocil IB was tested to a high dose (1500 mg/kg) that produced overt toxicity in the treated animals but failed to induce target cell cytotoxicity or genotoxicity in two independently performed trials. Results with the positive control (6BT at 40 mg/kg --both cell harvest times) confirmed the sensitivity of the test system to detect genotoxicity. We conclude, therefore, that the study provided acceptable evidence that Vantocil IB was negative in this whole animal test system.

E. STUDY DEFICIENCIES: NONE

TABLE 1. Representative Combined Results of the In Vivo UDS Assays in Male Rats Administered Vantocil IB by Oral Gavage

Treatment	Dose/kg	No. of Animals Treated	Harvest Time (Hours)	Total No. of Cells Scored	Mean Cytoplasmic Grain Counts[a]	Mean Nuclear Grain Counts[a]	Mean Net Nuclear Grains[a]	Mean % Cells with >5 NNG
Solvent Control								
Deionized Water	10 mL	2	4	200	9.19	5.05	-4.14	0
		2	12	200	12.48	7.65	-4.84	0
Positive Control								
6-p-Dimethylamino-phenylazobenzthiazole	40 mg	2	4	200	9.07	14.34	5.27	53
		2	12	200	13.71	20.80	7.08	61
Test Material								
Vantocil IB	750 mg	5	4	500	11.05	7.75	-3.30	0.2
		4	12	400	13.51	8.86	-4.65	0.2
	1500 mg	5	4	500	12.45	8.42	-4.03	0
		5	12	500	12.03	7.07	-4.96	0

[a]Mean values from the count of 100 cells/animal (50 cells/slide) for individual animals were presented.

Abbreviations used: NNG = Net nuclear grains

Note: Data were extracted from the study report, Tables 1-3; pp. 16-18.

VANTOCIL IB

SALMONELLA (84-2)

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED (7509C)
Signature: Nancy S. McCarroll
Date: 10/21/97

EPA Reviewer: Tim McMahon, Ph.D.
Review Section I,
Toxicology Branch II/HED (7509C)
Signature:
Date: 10/21/97

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Salmonella typhimurium/mammalian microsome mutagenicity assay; OPPTS 870.5265 [§84-2]

DP BARCODE: D220840 SUBMISSION NO.: S496691

PC CODE: 111801 TOX. CHEM. NO.: MRID NO: 41687004

TEST MATERIAL (PURITY): Vantocil IB (19.6% a.i.in water)

SYNONYM(S): Polyhexamethylene biguanide hydrochloride

CITATION: Callander, R.D (1989) Vantocil IB: An Evaluation in the Salmonella Mutation Assay; ICI Central Toxicology Laboratory: Alderley Park, Macclesfield, Cheshire, UK; Report No. CTL/P/2406; Study Completion Date: June 7, 1989. (Unpublished) MRID NUMBER: 41687004

SPONSOR: ICI Americas Inc., Wilmington, DE

EXECUTIVE SUMMARY: In two independently performed microbial gene mutation assays (MRID No. 41687004), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 were exposed to 0.32-500 µg/plate Vantocil IB (19.6% a.i.in water) in the absence or presence of S9 activation. Additional testing was carried out using comparable doses with and without S9 in TA1537 and TA98. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

The utility of testing a bactericidal agent in a microbial mutation assay is questionable. Nevertheless, cytotoxicity was observed for the majority of strains at ≥200 µg/plate +/-S9. All strains responded in the expected manner to the nonactivated and S9-activated positive controls. **There was, however, no evidence that the Vantocil IB induced a mutagenic response in any strain at any nonactivated or S9-activated dose.**

The study is classified as **Acceptable** and satisfies the guideline requirement for a microbial gene mutation assay (84-2).

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

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I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Vantocil IB

Description: Pale yellow liquid
Lot/batch number: Batch Reference No. Bx2125
Purity: 19.6% a.i. in water
Receipt date: Not reported
Stability: Considered to be stable under "normal storage" conditions and under the conditions of use.
CAS number: Not listed
Structure:

Vehicle used: Dimethyl sulfoxide (DMSO)
Other provided information: Neither the test material storage conditions nor the frequency of dosing solution preparation were reported. The study author stated, however, that "fresh stock solutions and dilutions were prepared as necessary for each experiment." Solutions were not adjusted to 100% a.i and achieved concentrations were not verified.

2. Control Materials:

Negative: None

Solvent/final concentration: DMSO/100 µL/plate

Positive:
Nonactivation:

N-Methyl-N'-nitro-n-nitroso-guanidine (MNNG)	1, 2, 5 µg/plate TA1535, TA100
ICR 191	0.5, 1, 2 µg/plate TA1537
Daunomycin (DR)	0.2, 0.5, 1 µg/plate TA98
4-Nitro-o-phenylenediamine (4NPD)	1, 2, 5 µg/plate TA1538

Activation:

2-Aminoanthracene (2-AA)	0.5, 1, 2 µg/plate TA1535, TA1537 0.2, 0.5, 1 µg/plate TA1538, TA98, TA100

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3. Activation: S9 derived from male Alderley Park (Alpk:APfSD) rats

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

The rat liver S9 homogenate was prepared by the performing laboratory and the S9 mix contained the following components:

Component:	Concentration
Na_2HPO_4	100 mM
KCl	33 mM
Glucose-6-phosphate	5 mM
NADP (NA salt)	4 mM
$MgCl_2$	8 mM
S9 homogenate	10 %

4. Test Organism Used: S. typhimurium strains

_____ TA97	x TA98	x TA100	_____ TA102
_____ TA104	x TA1535	x TA1537	x TA1538

list any others:

Test organisms were properly maintained? Yes.
Checked for appropriate genetic markers (rfa mutation, R factor)? Yes.

5. Test Compound Concentrations Used:

a. Preliminary cytotoxicity assay: Six doses (1.6, 8, 40, 200, 1000 and 5000 µg/plate were evaluated with and without S9 activation using strain TA 100. Triplicate plates were prepared per dose per condition.

b. Mutation assay: Three trials of the mutation assay were conducted using the following doses and strains:

Trials 1 and 2: 0.32, 1.6, 8, 40, 200 or 500 µg/plate +/-S9 --all strains

Repeat trial: Owing to contamination and/or slight increases in revertant colonies, the test was repeated using comparable nonactivated and S9-activated doses with strains TA1537 and TA98.

In all trials, triplicate plates were prepared per dose per strain per condition. Duplicate plates were prepared for each positive control set and five replicates were prepared for each negative/solvent control set.

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B. TEST PERFORMANCE:

1. Type of Salmonella Assay:
   - x Standard plate test
   - ___ Pre-incubation (__) minutes
   - ___ "Prival" modification
   - ___ Spot test
   - ___ Other (describe)

2. Preliminary Cytotoxicity/Mutation Assays: Similar procedures were used for the preliminary assessment of cytotoxicity and the mutation assays. Briefly, 2-mL volumes of top agar were added to bijou bottles containing 0.1 mL of an overnight broth culture of the appropriate tester strain, 0.5 mL of S9 cofactor/buffer (nonactivated series) or 0.5 mL of S9 mix (S9-activated series) and 0.1 mL of the appropriate test material dose, solvent, or positive control. The contents of the bottles were mixed, poured over Vogel-Bonner minimal medium, and incubated at 37°C for 66 hours. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were determined.

3. Evaluation Criteria:

   (a) Assay validity: The assay was considered valid if the following criteria were met: (1) the concurrent solvent control data were "acceptable"; (2) the positive control data showed unequivocal positive results and (3) the lowest dose showed no evidence of cytotoxicity and at least three doses showed no significant cytotoxicity.

   (b) Positive response: The test material was considered positive if: (1) there was a reproducible statistically significant dose-related increase in mean revertant colonies/plate and (2) there was a reproducible statistically significant, ≥2-fold increase in revertant colonies for at least one dose level.

4. Statistical Methods: The data were evaluated for statistical significance at $p<0.01$ using a one-tailed Student's t-test.

C. REPORTED RESULTS:

(a) Preliminary cytotoxicity assay: Doses ranging from 1.6-5000 µg/plate +/-S9 were tested using strain TA100. Severe cytotoxicity (i.e, <10% survival) was observed at the two highest levels (1000 and 5000 µg/plate +/-S9). At 200 µg/plate -S9, an ≈50% reduction in histidine (his^+) revertant colonies was recorded; nonactivated levels ≤40 µg/plate did not adversely affect the number of revertant colonies. The remaining S9-activated doses (1.6-200 µg/plate) were also not cytotoxic. Based on these data, doses selected for the mutation study ranged from 0.32-500 µg/plate with or without S9 activation.

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(b) Mutation assays: Selected data from the mutagenicity trials conducted with Vantocil IB are presented in Tables 1 and 2. Overall, the results indicate that the highest dose tested (500 µg/plate +/-S9) was severely cytotoxic for all strains and 200 µg/plate +/-S9 was generally cytotoxic in the majority of strains. As shown in Table 1, results for the first trial did not suggest a mutagenic effect. Although significant increases in his^+ revertants of strain TA98 were seen in the S9-activated phase of Trial 2 (Table 2), the increases were not seen in Trial 1 or reproduced in the repeat trial with this strain. Other significant increases in revertant colonies were sporadic and not indicative of a positive response. Owing to contamination in Trial 2, a repeat test was also conducted with strain TA1537 (with and without S9 activation) and TA 98 (without S9 activation). The findings from this additional test were negative. Our reviewers noted that the spontaneous revertant frequency for the majority of strains was low in all trials; nevertheless, all tester strains responded in the expected manner to the corresponding nonactivated or S9-activated positive control.

Based on the overall results, the study author concluded that Vantocil IB was negative in this microbial test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: While the utility of testing a bactericidal agent in a microbial mutation assay is questionable, the data do support a negative conclusion for Vantocil IB. We assess, therefore, that the test material was investigated to cytotoxic levels (≥200 µg/plate with or without S9 activation) and failed to induce a mutagenic response in any of the tester strains. Findings with the positive controls indicate that the test system was adequately sensitive for the detection of a mutagenic response. Based on the above considerations, we conclude that the study provided acceptable evidence that Vantocil IB is not mutagenic in this assay system.

E. STUDY DEFICIENCIES: NONE.

TABLE 1. Representative Results of the Microbial/Mammalian Microsome Mutation Assay with Vantocil IB -- Trial 1

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Microbial Tester Strains[a] S. typhimurium TA1535	TA1537	TA1538	TA98	TA100
Solvent Control							
DMSO	100 µL	-	9.6	3.2	3.4	13.8	73.2
	100 µL	+	10.4	4.6	6.5	18.8	87.4
Positive Controls[b]							
MNNG	2 µg	-	1319.5	--	--	--	1303.0
ICR 191	1 µg	-	--	109.0	--	--	--
4NPD	2 µg	-	--	--	118.0	--	--
DR	1 µg	-	--	--	--	1062.5	--
2AA	2 µg	+	137.0	46.5	--	--	--
	1 µg	+	90.0	14.0	61.0	445.5	1041.0
Test Material							
Vantocil IB	40 µg[c]	-	9.0	2.0	3.0	10.3	69.3
metabolite	200 µg[d,e]	-	3.0	2.3	1.7	4.7	28.3
	40 µg[c]	+	13.0	3.0	3.3	14.7	102.7
	200 µg[d]	+	7.3	2.3	2.7	21.0	82.3

[a]Means and standard deviations of counts from five plates--solvent control, duplicate plates--positive controls, and triplicate plates--test material doses
[b]Three levels of each positive control were assayed; results for all strains were generally significant ($p<0.01$) at the majority of doses. The presented data were selected as representative.
[c]Results for lower doses (0.32, 1.6 or 8.0 µg/plate +/-S9) did not suggest a mutagenic effect.
[d]Highest assayed dose (500 µg/plate +/-S9) was severely cytotoxic in all strains.
[e]Reduced background lawn of growth for all strains at these doses.

Abbreviations:

DMSO = Dimethyl sulfoxide
MNNG = N-Methyl-N'-nitro-n-nitrosoguanadine
4NPD = 4-Nitro-O-phenylenediamine
DR = Daunomycin
2AA = 2-Aminoanthracene

Note: Data were extracted from the study report, Tables 2 and 6; pp. 18, 19 and 24-26.

TABLE 2. Representative Results of the Microbial/Mammalian Microsome Mutation Assay with Vantocil IB -- Trial 2 and Repeat Tests

Substance	Dose per Plate	S9 Activation	Revertants per Plate of Microbial Tester Strains[a] S. typhimurium TA1535	TA1537[b]	TA1538	TA98	TA98	TA100
Solvent Control								
DMSO	100 µL	-	6.8	3.4	4.0	--[c]	5.6[d]	62.0
	100 µL	+	7.2	5.6	6.2	7.6	10.8	73.4
Positive Controls[e]								
MNNG	2 µg	-	1713.5	--	--	--	--	1228.5
ICR 191	1 µg	-	--	45.0	--	--	--	--
4NPD	2 µg	-	--	--	40.5	--	--	--
DR	1 µg	-	--	--	--	--[c]	223.0	--
2AA	2 µg	+	145.5	195.0	--	--	--	--
	1 µg	+	99.5	44.0	301.0	443.5	232.5	1474.5
Test Material								
Vantocil IB	40 µg[f]	-	5.3	4.0	0.3	--[c]	4.7	50.0
metabolite	200 µg[g,h]	-	1.0	1.3	1.7	--	0.7	3.7
	8 µg[f]	+	7.7	5.7	8.7 (1.4)[i]	14.7*(1.9)[i]	13.7	70.0
	40 µg	+	5.3	5.3	10.7*(1.7)	16.3**(2.1)	12.7	80.7
	200 µg[g]	+	4.3	2.7	9.3 (1.5)	4.7 (0.6)	2.2	47.7

[a]Means and standard deviations of counts from five plates--solvent control, duplicate plates--positive controls, and triplicate plates--test material doses
[b]Results from the repeat trial; due to contamination, this phase of the assay was repeated with strain TA1537.
[c]Data are from the initial phase of Trial 2 with strain TA98. The nonactivated portion of Trial 2 was contaminated; therefore, no data were available.
[d]Results from the repeat test with strain TA98.
[e]Three levels of each positive control were assayed; results for all strains were generally significant at the majority of doses. The presented data were selected as representative.
[f]Results for lower doses (0.32 or 1.6 µg/plate +/-S9 or 8.0 µg/plate -S9) did not suggest a mutagenic effect.
[g]Highest assayed dose (500 µg/plate +/-S9) was severely cytotoxic in all strains.
[h]Reduced background lawn of growth for all strains at this dose.
[i]Values in () are the fold increase over background. For significant increases, * = $p<0.05$ or ** = $p<0.01$.

Abbreviations:

DMSO = Dimethyl sulfoxide
MNNG = N-Methyl-N'-nitro-n-nitrosoguanadine
4NPD = 4-Nitro-O-phenylenediamine
DR = Daunomycin
2AA = 2-Aminoanthracene

Note: Data were extracted from the study report, Tables 3, 4, 7 and 8; pp. 20-22 and 27-30.

VANTOCIL IB

IN VITRO MAMMALIAN CELL CYTOGENETICS (84-2)

EPA Reviewer: Nancy McCarroll
Toxicology Branch 1/HED (7509C)

Signature: Nancy L. McCarroll
Date: 10/21/97

EPA Secondary Reviewer: Tim McMahon, Ph.D.
RASSB/AD (7510W)

Signature:
Date: 10/24/97

DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: In vitro cytogenetic assay with human lymphocytes; OPPTS 870.5375 [§84-2]

DP BARCODE: D220840 SUBMISSION NO.: S496691
PC CODE: 111801 TOX. CHEM. NO.: MRID NO: 41404501/42149905[1]

TEST MATERIAL (PURITY): Vantocil IB (19.6% a.i.in water)

SYNONYM(S): Polyhexamethylene biguanide hydrochloride

CITATION: Howard, C.A. (1989) Vantocil IB: An Evaluation in the In Vitro Cytogenetic Assay in Human Lymphocytes; ICI Central Toxicology Laboratory, Alderley Park, Nr. Macclesfield, Cheshire, UK; Report No. CTL/P/2582; Study Completion Date: July 26, 1989. (Unpublished) MRID NUMBER: 41404501/42149905

SPONSOR: ICI Americas Inc., Wilmington, DE

EXECUTIVE SUMMARY: In an in vitro mammalian cell cytogenetic assays (MRID No: 41404501/42149905), human lymphocytes derived from male and female donors were exposed to Vantocil IB (19.6% a.i.in water) doses of 5, 25 or 50 µg/mL without S9 activation (both donors) and levels of 25, 100 or 187.5 µg/mL (male donor) or 25, 100 or 250 µg/mL (female donor) with S9 activation for approximately 2.5-3.5 hours. The S9 liver homogenate was derived from Aroclor 1254 induced Sprague-Dawley rat livers and the test material was delivered to the test system in physiological saline.

A _50% reduction in the mitotic index occurred at 50 µg/mL -S9 (both donors) and at 100 µg/mL +S9 (male donor) or at 250 µg/mL +S9 (female donor). The positive controls induced the expected high yield of chromosome aberrations in the lymphocytes derived from the male and female donors. **There was, however, no evidence that Vantocil IB induced a clastogenic effect.** This study is classified as Acceptable and satisfies the guideline requirement for an in vitro cytogenetic assay.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

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[1]A Phase 3 Reformat of the study was also submitted and was assigned MRID No. 42149905.

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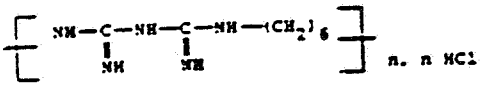
I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: Vantocil IB

Description: Colorless liquid
Lot/batch number: Sample reference No. BX2125
Purity: 19.6% a.i. in water
Receipt date: Not reported
Stability: Considered to be stable for at least 2 years (see MRID No. 41404502/42149903)
CAS number: Not listed
Structure:



Vehicle used: Physiological saline (0.85%)

Other provided information: Neither the test material storage conditions nor the frequency of dosing solution preparation were reported. Stability of the test material has been verified in previous work conducted by the registrant (HED Document No. 010677). The technical grade of the active ingredient for Vantocil IB is considered to be 20%, based on previous discussions with the registrant. However, achieved concentrations were not verified.

2. Control Materials:

Negative: None

Solvent/final concentration: Physiological saline/10 µL/mL

Positive:

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

Activation (concentrations, solvent): Cyclophosphamide (CP) was prepared in physiological saline to yield final concentrations of 50 and 100 µg/mL. Cells treated with 100 µg/mL were scored for chromosome aberrations.

3. Activation: S9 derived from male AlpK:APfSD

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: Nine concentrations ranging from 50-9990 µg/mL were examined with and without S9 activation. Duplicate cultures from two separate donors were used per dose per condition.

(b) Cytogenetic assay: Three nonactivated doses (5, 25 and 50 µg/mL) were evaluated in lymphocytes derived from a male and a female donor. Three S9-activated doses/donor (25, 100 and 187.5 µg/mL--male donor and 25, 100 and 250 µg/mL--female donor) were also tested. Duplicate cultures per donor were prepared for each experimental point.

5. Test Cells: Human lymphocytes were obtained from the blood of two healthy subjects (one male and one female) with established histories of a low incidence of chromosome damage. Lymphocyte cultures were initiated 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 to 3 hours and 40 minutes (nonactivated)
2 hours and 20 minutes to 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.

Treatment: At _44 hours after initiation, replicate cultures (two/sex), were exposed to the selected test material doses, the solvent control (physiological 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 and reincubated in fresh culture medium. 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 0.075 M KCl, and fixed in glacial acetic acid:methanol (1:3). Slides were prepared, stained with 10% Giemsa, and coded.

Metaphase analysis: When possible, one hundred metaphase cells per donor in treatment, solvent and positive control groups (50 cells/ culture) were scored for structural chromosome aberrations. The mitotic index (MI) was determined for each treatment group from the analysis of an unspecified number of lymphocytes per culture.

Statistical methods: The percentage of cells with chromosome aberrations (excluding gaps) was evaluated using the Fisher's exact test ($p<0.01$).

Evaluation criteria: No criteria were provided to establish the validity of the assay, a positive response or the biological significance of the results.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Test: The details of the preliminary cyto-toxicity assay were not furnished. Based on the findings, the study author indicated that nonactivated concentrations of 5-50 µg/mL (both donors) and S9-activated concentrations of 25-187.5 µg/mL (male donor) or 25-250 µg/mL (female donor) were selected for investigation in the cytogenetic assay.

2. Cytogenetic Assays:

Nonactivated conditions: MIs for lymphocyte cultures from both donors in the high-dose group were reduced (by _50% or greater) compared to the solvent control. Data from individual donors further indicated that the three nonactivated levels were not clastogenic in lymphocytes obtained from either donor (Table 1).

S9-activated conditions: An approximately _50% reduction in the MIs was achieved at _100 µg/mL (male donor) and at 250 µg/mL (female donor). In agreement with the nonactivated results, there was no indication of a clastogenic response in cell cultures from either donor.

By contrast to the negative results with the test substance, the nonactivated (0.5 µg/mL Mit C) and the S9-activated (100 µg/mL CP) positive controls induced significant ($p<0.01$) increases in the yield of cells with abnormal chromosome morphology. From the overall findings, the study author concluded that nonactivated and S9-activated Vantocil IB was not clastogenic in cultured human lymphocytes.

D. REVIEWERS' DISCUSSION: 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 levels (50 µg/mL -S9; µ100 µg/mL +S9) that induced clear cytotoxic responses but failed to increase the frequency of cells with structural chromosomal aberrations in either donor. The ability of the test system to detect clastogenesis was adequately demonstrated by the results obtained with the positive controls. We conclude, therefore, that the study provided acceptable evidence that the test substance was negative in this assay system.

E. STUDY DEFICIENCIES: NONE.

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

Substance	Dose/mL	No. of Cells Scored	Mitotic Index (%)	Total No. Of Aberrations[a]	Aberrations per cell[a]	Percent Cells with Aberrations[a]	Biologically Significant Aberrations[b] (No./Type)
Solvent Control							
Physiological saline	10 µL	200[c]	8.8	0	0.000	0.0	--
	10 µL	200[d]	7.0	2	0.010	1.0	2F-M
Positive Control							
Mitomycin C	0.5 µg	100[c]	2.7[e]	22	0.220	20.0*	13B;3F-M;6I
	0.5 µg	59[d]	2.4[e]	19	0.322	25.5*	14B;3F-M;2I
Test Material							
Vantocil IB	5.0 µg	200[c]	6.6	2	0.010	1.0	2B
	25.0 µg	200	5.6	3	0.015	1.5	2B;1F-M
	50.0 µg	200	2.2	0	0.000	0.0	--
	5.0 µg	200[d]	4.6	1	0.005	0.5	1B
	25.0 µg	200	6.6	2	0.010	1.0	2B
	50.0 µg	145	3.7	1	0.007	0.5	1B

[a]Gaps excluded.
[b]Abbreviations used:

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

[c]Lymphocytes obtained from male donor.
[d]Lymphocytes obtained from female donor.
[e]Values were determined from a single culture/donor.

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

Note: Data were extracted from the study report Table 1 and Appendix C pp. 17, 26 and 27.

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TABLE 2. Results from the S9-activated Human Lymphocyte *In Vitro* Cytogenetic Assay with Vantocil IB

Substance	Dose/mL	No. of Cells Scored	Mitotic Index (%)	Total No. Of Aberrations[a]	Aberrations per cell[a]	Percent Cells with Aberrations[a]	Biologically Significant Aberrations[b] (No./Type)
Solvent Control							
Physiological saline	10 µL	200[c]	12.3	0	0.000	0.0	--
	10 µL	200[d]	8.9	1	0.005	0.5	1F-M
Positive Control							
Cyclophosphamide	100.0 µg	100[c]	4.3[e]	30	0.300	26.0*	22B;1F-M;7I
	100.0 µg	100[d]	4.1[e]	16	0.160	14.0*	14B;1F-M;1I
Test Material							
Vantocil IB	25.0 µg	200[c]	8.2	2	0.010	1.0	2B
	100.0 µg	200	6.1	4	0.020	2.0	3B;1F-M
	187.5 µg	200	5.5	1	0.005	0.5	1B
	25.0 µg	200[d]	16.1	2	0.010	1.0	1B;1 F-M
	100.0 µg	93	6.8	0	0.000	0.0	--
	250.0 µg	111	4.8	1	0.009	0.9	1F-M

[a]Gaps excluded.
[b]Abbreviations used:

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

[c]Lymphocytes obtained from male donor.
[d]Lymphocytes obtained from female donor.
[e]Values were determined from a single culture/donor.

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

Note: Data were extracted from the study report Table 2 and Appendix D pp. 18, 28 and 29.

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