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To whom it may concern:

The following information is submitted in accordance with TSCA section 8(e).

Submitted by: NICCA USA Inc.
5000 Nelson Road
Fountain Inn, SC 29644
(864) 862-1426



Name and CAS # of Chemical Substance:

Phenol, 2-[(4-hydroxyphenyl)sulfonyl] (CAS# 5397-34-2)

Test Data submitted:

Chromosomal aberrations assay (Chinese hamster ovary cell)

Testing for Mutagenic Activity with *Salmonella typhimurium*

Testing Laboratory:

Inveresk Research International Limited
Tranent EH33 2NE
Scotland
01875 614545



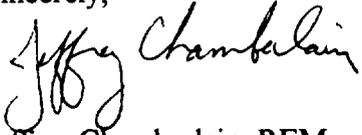
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Enclosed is a draft report of the above mentioned studies. If you have any questions regarding this matter please do not hesitate to contact me at (864) 862-1426 ext. 112.

Sincerely,

Handwritten signature of Jeffrey Chamberlain in black ink.

Jeffrey Chamberlain, REM
Environmental & Quality Control Manager

JSC/mr

Enclosure

cc: Mr. William Grabowski - NICCA USA
Mr. Shinichi Masuda - NICCA USA
file 6023

IRI Report No. 12692

BPS-24
CHROMOSOMAL ABERRATIONS ASSAY WITH
CHINESE HAMSTER OVARY CELLS *in vitro*
(COMPLYING WITH EC (ANNEX V) AND OECD 473 GUIDELINES)

IRI Project No. 757746

DRAFT



INVERESK RESEARCH INTERNATIONAL LIMITED
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CONFIDENTIAL

Inveresk Research International
Report No. 12692

BPS-24
CHROMOSOMAL ABERRATIONS ASSAY WITH
CHINESE HAMSTER OVARY CELLS *in vitro*
(COMPLYING WITH EC (ANNEX V) AND OECD 473 GUIDELINES)

IRI Project No. 757746

Author:

E Murie

Sponsor:

Nicca Chemical Company Limited
23-1, Bunkyo 4-chome
Fukui City
910 Japan

Performing Laboratory:

Inveresk Research International
Tranent, EH33 2NE
Scotland

Total Number of Pages: 38

AUTHENTICATION

'I, the undersigned, hereby declare that this work was performed under my direction and in accordance with the principles of Good Laboratory Practice. The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained.'

E Murie BSc
Study Director

Date:

Report No. 12692

DRAFT REPORT No. 12692

On receipt of approval or amendments, or 16 weeks from today's date if no amendments have been requested, IRI reserves the right to despatch the final report.

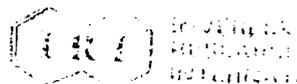
IRI reserves the right to make additional charges for a review of data, amendments or for corrections of minor errors following issue of the final report.

For the final report, this page will be replaced by the Quality Assurance Statement.

Inveresk Research International Limited

ISSUED

19 DEC 1995



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PERSONNEL INVOLVED IN PROJECT 757746

Study Director/Project Leader: E Murie BSc

Test Personnel:

Main Test: E Murie BSc
J M Cameron BSc
D C Innes BSc

Assessment: E Murie BSc
J M Cameron BSc

S9 Preparation: C G Riach BSc HNC

S9 Activity: S E Willington BSc

Quality Assurance: E M Donald BSc

SUMMARY

BPS-24 was submitted for testing and evaluation of clastogenic potential. Chromosomal aberrations assays were performed with duplicate, Chinese hamster ovary (CHO) cell cultures. This study was conducted incorporating 2 independent tests. Dimethylsulphoxide (DMSO) was the vehicle and cyclophosphamide and methyl methanesulphonate were the positive controls used in both experiments.

Both tests were conducted in both the presence and absence of a post-mitochondrial supernatant fraction obtained from the livers of adult, male rats treated with Aroclor 1254 (S9) and an NADPH-generating system.

Cultures, established approximately 20 hours before testing, were treated for 6 h in the presence or 22 hours in the absence of S9 mix. Cultures were harvested at 24 h (Test 1 and 2) or 48 h (Test 2) post treatment.

BPS-24 was toxic to Chinese hamster ovary cells *in vitro* when tested to the maximum permitted concentration of 5000 $\mu\text{g}.\text{ml}^{-1}$ in Test 1. Toxicity was noted in cultures treated with 313-5000 $\mu\text{g}.\text{ml}^{-1}$ in the presence and 625-5000 $\mu\text{g}.\text{ml}^{-1}$ in the absence of S9 mix. In Test 2, cultures were tested with 78-625 $\mu\text{g}.\text{ml}^{-1}$ in both the presence and absence of S9 mix. Toxicity was noted in cultures treated with 313-625 $\mu\text{g}.\text{ml}^{-1}$ in the presence and 234-625 $\mu\text{g}.\text{ml}^{-1}$ in the absence of S9 mix.

BPS-24 induced structural chromosomal aberrations in both the presence and absence of S9 mix.

It was concluded that BPS-24 was clastogenic when tested with Chinese hamster ovary cells *in vitro*.

INTRODUCTION

The test material was BPS-24.

The objective of the study was to determine the potential of the test material to induce chromosomal aberrations in Chinese hamster ovary (CHO) cells.

Many mutagens are clastogenic in higher organisms both *in vivo* and *in vitro*, cytological test systems are useful in the pre-screening of mutagens and carcinogens.

Chinese hamster ovary (CHO) cells possess characteristics which make them suitable for cytogenetic testing of clastogens. They can be subcultured indefinitely without ageing, generally maintaining a stable pseudo-diploid karyotype with a low and easily analysable chromosome number. The short cell cycle of CHO cells also allows easy propagation and a high mitotic rate (Hsu *et al* (1977)). Furthermore, CHO cells have been comprehensively validated in various cytogenetic trials and their sensitivity to a wide range of genotoxins documented (Galloway *et al* (1987) Ishidate *et al* (1988), Tezuka *et al* (1980)).

S9 mix (prepared from rat liver) provided an exogenous metabolic activation system, increasing the detection capability of the test system for pre-mutagens (Natarajan *et al* (1976)).

Many clastogens are active only when tested to toxic or subtoxic concentrations. In the first experiment, the wide range of concentrations used provided both toxic and non-toxic cultures for assessment. Concentrations chosen in the second assay attempted to optimise any toxic or clastogenic effects observed.

This study was authorised by Nicca Chemical Company Limited on 25 July 1995.

The experimental work of these tests was conducted at the Elphinstone Research Laboratories of Inveresk Research International Limited.

The dates for key stages in the conduct of this study were:

Stage	Description	Date
Study initiation	The date the protocol is signed by the Study Director	19 July 1995
Experimental start date	The date the test material is first applied to the test system	3 August 1995
Experimental completion date	Slide reading completed	7 November 1995
Study completion date	The date of signature of the final report by Study Director	See Authentication page for date of Study Director's signature

All data generated and recorded during this study, including a copy of the final report, will be stored in the Scientific Archives of Inveresk Research International Limited for 5 years after issue of the final report. At the end of the 5 year period the Sponsor will be consulted regarding the disposal or continued storage of raw data.

EXPERIMENTAL PROCEDURE

TEST MATERIAL

The test material, BPS-24 , Batch No. A050131 was received from Nicca Chemical Company Limited on 27 July 1995. BPS-24, a white powder, was kept at ambient temperature, and in the dark, when not in use.

METHODS

All experimental procedures, up to harvesting, were conducted using aseptic technique and under amber light.

Cells

The cell line used was Chinese hamster ovary (CHO) cells, obtained from the University of Leiden in 1987. The cells were grown as monolayers, and have a generation time of approximately 12 hours.

Culture Medium

The basic medium (Ham's F-10) containing HEPES buffer, was supplemented with the antibiotic minocycline. For cell growth and treatment in the absence of S9 mix, foetal bovine serum (10% v/v) was added. The medium used for treatment in the presence of S9 mix and for washing cultures before or after treatment, was serum free.

Cell Cultures

Cells were trypsinised from stock flasks and resuspended in fresh culture medium at densities of 0.1×10^6 or 0.05×10^6 cells.ml⁻¹. These cells, in 5 ml volumes, were

dispensed into 25 cm² tissue culture flasks. The high and low cell densities were for cultures harvested at 24 or 48 h post treatment respectively.

S9 Mix (McGregor *et al* (1988))

S9 enzymes (S9) were prepared from the livers of adult, male Fischer rats, as described by Ames *et al* (1975). S9 was stored, as 2 or 5 ml samples, in sterile plastic tubes immersed in liquid nitrogen (-196°C). Enzymic activity of each batch of S9 was characterised by testing selected pre-mutagens in an Ames test with *S. typhimurium* TA 1538 (Appendix 7). S9 batches used demonstrated, within each test, a satisfactory clastogenic response in cells treated with cyclophosphamide (CPH).

To prepare S9 mix, Ham's F-10 was added to pre-weighed cofactors: nicotinamide adenine dinucleotide phosphate (NADP) disodium salt and glucose-6-phosphate (G-6-P) disodium salt, giving final concentrations in the 'S9 mix' of:

NADP di-Na salt	4 mM (= 3.150 mg.ml ⁻¹)
Glucose-6-phosphate di-Na salt	25 mM (= 7.605 mg.ml ⁻¹)

This solution was immediately filter-sterilised by passage through a 0.2 µm disposable filter assembly and mixed 9:1 (v/v) with the S9.

Solubility and Physical Characteristics of the Test Material

The test material was freely soluble in dimethylsulphoxide (DMSO) when warmed to 37°C.

During the first test, the osmolality of selected concentrations of the test material was measured (Appendix 6). BPS-24 did not affect the osmolality of the culture medium, therefore no further measurements were made.

Observations of precipitation were made before cultures were washed out at the end of the treatment period. Precipitation was noted in cultures treated with 5000 $\mu\text{g}.\text{ml}^{-1}$ in the presence of S9 mix.

BPS-24 did not change the colour of the culture medium, therefore no pH measurements were made.

Dose Selection

For the first experiment, 9 dose levels, covering a wide concentration range, were tested. The highest dose was 5000 $\mu\text{g}.\text{ml}^{-1}$, the maximum allowable concentration. Subsequent dose levels were halving dilutions of this dose.

BPS-24 was toxic and showed clastogenic activity in Test 1. In the second experiment, the dose levels selected were:

78, 156, 234, 313, 469 and 625 $\mu\text{g}.\text{ml}^{-1}$

Aberration Tests

Vehicle and Untreated Controls: all experiments included vehicle control cultures. These cultures were subjected to the same experimental manipulations as the treated cultures. The vehicle control was tissue culture medium therefore untreated control cultures were not required.

Positive Control Cultures: the positive controls used were methyl methanesulphonate (MMS), in the absence of S9 mix and cyclophosphamide (CPH), in the presence of S9 mix. These substances were tested at the following concentrations:

CPH: 20-60 $\mu\text{g}.\text{ml}^{-1}$

MMS: 10-40 $\mu\text{g}.\text{ml}^{-1}$

The positive control cultures were subjected to the same experimental manipulations as the test material treated cultures.

Treatment of Cultures

The following table shows the treatment schedules for this study.

S9 Mix	Test	Treatment Period	Recovery Period	Colcemid	Harvest
Presence of S9 mix	Tests 1 and 2	0-6 h	6-22 h	22-24 h	24 h
	Test 2 only		6-46 h	46-48 h	48 h
Absence of S9 Mix	Tests 1 and 2	0-22 h	none	22-24 h	24 h
	Test 2 only		22-46 h	46-48 h	48 h

Tests were conducted both in the presence and absence of S9 mix. Treatments with test material or vehicle control substances were performed on duplicate cell cultures. Several concentrations of the positive controls were tested using single cultures.

Test cultures were established from the stock flask about 20 h before testing.

Cultures to be treated in the presence of S9 mix were washed before treatment with serum free medium.

Exposure medium was prepared, immediately before dosing, in sterile containers.

Exposure medium consisted of:

Presence of S9 mix: serum free medium, dosing solution, and S9 mix
Absence of S9 mix: growth medium and dosing solution

After treatment, cells were washed twice with serum free medium, then full growth medium added, for the recovery period and colcemid treatment. The volume of medium for the recovery period was 5 ml, except for cultures treated in the presence of S9 mix and harvested at 48 h. These had a volume of 10 ml, ensuring sufficient nutrients for the extended recovery period.

Living cultures were examined for evidence of changes to cell morphology. Once at the end of the treatment period and again before harvesting of cultures.

Harvesting of Cultures and Slide Preparation

Colcemid was added to all cultures at a final concentration of $0.1 \mu\text{g}.\text{ml}^{-1}$. Culturing the cells in medium containing colcemid for 2 h accumulated cells in metaphase; the stage of cell division at which chromosomes can be examined using light microscopy.

Mitotic cells were harvested by gently tapping flasks to release these cells from the monolayer. Cells were sedimented by centrifugation (approximately 210 g), and treated with hypotonic solution (1% trisodium citrate) for 15 min at room temperature. The cells were then fixed (after sedimentation as before) using 4 ml of freshly prepared fixative (methanol:glacial acetic acid, 3:1). Two further changes of fixative were made.

Monolayer cells were trypsinised, counted and discarded. This provided a quantitative measure of toxicity.

Slides were prepared by dropping the cell suspension on to clean, grease-free slides.

For both experiments, 3 slides per culture were made. All slides were marked with the project number and assigned a unique, coded number from a computer generated sequence.

Slide Assessment

Slides were examined for evidence of metaphase cells and signs of cellular necrosis.

Based on the toxicity and osmolality, 3 concentration levels were selected for assessment of chromosomal aberrations.

From 2 or 3 slides per culture, up to 50 metaphase cells per slide, a total of 100 metaphase cells per culture, were examined. Slides were scored in order of coded number. A reduced number of metaphases were scored if a high proportion ($\geq 40\%$) of metaphase cells were found to be damaged.

A Leitz-Dialux 20 microscope was used for this assessment, the magnification used being x 1000 or x 1250, achieved with x 10 or x 12.5 eyepieces and x 100 objective.

The number of chromosomes in each metaphase cell and all abnormalities, using the nomenclature of Gebhart (1970) were recorded.

The types of structural and numerical aberrations recorded are listed in Appendix 8.

The positions on the slides of any structurally aberrant cells were recorded using the Vernier scale on the microscope stage.

EVALUATION OF RESULTS

Calculations

Toxicity: from the cell counts, the number of cells recovered per culture, was calculated. This was then compared with the number of cells (mean of 2 cultures) recovered from the vehicle control cultures.

Structural and Numerical Chromosomal Aberrations: from the results, 5 parameters were calculated, and judged as negative, suspicious or positive. These parameters were:

1. Lesions per cell
2. Percentage of aberrant cells including cells with gaps only
3. Percentage of aberrant cells excluding cells with gaps only
4. Percentage of aneuploid cells
5. Percentage of polyploid cells (normal and endoreduplicated)

The third parameter is considered the most important in judging the true clastogenicity of a test material.

Historical Control Data

The results obtained were compared with the historical control data. Historical control data is given in Appendix 1.

Acceptance Criteria

The experiments in this study were deemed to be valid because they fulfilled the following criteria:

There was no evidence of contamination

Cells in vehicle control cultures had normal growth

The results of vehicle and positive control cultures were typical

The test material had 3 acceptable dose levels for assessment.

Interpretation of Toxicity

A dose level was considered to be toxic if the cell count was reduced to less than 60% of the vehicle control cultures or if consistent evidence of changes to cell morphology was observed.

Interpretation of Clastogenicity

A negative response was recorded if responses from the test material treated cultures are within the 95% confidence limits for historical negative controls.

The response at a single dose was classified as significant if the percent of aberrant cells is consistently greater than the 99% confidence limits for a negative historical negative controls or greater than double the frequency of an elevated vehicle control culture if appropriate.

An experiment was positive if the response in at least the one acceptable dose level is significant by the criterion described above and is associated with an increase in aberrant cells in at least one other dose level.

A test material was positive if both experiments were positive, as described above or if the second test was positive after the first test gave indications of activity. These indications may be suspicious levels of aberrant cells (between 95% and 99% confidence limits) at extreme or sub toxic dose levels.

An inconclusive response was recorded for experiments that met, in part or marginally, the criteria for a positive response.

RESULTS AND DISCUSSION

TOXICITY DATA (Appendices 2-5)

In Test 1 BPS-24 was toxic to cultures treated with 313-5000 $\mu\text{g}\cdot\text{ml}^{-1}$ in the presence of S9 mix. Concentrations of 625-5000 $\mu\text{g}\cdot\text{ml}^{-1}$ were very toxic to the cells, with no metaphases for assessment. This was also noted in the absence of S9 mix at these concentrations but there was no indication of toxicity at 313 $\mu\text{g}\cdot\text{ml}^{-1}$.

In Test 2, in the presence of S9 mix, the toxicity was similar to that seen in Test 1 with toxicity in cultures treated with 313-625 $\mu\text{g}\cdot\text{ml}^{-1}$. The cultures treated with 313 $\mu\text{g}\cdot\text{ml}^{-1}$ were more toxic at the 48 h harvest than the 24 h harvest. Reduced cell counts (below 60% of the vehicle control) were noted in the cultures harvested at 48 h. At the 24 h harvest toxicity was evident only from culture and slide observations.

In the absence of S9 mix there was a slight shift in toxicity in Test 2 and cultures treated with 234-625 $\mu\text{g}\cdot\text{ml}^{-1}$ had reduced cell counts. This was noted at both harvest times. In the 48 h cultures, however, 469 $\mu\text{g}\cdot\text{ml}^{-1}$ could be assessed for chromosomal aberrations.

CHROMOSOMAL ABERRATIONS RESULTS (Tables 1-6)

Vehicle Control Cultures

All vehicle control cultures had levels of structural and numerical aberrations within the 95% confidence limits of the historical negative control data.

Positive Control Treated Cultures

The positive control substances, CPH in the presence and MMS in the absence of S9 mix, induced positive frequencies of structural aberrations. These results demonstrated the sensitivity of the test system.

BPS-24 Treated Cultures

In Test 1, in the presence of S9 mix, BPS-24 induced a positive response in one culture treated with $313 \mu\text{g}.\text{ml}^{-1}$ (number of cells excluding gaps) and a suspicious response in the duplicate. In the absence of S9 mix all cultures were within the 95% confidence limits for a negative response.

In Test 2, the same pattern was seen in the presence of S9 mix in the 24 h harvest cultures. BPS-24 again induced one positive response and one suspicious response in cultures treated with $313 \mu\text{g}.\text{ml}^{-1}$. In cultures harvested at 48 h, however, a positive response was noted in both cultures treated with $313 \mu\text{g}.\text{ml}^{-1}$ and a suspicious response (number of cells excluding gaps) in both cultures treated with $234 \mu\text{g}.\text{ml}^{-1}$.

In the absence of S9 mix, all cultures harvested at 24 h were within the 95% confidence limits for a negative response. In the 48 h harvest, however, $469 \mu\text{g}.\text{ml}^{-1}$ could be assessed for chromosomal aberrations (insufficient metaphases in 24 h harvest cultures) and a positive response was induced in both cultures. The results from this assay fulfil the criteria to judge BPS-24 as a clastogen in CHO cells.

CONCLUSION

It was concluded that BPS-24 was clastogenic when tested with Chinese hamster ovary cells *in vitro*.

REFERENCES

- Ames B N, McCann J and Yamasaki E (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. *Mutation Res.*, 31, 347-404.
- Galloway S M, Armstrong M A, Reuben C, Colman S, Brown C, Cannon A D, Bloom A D, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin B H, Resnick M A, Anderson B, Zeiger E (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ Mol Mutagen*, 10 (Suppl 10), 1-175.
- Gebhart E, (1970). The Treatment of Human Chromosomes *in vitro*, in Vogel F and Rohrborn G, *Chemical Mutagenesis in Mammals and Man*, Springer-Verlag, Berlin.
- Hsu T L, Collie C J, Lusley A F and Johnston D A (1977). Cytogenetic assays of chemical clastogens using mammalian cells in culture. *Mutat Res*, 45, 233-247.
- Ishidate M Jr, Harnois M C and Sofuni T (1988). A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. *Mutat Res*, 195, 151-213.
- McGregor D B, Edwards I, Riach C G, Cattanach P, Martin R, Mitchell A and Caspary W J (1988). Studies of an S9-based metabolic activation system used in the mouse lymphoma L5178Y cell mutation assay. *Mutagenesis*, Vol. 3, No. 6, 485-490.
- Natarajan A T, Tates A D, van Buul P P W, Meijers M and De Vogel N (1976). Cytogenetic effects of mutagens/carcinogens after activation in a microsomal system *in vitro*, I. Induction of chromosome aberrations and sister chromatid exchanges by diethylnitrosamine (DEN) and dimethylnitrosamine (DMN) in CHO cells in the presence of rat-liver microsomes. *Mutat Res*, 37, 83-90.

REFERENCES (continued)

Tezuka H, Ando N, Suzuki R, Terahata M, Moriya M, Shirasu Y (1980). Sister-chromatid exchanges and chromosomal aberrations in cultured Chinese hamster cells treated with pesticides positive in microbial reversion assays. *Mutat Res*, 78, 177-191.

TABLES 1-6 AND APPENDICES 2-6BPS-24: Chromosomal Aberrations Assay
with Chinese Hamster Ovary Cells *in vitro*Abbreviations and Footnotes

+	=	Positive aberration frequency
+ -	=	Suspicious aberration frequency
-	=	Negative aberration frequency
t	=	Toxicity evident from morphological changes
tt	=	Toxicity evident from reduced cell count (< 60% of vehicle)
ttt	=	Too toxic for metaphase assessment
r	=	Reduced number of cells scored due to extensive chromosome damage
Index	=	Expressed as an index of the vehicle control
Conc.	=	Concentration

TABLE 1
BPS-24
 Chromosomal Aberrations Assay with Chinese Hamster Ovary Cells *in vitro*
 Test 1, With S9 Mix, 24 h Harvest

Treatment Group	Conc. (µg.ml ⁻¹)	Decoded Culture No.	No. of Cells Scored	Structural Aberrations										Aberration Frequency		Aberrant Cell Frequency		Numerical Aberrations						
				Chromatid			Chromosome			Complex		Multi-ple	Other	Lesions/Cell Judge	Including Gaps	Excluding Gaps	% Judge	AE	ER	PP				
				G	B	F	G	B	F	E	D										R	% Judge	% of Cells with	
Dimethyl-sulphoxide	1%	1	100	2	0	0	1	0	0	0	0	0	0	0	0	0.03	-	3	-	0	-	0	1	1
		2	100	2	0	0	0	0	0	0	0	0	0	0	0	0.02	-	2	-	0	-	0	1	0
	78	7	100	1	0	1	0	0	0	0	0	0	0	0	0	0.02	-	2	-	1	-	1	0	2
		8	100	1	0	0	0	0	0	0	0	0	0	0	0	0.01	-	1	-	0	-	0	0	1
BPS-24	156	9	100	1	0	0	0	0	0	0	0	0	0	0	0	0.01	-	1	-	0	-	0	0	0
		10	100	2	0	1	0	0	0	1	0	0	0	0	0	0.05	-	4	-	2	-	2	1	1
	313	11	100	0	1	1	0	0	0	4	0	0	0	0	0	0.10	+-	6	-	6	+	0	0	1
		12	100	3	3	0	0	1	0	0	0	0	0	0	0	0.07	-	6	-	4	+-	0	0	1
Cyclophosphamide	30	22	100	8	2	1	1	0	0	8	0	0	0	0	0	0.28	+	14	+	9	+	1	0	1
		23	100	5	6	0	0	1	0	7	0	0	0	0	0	0.26	+	13	+	11	+	0	0	0

TABLE 2

BPS-24
 Chromosomal Aberrations Assay with Chinese Hamster Ovary Cells *in vitro*
 Test 1, Without S9 Mix, 24 h Harvest

Treatment Group	Conc. ($\mu\text{g.ml}^{-1}$)	Decoded Culture No.	No. of Cells Scored	Structural Aberrations										Aberration Frequency		Aberrant Cell Frequency		Numerical Aberrations				
				Chromatid			Chromosome			Complex		Multiple	Other	Lesions/Cell	Judge	Including Gaps	Excluding Gaps	% Judge	% Judge	AE	ER	PP
				G	B	F	G	B	F	E	D											
Dimethyl-sulphoxide	1%	25	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3		
		26	100	1	0	1	0	0	0	0	0	0	0	0	2	1	0	0	2	0		
	78	31	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		32	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	
BPS-24	156	33	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1		
		34	100	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	3		
	313	35	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1		
		36	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Methylmethane-sulphonate	20	45	100	2	0	0	2	0	0	0	0	0	0	0	4	0	0	0	1	0		
		46	100	5	5	0	0	0	0	0	14	0	0	0	19	16	0	0	0	0		

TABLE 6

BPS-24
 Chromosomal Aberrations Assay with Chinese Hamster Ovary Cells *in vitro*
 Test 2, Without S9 Mix, 48 h Harvest

Treatment Group	Conc. ($\mu\text{g. ml}^{-1}$)	Decoded Culture No.	No. of Cells Scored	Structural Aberrations										Aberration Frequency		Aberrant Cell Frequency		Numerical Aberrations			
				Chromatid		Chromosome			Complex		Multiple	Other	Lesions/Cell	Judge	Including Gaps	Excluding Gaps	% Judge	% Judge	AE	ER	PP
				G	B	F	G	B	F	E											
Dimethylsulphoxide	1%	103	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		104	100	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	
BPS-24	156	107	100	0	0	1	1	0	0	0	0	0	0	0	0	2	1	1	0	0	1
		108	100	2	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1
	313	111	100	0	0	1	0	0	0	1	0	0	0	0	0	2	2	0	0	8	2
		112	100	0	4	0	0	0	0	1	0	0	0	0	0	1	1	0	0	5	0
Methylmethanesulphonate	30	113	100	0	10	1	0	0	0	13	0	0	1	0	8	8	+	+	0	5	1
		114	100	3	16	4	0	0	0	13	0	0	0	0	18	16	+	+	0	10	2
Methylmethanesulphonate	40	119	100	3	15	2	0	0	0	18	0	0	1	0	24	22	+	+	1	1	0
		120	50r	4	22	1	0	0	0	31	0	0	0	0	44	44	+	+	0	0	0

APPENDIX 1

Historical Negative Control Data for Chromosomal Aberrations in Chinese Hamster Ovary Cells *in vitro*

	Number of Records	Parameter	Confidence Levels for Negative Results		
			0-95%	>95-≤99%	>99%
Structural Aberrations	312	Lesions/cell	0.00-0.07	>0.07-≤0.10	>0.10
	324	ACG (%)	0-6	>6-≤9	>9
		AC (%)	0-2	>2-≤5	>5
Numerical Aberrations ¹	284	AE (%)	0-8	>8-≤10	>10
Judgement of test material or positive control culture aberration values			Negative (-)	Suspicious (+-)	Positive (+)
QUALITY ASSURANCE		Last date, data added to database: 20 June 1994 Auditor: E M Donald Date Audited: 20 July 1994			

¹ = Aneuploidy (AE) measured in 100 cells assessed for structural aberrations.

95% confidence limits = 95% of values from negative controls fall on or within the values given.

99% confidence limits = 99% of values from negative controls fall on or within the values given.

APPENDIX 2

BPS-24
 Chromosomal Aberrations Assay with Chinese Hamster Ovary Cells *in vitro*
 Toxicity Data
 Test 1, With S9 Mix, 24 h Harvest

Treatment Group	Conc. ($\mu\text{g} \cdot \text{ml}^{-1}$)	De-coded No.	Cells Count Data		Observations		Toxic Judge		
			No of Cells ($\times 10^6$)	Index	Culture	Slide			
Dimethyl-sulphoxide	1%	1	1.15	1.05	Nil toxicity	Nil toxicity	-		
		2	1.05	0.95			-		
BPS-24	20	3	1.25	1.14			-		
		4	1.30	1.18			-		
	39	5	1.13	1.03			-		
		6	1.20	1.09			-		
	78	7	1.28	1.16			-		
		8	1.13	1.03			-		
	156	9	1.23	1.12			-		
		10	1.18	1.07			-		
	313	11	0.75	0.68			Sparse metaphases, small dark interphase cells	t	
		12	0.58	0.53				tt	
625	625	13	0.23	0.21			Rounded cells	ttt	
		14	0.25	0.23				ttt	
1250	1250	15	0.00	0.00			No cells	No metaphases, cell debris	ttt
		16	0.00	0.00					ttt
2500	2500	17	0.00	0.00					ttt
		18	0.00	0.00					ttt
5000	5000	19	0.00	0.00			Cells fixed to flask	ttt	
		20	0.00	0.00				ttt	

APPENDIX 3

BPS-24
 Chromosomal Aberrations Assay with Chinese Hamster Ovary Cells *in vitro*
 Toxicity Data
 Test 1, Without S9 Mix, 24 h Harvest

Treatment Group	Conc. ($\mu\text{g} \cdot \text{ml}^{-1}$)	De-coded No.	Cells Count Data		Observations		Toxic Judge	
			No of Cells ($\times 10^6$)	Index	Culture	Slide		
Dimethyl-sulphoxide	1X	25	1.68	1.01	Nil toxicity	Nil toxicity	-	
		26	1.65	0.99			-	
20	27	1.98	1.19	-				
	28	2.08	1.25	-				
39	29	1.70	1.02	-				
	30	2.03	1.22	-				
78	31	1.53	0.92	-				
	32	1.53	0.92	-				
156	33	1.53	0.92	-				
	34	1.43	0.86	-				
313	35	1.05	0.63	-				
	36	1.03	0.62	-				
625	37	0.53	0.32	Rounded cells			Very sparse metaphase cells, cell debris	ttt
	38	0.65	0.39					ttt
1250	39	0.00	0.00	Cells floating in medium	No metaphases, cell debris	ttt		
	40	0.00	0.00			ttt		
2500	41	0.00	0.00			ttt		
	42	0.00	0.00			ttt		
5000	43	0.00	0.00	Cells fixed to flask		ttt		
	44	0.00	0.00			ttt		

APPENDIX 4

BPS-24
 Chromosomal Aberrations Assay with Chinese Hamster Ovary Cells *in vitro*
 Toxicity Data
 Test 2, With S9 Mix

Harvest Time	Treatment Group	Conc. ($\mu\text{g}.\text{ml}^{-1}$)	De-coded No.	Cells Count Data		Observations		Toxic Judge	
				No of Cells ($\times 10^6$)	Index	Culture	Slide		
24 h	Dimethyl-sulphoxide	1%	49	0.88	0.85	Nil toxicity	Nil toxicity	-	
			50	1.18	1.15			-	
	78	51	1.13	1.10	-				
		52	1.15	1.12	-				
	156	53	0.98	0.95	-				
		54	0.95	0.92	-				
	234	55	0.98	0.95	-				
		56	1.20	1.17	-				
	313	57	0.70	0.68	Slightly rounded cells			Slightly sparse metaphase cells	t
		58	0.75	0.73					t
	469	59	0.15	0.15	Rounded cells			No metaphase cells	ttt
		60	0.30	0.29					ttt
	625	61	0.00	0.00	No cells				ttt
		62	0.00	0.00					ttt
48 h	Dimethyl-sulphoxide	1%	67	1.68	0.98	Nil toxicity	Nil toxicity	-	
			68	1.75	1.02			-	
	78	69	1.50	0.87	-				
		70	1.78	1.04	-				
	156	71	1.30	0.76	-				
		72	1.35	0.79	-				
	234	73	1.18	0.69	-				
		74	1.30	0.76	-				
	313	75	0.70	0.41	Sparse metaphase cells			tt	
		76	0.33	0.19				tt	
	469	77	0.08	0.05	Rounded cells			No metaphase cells	ttt
		78	0.03	0.02					ttt
	625	79	0.00	0.00	No cells				ttt
		80	0.00	0.00					ttt

APPENDIX 5

BPS-24
 Chromosomal Aberrations Assay with Chinese Hamster Ovary Cells *in vitro*
 Toxicity Data
 Test 2, Without S9 Mix

Harvest Time	Treatment Group	Conc. ($\mu\text{g}\cdot\text{ml}^{-1}$)	De-coded No.	Cells Count Data		Observations		Toxic Judge			
				No of Cells ($\times 10^6$)	Index	Culture	Slide				
24 h	Dimethyl-sulphoxide	1%	85	1.70	0.96	Nil toxicity	Nil toxicity	-			
			86	1.85	1.04			-			
	78	87	1.55	0.87	-						
		88	1.90	1.07	-						
	156	89	1.65	0.93	-						
		90	1.30	0.73	-						
	234	91	0.93	0.52	tt						
		92	0.95	0.54	tt						
	313	93	0.65	0.37	Slightly rounded cells			Sparse metaphase cells	tt		
		94	0.78	0.44				Very sparse metaphase cells	tt		
	469	95	0.60	0.34	Cells floating in medium			No metaphase cells	ttt		
		96	0.50	0.28					ttt		
	625	97	0.00	0.00					ttt		
		98	0.00	0.00					ttt		
	48 h	Dimethyl-sulphoxide	1%	103	1.83			0.95	Nil toxicity	Nil toxicity	-
				104	2.03			1.05			-
78		105	1.65	0.85	-						
		106	1.83	0.95	-						
156		107	1.35	0.70	-						
		108	1.55	0.80	-						
234		109	1.00	0.52	tt						
		110	0.90	0.47	tt						
313		111	1.03	0.53	tt						
		112	1.10	0.57	tt						
469		113	0.28	0.15	Slightly rounded cells	Sparse metaphase cells	tt				
		114	0.35	0.18			tt				
625		115	0.00	0.00	Very few, round cells	No metaphase cells	ttt				
		116	0.00	0.00			ttt				

APPENDIX 6

BPS-24
Chromosomal Aberrations Assay with Chinese Hamster Ovary Cells *in vitro*
Osmolality and Precipitation Data
Test 1, with S9 Mix

Treatment Group	Conc. ($\mu\text{g} \cdot \text{ml}^{-1}$)	Osmotic Pressure ($\text{mmol} \cdot \text{kg}^{-1}$)	Precipitation
Dimethylsulphoxide	1%	410	Nil precipitation
BPS-24	78	399	
	625	402	
	5000	388	Cloudy

APPENDIX 8

Summary of Types of Structural and Numerical Aberrations Recorded in Chromosomal Aberrations Assays with Chinese Hamster Ovary Cells *in vitro*

Type	Description	Abbreviation in Tables	Number of Lesions	
Chromatid: Affecting one chromatid	Gap	Achromatic region	G	1
	Break	Discontinuation with dislocation of chromatid section or clearly reduced chromatid arm length	B	1
	Fragment	Chromatid fragment unassociated with chromosome	F	1
Chromosome: Affecting both chromatids at identical sites	Gap	Achromatic region	G	1
	Break	Discontinuation with dislocation of chromosome section or clearly reduced chromosome arm length	B	1
	Fragment	Acentric chromosome fragment unassociated with chromosome	F	1
Complex	Exchange	Two or more chromosomes clearly joined in a complex figure	E	2
	Dicentric	Chromosome with 2 centromeres	D	2
	Ring	Circular chromosome; with or without a centromere	R	2
Double minutes	Very small pairs of chromatin, appear as dots	DM	None	
Uncondensed chromosome	Extended chromosome region often with a banded appearance	UC	None	
Reciprocal translocation	Complete exchange of material between chromosomes	TR	2	
Robertsonian translocation	Fusion of 2 telocentric chromosomes	RTR	1	
Pericentric inversion	Change in position of centromere within chromosome	INV	2	
Pulverised chromosome	Several lesions usually breaks and gaps in one chromosome	PC	5	
Multiple aberration	A metaphase cell with more than 10 lesions	MDC	10	
Aneuploid	Metaphase cell with 23-25 chromosomes	AE	No lesions	
Endoreduplicated	Characteristic paired chromosomes	ER		
Polyploidy	26 and more chromosomes	PP		

IRI Report No. 12754

BPS-24

TESTING FOR MUTAGENIC ACTIVITY WITH *Salmonella typhimurium*

TA 1535, TA 1537, TA 1538, TA 98 AND TA 100

IRI Project No. 757730

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Inveresk Research International
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BPS-24

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IRI Project No. 757730

Author:

S E Willington

Sponsor:

Nicca Chemical Company Ltd

23-1, Bunkyo 4-Chome

Fukui City

910 Japan

Performing Laboratory:

Inveresk Research International

Tranent, EH33 2NE

Scotland

Total Number of Pages: 40

AUTHENTICATION

'I, the undersigned, hereby declare that this work was performed under my direction and in accordance with the principles of Good Laboratory Practice. The study was conducted according to the procedures herein described and this report represents a true and accurate record of the results obtained.'

S E Willington BSc
Study Director

Date:

Report No. 12754

QUALITY ASSURANCE STATEMENT

The execution of this type of short-term study is not individually inspected. The processes involved are inspected at intervals according to a predetermined schedule.

This report has been audited by IRI Quality Assurance Personnel according to the appropriate Standard Operating Procedure and is considered to describe the methods and procedures used in the study. The reported results accurately reflect the original data of the study.

IRI Project No. 757730

Report No. 12754

Signed: _____

(Quality Assurance)

Date: _____

ISSUED

20 DEC 1995



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PERSONNEL INVOLVED IN PROJECT 757730

Study Director: S E Willington BSc

Project Leader: S E Willington BSc

Technical Assistance: D Self
P J Cattanach BSc
W C Zajac

Quality Assurance: E M Donald BSc

SUMMARY

BPS-24 was tested for mutagenic activity in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 at concentrations ranging from 3 to 2500 μg per plate.

The tests were conducted on agar plates in the presence and absence of an Aroclor 1254 induced rat liver preparation and co-factors (S9 mix) required for mixed-function oxidase activity.

Concurrent positive controls demonstrated the sensitivity of the assay and the metabolising activity of the S9 mix.

Reproducible mutagenic activity was observed in strain TA 1537 only. Interpretation of the response was complicated by the toxicity; specific toxicity to the mutant colonies was noted in addition to toxicity to the general population of bacteria (as noted by examination of the background lawns). There were shifts in the toxicity which meant it was difficult to test into the range of mutagenic activity without causing excessive toxicity.

No precipitation of the test material was observed.

It was concluded that BPS-24 was mutagenic to *Salmonella typhimurium* when tested in dimethylsulphoxide into the toxic range.

INTRODUCTION

The purpose of this study was to establish the potential of BPS-24 to induce gene mutation in the bacterium *Salmonella typhimurium*, causing the histidine-dependent strains to revert to the wild-type phenotype. The particular damage induced to DNA by a mutagen may cause reversions to occur which are observable in certain strains of *Salmonella typhimurium*, but not in others. It is necessary, therefore, to use a variety of bacterial strains in order to test for a broad range of chemical mutagens. At the present time, available data suggest that the use of the 5 strains used in this project permits the detection of a wide spectrum of mutagens.

It is well recognised, however, that many chemicals which may be reactive in a mammalian cell, following metabolic activation, are quite inactive in bacterial cells. Extracts of mammalian cells are combined, therefore, with the bacterial indicator cells in a tissue mediated assay to increase the relevance of the test in assessing the mutagenicity of chemicals to man.

The methods used in this study conform to OECD Guideline No. 471.

This report describes the methods used and the results obtained in tests carried out at the Elphinstone Research Centre laboratories of Inveresk Research International Limited, Tranent.

Key dates in the conduct of the study:

Study Initiation:	19 July 1995
Experimental Start Date:	15 September 1995
Experimental Completion Date:	26 October 1995

Study Completion Date:

Please see Authentication page for date of Study Director's signature (Final reports only).

All data generated and recorded during this study will be stored in the Scientific Archives of Inveresk Research International Limited for 5 years after issue of the final report. At the end of the 5 year period the Sponsor will be consulted regarding the disposal or continued storage of raw data.

EXPERIMENTAL PROCEDURE

The procedures used are based on the method of Ames *et al* (1975). Aseptic techniques were used throughout.

MATERIALS

Test Substance

BPS-24, Batch No. A050131, a white powder, was received from Nicca Chemical Company on 27 July 1995 and was stored in the dark at ambient temperature. The purity of BPS-24 (2,4'-dihydroxy diphenyl sulfone) was quoted as > 95%.

Positive Controls

Positive control substances used were 2-aminoanthracene (2-AAN) (Lot No. 33F-0816) from the Sigma Chemical Company Limited, Poole, Dorset, England; sodium azide (NaN_3) (Lot No. 6529052) from BDH Limited, Poole, Dorset, England; 9-aminoacridine (9-AA) (Lot No. N333JE) and 2-nitrofluorene (2-NF) (Lot No. A3A) from IIT Research Institute, Chicago, Illinois 60616, USA.

The positive control substances, except sodium azide, were dissolved in dimethylsulphoxide ('AnalaR' grade from BDH Limited, Poole, Dorset, England). Sodium azide was dissolved in sterile, ultra-pure water.

Test Solution

Dimethylsulphoxide (DMSO) was used to dissolve and dilute BPS-24.

Vehicle Control

Dimethylsulphoxide (DMSO) was used as the vehicle control.

Inducer

The polychlorinated biphenyl mixture, Aroclor 1254, was obtained from Monsanto (UK) Limited, Newport, Wales.

Agar Plates

The Vogel-Bonner Medium E agar plates with 2% glucose used in the Ames test were prepared in-house using BBL purified agar obtained from Becton Dickinson Limited, Oxford, England.

Animals

Male Fischer 344 rats were obtained from Charles River (UK) Limited, Margate, Kent.

Bacteria

Five strains of *Salmonella typhimurium* were used:

S. typhimurium TA 1535

S. typhimurium TA 1537

S. typhimurium TA 1538

S. typhimurium TA 98

S. typhimurium TA 100

They were obtained in 1976 from Professor B N Ames, Department of Biochemistry, University of California, Berkeley, CA, USA, and stored in liquid nitrogen since that time until used.

All these strains contain mutations in the histidine operon, thereby imposing a requirement for histidine in the growth medium. Three mutations in the histidine operon are involved:

his G 46 in TA 1535 and TA 100

his C 3076 in TA 1537

his D 3052 in TA 1538 and TA 98

his G 46 is a mis-sense mutation which is reverted to prototrophy by a variety of mutagens that cause base-pair substitutions.

his C 3076 contains a frameshift mutation which appears to have added a $\begin{matrix} -G- \\ -C- \end{matrix}$ base-pair resulting in $\begin{matrix} -GGGG- \\ -CCCC- \end{matrix}$. This mutation is reverted by 9-aminoacridine, ICR-191 and epoxides of polycyclic hydrocarbons.

his D 3052 also contains a frameshift mutation with the sequence $\begin{matrix} -CGCGCG- \\ -GCGCGC- \end{matrix}$ which is reverted with the deletion of 2 base-pairs, $\begin{matrix} -CG- \\ -GC- \end{matrix}$. It is readily reverted by aromatic amines and derivatives.

All 5 strains contain the deep rough (*rfa*) mutation, which deletes the polysaccharide side chain of the lipopolysaccharide coat of the bacterial cell surface. This deletion increases cell permeability to more hydrophobic substances and, furthermore, greatly decreases the pathogenicity of these organisms.

The second deletion, through *uvrB*, renders the organisms incapable of DNA excision repair and thus more susceptible to mutagenicity. These 2 deletions include the nitrate reductase (*chl*) and biotin (*bio*) genes also.

Differences between TA 1535 and TA 1538, on the one hand, and the corresponding TA 100 and TA 98 strains, on the other hand, are due to a plasmid the latter pair contains. A plasmid, R-Utrecht (pKM101), was originally shown to increase the sensitivity of the *his G 46* mutation in *S. typhimurium* to methyl methanesulphonate and trimethyl phosphate. The particular R-factor in TA 100 and TA 98 (pKM101) carries resistance to ampicillin. It is not yet clear why the presence of this particular R-factor should increase the sensitivity of strains TA 1535 and TA 1538 to the mutagenicity of certain chemicals. The involvement of an error-prone repair mechanism has been postulated.

METHODS

Preparation of the Metabolic Activation System

Animals

Male Fischer 344 rats (average weight 255 g) were injected once intraperitoneally with Aroclor 1254 (diluted in corn oil to a concentration of 200 mg.ml⁻¹) at a dosage of 500 mg.kg⁻¹. They were allowed drinking water continuously, but food was withheld for 16 h before they were killed in an atmosphere of carbon dioxide 5 days after injection.

Preparation of 9,000 g Supernatant Fluid (S9 Mix) from Livers

Freshly killed animals were totally immersed in cold 2% Tego (an ampholytic detergent), then excess fluid was wiped off. The abdomen was opened and the liver removed, taking special care not to cut the gastro-intestinal tract. Livers from several animals were collected in a tared beaker containing ice-cold 0.15 M KCl.

The beaker was weighed and the livers transferred to the homogenisation vessel. A volume of ice-cold 0.15 M KCl equivalent to 3 times the weight of the liver was added to the vessel and the livers chopped using long-handled scissors and homogenised by 8 strokes of a glass tube vessel while the Teflon pestle (radial clearance 0.14-0.15 mm) rotated at about 1,200 r.p.m. The homogenate was transferred to sterile polypropylene centrifuge tubes and spun at a relative centrifugal force of 9,000 g for 10 min at 0° to +2°C. The supernatant fluid was decanted leaving behind a thick pellet of (mainly) whole cells, nuclei and mitochondria.

Post-mitochondrial supernatant fluid was prepared in sufficient quantity for the experiment and stored, as 2 ml and 5 ml samples in sterile plastic tubes, immersed in liquid nitrogen (-196°C).

Enzyme Properties of the 9,000 g Supernatant Fluid

Details of the batch of S9 mix used in the mutation experiments are shown in Appendix 4 together with responses from positive control pre-mutagens in the Ames test using *S. typhimurium* strain TA 1538.

Preparation of S9 Mix

Ice-cold 0.05 M phosphate buffer, pH 7.4, was added to the following pre-weighed reagents to give final concentrations in S9 mix of:

NADP di-Na salt	4 mM (= 3.150 mg.ml ⁻¹)
Glucose-6-phosphate di-Na salt	25 mM (= 7.605 mg.ml ⁻¹)
MgCl ₂ .6H ₂ O	8 mM (= 1.626 mg.ml ⁻¹)
KCl	33 mM (= 2.460 mg.ml ⁻¹)

This solution was immediately filter-sterilised by passage through a 0.45 μ m Millipore filter and mixed with the liver 9,000 g supernatant fluid in the following proportion:

co-factor solution	9 parts
liver preparation	1 part

This combination of co-factors and liver preparation was called the S9 mix.

Preparation of Bacteria

Samples of each strain were grown by culturing for 16 h at 37°C in nutrient broth (25 g Oxoid Nutrient Broth No. 2.litre⁻¹). These cultures were kept for up to 2 days at +4°C to allow relevant checks to be performed but fresh cultures were used for the experiments.

Preparation of the Assay Plates

Diluted agar (0.6% Difco Bacto-agar, 0.6% NaCl) was autoclaved and, just before use, 5 ml of sterile 1.0 mM L-histidine.HCl, 1.0 mM biotin solution was added to each

100 ml of soft agar and thoroughly mixed. This molten agar, maintained in a water bath at 45°C, was dispensed in 2 ml volumes into small sterile tubes to which were added in order:

0.5 ml S9 mix or 0.05 M phosphate buffer, pH 7.4

0.1 ml bacteria (*ca* 2×10^9 cells.ml⁻¹)

100 µl solvent or test solution

The tube contents, which were continually cooling, were mixed and then poured in minimal medium plates prepared in-house. These plates contained 25 ml of 1.5% BBL purified agar in Vogel-Bonner Medium E (Vogel *et al* (1956)) with 2% glucose. When the soft agar had set, the plates were inverted and incubated at 37°C for 2 days whereupon colonies were counted using a Biotran III automated counter (New Brunswick Incorporated, NJ, USA) at maximum sensitivity ie colonies of 0.1 mm or more in diameter counted. The plates were also examined for precipitates and, microscopically, for microcolony growth.

Toxicity Test

A toxicity test using strain TA 100 only was performed in the presence and absence of S9 mix to establish suitable dose levels for the mutation tests. One plate of each of the following concentrations of BPS-24 was used:

33, 100, 333, 1000, 3333 and 10000 µg per plate.

Mutation Tests

Two independent mutation tests were conducted using 5 bacterial strains (TA 1535, TA 1537, TA 1538, TA 98 and TA 100). The dose levels used in the first of these assays, based on the results of the toxicity test, were:

8, 25, 83, 250, 833 and 2500 μg per plate.

The dose levels were adjusted for the second assay due to the test material toxicity. The following doses were used (except for TA 100 in the absence of S9 mix):

3, 10, 33, 100, 333 and 1000 μg per plate.

The following doses were used for the second test with TA 100 in the absence of S9 mix:

100, 400, 700, 1000, 1300 and 1600 μg per plate.

Triplicate plates were prepared for each bacterial strain and dose level in both the presence and absence of S9 mix.

Quality Control

At the times that the experiments were conducted, each strain was tested for its resistance to ampicillin (indicating the presence of pKM101) and sensitivity to ultraviolet (u.v.) light and crystal violet (indicating persistence of the *uvrB* and *rfa* mutations). In addition, the following control groups were established, triplicate plates being poured for each mean datum point.

1. Dimethylsulphoxide (DMSO), 100 μl , used as the test compound vehicle, in both the presence and the absence of S9 mix.

2. With S9 Mix

2-Aminoanthracene (2-AAN), 2 μg per plate with TA 1535 and TA 1537 and 0.5 μg per plate with TA 1538, TA 98 and TA 100, used to demonstrate activity of the S9 mix and the mutability of the bacteria.

3. Without S9 Mix

Sodium azide (NaN_3), 1 μg per plate, with TA 1535 and TA 100; 2-nitrofluorene (2-NF), 1 μg per plate, with TA 1538 and TA 98; 9-aminoacridine (9-AA), 80 μg per plate, with TA 1537. These substances served as an aid to strain identification and to demonstrate the mutability of the bacteria.

Evaluation of Results

A test was considered acceptable if for each strain:

- i) the bacteria demonstrated their typical responses to crystal violet, ampicillin and u.v. light.
- ii) at least 2 of the vehicle control plates were within the following ranges:
TA 1535, 4-30; TA 1537, 1-20; TA 98, 10-60; TA 100, 60-200 and TA 1538, 5-35.
- iii) on at least 2 of the positive control plates there were $\times 2$ the mean vehicle control mutant numbers per plate, or in the case of TA 100, $\times 1.5$ the mean vehicle control mutant numbers per plate.

If the mean colony count on the vehicle control plates was less than 10 then a value of 10 was assumed for assessment purposes. In such cases a minimum count of 20 was required on at least 2 of the positive control plates.

- iv) no toxicity or contamination was observed in at least 4 dose levels.
- v) in cases where a mutagenic response was observed, that no more than one dose level was discarded before the dose which gave the highest significant mean colony number.

Where these criteria were met, a significant mutagenic response was recorded if there was:

- i) for *S. typhimurium* strains TA 1535, TA 1537, TA 1538 and TA 98, at least a doubling of the mean concurrent vehicle control values at some concentration of the test substances and, for *S. typhimurium* strain TA 100, a 1.5-fold increase over the control value. If the mean colony count on the vehicle control plates was less than 10 then a value of 10 was assumed for assessment purposes. In such cases a minimum count of 20 was required before a significant mutagenic response was identified.
- ii) a dose related response, although at high dose levels this relationship could be inverted because of, for example, (1) toxicity to the bacteria generally, (2) specific toxicity to the mutants and (3) inhibition of foreign compound metabolising enzymes where mutagens require metabolic activation by the liver.
- iii) a reproducible effect in independent tests.

RESULTS AND DISCUSSION

Toxicity Test

The results of the toxicity test on BPS-24 are shown in Table 1.

Toxicity to the bacteria as shown by thinning of the background lawn of microcolonies was observed at 1000 μg per plate (slightly thin lawn), 3333 μg per plate (thin lawn) and 10000 μg per plate (no background lawn), both in the presence and absence of S9 mix.

There was a significant drop in mutant colony number at the 333 μg per plate dose level. There was then an increase in mutant colony number at the next dose level, 1000 μg per plate, but no mutant colonies were present at the two highest dose levels. These variations in mutant colony number indicate a combination of a possible mutagenic response and specific toxicity to the mutant colonies.

There was no precipitation of the test material.

Mutation Tests

The average numbers of *his*⁺ revertant colonies per dose level obtained in the main tests are shown in Tables 2-8 whilst the individual plate counts are displayed in Appendices 1-3.

Quality Control

All strains of *S. typhimurium* were sensitive to crystal violet, whereas only the plasmid-containing strains, TA 98 and TA 100, were resistant to ampicillin. The strains were also tested for sensitivity to u.v. light emitted over a period of 10 s

from a CAMAG u.v. lamp set at 254 nm. Increased sensitivity to u.v. light was demonstrated. These results are consistent with the known properties of these bacteria.

Vehicle Control Groups

The vehicle control values were generally within the normal ranges experienced in this laboratory and reported in the literature with these strains of *S. typhimurium*.

Positive Control Groups

The results obtained in the positive control groups were within the normal ranges expected for each bacterial strain and activation condition.

Test Rejection

All tests were acceptable according to the study criteria.

BPS-24

No mutagenic activity was noted in the first assay, however there was considerable toxicity in both the presence and absence of S9 mix. Mutant colony counts were almost zero at the two highest doses (833 and 2500 μg per plate) and there was a significant drop in mutant colony number at the next highest dose (250 μg per plate) with all strains except TA 1535. Toxicity to the background lawns of microcolonies was also observed at the two highest doses.

The dose levels were reduced for the second mutation assay in view of this toxicity. In the presence of S9 mix, at 333 μg per plate, significant reductions in mutant colony number were observed with all strains except TA 1535 and slightly thin background

lawns were noted with TA 1537 and TA 1538. At the highest dose (1000 μg per plate) there was a significant drop in mutant colony number with all strains except TA 1537 - where the number of colonies was 3x the vehicle control value. At this dose there was also toxicity to the background lawns (thin lawn - TA 1537 and TA 1538, slightly thin lawn - other strains). In the absence of S9 mix BPS-24 produced a similar pattern of toxicity. However the number of mutant colonies at 333 μg per plate was greatly increased with TA 1537 (11.5x the vehicle control value). At the highest dose (1000 μg per plate) the TA 1537 mutant colony count showed signs of toxicity, being reduced to less than 20. Different (higher) doses were used with TA 100 to investigate the possible mutagenic effect noted in the toxicity test. No mutagenic response was observed with TA 100, thin background lawns were noted at the two highest doses (1300 and 1600 μg per plate).

A third assay was conducted with TA 1537 in both the presence and absence of S9 mix using the same doses as those used for the second assay. In the presence of S9 mix the mutant colony count was reduced at the 333 μg per plate dose level and increased (5.5x the vehicle control value) at the 1000 μg per plate dose level. In the absence of S9 mix the mutant count was double the vehicle control value (and just above the 20 colony per plate limit) at 33 and 100 μg per plate. At 333 μg per plate the mutant colony count dropped but increased again at 1000 μg per plate. Toxicity to the background lawn was again noted at the two highest doses.

Interpretation of the results of this study are complicated by the toxicity of BPS-24 to the bacteria. The toxicity to the background lawns of microcolonies was fairly consistent, but there were shifts in the specific toxicity to the mutant colonies which meant that it was difficult to test up to the range of mutagenic activity without causing excessive toxicity. A reproducible mutagenic response was obtained with TA 1537; this was not dose-related and the response varied between assays because of the toxicity.

No precipitation of the test material was observed.

CONCLUSION

It was concluded that BPS-24 was mutagenic to *Salmonella typhimurium* when tested in dimethylsulphoxide into the toxic range.

REFERENCES

Ames B N, McCann J and Yamasaki E (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. *Mutation Res*, 31, 347-364.

Vogel H J and Bonner D M (1956). Acetylornithinase of *E. coli*: partial purification and some properties. *J Biol Chem*, 218, 97-106.

TABLE 1

Test 1

BPS-24 - revertant colony numbers obtained per plate using
bacterial strains :- TA 100

Strain	Dose level µg/plate	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 100	Solvent	-	109	-	109
	33	-	119	-	119
	100	-	103	-	103
	333	-	34	-	34
	1000	-	280	-	280 STL
	3333	-	0	-	0 TL
	10000	-	0	-	0 NL
	Solvent	+	122	-	122
	33	+	119	-	119
	100	+	109	-	109
	333	+	34	-	34
	1000	+	83	-	83 STL
	3333	+	0	-	0 TL
	10000	+	0	-	0 NL

SD : Standard Deviation

- : Absence
+ : Presence

STL : SLIGHTLY THIN LAWN

NL : NO LAWN.

TL : THIN LAWN

End of final report.

TABLE 2

Test 2
 Mean Number of his⁺ Revertant Colonies Obtained when 5 Strains of S. typhimurium
 were Treated with BPS-24 in the Presence of a Post-mitochondrial Fraction
 (S9 Mix) from the Livers of Male Rats Treated with Aroclor 1254 (FLI 079)

Substance	Dose Level μg per plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
DMSO	100 μl	12 ± 1	7 ± 1	20 ± 3	51 ± 7	95 ± 4
BPS-24	8	7 ± 1	8 ± 4	20 ± 7	49 ± 7	103 ± 12
	25	7 ± 3	9 ± 5	18 ± 3	48 ± 3	104 ± 12
	83	7 ± 3	7 ± 2	20 ± 2	44 ± 6	89 ± 7
	250	11 ± 5	0 ± 0	11 ± 4	39 ± 3	13 ± 9
	833	0 ± 0 (TL)	0 ± 1 (TL)	0 ± 0 (TL)	0 ± 0 (STL)	0 ± 0 (TL)
	2500	0 ± 0 (VTL)	0 ± 0 (TL)	0 ± 0 (VTL)	0 ± 0 (VTL)	0 ± 0 (TL)
Positive Controls	Compound	2AAN	2AAN	2AAN	2AAN	2AAN
	Dose Level	2 μg	2 μg	0.5 μg	0.5 μg	0.5 μg
	Mean ± S.D.	200 ± 2	208 ± 64	612 ± 43	499 ± 153	1086 ± 69

S.D. Standard Deviation

2AAN 2-Aminoanthracene

STL : SLIGHTLY THIN LAWN

TL : THIN LAWN

VTL : VERY THIN LAWN

N.B. The mean values were generally calculated from triplicate plate counts.

TABLE 3

Test 2
 Mean Numbers of his+ Revertant Colonies Obtained when 5 Strains of S. typhimurium
 were Treated with BPS-24 in the Absence of S9 Mix

Substance	Dose Level μg per plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100
		Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.
DMSO	100 μl	6 \pm 2	7 \pm 2	13 \pm 1	48 \pm 7	103 \pm 7
BPS-24	8	11 \pm 2	7 \pm 4	14 \pm 1	34 \pm 6	88 \pm 10
	25	10 \pm 5	4 \pm 2	15 \pm 1	37 \pm 10	104 \pm 13
	83	10 \pm 3	5 \pm 3	9 \pm 4	39 \pm 11	87 \pm 8
	250	12 \pm 1	0 \pm 1	7 \pm 3	32 \pm 5	65 \pm 2
	833	0 \pm 0 (VTL)	0 \pm 1 (TL)	0 \pm 0 (TL)	0 \pm 0 (STL)	0 \pm 1 (TL)
	2500	0 \pm 0 (VTL)	0 \pm 0 (TL)	0 \pm 0 (VTL)	0 \pm 1 (TL)	0 \pm 0 (TL)
Positive Controls	Compound	NaN3	9AA	2NF	2NF	NaN3
	Dose Level	1 μg	80 μg	1 μg	1 μg	1 μg
	Mean \pm S.D.	265 \pm 27	1031 \pm 67	301 \pm 40	299 \pm 9	692 \pm 12

S.D. Standard Deviation

NaN3 Sodium azide
 2NF 2-Nitrofluorene

9AA 9-Aminoacridine

STL : SLIGHTLY THIN LAWN TL : THIN LAWN VTL : VERY THIN LAWN

N.B. The mean values were generally calculated from triplicate plate counts.

TABLE 4

Test 3

Mean Number of his+ Revertant Colonies Obtained when 5 Strains of *S. typhimurium* were Treated with BPS-24 in the Presence of a Post-mitochondrial Fraction (S9 Mix) from the Livers of Male Rats Treated with Aroclor 1254 (FLI 079)

Substance	Dose Level μg per plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100
		Mean ± S.D.				
DMSO	100 μl	10 ± 2	7 ± 3	17 ± 0	23 ± 9	94 ± 5
BPS-24	3	11 ± 4	12 ± 2	24 ± 4	30 ± 6	107 ± 7
	10	13 ± 4	14 ± 1	15 ± 3	22 ± 8	100 ± 14
	33	10 ± 2	15 ± 2	19 ± 7	22 ± 6	89 ± 6
	100	15 ± 4	7 ± 2	16 ± 2	20 ± 4	96 ± 8
	333	11 ± 2	1 ± 0 (STL)	5 ± 4 (STL)	6 ± 4	4 ± 2
	1000	1 ± 1 (STL)	22 ± 1 (TL)	4 ± 2 (TL)	1 ± 1 (STL)	11 ± 4 (STL)
Positive Controls	Compound	2AAN	2AAN	2AAN	2AAN	2AAN
	Dose Level	2 μg	2 μg	0.5 μg	0.5 μg	0.5 μg
	Mean ± S.D.	205 ± 14	157 ± 46	574 ± 45	349 ± 55	814 ± 57

S.D. Standard Deviation

2AAN 2-Aminoanthracene

STL : SLIGHTLY THIN LAWN TL : THIN LAWN

N.B. The mean values were generally calculated from triplicate plate counts.

TABLE 5

Test 3
 Mean Numbers of his⁺ Revertant Colonies Obtained when 5 Strains of S. typhimurium
 were Treated with BPS-24 in the Absence of S9 Mix

Substance	Dose Level μg per plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
DMSO	100 μl	12 ± 1	6 ± 2	13 ± 4	13 ± 3	-
BPS-24	3	12 ± 3	8 ± 1	11 ± 1	23 ± 2	-
	10	8 ± 1	6 ± 1	19 ± 7	27 ± 4	-
	33	11 ± 6	9 ± 3	18 ± 6	17 ± 8	-
	100	14 ± 2	10 ± 3	8 ± 3	22 ± 5	-
	333	12 ± 5	69 ± 8 (TL)	5 ± 4	7 ± 3	-
	1000	0 ± 1 (STL)	17 ± 2 (TL)	1 ± 1 (TL)	1 ± 1 (STL)	-
Positive Controls	Compound	NaN ₃	9AA	2NF	2NF	NaN ₃
	Dose Level	1 μg	80 μg	1 μg	1 μg	1 μg
	Mean ± S.D.	317 ± 20	1182 ± 235	431 ± 10	325 ± 34	-

S.D. Standard Deviation

NaN₃ Sodium azide
 2NF 2-Nitrofluorene

9AA 9-Aminoacridine

STL : SLIGHTLY THIN LAWN TL : THIN LAWN

N.B. The mean values were generally calculated from triplicate plate counts.

TABLE 6

Test 3
 Mean Numbers of *his*⁺ Revertant Colonies Obtained when 5 Strains of *S. typhimurium*
 were Treated with BPS-24 in the Absence of S9 Mix

Substance	Dose Level μg per plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
DMSO	100 μl	-	-	-	-	84 ± 7
BPS-24	100	-	-	-	-	95 ± 10
	400	-	-	-	-	11 ± 9
	700	-	-	-	-	11 ± 1
	1000	-	-	-	-	7 ± 3
	1300	-	-	-	-	8 ± 5 (TL)
	1600	-	-	-	-	3 ± 2 (TL)
Positive Controls	Compound	NaN ₃	9AA	2NF	2NF	NaN ₃
	Dose Level	1 μg	80 μg	1 μg	1 μg	1 μg
	Mean ± S.D.	-	-	-	-	681 ± 5

S.D. Standard Deviation

NaN₃ Sodium azide
 2NF 2-Nitrofluorene

9AA 9-Aminoacridine

TL : THIN LAWN

N.B. The mean values were generally calculated from triplicate plate counts.

TABLE 7

Test 4

Mean Number of his+ Revertant Colonies Obtained when 5 Strains of S. typhimurium were Treated with BPS-24 in the Presence of a Post-mitochondrial Fraction (S9 Mix) from the Livers of Male Rats Treated with Aroclor 1254 (FLI 079)

Substance	Dose Level μg per plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100
		Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.	Mean \pm S.D.
DMSO	100 μl	-	13 \pm 4	-	-	-
BPS-24	3	-	12 \pm 4	-	-	-
	10	-	14 \pm 3	-	-	-
	33	-	14 \pm 3	-	-	-
	100	-	11 \pm 1	-	-	-
	333	-	1 \pm 1 (STL)	-	-	-
	1000	-	72 \pm 10 (TL)	-	-	-
Positive Controls	Compound	2AAN	2AAN	2AAN	2AAN	2AAN
	Dose Level	2 μg	2 μg	0.5 μg	0.5 μg	0.5 μg
	Mean \pm S.D.	-	176 \pm 8	-	-	-

S.D. Standard Deviation

2AAN 2-Aminoanthracene

STL : SLIGHTLY THIN LAWN TL : THIN LAWN

N.B. The mean values were generally calculated from triplicate plate counts.

TABLE 8

Test 4

Mean Numbers of his⁺ Revertant Colonies Obtained when 5 Strains of S. typhimurium were Treated with BPS-24 in the Absence of S9 Mix

Substance	Dose Level μg per plate	TA 1535	TA 1537	TA 1538	TA 98	TA 100
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
DMSO	100 μl	-	8 ± 2	-	-	-
BPS-24	3	-	17 ± 5	-	-	-
	10	-	12 ± 2	-	-	-
	33	-	21 ± 6	-	-	-
	100	-	21 ± 7	-	-	-
	333	-	3 ± 1 (TL)	-	-	-
	1000	-	17 ± 4 (TL)	-	-	-
Positive Controls	Compound	NaN ₃	9AA	2NF	2NF	NaN ₃
	Dose Level	1 μg	80 μg	1 μg	1 μg	1 μg
	Mean ± S.D.	-	971 ± 41	-	-	-

S.D. Standard Deviation

NaN₃ Sodium azide
2NF 2-Nitrofluorene

9AA 9-Aminoacridine

TL : THIN LAWN

N.B. The mean values were generally calculated from triplicate plate counts.

APPENDIX 1

Test 2

BPS-24 - revertant colony numbers obtained per plate using bacterial strains :- TA 1535, TA 1537, TA 1538, TA 98 and TA 100

Strain	Dose level µg/plate	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	Solvent	-	6	2	9, 5, 5
	8	-	11	2	13, 10, 9
	25	-	10	5	12, 13, 4
	83	-	10	3	10, 13, 7
	250	-	12	1	11, 12, 12
	833	-	0	0	0 VTL, 0 VTL, 0 VTL
	2500	-	0	0	0 VTL, 0 VTL, 0 VTL
	Solvent	+	12	1	13, 12, 11
	8	+	7	1	7, 6, 7
	25	+	7	3	7, 5, 10
	83	+	7	3	4, 8, 9
	250	+	11	5	9, 17, 8
	833	+	0	0	0 TL, 0 TL, 0 TL
	2500	+	0	0	0 VTL, 0 VTL, 0 VTL
TA 1537	Solvent	-	7	2	7, 9, 5
	8	-	7	4	5, 4, 12
	25	-	4	2	5, 2, 6
	83	-	5	3	6, 7, 2
	250	-	0	1	0, 1, 0
	833	-	0	1	0 TL, 1 TL, 0 TL
	2500	-	0	0	0 TL, 0 TL, 0 TL
	Solvent	+	7	1	7, 8, 7
	8	+	8	4	5, 7, 13
	25	+	9	5	14, 8, 5
	83	+	7	2	8, 8, 4
	250	+	0	0	0, 0, 0
	833	+	0	1	1 TL, 0 TL, 0 TL
	2500	+	0	0	0 TL, 0 TL, 0 TL
TA 1538	Solvent	-	13	1	12, 14, 14
	8	-	14	1	14, 14, 15
	25	-	15	1	15, 15, 14
	83	-	9	4	12, 11, 4
	250	-	7	3	9, 4, 8
	833	-	0	0	0 TL, 0 TL, 0 TL
	2500	-	0	0	0 VTL, 0 VTL, 0 VTL
	Solvent	+	20	3	19, 17, 23
	8	+	20	7	14, 27, 20
	25	+	18	3	19, 21, 15
	83	+	20	2	20, 22, 19
	250	+	11	4	6, 14, 12
	833	+	0	0	0 TL, 0 TL, 0 TL
	2500	+	0	0	0 VTL, 0 VTL, 0 VTL

SD : Standard Deviation

- : Absence
+ : Presence

TL : THIN LAWN

VTL : VERY THIN LAWN

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APPENDIX 1 (Continued)

Test 2

BPS-24 - revertant colony numbers obtained per plate using
bacterial strains :- TA 1535, TA 1537, TA 1538, TA 98 and TA 100

Strain	Dose level µg/plate	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts	
TA 98	Solvent	-	48	7	50, 40, 54	
	8	-	34	6	40, 29, 33	
	25	-	37	10	48, 28, 34	
	83	-	39	11	47, 43, 26	
	250	-	32	5	26, 34, 36	
	833	-	0	0	0 STL, 0 STL, 0 STL	
	2500	-	0	1	1 TL, 0 TL, 0 TL	
	Solvent	+	51	7	54, 43, 55	
	8	+	49	7	45, 46, 57	
	25	+	48	3	45, 50, 49	
	83	+	44	6	49, 45, 37	
	250	+	39	3	36, 42, 39	
	833	+	0	0	0 STL, 0 STL, 0 STL	
	2500	+	0	0	0 VTL, 0 VTL, 0 VTL	
	TA 100	Solvent	-	103	7	98, 99, 111
		8	-	88	10	80, 84, 99
25		-	104	13	101, 93, 119	
83		-	87	8	94, 88, 79	
250		-	65	2	63, 66, 67	
833		-	0	1	1 TL, 0 TL, 0 TL	
2500		-	0	0	0 TL, 0 TL, 0 TL	
Solvent		+	95	4	92, 99, 93	
8		+	103	12	109, 90, 111	
25		+	104	12	103, 116, 92	
83		+	89	7	97, 84, 86	
250		+	13	9	21, 14, 3	
833		+	0	0	0 TL, 0 TL, 0 TL	
2500		+	0	0	0 TL, 0 TL, 0 TL	

SD : Standard Deviation

- : Absence

+ : Presence

STL : SLIGHTLY THIN LAWN

TL : THIN LAWN

VTL : VERY THIN LAWN

End of final report.

APPENDIX 1 (Continued)

Test 2

Mutability test with bacterial strains :-
TA 1535, TA 1537, TA 1538, TA 98 and TA 100

Strain	Compound	Dose level per plate	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	NaN3	1 μ g	-	265	27	235, 288, 272
TA 1537	9AA	80 μ g	-	1031	67	1044, 958, 1091
TA 1538	2NF	1 μ g	-	301	40	277, 279, 348
TA 98	2NF	1 μ g	-	299	9	297, 291, 309
TA 100	NaN3	1 μ g	-	692	12	699, 678, 698
TA 1535	2AAN	2 μ g	+	200	2	200, 201, 198
TA 1537	2AAN	2 μ g	+	208	64	135, 238, 252
TA 1538	2AAN	0.5 μ g	+	612	43	638, 636, 563
TA 98	2AAN	0.5 μ g	+	499	153	665, 468, 363
TA 100	2AAN	0.5 μ g	+	1086	69	1165, 1042, 1051

SD : Standard Deviation

- : Absence

+ : Presence

NaN3 Sodium azide

9AA 9-Aminoacridine

2NF 2-Nitrofluorene

2AAN 2-Aminoanthracene

APPENDIX 2

Test 3

BPS-24 - revertant colony numbers obtained per plate using bacterial strains :- TA 1535, TA 1537, TA 1538, TA 98 and TA 100

Strain	Dose level µg/plate	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	Solvent	-	12	1	12, 11, 13
	3	-	12	3	14, 12, 9
	10	-	8	1	9, 8, 8
	33	-	11	6	6, 10, 17
	100	-	14	2	16, 13, 13
	333	-	12	5	11, 7, 17
	1000	-	0	1	0 STL, 0 STL, 1 STL
	Solvent	+	10	2	10, 11, 8
	3	+	11	4	13, 13, 6
	10	+	13	4	8, 16, 14
	33	+	10	2	8, 12, 11
	100	+	15	4	14, 19, 11
	333	+	11	2	9, 11, 12
	1000	+	1	1	0 STL, 0 STL, 2 STL
TA 1537	Solvent	-	6	2	6, 4, 8
	3	-	8	1	8, 7, 8
	10	-	6	1	7, 5, 6
	33	-	9	3	13, 8, 7
	100	-	10	3	13, 8, 9
	333	-	69	8	71 TL, 61 TL, 76 TL
	1000	-	17	2	19 TL, 15 TL, 17 TL
	Solvent	+	7	3	8, 3, 9
	3	+	12	2	12, 14, 10
	10	+	14	1	14, 15, 13
	33	+	15	2	15, 13, 16
	100	+	7	2	6, 9, 7
	333	+	1	0	1 STL, 1 STL, 1 STL
	1000	+	22	1	22 TL, 23 TL, 22 TL
TA 1538	Solvent	-	13	4	17, 12, 9
	3	-	11	1	10, 10, 12
	10	-	19	7	18, 26, 13
	33	-	18	6	17, 24, 13
	100	-	8	3	6, 11, 6
	333	-	5	4	6, 8, 1
	1000	-	1	1	1 TL, 1 TL, 2 TL
	Solvent	+	17	0	17, 17, 17
	3	+	24	4	28, 20, 23
	10	+	15	3	13, 19, 14
	33	+	19	7	27, 18, 13
	100	+	16	2	14, 18, 15
	333	+	5	4	4 STL, 9 STL, 1 STL
	1000	+	4	2	2 TL, 6 TL, 3 TL

SD : Standard Deviation

- : Absence

+ : Presence

STL : SLIGHTLY THIN LAWN

TL : THIN LAWN

Continued on next page.

APPENDIX 2 (Continued)

Test 3

BPS-24 - revertant colony numbers obtained per plate using
bacterial strains :- TA 1535, TA 1537, TA 1538, TA 98 and TA 100

Strain	Dose level µg/plate	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 98	Solvent	-	13	3	17, 11, 11
	3	-	23	2	21, 24, 23
	10	-	27	4	23, 30, 27
	33	-	17	8	26, 10, 15
	100	-	22	5	26, 22, 17
	333	-	7	3	8, 9, 4
	1000	-	1	1	0 STL, 1 STL, 1 STL
	- Solvent	+	23	9	33, 19, 17
	3	+	30	6	24, 35, 30
	10	+	22	8	30, 23, 14
	33	+	22	6	25, 25, 15
	100	+	20	4	24, 20, 17
	333	+	6	4	6, 3, 10
	1000	+	1	1	1 STL, 1 STL, 0 STL
TA 100	Solvent	-	84	7	88, 89, 76
	100	-	95	10	106, 87, 91
	400	-	11	9	19, 1, 13
	700	-	11	1	11, 10, 11
	1000	-	7	3	10, 5, 7
	1300	-	8	5	11 TL, 4 TL, C+TL
	1600	-	3	2	4 TL, 1 TL, 3 TL
	Solvent	+	94	5	98, 89, 94
	3	+	107	7	103, 115, 102
	10	+	100	14	113, 103, 85
	33	+	89	6	85, 95, 86
	100	+	96	8	92, 105, 90
	333	+	4	2	1, 5, 5
	1000	+	11	4	15 STL, 12 STL, 7 STL

SD : Standard Deviation

- : Absence

+ : Presence

STL : SLIGHTLY THIN LAWN

TL : THIN LAWN

End of final report.

APPENDIX 2 (Continued)

Test 3

Mutability test with bacterial strains :-
TA 1535, TA 1537, TA 1538, TA 98 and TA 100

Strain	Compound	Dose level per plate	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	NaN3	1 μ g	-	317	20	304, 307, 340
TA 1537	9AA	80 μ g	-	1182	235	941, 1410, 1196
TA 1538	2NF	1 μ g	-	431	10	420, 437, 436
TA 98	2NF	1 μ g	-	325	34	330, 288, 356
TA 100	NaN3	1 μ g	-	681	5	681, 676, 685
TA 1535	2AAN	2 μ g	+	205	14	192, 202, 220
TA 1537	2AAN	2 μ g	+	157	46	107, 165, 198
TA 1538	2AAN	0.5 μ g	+	574	45	549, 547, 626
TA 98	2AAN	0.5 μ g	+	349	55	286, 386, 376
TA 100	2AAN	0.5 μ g	+	814	57	758, 812, 871

SD : Standard Deviation

- : Absence

+ : Presence

NaN3 Sodium azide

9AA 9-Aminoacridine

2NF 2-Nitrofluorene

2AAN 2-Aminoanthracene

APPENDIX 3

Test 4

BPS-24 - revertant colony numbers obtained per plate using
bacterial strain:- TA 1537

Strain	Compound	Dose level µg/plate	Liver S-9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1537	BPS-24	Solvent	-	8	2	10, 7, 8
		3	-	17	5	17, 21, 12
		10	-	12	2	11, 14, 10
		33	-	21	6	23, 26, 15
		100	-	21	7	18, 29, 15
		333	-	3	1	2 TL, 3 TL, 3 TL
		1000	-	17	4	19 TL, 20 TL, 12 TL
		Solvent	+	13	4	17, 9, 14
		3	+	12	4	16, 8, 12
		10	+	14	3	12, 14, 17
		33	+	14	3	14, 16, 11
		100	+	11	1	11, 10, 12
		333	+	1	1	0 STL, 0 STL, 2 STL
		1000	+	72	10	80 TL, 75 TL, 60 TL
		9AA	80	-	971	41
2AAN	2	+	176	8	185, 173, 169	

SD : Standard Deviation

- : Absence

+ : Presence

STL : SLIGHTLY THIN LAWN

TL : THIN LAWN

9AA : 9-Aminoacridine

2AAN : 2-Aminoanthracene

Triage of 8(e) Submissions

Date sent to triage: 7/17/96

NON-CAP

CAP

Submission number: 13605A

TSCA Inventory: (Y) N D

Study type (circle appropriate):

Group 1 - Gordon Cash (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - HERD (1 copy each)

STOX CTOX EPI RTOX

STOX/ONCO CTOX/ONCO IMMUNO CYTO

~~GTOX~~
NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

- This is the **original** 8(e) submission; refile after triage evaluation.
- This **original** submission has been **split**; rejoin after triage evaluation.
- Other:

Photocopies Needed for Triage Evaluation

entire document: (0) 1 2 3

front section and CECATS: (0) 1 2 3

Initials: JW

Date: 7/18/96

CECATS TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA: Submission # 8E11Q-0396-13605 SEQ. A

TYPE: INT/SUPP FLWP

SUBMITTER NAME: NISCA USA, INC.

INFORMATION REQUESTED: FLWP DATE:

- 0501 NO INFO REQUESTED
 - 0502 INFO REQUESTED (TECH)
 - 0503 INFO REQUESTED (VOL ACTIONS)
 - 0504 INFO REQUESTED (REPORTING RATIONALE)
- DISPOSITION:
 0639 REFER TO CHEMICAL SCREENING
 0678 CAP NOTICE

- ~~VOLUNTARY ACTIONS:~~
 0401 NO ACTION REPORTED
 0402 STUDIES PLANNED/UNDERWAY
 0403 NOTIFICATION OF WORKER/OTHERS
 0404 LABEL/MSDS CHANGES
 0405 PROCESS/HANDLING CHANGES
 0406 APP/USE DISCONTINUED
 0407 PRODUCTION DISCONTINUED
 0408 CONFIDENTIAL

SUB. DATE: 03/05/96 OTS DATE: 03/12/96 CSRAD DATE: 04/10/96

CHEMICAL NAME: _____ CAS# 5397-34-2

INFORMATION TYPE:	P	F	C	INFORMATION TYPE:	P	F	C	INFORMATION TYPE:	P	F	C
0201 ONCO (HUMAN)	01	02	04	0216 EPI/CLIN	01	02	04	0241 IMMUNO (ANIMAL)	01	02	04
0202 ONCO (ANIMAL)	01	02	04	0217 HUMAN EXPOS (PROD CONTAM)	01	02	04	0242 IMMUNO (HUMAN)	01	02	04
0203 CELL TRANS (IN VITRO)	01	02	04	0218 HUMAN EXPOS (ACCIDENTAL)	01	02	04	0243 CHEM/PHYS PROP	01	02	04
0204 MUTA (IN VITRO)	01	02	04	0219 HUMAN EXPOS (MONITORING)	01	02	04	0244 CLASTO (IN VITRO)	01	02	04
0205 MUTA (IN VIVO)	01	02	04	0220 ECO/AQUA TOX	01	02	04	0245 CLASTO (ANIMAL)	01	02	04
0206 REPRO/TERATO (HUMAN)	01	02	04	0221 ENV. OCC/REL/FATE	01	02	04	0246 CLASTO (HUMAN)	01	02	04
0207 REPRO/TERATO (ANIMAL)	01	02	04	0222 EMER INCI OF ENV CONTAM	01	02	04	0247 DNA DAM/REPAIR	01	02	04
0208 NEURO (HUMAN)	01	02	04	0223 RESPONSE REQEST DELAY	01	02	04	0248 PROD/USE/PROC	01	02	04
0209 NEURO (ANIMAL)	01	02	04	0224 PROD/COMP/CHEM ID	01	02	04	0251 MSDS	01	02	04
0210 ACUTE TOX. (HUMAN)	01	02	04	0225 REPORTING RATIONALE	01	02	04	0299 OTHER	01	02	04
0211 CHR. TOX. (HUMAN)	01	02	04	0226 CONFIDENTIAL	01	02	04				
0212 ACUTE TOX. (ANIMAL)	01	02	04	0227 ALLERG (HUMAN)	01	02	04				
0213 SUB ACUTE TOX (ANIMAL)	01	02	04	0228 ALLERG (ANIMAL)	01	02	04				
0214 SUB CHRONIC TOX (ANIMAL)	01	02	04	0239 METAB/PHARMACO (ANIMAL)	01	02	04				
0215 CHRONIC TOX (ANIMAL)	01	02	04	0240 METAB/PHARMACO (HUMAN)	01	02	04				

TRIAJE DATA:	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
<input checked="" type="radio"/> YES (CONTINUE)	<input type="radio"/> NO (DROP)	<input type="radio"/> YES (DROP/REFER)	<u>IN VITRO</u>	<u>LOW</u>		
<input type="radio"/> NO (DROP)	<input type="radio"/> NO (CONTINUE)	<input type="radio"/> NO (CONTINUE)		<u>MED</u>		
<input type="radio"/> DETERMINE	<input type="radio"/> REFER:	<input type="radio"/> REFER:		<u>HIGH</u>		

COMMENTS:

12)

8EHQ-0396-13605: Rank - medium.

Chemical: 2-[(4-hydroxyphenyl)sulfonyl]phenol (CAS# 5397-34-2).

Testing for mutagenic activity with Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 and TA 100, Inveresk Research International, Tranent, Scotland, not dated, cover letter dated March 5, 1996: Weakly positive for gene mutations in the Salmonella typhimurium/mammalian microsomal (Ames) assay in strain TA1537 both without and with metabolic activation at toxic dose levels.

Chromosomal aberrations assay with Chinese hamster ovary cells in vitro, Inveresk Research International, Tranent, Scotland, not dated, cover letter dated March 5, 1996: Positive for chromosome mutations (aberrations) in Chinese hamster ovary (CHO) cells in vitro both without and with metabolic activation.