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Arkema Inc.

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November 27, 2006

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Attn: Section 8(d) Health and Safety Reporting Rule (Notification/Reporting)
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1201 Constitution Ave., NW
Washington, DC 20004-3302

Subject: Health and Safety Data Reporting, Docket No. EPA-HQ-OPPT-2005-0055, RIN 2070-AB11 (71 Fed. Reg. 47130, August 16, 2006)

Dear Sir/Madam:

Pursuant to the August 16, 2006, *Federal Register* notice referenced above and 40 CFR § 716.35, Arkema Inc. is submitting to EPA final reports of the following studies conducted on methane sulfonyl chloride (CASRN 124-63-0) and octane sulfonyl chloride (CASRN 7795-95-1).

Methane Sulfonyl Chloride (CASRN 124-63-0)

1. Methane Sulfonyl Chloride 1491 Toxicology Report, Mice. Pharmacology Research Inc. September 1976.
2. Methane Sulfonyl Chloride 1760 Mice Toxicology Report. Pharmacology Research Inc. September 1976.
3. Toxicological Properties of Methane Sulfonyl Chloride #1491. Pharmacology Research Inc. November 8, 1977.
4. Inhalation Toxicity of Methane Sulfonyl Chloride. Bio/dynamics Inc. Report No. 85-7854. October 8, 1986.
5. Methane Sulfonyl Chloride Inhalation Toxicity in Rats 4-Hour Exposure. Report No. PWT 45/861670. Huntingdon Research Centre. February 23, 1987.
6. Toxicity to Fish. Report No. 86.0628-A. Krachtwerkhuizen Laboratorium. March 1, 1988.
7. Methane Sulphonyl Chloride: Assessment of Respiratory Irritancy in Mice. Study No. HM0464. Imperial Chemical Industries PLC, Central Toxicology Laboratory. May 23, 1983.
8. Methane Sulfonyl Chloride: Reverse mutation in two Histidine-requiring strains of *Salmonella typhimurium*. Report No. 514/48-1052 March 1998.
9. Methane Sulphonyl Chloride: induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes. Report No. 514/30-1052. Covance Laboratories Limited. March 1997.
10. Methane Sulfonyl Chloride: Reverse Mutation in five Histidine-requiring strains of *Salmonella typhimurium*. Report No. 514/29-1052. Covance Laboratories Limited. January 1997.
11. Bone Marrow Micronucleus Test by Intraperitoneal Route in Mice. Report No. 25873 MAS. CIT. November 7, 2003.
12. Skin Sensitization Test in Guinea Pigs. Report No. 25982 TSG. CIT. December 2, 2003.

300480

November 27, 2006
Page 2
Health and Safety Data Reporting

Octane Sulfonyl Chloride (CASRN 7795-95-1)

1. Skin Irritant Properties and Vapor Toxicity of Octanesulfonyl Chloride. Pharmacology Research, Inc. October 6, 1955.
2. Toxicology Report Octane Sulfonyl Chloride. Pharmacology Research, Inc. October 8, 1979.
3. Toxicity Studies with Octane Sulfonyl Chloride. Pharmacology Research, Inc. March 25, 1957.

Questions regarding this submission may be directed to me at 215-419-5890 or via e-mail at debra.randall@arkema.com.

Sincerely,



Debra Randall, DABT
Manager, Product Safety

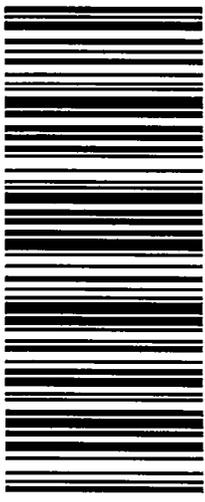
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Methane Sulfonyl Chloride Studies

MN 1256-35

Case 124-63-0KW

K 76-178

A. R. LATVEN
Director

On Microfilm

BOX 70, 21 MAIN STREET
DARBY, PA 19023
(215) 586-0707

PHARMACOLOGY RESEARCH, INC.

5877pt1

1 SEPTEMBER 1976

PENNWALT CORPORATION

MR. GEORGE I. ABBOTT
CORPORATE INDUSTRIAL HYGIENIST
PENNWALT CORPORATION
900 FIRST AVENUE
KING OF PRUSSIA, PA. 19406

REC'D SEP 2 1976
READ BY JA DATE 4/1
ANS. BY _____ DATE _____

DEAR MR. ABBOTT:

ENCLOSED ARE REPORTS OF OUR FINDINGS WITH THE THREE METHANE SULFONIC ACID (MSA) SAMPLES AND THE TWO METHANE SULFONYL CHLORIDE (MSC) SAMPLES.

EACH OF THESE PRODUCTS WAS CORROSIVE TO THE SKIN WHEN TESTED BY THE MOUSE TAIL METHOD.

THREE OF THE SAMPLES WERE EVALUATED FOR INHALATION TOXICITY. MSA ANHYDROUS WAS NONTOXIC BECAUSE OF INSUFFICIENT VOLATILITY. BOTH MSC SAMPLES WERE TOXIC: THE LC₅₀ OF MSC-1491 WAS FOUND TO BE 1.5 MG/LITER AND THAT OF MSC-1760 TO BE 1.9 MG PER LITER. THE DIFFERENCE BETWEEN THESE TWO VALUES IS NOT STATISTICALLY SIGNIFICANT. HEMORRHAGIC LUNGS WERE FOUND IN THE ANIMALS WHICH DIED SUBSEQUENT TO EXPOSURE. THE SURVIVORS SHOWED PROFOUND AND PROLONGED LOSSES IN BODY WEIGHT; THE LATTER WAS PROBABLY DUE TO PULMONARY DAMAGE DURING INHALATIONAL EXPOSURE WHICH WAS FOLLOWED BY PROLONGED RECOVERY.

SINCERELY YOURS,
A. R. Latven
A. R. LATVEN
PHARMACOLOGY RESEARCH, INC.

ENC: 5 REPORTS + 3 CC EACH
STATEMENT + 3 CC

DESCRIPTION	IDENTIFICATION NO.	DATE
Methane Sulfonic Acid #1751 (7040)	76-175	1873
40% Methane Sulfonic Acid #1759	76-176	1873
Methane Sulfonic Acid an. (anhydrous)	76-177	178
Methane sulfonyl chloride 1491	76-178	1256-83
Methane sulfonyl chloride 1760 (CONSTRUCTED)	76-179	125

TOXICOLOGY REPORT
FOR PENNALT CORPORATION

RE: METHANE SULFONYL CHLORIDE 1491.

A CLEAR, TINTED LIQUID; AMBIENT D = 1.475 G/ML.

SUMMARY. (1) SKIN CORROSIVITY, MOUSE TAIL METHOD: CORROSIVE.
(2) ONE-HOUR INHALATION TOXICITY IN MICE: $LC_{50} = 1.5$ MG/LITER.

(1) SKIN CORROSIVITY, MOUSE TAIL METHOD.

METHOD. THE TAILS OF FOUR ANESTHETIZED MICE WERE IMMERSSED IN THE SAMPLE TO A DEPTH OF 4 CM. ONE HOUR LATER THE TISSUE REACTION WAS TERMINATED BY PLUNGING THE APPENDAGES INTO A SATURATED SOLUTION OF SODIUM BICARBONATE FOR A PERIOD OF 30 SECONDS; EACH TAIL WAS THEN RINSED WITH WATER AND BLOTTED DRY.

RESULTS. THE TAIL OF EACH ANIMAL WAS WHITE (NOT PINK) WHEN EXPOSURE WAS TERMINATED AND THE APPENDAGE FELL OFF IN A DAY OR TWO.

(2) ONE-HOUR INHALATION TOXICITY IN MICE.

METHOD. FIVE MICE (WBS/S, $32 \pm$ G BW) WERE PLACED IN EACH OF A SERIES OF 20-LITER EXPOSURE CHAMBERS. A MEASURED VOLUME OF THE SAMPLE WAS PLACED UPON A DISC OF FILTER PAPER SUSPENDED FROM THE ROOF OF EACH CHAMBER; THE LATTER WAS IMMEDIATELY SEALED AIRTIGHT. INHALATIONAL EXPOSURE WAS TERMINATED ONE HOUR LATER AND SURVIVING ANIMALS WERE OBSERVED UNTIL LOST BODY WEIGHT WAS RESTORED (TO THREE WEEKS).

RESULTS.

ML SAMPLE IN CHAMBER	VAPOR CONC. MG/LITER	NO. MICE DEAD/TOTAL	MORTALITY	TIME FOR DEATH			
				MINUTES	HOURS	DAYS	
0.0095	0.7	0 / 5	0 %	-	-	-	-
0.0136	1.0	0 / 5	0 %	-	-	-	-
0.019	1.4	2 / 5	40 %	-	-	-	>4 ^H , 8 ^D
0.027	2.0	5 / 5	100 %	45 ^M	3 ^D	4 ^D	9 ^D , 10 ^D
0.136	<10.0*	5 / 5	100 %	8 ^M	9 ^M	11 ^M	16 ^M , 23 ^M

* SATURATED.

$LC_{50} = 1.5$ MG/LITER

(CONTINUED)

(METHANE SULFONYL CHLORIDE 149) CONCLUDED)

-2-

SYMPTOMATOLOGY: SEVERE SENSORY IRRITATION (EYES, NOSE), MILD LACRIMATION, PERIPHERAL VASODILATION, AND BRONCHOSPASTIC GASPING; RECOVERY WAS VIRTUALLY COMPLETE ONE OR TWO HOURS AFTER EXPOSURE. PRONOUNCED AND PROLONGED LOSSES IN BODY WEIGHT OCCURRED POST-EXPOSURE.

HEMORRHAGIC LUNGS WERE FOUND IN THE ANIMALS WHICH SUCCEDED AFTER EXPOSURE WAS TERMINATED.

PHARMACOLOGY RESEARCH, INC.

By *A. R. Latven*
A. R. LATVEN 9/01/76

PROTOCOL REFS: PR#76.5271; (1) ARL 32, 33; (2) do 35.

A. R. LATVEN
Director

S 877 pt 2

1359
PHARMACOLOGY RESEARCH,
INC.

12 76 179
BOX 70, 21 MAIN STREET
DARBY, PA 19023
(215) 586-0707

1 SEPTEMBER 1976

PENNWALT CORPORATION

MR. GEORGE I. ABBOTT
CORPORATE INDUSTRIAL HYGIENIST
PENNWALT CORPORATION
900 FIRST AVENUE
KING OF PRUSSIA, PA. 19406

REC'D SEP 2 1976

READ BY JA DATE 4/12

ANS. BY _____ DATE _____

DEAR MR. ABBOTT:

ENCLOSED ARE REPORTS OF OUR FINDINGS WITH THE THREE METHANE SULFONIC ACID (MSA) SAMPLES AND THE TWO METHANE SULFONYL CHLORIDE (MSC) SAMPLES.

EACH OF THESE PRODUCTS WAS CORROSIVE TO THE SKIN WHEN TESTED BY THE MOUSE TAIL METHOD.

THREE OF THE SAMPLES WERE EVALUATED FOR INHALATION TOXICITY. MSA ANHYDROUS WAS NONTOXIC BECAUSE OF INSUFFICIENT VOLATILITY. BOTH MSC SAMPLES WERE TOXIC: THE LC₅₀ OF MSC-1491 WAS FOUND TO BE 1.5 MG/LITER AND THAT OF MSC-1760 TO BE 1.9 MG PER LITER. THE DIFFERENCE BETWEEN THESE TWO VALUES IS NOT STATISTICALLY SIGNIFICANT. HEMORRHAGIC LUNGS WERE FOUND IN THE ANIMALS WHICH DIED SUBSEQUENT TO EXPOSURE. THE SURVIVORS SHOWED PROFOUND AND PROLONGED LOSSES IN BODY WEIGHT; THE LATTER WAS PROBABLY DUE TO PULMONARY DAMAGE DURING INHALATIONAL EXPOSURE WHICH WAS FOLLOWED BY PROLONGED RECOVERY.

SINCERELY YOURS,

A. R. Latven

A. R. LATVEN
PHARMACOLOGY RESEARCH, INC.

ENC: 5 REPORTS + 3 CC EACH
STATEMENT + 3 CC

Sample Description	File No.	Reference No.
Methane Sulfonic Acid #1751 (7040)	76-175	1893
10% Methane Sulfonic Acid #1759	76-176	1895
Methane Sulfonic Acid anhydrous	76-177	1891
Methane sulfonyl chloride 1491	76-178	1256-5
Methane sulfonyl chloride 1760 (constrained)	76-179	125

017-200-55

76-149
~~44-2013~~
MN 1256

TOXICOLOGY REPORT
FOR PENNWALT CORPORATION

RE: METHANE SULFONYL CHLORIDE 1760 (UNSTRIPPED).

A CLEAR, TINTED LIQUID; AMBIENT D = 1.472 G/ML.

SUMMARY. (1) SKIN CORROSIVITY, MOUSE TAIL METHOD: CORROSIVE.
(2) ONE-HOUR INHALATION TOXICITY IN MICE: LC₅₀ = 1.9 MG/LITER.

(1) SKIN CORROSIVITY, MOUSE TAIL METHOD.

METHOD. THE TAILS OF FOUR ANESTHETIZED MICE WERE IMMERSERD IN THE SAMPLE TO A DEPTH OF 4 CM. ONE HOUR LATER THE TISSUE REACTION WAS TERMINATED BY PLUNGING THE APPENDAGES INTO A SATURATED SOLUTION OF SODIUM BICARBONATE FOR A PERIOD OF 30 SECONDS; EACH TAIL WAS THEN RINSED WITH WATER AND BLOTTED DRY.

RESULTS. THE TAIL OF EACH ANIMAL WAS WHITE (NOT PINK) WHEN EXPOSURE WAS TERMINATED AND THE APPENDAGE FELL OFF IN A DAY OR TWO.

(2) ONE-HOUR INHALATION TOXICITY IN MICE.

METHOD. FIVE MICE (WBS/S, 32± G BW) WERE PLACED IN EACH OF A SERIES OF 20-LITER EXPOSURE CHAMBERS. A MEASURED VOLUME OF THE SAMPLE WAS PLACED UPON A DISC OF FILTER PAPER SUSPENDED FROM THE ROOF OF EACH CHAMBER; THE LATTER WAS IMMEDIATELY SEALED AIRTIGHT. INHALATIONAL EXPOSURE WAS TERMINATED ONE HOUR LATER AND SURVIVING ANIMALS WERE OBSERVED UNTIL LOST BODY WEIGHT WAS RESTORED (TO THREE WEEKS).

RESULTS.

ML SAMPLE IN CHAMBER	VAPOR CONC. MG/LITER	NO. MICE DEAD/TOTAL	MORTALITY	TIME FOR DEATH		
				MINUTES	HOURS	DAYS
0.0095	0.7	0 / 5	0 %	-	-	-
0.0136	1.0	0 / 5	0 %	-	-	-
0.019	1.4	3 / 5	60 %	-	-	2 ^D , 2 ^D , 10 ^D
0.027	2.0	2 / 5	40 %	-	-	4 ^H , 2 ^D
0.038	2.8	3 / 5	60 %	-	-	4 ^H , 2 ^D , 8 ^D
0.054	4.0	5 / 5	100 %	23 ^M , 3 ^H	4 ^H , 4 ^H	2 ^D

LC₅₀ = 1.9 MG/LITER

(CONTINUED)

(METHANE SULFONYL CHLORIDE 1760 CONCLUDED)

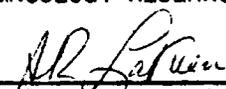
-2-

SYMPTOMATOLOGY: SEVERE SENSORY IRRITATION (EYES, NOSE), MILD LACRIMATION, PERIPHERAL VASODILATION, AND BRONCHOSPASTIC GASPING; RECOVERY WAS VIRTUALLY COMPLETE ONE OR TWO HOURS AFTER EXPOSURE. PRONOUNCED AND PROLONGED LOSSES IN BODY WEIGHT OCCURRED POST-EXPOSURE.

HEMORRHAGIC LUNGS WERE FOUND IN THE ANIMALS WHICH SUCCEDED AFTER EXPOSURE WAS TERMINATED.

PHARMACOLOGY RESEARCH, INC.

By


A. R. LATVEN 9/01/76

PROTOCOL REFS: PR#76.5272; (1) ARL 32, 33; (2) oo 37.

Filed: MN 1256 - 85 (TMC Vault)

(215) 586-0707

TR 77-226

PHARMACOLOGY RESEARCH, INC. On Microfilm

S-1150

CAS 124-63-0

8 NOVEMBER 1977

S1150

MR. GEORGE I. ABBOTT
CORPORATE INDUSTRIAL HYGIENIST
PENNWALT CORPORATION
900 FIRST AVENUE
KING OF PRUSSIA, PA. 19406

DEAR MR. ABBOTT:

ENCLOSED ARE REPORTS CONCERNING THE REQUESTED TOXICOLOGICAL PROPERTIES OF METHANE SULFONYL CHLORIDE #1491 AND OF ENDOTHALL ACID #3441. COMMENTS ARE AS FOLLOWS.

- 85

MSC #1491. THIS FUMING LIQUID IS CORROSIVE TO ALL LIVING TISSUES EXAMINED (EYE, SKIN, STOMACH). FORTUNATELY IT IS A POWERFUL VAPOR IRRITANT AND LACRIMATOR, EFFECTIVELY ANNOUNCING POTENTIAL OR IMMINENT INJURY. THUS HUMAN INJURY IS CONCEIVABLE ONLY UNDER EXTRAORDINARY CONDITIONS (CONFINED SPILLAGE, ETC.).

TR 77-226
MN: 1256

EA #3441. THIS PRODUCT IS HIGHLY TOXIC WHICH, OF COURSE, IS NOT NEWS. ALTHOUGH THE DOT SKIN CORROSIVITY TEST SHOWED IT TO BE NONCORROSIVE AND NONTOXIC UNDER THE PRESCRIBED EXPERIMENTAL CONDITIONS, THESE RESULTS MAY NOT APPLY TO HUMANS BECAUSE RABBITS DO NOT SWEAT WHEREAS HUMANS DO. IN MY OPINION, THIS PRODUCT IS VERY HAZARDOUS TOXICOLOGICALLY.

TR 77-224
MN: 4304

SINCERELY YOURS,

A. R. Latven

A. R. LATVEN
PHARMACOLOGY RESEARCH, INC.

ENC: 2 REPORTS + 3 COPIES EACH.
STATEMENT + 3 CC.
2 REQUEST FORMS.

TOXICOLOGY REPORT
FOR PENNWALT CORPORATION

RE: METHANE SULFONYL CHLORIDE #1491.

A CLEAR, COLORLESS LIQUID; AMBIENT D = 1.471 G/ML.

- SUMMARY. (1) EYE IRRITANCY IN RABBITS: CORROSIVE.
 (2) ACUTE ORAL TOXICITY IN RATS: LD₅₀ = 255 MG/KG.
 (3) DERMAL TOXICITY IN RABBITS: TOXIC AT 2000 MG/KG,
 NONTOXIC AT 200 MG/KG.

(1) EYE IRRITANCY IN RABBITS.

METHOD. ONE-TENTH ML OF THE SAMPLE WAS PLACED IN THE CONJUNCTIVAL SAC OF ONE EYE OF EACH OF SIX ALBINO RABBITS. WITH THREE OF THESE ANIMALS, THE TREATED EYE WAS WASHED WITH FLOWING WATER INITIATED 20 TO 30 SECONDS AFTER INSTILLATION AND CONTINUED FOR ONE MINUTE. THE RESULTING REACTIONS WERE SCORED FOR SEVEN DAYS.

RESULTS. UNWASHED. ALL ANIMALS SCREAMED IN PAIN FOLLOWING INSTILLATION. OPACIFICATION OF THE CORNEA COMPLETELY OBSCURED THE IRIS AND THE CONJUNCTIVAE BECAME NECROTIC WHITE (DEAD) WITHOUT CHEMOSIS. THESE EFFECTS APPEARED PROMPTLY AND REMAINED UNALTERED THEREAFTER.

WASHED. ALL ANIMALS SCREAMED IN PAIN FOLLOWING INSTILLATION. THE CONJUNCTIVAE BECAME SEVERELY INFLAMED AND WERE SWOLLEN TO SUCH AN EXTENT THAT NEITHER THE ORB NOR THE NICTITATING MEMBRANE COULD BE EXAMINED DURING THE FIRST 48 HOURS. THE ORB BECAME VISIBLE ON THE THIRD DAY REVEALING OPACIFIED AREAS ON THE CORNEA AND SEVERE CONGESTION OF AN IRIS WHICH DID NOT REACT TO LIGHT. THESE EFFECTS PERSISTED UNCHANGED THEREAFTER.

AVERAGE SCORES WERE AS FOLLOWS (C = CORNEA, I = IRIS, R = CONJUNCTIVAL REDNESS, CH = CHEMOSIS):

TIME	UNWASHED				WASHED			
	C	I	R	CH	C	I	R	CH
1 HR	4	?	W	0	?	?	2	>4
2 HRS	4	?	W	0	?	?	2	>4
3 HRS	4	?	W	0	?	?	2	>4
4 HRS	4	?	W	0	?	?	2	>4
24 HRS	4	?	W	0	?	?	2	>4
48 HRS	4	?	W	0	?	?	2	>4
72 HRS	4	?	W	0	3	2*	2	4
4 DA	4	?	W	0	3	2*	2	4
5 DA	4	?	W	0	3	2*	2	4
6 DA	4	?	W	0	3	2*	2	4
7 DA	4	?	W	0	4	2*	2	4

W = WHITE NECROSIS. * EYE REFLEX ABSENT.

(CONTINUED)

(2) ACUTE ORAL TOXICITY IN RATS.

METHOD. A 10% DILUTION OF THE SAMPLE IN PEG 400 WAS ADMINISTERED BY STOMACH TUBE TO ♂ WBS/W RATS, 240+ G BW. SURVIVING ANIMALS WERE OBSERVED FOR AT LEAST SEVEN DAYS. (NOTE. THE UNDILUTED SAMPLE COULD NOT BE ADMINISTERED BECAUSE IT CAUSED IRREPARABLE PLUNGER/BARREL SEIZURE OF THE REQUIRED MICROSYRINGE.)

RESULTS.	ORAL DOSE MG/KG	NO. RATS DEAD/TOTAL	MORTALITY	TIME FOR DEATH HOURS
	100	0 / 5	0 %	- - - - -
	141	0 / 5	0 %	- - - - -
	200	1 / 5	20 %	- - - - 4
	283	3 / 5	60 %	- - 3, 3, 4
	400	5 / 5	100 %	3, 3, 3, 4

LD₅₀ = 255 MG/KG (205-317 = 95% CONFIDENCE LIMITS).

SYMPTOMATOLOGY: GENERAL DISTRESS (IMMEDIATE) AND HYPOTONIA. SURVIVING ANIMALS SHOWED SIGNIFICANT LOSSES IN BODY WEIGHT FOR ONE TO 5 DAYS BUT THESE WERE RECOVERED 2 TO 7 DAYS AFTER TREATMENT.

AUTOPSIES REVEALED THE GLANDULAR GASTRIC MUCOSA TO BE SEVERELY HEMORRHAGIC (BLACK).

(3) DERMAL TOXICITY IN RABBITS.

METHOD. THREE ALBINO RABBITS WERE TREATED DERMALLY WITH A SINGLE DOSE OF 2000 MG/KG (1.36 ML/KG) AND SIX ADDITIONAL RABBITS WERE TREATED WITH A SINGLE DOSE OF 200 MG/KG (2.0 ML/KG OF A 10% W/V DILUTION IN PEG 400). INDIVIDUAL DOSES WERE APPLIED TO THE FUR-CLIPPED SKIN OF THE TRUNK UNDER A PRE-FITTED IMPERVIOUS SLEEVE ON EACH ANIMAL. AFTER A SKIN-CONTACT PERIOD OF 24 HOURS, THE SLEEVES WERE REMOVED AND SURVIVING ANIMALS WERE OBSERVED FOR SEVEN DAYS.

RESULTS. 2000 MG/KG (UNDILUTED SAMPLE). INITIAL SKIN CONTACT EVOKED AN INTENSE PAIN REACTION. ONE OF THE THREE RABBITS DIED 30 MINUTES AFTER EXPOSURE WAS TERMINATED AND THE OTHER TWO RABBITS DIED DURING THE FOLLOWING NIGHT. IN EACH CASE THE SKIN OF THE ENTIRE TRUNK WAS NECROTIC GRAY IN APPEARANCE AND THE EYES WERE IRRITATED (R-1).

200 MG/KG (10% DILUTION). WHEN EXPOSURES WERE TERMINATED, A WELL-DEFINED ERYTHEMA (SCORE 2) WAS PRESENT OVER THE ENTIRE TRUNK OF EACH RABBIT; THIS DISSIPATED COMPLETELY ONE OR TWO DAYS LATER. ALL OF THE ANIMALS REMAINED ASYMPTOMATIC AND GAINED BODY WEIGHT DURING THE OBSERVATION PERIOD.

PROTOCOL REFS: #77.5387;
(1) RS 8, 17;
(2) ARL 35, 31;
(3) RS 8, 21.

PHARMACOLOGY RESEARCH, INC.

BY A. R. Latven
A. R. LATVEN 11/08/77

S1241

I. INTRODUCTION:

A series of single one-hour whole-body inhalation exposure was performed for Pennwalt Corporation using CD® (Sprague-Dawley derived) rats (5/sex) to determine the acute inhalation toxicity of methane sulfonyl chloride. The test substance was administered into the breathing zone of the animals as a vapor.

Species and strain of the test animal, method and route of test substance administration and target exposure level were determined by the sponsor. The test procedures followed guidelines described in the U.S. Department of Transportation Regulation 49 CFR Parts 172 and 173. Packaging Requirements for Liquids Toxic by Inhalation; Final Rule. Fed. Reg. Vol 50, No. 195, October 8, 1985, pp. 41092-41097. This study was conducted at Bio/dynamics, Inc., Mettlers Road, East Millstone, New Jersey 08873. All raw data, specimens, a sample of the test substance, the original study protocol and the original final report are stored in the Archives of Bio/dynamics, Inc.

II. MATERIALS AND METHODS:

A. Test Substance:	Methane sulfonyl chloride	<i>NN 1256</i>
Supplier:	Organic Chemicals Division Pennwalt Corporation Philadelphia, Pennsylvania 19102	
Lot No.:	1491	
Batch No.:	B-27-F	
Concentration:	99.6% Active Ingredient	
Description:	Liquid	
Date Received:	11 October 1985 (4 containers)	
Label Information:	CAS #124-63-0	
Expiration Date:	>1 year	
Storage:	Temperature monitored room (60-85°F).	
Analysis:	The identity, strength, purity and composition; and synthesis, fabrication, and/or derivation of the test substance have been documented by the sponsor.	

II. MATERIALS AND METHODS (cont.):

A. Test Substance (cont.):

Stability: The stability of the test substance has been determined by the sponsor.

Sampling: An archival sample of approximately 10 grams of methane sulfonyl chloride is stored in the Archives of Bio/dynamics, Inc.

Disposition: All remaining containers of the test substance will be returned to the sponsor after issuance of the final report.

B. Test Animals:

Rats

Strain: CD® (Sprague-Dawley derived)

Justification for Animal Selection: Standard laboratory animal for inhalation toxicity studies. The Sprague-Dawley rat was used due to its availability and due to the existing historical data base for comparative evaluation.

Number of Animals Placed on Test: 3 groups of 5 animals/sex/group

Supplier: Charles River Breeding Laboratories, Inc. Kingston, New York 12484

	<u>Group I</u>	<u>Group II</u>	<u>Group III</u>
Date Received:	10/22/85	4/1/86	4/8/86
Date of Birth:			
Males:	9/17/85	2/16/86	3/6/86
Females:	8/31/85	2/6/86	2/27/86
Date of Exposure:	11/1/85	4/16/86	4/24/86
Acclimation Period:	2 weeks	2 weeks	2 weeks
Age at Exposure (approx.):			
Males:	6 weeks	8 weeks	7 weeks
Females:	9 weeks	10 weeks	8 weeks

II. MATERIALS AND METHODS (cont.):

B. Test Animals (cont.):

	<u>Group I</u>	<u>Group II</u>	<u>Group III</u>
Body Weights on Day of Exposure (grams): ¹			
Males:			
Range:	202-210	217-293	225-279
Mean:	206	266	251
Females:			
Range:	200-209	215-236	202-221
Mean:	203	226	210

C. Selection and Group Assignment:

Prior to the initiation of exposure to the test substance, animals were arbitrarily selected from the Bio/dynamic's in-house population based on acceptable pretest physical examinations and body weights (S.O.P. E.3.2.12).

D. Animal Identification:

Each rat was identified after selection with a metal ear tag bearing its unique Bio/dynamics, Inc. animal number.

E. Experimental Outline:

Initially, one group (Group I) consisting of five male and five female CD® (Sprague-Dawley derived) rats was exposed to an atmosphere containing methane sulfonyl chloride for one hour at an analytical concentration of 174 parts per million (ppm). Two additional groups consisting of five male and five female CD® rats were also exposed to the test substance for one hour. All survivors were held for a 14-day post-exposure observation period. A gross necropsy examination was performed on all animals dying spontaneously during the study and on all survivors sacrificed at the end of the post-exposure observation period.

¹Represents a body weight measurement obtained immediately prior to exposure.

II. MATERIALS AND METHODS (cont.):

F. Husbandry:

During Non-Exposure
Periods:

Housing: Animals were doubly-housed in suspended stainless steel wire mesh cages during the first week of the acclimation period and individually during the remainder of the acclimation period and all other non-exposure periods.

Food: ad libitum; standard laboratory diet (Purina® Rodent Laboratory Chow #5001).² Fresh food presented as required.

Water: ad libitum; by automated watering system (Elizabethtown Water Company).

Temperature: Acceptable range: 67-76°F
Monitored and recorded twice daily; maintained within this range to the maximum extent possible.

Relative Humidity: Acceptable range: 30-70%
Monitored and recorded twice daily; maintained within this range to the maximum extent possible.

Environmental Conditions: 12 hour light/dark cycle (7 AM to 7 PM) via automatic timer.

During Exposure Periods:

Housing: Animals were individually housed in a 100 liter Plexiglas® exposure chamber.

Food: None

Water: None

Temperature: Recorded twice during exposure.³
Acceptable range: 67-76°F
Actual range: 68-75°F

Relative Humidity: Recorded twice during exposure.³
Acceptable range: 30-70%
Actual range: 46-68%

²For Group II only, Purina® Certified Rodent Chow #5002 was used during part of the acclimation period.

³Parameter not recorded for Group II only; gauge not placed in chamber prior to exposure of animals.

II. MATERIALS AND METHODS (cont.):

G Test Substance Administration
and Chamber Operation
(Figures 1-2):

Chamber Operation:

The Plexiglas® exposure chamber had a total volume of 100 liters. The chamber was operated dynamically at a calibrated airflow rate of 25 liters per minute (lpm) for Groups I and III. This flow rate was calculated to provide one complete air change every 4.0 minutes and a 99% equilibrium time of 18.4 minutes. For Group II, the chamber was operated dynamically at a calibrated airflow rate of 26.1 liters per minute (lpm). This flow rate was calculated to provide one complete air change every 3.8 minutes and a 99% equilibrium time of 17.6 minutes.

Exposure Procedure:

For exposure of Group I, approximately 15 milliliters (mls) of test substance were placed into a 30 ml bubbler fitted with an impinger. House-supply air was delivered through a 0-4 lpm Dwyer flowmeter and a Nupro metering valve into the bubbler then into a 3-neck flask containing glass wool. Additional dilution air was delivered to the same 3-neck flask via a Nupro metering valve and a 0-30 lpm Dwyer flowmeter. The resultant vapor-laden airstream was directed into the 100 liter Plexiglas® exposure chamber which housed the animals.

For exposure of Groups II and III, approximately 200 mls of test substance were placed into a 500 ml bubbler. House-supply air was delivered through a drying tube, a 0-4 lpm Dwyer flowmeter and regulated by a Nupro metering valve, into the bubbler then into a 3-neck flask containing glass wool, serving as a trap and mixing vessel. Additional dilution air was delivered to the same 3-neck flask via a 0-30 lpm Dwyer flowmeter a pressure gauge and a Nupro metering valve. The resultant vapor-laden airstream was directed into the 100 liter Plexiglas® exposure chamber which housed the animals. The initial generation airflow rate, dilution airflow rate and total airflow rate are summarized on the following page.

II. MATERIALS AND METHODS (cont.):

G. Test Substance Administration and Chamber Operation (cont.):

Group	Generation Airflow Rate (lpm)	Dilution Airflow Rate (lpm)	Total Airflow Rate (lpm)
I	4.0	21.0	25.0
II	2.1	24.0	26.1
III	4.0	21.0	25.0

H. Exposure Chamber Sampling and Monitoring:

Samples for determination of the methane sulfonyl chloride exposure level were taken using two midget impingers/bubblers (T-1 and T-2), each containing 15 mls of hexane. The two impingers were connected in tandem using glass elbow connectors. A Dwyer 0-4 lpm flowmeter was attached to the exhaust end of the impinger system and a Nupro metering valve was connected to the exhaust end of the flowmeter to adjust the airflow rate. Sample air was drawn through the system via a Neptune Dyna-pump, at an airflow rate of 1.0 lpm for 10 minutes for Group I and at an airflow rate of 1.2 lpm for 10 minutes for Groups II and III. After sample collection, the samples were poured into labelled scintillation vials; between samples, the impingers were rinsed twice with approximately 15 mls of hexane. The samples were then assayed by the Department of Metabolic and Analytical Chemistry of Bio/dynamics, Inc. (see Appendix A, pages A-2 to A-7).

Samples for particle size distribution assessment of the chamber and room air were drawn twice during the exposure from the H-1 sampling portal. Measurements were performed using a Royco Portable Particle Monitor (Model #227).

II. MATERIALS AND METHODS (cont.):

H. Exposure Chamber Sampling and Monitoring (cont.):

The nominal concentration was determined by weighing the generation apparatus containing test substance before and after the exposure and dividing the difference in these weights by the total volume of air used during exposure.

An Airguide humidity indicator was used to continuously monitor relative humidity and a Taylor thermometer was used to continuously monitor air temperature in the exposure chamber. Recordings of temperature, relative humidity and airflow rate readings were made twice during exposure.

I. In-Life Observations:

Day 1 (Day of Exposure):

All animals were observed individually, immediately prior to exposure and as a group at approximately fifteen-minute intervals during exposure. All survivors were observed individually upon removal from the chamber (half-hour after exposure was completed) and at hours two and five post-exposure. Detailed physical observations were recorded at each interval.

Days 2 through 15
(Post-exposure):

Detailed observations were recorded for survivors twice daily; viability was assessed twice daily.

J. Body Weight:⁴

Day 1 (immediately prior to exposure) and on Days 2, 3, 4, 8, 11 and 15 (just prior to sacrifice).

K. Postmortem:

A complete gross postmortem examination was performed on all animals dying spontaneously during the course of the study as well as on those animals surviving to the end of the 14-day post-exposure observation period. The gross postmortem examinations included the nasal passages, trachea, external

⁴Group I body weights were taken Day 5 rather than Day 4.

II. MATERIALS AND METHODS (cont.):

K. Postmortem (cont.):

surface, all orifices, the cranial cavity, carcass, the brain and spinal cord, the thoracic, abdominal and pelvic cavities and their viscera, and the cervical tissues and organs.

Terminal Necropsy:

Post-exposure Day 15 (see Animal Termination History for actual dates of sacrifice).

Method of Sacrifice:

Exsanguination under ether anesthesia.

III. RESULTS AND DISCUSSION:

A. Chamber Monitoring (Tables 1, 2 and 3):

The mean analytically determined vapor exposure chamber concentrations were 174, 165 and 300 ppm of methane sulfonyl chloride for Groups I, II and III, respectively. Variability of the two samples taken during each exposure was negligible for Group I, less than 8% for Group II and less than 16% for Group III (see Table 2). The differences between nominal and analytical concentrations was undoubtedly due to impaction on chamber walls; pre-exposure chamber trials without animals gave similar results.

In-chamber particle count determinations (see Table 3), suggested the presence of only very small amounts of aerosol during exposure with the majority of particles at 1.4 microns or less. It was considered that the mass of particulate during exposure was minimal and of no consequence to the results of exposure.

Chamber temperature and relative humidity measurements were considered to be within an acceptable range for Groups I and III. Data was inadvertently not available for the Group II exposure.

B. Mortality (Table 4):

Some mortality was observed at each exposure level as shown below:

<u>Group</u>	<u>Concentration</u> (ppm)	<u>Mortality</u>	
		<u>#Dead/#Exposed</u> <u>Males</u>	<u>Females</u>
II	165	1/5	0/5
I	174	1/5	1/5
III	300	5/5	5/5

All deaths occurred within three days of exposure. Data does not allow for the calculation of an LC50, but the LC50 is felt to most likely be in the range of 175 to 250 ppm for a single one-hour exposure.

III. RESULTS AND DISCUSSION (cont.):

C. Physical Observations in Test Animals:

1. During Exposure and Within 5-Hours Post-Exposure (Table 5):

Secretory and pulmonary responses as well as reduced activity were common findings during exposure and at each concentration level. In addition, most of the animals at the high level were noted to have eyes closed and a few showed evidence of prostration. The secretory and pulmonary responses continued throughout the 5-hour post-exposure period.

2. During the 14-Day Post-Exposure Observation Period (Table 6):

Survivors of the high exposure (300 ppm) showed secretory and pulmonary responses and poor condition until death intervened by the afternoon of the day following exposure. Survivors of exposure to either 165 or 174 ppm commonly showed secretory and pulmonary responses through Days 4-5; these signs became sporadic thereafter. An incidence of corneal irregularities/opacities became evident following exposure. At the end of the observation period, the incidence was 3/9 for the 165 ppm exposure and 8/8 for the 174 ppm exposure. A number of these animals showed this effect to only a minor degree (10-25% of the corneal surface). At necropsy, some of these small effects were not noted, and the one animal which died on Day 3 following exposure to 165 ppm was noted at necropsy to have this effect; therefore, a different incidence is calculated based on final gross observations than noted on gross necropsy.

D. Body Weights (Table 7):

A marked weight loss was observed following exposure for all exposure levels. Survivors generally began to regain weight after the third day, most had regained the starting body weight by the eleventh day, and all were at or in excess of their original weight by the end of the observation period.

III. RESULTS AND DISCUSSION (cont.):

E. Gross Postmortem Observations (Appendix B):

Ocular opacities were observed in seven males (Group I - 3/5 and Group II - 4/5); this was not seen in the females.

The lungs of numerous animals which died spontaneously were red (various intensities). Discoloration of the skin/fur, seen in animals which died, were primarily dark red material in the facial area (snout) and yellow material in the ano-genital area. These findings were not considered to be unusual in animals which died spontaneously and their toxicological significance, if any, cannot be determined on the basis of gross examination, only.

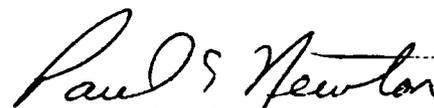
IV. CONCLUSION:

Single one-hour exposure of rats to vapors of methane sulfonyl chloride in the range of 165-300 ppm produced partial and complete mortality. The LC50 most likely is between 175 and 250 ppm. Secretory and pulmonary responses indicative of lung irritation, were commonly noted and ocular irregularities/opacities developed following exposure. Body weight losses were marked following exposure but recovery was noted for all survivors by the end of the observation period. Gross postmortem findings showed ocular opacities in seven males (4/5 exposed to 165 ppm and 3/5 exposed to 174 ppm); this was not seen in the females.

The lungs of numerous animals which died spontaneously were red (various intensities). Discoloration of the skin/fur, seen in animals which died, were primarily dark red material in the facial area (snout) and yellow material in the ano-genital area. These findings were not considered to be unusual in animals which died spontaneously and their toxicological significance, if any, cannot be determined on the basis of gross examination, only.



William E. Rinehart, Sc.D.
Study Director 10/8/86
Date



Paul E. Newton, Ph.D., D.A.B.T.
Director of Inhalation Toxicology 10/8/86
Date

S-1258

S-1258



REPORT

METHANE SULFONYL CHLORIDE
ACUTE INHALATION TOXICITY
IN RATS
4-HOUR EXPOSURE

HUNTINGDON RESEARCH CENTRE

Huntingdon England

Filed: MN 1256-85
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copy 1 of 2

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PWT 45/861670

Pennwalt Corporation
Technical Division
Safety, Health & Environmental Affairs
Rec'd 3/2/87
Noted _____ Date _____
File _____

12585

METHANE SULFONYL CHLORIDE
ACUTE INHALATION TOXICITY
IN RATS
4-HOUR EXPOSURE

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We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

C. Hardy.

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HRC REPORT No. PWT 45/861670

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

To the best of my knowledge and belief the study described in this report was conducted in compliance with the following Good Laboratory Practice Standards:

United States Environmental Protection Agency,
Title 40 Code of Federal Regulations Part 792,
Federal Register, 29 November 1983

Organization for Economic Co-operation and Development
ISBN 92-64-12367-9, Paris 1982

C. Hardy
C.J. Hardy, B.Sc., Ph.D., M.I.Biol., Dip.R.C.Path.,
Study Director

2/4/86
Date

QUALITY ASSURANCE STATEMENT

Certain studies of short duration, such as that described in this report, are conducted at HRC in a setting which involves frequent repetition of similar or identical procedures. At or about the time the study described in this report was in progress, 'process-based' inspections were made by the Quality Assurance Unit of critical procedures relevant to this study type. For the inspection of any given procedure, at least one study was selected without bias. The findings of these inspections were reported promptly to the Study Director and to HRC management.

This report has been audited by the HRC Quality Assurance Unit. It is considered to be an accurate presentation of the procedures and practices employed during the course of the study and an accurate presentation of the findings.

P. Richold
Peter H.C.V. Richold, B.Sc.,
Systems Compliance Auditor,
Quality Assurance Unit.

18-2-87
Date

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PWT/45

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APPENDICES

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Test substance: METHANE SULFONYL CHLORIDE.

Test animals: Albino rats, (Sprague-Dawley).

Route of administration: By inhalation of test atmospheres containing vapour of the test substance. Each test group was subjected to a single 4-hour continuous whole-body exposure.

Observation period: 14 days post exposure.

Results

Atmosphere concentrations and mortality:

Group	Exposure level (mg/m ³)	Number dead/ number exposed		
		Males	Females	Total
1	Control	0/5	0/5	0/10
2	95.5	1/5	0/5	1/10
3	251.6	5/5	5/5	10/10
4	130.6	4/5	5/5	9/10

Clinical signs:

(a) During exposure:

Signs observed in rats exposed to METHANE SULFONYL CHLORIDE, included closing or partial closing of the eyes, wet fur around mouth, disturbances of the respiratory pattern and adoption of a hunched body posture.

(b) During the observation period:

Signs observed in a proportion of test rats following exposure to METHANE SULFONYL CHLORIDE included lethargy and disturbances of the respiratory pattern. Signs indicative of an effect on the respiratory tract persisted for several days in rats that survived exposure.

Bodyweight: The rats that survived exposure to METHANE SULFONYL CHLORIDE lost weight or gained at a reduced rate for up to 2 days following exposure. Subsequently the rate of bodyweight gain was similar to that of the control rats.

Food and water consumption: In groups with rats surviving to Day 14 following exposure there was a marked to moderate reduction in food consumption for up to 1-2 days and slightly reduced consumption for 2-3 further days.

There was a marked to moderate reduction in water consumption for up to 4 days in groups exposed at 95.5 or 13.06 mg/m³.

Lung weight to bodyweight ratio: The ratio was higher than normal for most decedent rats.

Estimate of the LC₅₀ (4-hour) for METHANE SULFONYL CHLORIDE: 117.7 mg/m³ of air (25 ppm) (standard error 12.7 mg/m³)

Macroscopic pathology: Lung congestion and damage to the corneal surface of the eyes seen in a high proportion of the decedents were considered to be treatment- related findings.



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CHEMICALS • EQUIPMENT • HEALTH PRODUCTS

SAFETY, HEALTH & ENVIRONMENTAL AFFAIRS

SUMMARY OF TOXICOLOGY STUDY

PERFORMED FOR ORGANIC CHEMICALS DIVISION

<u>Test Material:</u>	Methane Sulfonyl Chloride
<u>Product Code:</u>	W-1491
<u>Study Type:</u> On Microfilm	Acute (4-hour) inhalation toxicity in the rat
<u>Testing Laboratory:</u>	Huntingdon Research Centre Huntingdon Cambs PE18 6ES, England
<u>Summary of Results:</u>	The LC50 was 25 ppm. Marked irritation of the eyes/nose/lungs was noted. Corneal opacities were noted at necropsy of decedants. Body weight increments, food intake and water intake were decreased for up to five days.
<u>Storage:</u>	A report is filed in the Technical Records Center at King of Prussia under Master No. 1256.
<u>Information for MSDS:</u>	LC50 = 25 ppm (4-hour, rat)-- marked irritation of the eyes/nose/lungs

S-1258

Signatures

Joseph F. Jadlocki, Jr.
Manager, Product Safety

3-20-87
Date

Joel A. Seckar, Ph.D.
Manager, Toxicology

3-19-87
Date

The acute inhalation toxicity of METHANE SULFONYL CHLORIDE was assessed by exposing groups of rats, each for a period of 4 hours, to atmospheres containing vapour of the test substance.

The study was conducted at the Huntingdon Research Centre during the period August - September 1986.

On completion of the study all data relating to the study, all preserved tissues and a copy of this report were lodged in the Huntingdon Research Centre Archives, Huntingdon, Cambridgeshire, England.

Other dates were:

Protocol Approval by Study Director: 3 February 1986.

Protocol Approval by HRC Management: 31 January 1986.

Protocol Approval by Sponsor: 26 February 1986.

Test substance

The test substance was a clear colourless liquid supplied by the Sponsor, in a container labelled:

METHANE SULFONYL CHLORIDE D-3-G

The test substance was received on 13 May 1986 and was stored at room temperature in a closed container.

The purity was stated to be >95% and the material was sufficiently stable for use in this study.

The test substance is referred to as METHANE SULFONYL CHLORIDE in this report.

Animals and maintenance

Forty (20 male and 20 female) albino rats (Sprague-Dawley CD strain), 6-8 weeks old, were obtained in one consignment from Charles River U.K. Limited, Manston Road, Margate, Kent, England, on 27 August 1986.

The rats were ordered so that they would be approximately 200 g weight on the day of exposure. The rats were allocated to one of 4 groups and each rat was identified by a number tattooed on the ears.

The rats were caged 5 males or 5 females to a cage and acclimatised to laboratory conditions for at least 5 days before exposure.

The cages were made of polypropylene (size 38 cm x 56 cm x 18 cm height) and had detachable wire mesh tops and floors. The cages were suspended on a movable rack. While in their cages all rats had free access to a measured amount of food (Labsure LAD 1) and tap water. Food and water supplies were analysed routinely to determine the levels of chemical or microbiological contaminants.

The rats remained in a holding room except for the 4-hour exposure and an overnight post exposure period when the rats were kept in a ventilated cabinet to allow dispersal of any residual test substance.

The mean maximum and minimum temperatures of the holding room during the periods of the study are shown below:

Dates	Groups	Mean maximum temperature °C	Mean minimum temperature °C	Relative humidity %
27.8.86 - 18.9.86	1-4	24.9(0.99)	20.3(1.04)	52(6.6)

The numbers in parentheses are standard deviations of the mean

Inhalation exposures

Three groups of 10 rats (5 male and 5 female), were exposed continuously for 4 hours to test atmospheres containing vapour of the test substance.

A group of 10 rats (5 male and 5 female) was exposed to air alone using the same type of exposure system.

The groups were exposed on the following dates:

Group	Date of exposure
1 (Control)	2 September 1986
2 (Test)	2 September 1986
3 (Test)	3 September 1986
4 (Test)	4 September 1986

Exposure systemVapour generator

The vapour generator, shown in Figure 1, was designed to produce and maintain an atmosphere containing vapour of the test substance only. All parts of the generator in contact with the test substance were made of glass.

The test substance was supplied to the generator from a syringe driven at a constant rate by a syringe pump. The compressed air supply to the generator was dried, filtered and oil-free.

Exposure chambers

The whole-body exposure chambers used for the exposures were of square section and were fitted with pyramidal tops. The chambers were made of perspex and had an internal volume of approximately 115 litres. Each chamber was divided by wire mesh partitions to provide 10 separate animal compartments.

The test atmosphere entered through a port at the base centre of the chamber and passed out through small holes in the lower edge of the square section. Each chamber was positioned inside a large cabinet equipped with an extract fan exhausting to atmosphere through a collection filter.

Procedure

A supply of clean dried air was connected to the vapour generator and a supply pressure was adjusted to give a flow rate of 25 litres per minute measured at the generator outlet tube. An in-line flow meter was used to monitor air flow throughout the exposure. The water bath around the generator was maintained at 50°C using a thermostatically controlled thermostirrer. The temperature of the water was recorded at 30 minute intervals.

A syringe filled with METHANE SULFONYL CHLORIDE was fitted to the syringe pump and connected to the generator with PTFE tubing.

The rats to be exposed were placed into separate compartments of the exposure chamber.

The syringe pump was switched on and the exposure timed for 4 hours, following a 10.5 minute¹ equilibration period. (¹) An appropriate feed rate, as determined in preliminary experiments, was selected.

After 4 hours the supply of test substance was discontinued and the exposure chamber was allowed to clear before the rats were removed for examination.

The procedure was repeated, with appropriate flow rates of METHANE SULFONYL CHLORIDE, for each of the other test groups.

The control group was treated similarly but exposed to air only.

Following exposure the rats were returned to the holding cages and food and water supplies were restored. The rats were kept in a ventilated cabinet overnight and then returned to the holding room for the remainder of the observation period.

Chamber atmosphere analyses

Five air samples were taken from the chamber during each exposure and analysed to determine the concentration of METHANE SULFONYL CHLORIDE in the chamber atmosphere. The method of analysis for METHANE SULFONYL CHLORIDE is described in Appendix 1.

The samples were drawn through 2 sintered glass bubblers, connected in series and containing hexane as the trapping agent, at 2 litres per minute. The volume of the air samples was measured with a wet-type gas meter.

The test atmosphere was monitored for the presence of droplets of the test substance by withdrawing samples into a Royco model 218 optical particle analyser at 1 hour 30 minutes and 3 hour 30 minutes into each exposure.

¹ 10.5 minutes is the theoretical time required for the concentration of aerosol in the chamber to reach 90% of its final value under the conditions of exposure employed

Chamber temperature

The temperature in each exposure chamber was measured with a mercury bulb thermometer and recorded at the start of the exposure and at 30-minute intervals during the exposure.

ObservationsClinical signs

The rats were observed continuously during exposure for signs of reaction to the test substance and at least twice daily during the observation period.

Bodyweight

All rats were weighed daily from the day of delivery to the Huntingdon Research Centre until the end of the observation period.

Terminal studies

At the end of the 14-day observation period the surviving rats were anaesthetised by intraperitoneal injection of pentobarbitone sodium and killed by exsanguination.

All rats dying as a result of exposure or killed at termination were subjected to a detailed macroscopic examination. The lungs were removed dissected clear of surrounding tissue and weighed in order to calculate the lung weight to bodyweight ratio.

The lungs were infused with and preserved in buffered 10% formalin together with samples of the liver and kidneys and stored for possible future microscopic examination.

Estimation of the LC₅₀ (4-hour) and standard error

The concentration of METHANE SULFONYL CHLORIDE likely to cause death in 50% of exposed rats within 14 days following a single 4-hour exposure was calculated by the log probit method of Miller and Tainter¹.

The standard error was calculated from the formula:

$$SE \text{ of } LC_{50} = \frac{2s}{\sqrt{2N}}$$

where 2s is the estimated increment in concentrations of the test substance between probits 4.0 and 6.0 corresponding to 16% and 84% mortality and N is the total number of rats in groups with mortality between 6.7% and 93.3% (Probits 3.5 and 6.5).

¹ Miller, L.C. and Tainter, M.L. Proc. Soc. Exp. Biol. Med. 57, (2), 1944, pp 261-264.

CHAMBER ATMOSPHERE CONDITIONSConcentrations of METHANE SULFONYL CHLORIDE

The analytical results for each air sample are shown in Table 1.

The mean concentrations determined for each exposure and the variations in concentration (range of values x 100/mean value) during the exposures were:

Group	Concentration (mg/m ³)	Variation
2	95.5	15.4%
3	251.6	22.7%
4	130.6	16.5%

The variations were within acceptable limits.

Particle size analysis

In all samples the particle count was less than that for the control chamber indicating that the test substance was all present as vapour.

Chamber air temperature

The means and standard deviation (SD) of the means for the temperatures recorded during the exposure of the groups were:

Group	Mean (°C)	SD
1 (Control)	28.1	1.17
2 (95.5 mg/m ³)	29.9	0.91
3 (251.6 mg/m ³)	27.9	1.10
4 (130.6 mg/m ³)	28.5	0.78

The differences in temperature were negligible.

CLINICAL OBSERVATIONSMortality

The mortality is summarised in the following table:

Group	Concentration of METHANE SULFONYL CHLORIDE (mg/m ³)	Mortality		
		♂	♀	Total
1	Control	0/5	0/5	0/10
2	95.5	1/5	0/5	1/10
3	251.6	5/5	5/5	10/10
4	130.6	4/5	5/5	9/10

In Group 2 (95.5 mg/m³) 1 male rat (63) died during exposure.

In Group 3 (251.6 mg/m³) 1 male rat (71) and 1 female rat (78) died during exposure; 4 male rats (72, 73, 74, 75) and 1 female rat (80) were found dead on the morning of Day 1 and 3 female rats (76, 77, 79) died on Day 1.

In Group 4 (130.6 mg/m³) 1 female rat (86) died during exposure; 2 male rats (83, 84) and 3 female rats (88, 89, 90) were found dead on the morning of Day 1, and 2 male rats (82, 85) and 1 female rat (87) died on Day 1.

Clinical signs(a) During the exposure

The incidence of clinical signs seen during the exposures to METHANE SULFONYL CHLORIDE is shown in Table 2. Column 0 of this table shows the observations made during the 10.5 minute equilibration period.

Closing or partial closing of the eyes, wet fur around the mouth (probably due to salivation), disturbances to the respiratory pattern and adoption of a hunched body posture were seen in rats exposed to METHANE SULFONYL CHLORIDE. These signs were considered to be consistent with exposure to an irritant vapour.

(b) During the observation period

The incidence of clinical signs seen during the observation period is shown in Table 3. Column 0 of this table shows the observations made when the rats were removed from the exposure chambers. Clinical signs evident at this time in rats exposed to METHANE SULFONYL CHLORIDE included lethargy and disturbances to the respiratory pattern.

Signs indicative of an effect of the test substance on the respiratory tract persisted for several days in rats that survived exposure to METHANE SULFONYL CHLORIDE

Bodyweight

The individual and group mean bodyweights are shown in Table 4.

The decedent rats lost bodyweight before death. The rats that survived the effects of exposure to METHANE SULFONYL CHLORIDE lost weight or gained at a reduced rate for up to 2 days following exposure. Subsequently the rate of bodyweight gain was similar to or exceeded that of the control rats.

Food and water consumption

The food and water consumption data are given in Tables 5 and 6 respectively.

Little or no food was consumed following exposure to METHANE SULFONYL CHLORIDE at 251.6 mg/m³ prior to death of all rats by Day 1. In other rats exposed to METHANE SULFONYL CHLORIDE a marked to moderate reduction in food consumption was observed over a period of 1-2 days. The food consumption for exposed groups was subsequently slightly reduced for 2-3 further days.

Little water was consumed following exposure to METHANE SULFONYL CHLORIDE at 251.6 mg/m³ prior to death of all rats by Day 1. In the other exposed groups there were marked to moderate reductions in water consumption for up to 4 days following exposure.

TERMINAL STUDIESLung weight to bodyweight ratio

The lung weight to bodyweight ratios for individual rats are presented in Table 7.

The ratio was higher than normal for most decedent rats and within normal limits for other rats.

Estimation of the LC₅₀ (4-hour) for METHANE SULFONYL CHLORIDE

From the mortality data for Groups 2, 3 and 4 the LC₅₀ (4-hour) for METHANE SULFONYL CHLORIDE was established at:

117.7 mg per m³ of air (25 ppm)

The standard error of the estimate was 12.7 mg/m³.

Macroscopic pathology

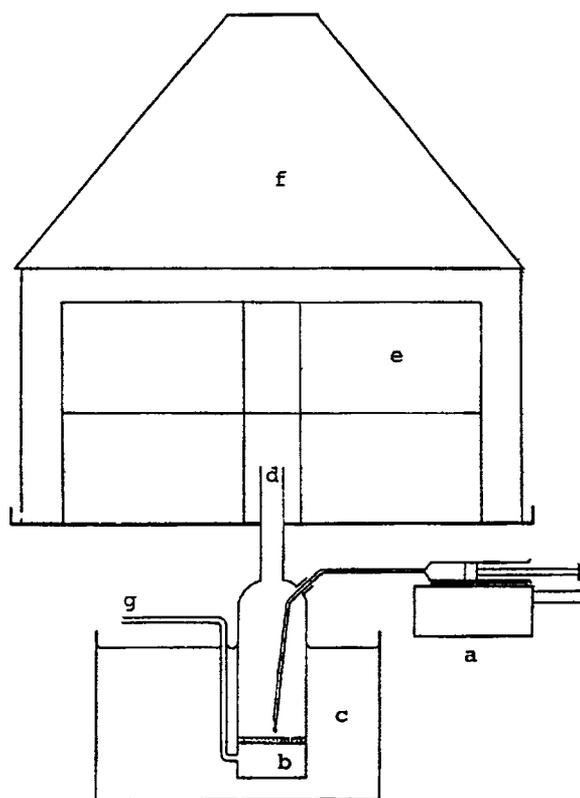
The macroscopic findings for individual rats are presented in Appendix 2.

The lungs of a high proportion of the rats that died as a result of exposure were congested. This was considered to be a treatment-related finding.

The eyes of 7 decedent rats had an opaque appearance and the eyes of 2 decedent rats had an apparently rough corneal surface. This was considered to be a treatment-related finding. The stomachs of several decedent rats were gas-filled. This was considered to be a secondary effect of the damage to the respiratory tract and was probably due to swallowing of air during attempts to breathe.

FIGURE 1
Exposure system

PWT/45



- a Syringe pump
- b Sintered glass disc
- c Water bath (50°C)
- d Vapour entry tube
- e Exposure holding cage
- f Exposure chamber
- g Air in (25 lpm)

TABLE 1

PWT/45

Concentration of METHANE SULFONYL CHLORIDE in air

Group	Sample	Time taken		METHANE SULFONYL CHLORIDE in air mg/m ³
		h	min	
2	2.1	0	30	93.3
	2.2	1	00	92.1
	2.3	2	00	95.5
	2.4	3	00	90.9
	2.5	3	50	105.6
				Mean 95.5
				*Variation 15.4%
3	3.1	0	30	291.8
	3.2	1	00	236.4
	3.3	2	00	250.4
	3.4	3	00	234.6
	3.5	3	50	245.0
				Mean 251.6
				Variation 22.7%
4	4.1	0	30	142.9
	4.2	1	00	134.8
	4.3	2	00	128.0
	4.4	3	00	125.9
	4.5	3	50	121.4
				Mean 130.6
				Variation 16.5%

* $\frac{\text{Range} \times 100}{\text{Mean}}$

TABLE 2

PWT/45

Clinical signs during exposure

Group	Signs	Number showing signs						
		Time in hours						
		E	0.25	0.5	1.0	2.0	3.0	4.0
1♂ (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5
1♀ (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5
2♂ (95.5 mg/m ³)	Eyes partially closed	5	5	5	5	5	5	4
	Wet around mouth		5	5	5	5	5	4
	Slow respiratory pattern	5	5	5	5	5	5	4
	Irregular respiratory pattern		5	5	5	5	5	
	Mouth opening upon inhalation					2	2	4
	Hunched posture		5	5	5	5	5	4
	Dead (Total)							1
2♀ (95.5 mg/m ³)	Eyes partially closed	5	5	5	5	5	5	5
	Wet around mouth		5	5	5	5	5	5
	Slow respiratory pattern	5	5	5	5	5	5	5
	Irregular respiratory pattern		5	5	5	5	5	2
	Mouth opening upon inhalation					2	2	5
	Hunched posture		5	5	5	5	5	5

E During the equilibration period

TABLE 2

PWT/45

(Clinical signs - continued)

Group	Signs	Number showing signs						
		Time in hours						
		E	0.25	0.5	1.0	2.0	3.0	4.0
3♂ (251.6 mg/m ³)	Wet around the eyes		5	5	5	5	5	4
	Eyes partially closed	5	5	5	5	5	5	4
	Wet around the mouth		5	5	5	5	5	4
	Slow respiratory pattern	5	5	5	5	5	5	4
	Irregular respiratory pattern		5	5	5	5	5	4
	Mouth opening upon inhalation			1	2	5	5	4
	Hunched posture	4	5	5	5	5	5	4
	Restless behaviour	1						
	Dead (Total)							1
3♀ (251.6 mg/m ³)	Wet around the eyes			5	5	5	5	4
	Eyes partially closed	5	5	5	5	5	5	4
	Wet around the mouth		5	5	5	5	5	4
	Slow respiratory pattern	5	5	5	5	5	5	4
	Irregular respiratory pattern		5	5	5	5	5	4
	Mouth opening upon inhalation	1		2	2	5	5	4
	Hunched posture	4	5	5	5	5	5	4
	Dead (Total)							1

E During the equilibration period

TABLE 2

PWT/45

(Clinical signs - continued)

Group	Signs	Number showing signs						
		Time in hours						
		E	0.25	0.5	1.0	2.0	3.0	4.0
4 [♂] (130.6 mg/m ³)	Wet around the eyes		5	5	5	5	5	5
	Eyes partially closed	5	5	5	5	5	5	5
	Wet around the mouth					5	5	5
	Slow respiratory pattern	5	5	5	5	5	5	5
	Irregular respiratory pattern		5	5	4	3	3	5
	Mouth opening upon inhalation				1	2	2	5
	Hunched posture		3	5	5	5	5	5
4 [♀] (130.6 mg/m ³)	Wet around the eyes		5	5	5	5	4	4
	Eyes partially closed	5	5	5	5	5	4	4
	Wet around the mouth					5	4	4
	Slow respiratory pattern	5	5	5	5	5	4	4
	Irregular respiratory pattern		5	5	4	3	2	4
	Mouth opening upon inhalation				1	2	2	4
	Hunched posture		3	5	5	5	4	4
	Dead (Total)						1	1

E During the equilibration period

TABLE 3
Clinical signs during observation period

Group	Signs	Number showing signs														
		Day of observation period														
		0+	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 ^o (Control)	Normal appearance and behaviour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Normal appearance and behaviour	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Normal appearance and behaviour			3		4	4	4	4	4	4	4	4	4	4	4
2 ^o (95.5 mg/m ³)	Lethargic	4	4		4											
	Brown staining around eyes		1	1												
	Slow and irregular respiratory pattern	4	4													
2 ^o (95.5 mg/m ³)	Dead (Total)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Normal appearance and behaviour		3	3	3	4	4	4	5	5	5	5	5	5	5	5
	Brown staining around snout and/or jaws	1														
	Hair loss from body					1	1									
	Slow and irregular respiratory pattern	5	5													
	Noisy respiration	1	2	2	1	1	1									
	Lethargic	5	5													

+ After exposure on the day of exposure

TABLE 3
(Clinical signs - continued)

Group	Signs	Number showing signs														
		Day of observation period														
		0+	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3♂ (251.6 mg/m ³)	Wet fur around jaws	4														
	Slow respiratory pattern	4														
	Exaggerated respiratory pattern	4														
	Noisy respiration	4														
	Lethargic	4														
	Dead (Total)	1	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Brown staining around snout and/or jaws		3													
3♀ (251.6 mg/m ³)	Wet fur around jaws	4														
	Slow respiratory pattern	4	3													
	Exaggerated respiratory pattern	4	3													
	Gasping		3													
	Noisy respiration	4	3													
	Lethargic	4	3													
	Dead (Total)	1	2	5	5	5	5	5	5	5	5	5	5	5	5	5

+ After exposure on day of exposure

TABLE 4
Individual and group mean bodyweight (g)

Group	Rat no.	Pre-exposure										Day of observation													
		-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14				
1 ^o (Control)	51	156	169	179	182	191	201	206	214	224	230	236	243	250	258	262	267	275	284	290	295				
	52	157	167	178	187	196	200	207	217	226	234	242	246	252	256	263	269	277	282	287	292				
	53	152	164	176	178	188	196	197	206	216	217	226	228	234	236	244	244	249	254	260	264				
	54	161	170	180	189	198	206	210	215	225	236	242	250	257	261	269	272	281	283	288	292				
	55	157	167	176	181	192	200	207	217	219	236	244	248	258	260	266	272	278	282	289	290				
	Mean	157	167	178	183	193	201	205	214	222	231	238	243	250	253	261	263	272	277	283	287				
1 ^o (Control)	56	184	189	194	190	194	192	195	195	200	204	207	212	212	212	214	214	218	212	218	218				
	57	181	186	192	190	193	204	197	204	206	212	208	217	220	220	222	228	232	233	230	232				
	58	173	169	178	185	190	191	192	204	208	208	218	219	222	222	222	228	232	224	230	238				
	59	179	184	190	191	196	203	204	207	209	214	212	217	223	223	225	225	231	230	232	231				
	60	173	181	186	188	192	192	194	197	200	203	204	209	207	206	210	210	210	204	220	221				
	Mean	178	182	188	189	193	196	196	201	205	208	210	215	217	217	219	221	225	221	226	228				

TABLE 4
(Bodyweight - continued)

Group	Rat no.	Pre-exposure											Day of observation													
		-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14					
2 ^a (95.5 mg/m ³)	61	153	157	162	171	181	188	168	167	182	188	196	206	212	220	226	230	238	246	248	252					
	62	160	164	175	185	194	200	181	176	184	205	212	223	229	232	240	246	252	260	262	265					
	63	150	159	169	179	188	200	DEAD																		
	64	156	162	170	177	186	192	167	154	174	186	190	201	210	216	217	222	230	238	240	244					
	65	156	166	174	183	194	200	181	182	196	203	213	222	230	237	244	250	258	264	265	271					
	Mean	155	162	170	179	189	196	174	170	184	196	203	213	220	226	232	237	245	252	244	258					
2 ^b (95.5 mg/m ³)	66	176	180	176	190	194	200	172	159	179	185	196	204	206	207	215	218	220	222	226	228					
	67	167	173	177	187	190	200	179	192	197	206	211	217	218	220	227	230	234	236	242	247					
	68	174	172	176	179	186	191	172	174	182	186	186	189	196	196	200	198	202	209	205	206					
	69	166	167	174	177	173	180	173	174	174	177	184	188	188	190	190	191	194	194	199	199	201				
	70	172	176	177	183	189	192	180	180	180	191	194	196	199	202	204	204	211	212	215	213	216				
	Mean	171	174	176	183	186	193	175	176	185	190	195	199	202	203	207	210	212	216	217	220					

TABLE 4
(Bodyweight - continued)

Group	Rat no.	Pre-exposure					Day of observation														
		-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3 ^d (251.6 mg/m ³)	71	153	164	177	188	195	198	DEAD													
	72	156	166	177	186	195	197	DEAD													
	73	154	162	174	185	192	198	DEAD													
	74	166	174	183	190	200	208	DEAD													
	75	159	169	181	190	200	205	DEAD													
	Mean	158	167	178	188	196	201														
3 ^g (251.6 mg/m ³)	76	172	180	187	190	200	202	176	DEAD												
	77	187	189	195	199	201	204	186	DEAD												
	78	178	180	181	192	195	195	DEAD													
	79	175	183	190	193	198	198	180	DEAD												
	80	179	184	192	195	200	194	DEAD													
	Mean	178	183	189	194	199	199	181													

TABLE 4
(Bodyweight - continued)

Group	Rat no.	Pre-exposure										Day of observation													
		-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14				
4 [♂] (130.6 mg/m ³)	81	165	172	181	189	198	200	170	163	165	172	182	188	194	206	215	223	230	239	244	252				
	82	162	170	180	190	200	202	176	DEAD																
	83	157	167	176	186	195	197	DEAD																	
	84	164	175	179	190	199	204	DEAD																	
	85	161	174	183	188	187	204	174	DEAD																
	Mean	162	172	180	189	196	201	173	163	165	172	182	188	194	206	215	223	230	239	244	252				
4 [♀] (130.6 mg/m ³)	86	180	184	188	197	200	196	DEAD																	
	87	170	174	180	185	190	191	166	DEAD																
	88	194	198	202	204	207	209	DEAD																	
	89	171	172	176	183	188	188	DEAD																	
	90	182	187	190	200	204	202	DEAD																	
	Mean	179	183	187	194	198	197	166																	

TABLE 5
Group mean daily food consumption (g/rat)

Group	Exposure level mg/m ³	Pre-exposure					Post-exposure													
		-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 ^a	Control	19	21	20	21	20	21	22	23	23	24	23	25	24	25	24	25	23	23	24
2 ^a	95.5	18	19	20	20	20	5	8	17	19	22	22	24	24	20	21	18	18	23	
3 ^a	251.6	20	19	21	22	21	0	0	0	0	0	0	0	0	0	0	0	0	0	
4 ^a	130.6	21	20	20	21	22	0	2	14	11	18	18	22	28	22	24	26	26	22	
1 ^b	Control	19	19	18	17	16	16	19	17	18	20	20	19	18	19	21	17	19	19	
2 ^b	95.5	17	17	18	17	17	6	9	14	15	19	18	19	18	20	21	17	19		
3 ^b	251.6	20	21	19	19	18	1	0	0	0	0	0	0	0	0	0	0	0		
4 ^b	130.6	18	19	18	17	17	17	0	0	0	0	0	0	0	0	0	0	0		

TABLE 6
Group mean daily water consumption (g/rat)

Group	Exposure level mg/m ³	Pre-exposure					Post-exposure													
		-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1♂	Control	24	25	24	26	25	27	26	27	28	27	28	28	29	28	29	29	32	30	28
2♂	95.5	21	23	22	22	23	5	11	28	27	28	26	24	28	27	27	25	27	26	26
3♂	251.6	24	21	24	23	26	0													
4♂	130.6	23	24	25	26	24	1	14	19	21	28	28	30	32	28	27	32	27	29	29
1♀	Control	20	24	22	21	23	22	24	23	23	24	24	24	26	25	26	22	24	25	25
2♀	95.5	22	23	25	24	25	9	16	27	28	28	27	24	25	27	26	23	26	26	26
3♀	251.6	26	24	26	25	26	4													
4♀	130.6	23	23	25	24	21	2													

TABLE 7

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Lung weight to bodyweight ratios

Group	Animal	Lung weight (g)	Bodyweight (g)	Lung to bodyweight ratio (LW/BW) x 100	
				Survivors	Decedents
1♂ (Control)	51	1.24	295	0.42	
	52	1.33	292	0.46	
	53	1.29	264	0.49	
	54	1.34	292	0.46	
	55	1.18	290	0.41	
			Mean	0.45	
			SD	0.033	
1♀ (Control)	56	1.24	218	0.57	
	57	1.19	232	0.51	
	58	1.28	238	0.54	
	59	1.28	231	0.55	
	60	1.17	221	0.53	
			Mean	0.54	
			SD	0.022	
2♂ (95.5 mg/m ³)	61	1.11	252	0.44	0.65
	62	1.15	265	0.43	
	63	1.29	200		
	64	1.18	244	0.48	
	65	1.31	271	0.48	
			Mean	0.46	
			SD	0.026	
2♀ (95.5 mg/m ³)	66	1.25	228	0.55	
	67	1.12	247	0.45	
	68	1.23	206	0.60	
	69	1.16	201	0.58	
	70	1.08	216	0.50	
			Mean	0.54	
			SD	0.061	

SD Standard deviation

TABLE 7

PWT/45

(Lung weight to bodyweight ratios - continued)

Group	Animal	Lung weight (g)	Bodyweight (g)	Lung to bodyweight ratio (LW/BW) x 100	
				Survivors	Decedents
3♂ (251.6 mg/m ³)	71	1.08	198		0.55
	72	1.48	197		0.75
	73	2.19	198		1.11
	74	1.57	208		0.75
	75	1.52	205		0.74
			Mean		0.78
			SD		0.203
3♀ (251.6 mg/m ³)	76	2.38	176		1.35
	77	2.37	186		1.27
	78	1.33	195		0.68
	79	1.87	180		1.04
	80	1.57	194		0.81
			Mean		1.03
			SD		0.288
4♂ (130.6 mg/m ³)	81	1.52	252	0.60	
	82	1.61	176		0.91
	83	1.60	197		0.81
	84	2.05	204		1.00
	85	1.18	174		0.68
			Mean		0.85
			SD		0.137
4♀ (130.6 mg/m ³)	86	1.73	196		0.88
	87	1.44	166		0.87
	88	1.99	209		0.95
	89	2.12	188		1.13
	90	2.28	202		1.13
			Mean		0.99
			SD		0.130

SD Standard deviation

Method of analysis for METHANE SULFONYL CHLORIDE

1. Instrumentation and apparatus

Gas chromatograph: Pye Unicam Series 304 with S8 autojector.

Integrator: Pye Unicam CDP4 computing integrator.

Apparatus: Volumetric flasks and pipettes.
Glass extraction columns.

2. Reagents

Hexane: 'Distol' grade Fisons.

METHANE SULFONYL CHLORIDE: Supplied by Sponsor.

3. Preparation of sample solutions

The contents of the bubblers were transferred to volumetric flasks and diluted to volume with hexane.

4. Gas chromatography4.1. GLC operating conditions

Column: 0.5 m x 3 mm i.d. pyrex glass packed with 1.5% OV 17 + 1.95% QF1.

Temperatures: Column - 60°C
Injector - 75°C
Detector - 120°C

Gasses: Helium 30 ml/minute
Hydrogen, 33 ml/minute
Air 300 ml/minute

Retention time for METHANE SULFONYL CHLORIDE: Typically 2.2 minutes.

(Method of analysis - continued)

4.2. Analysis of samples

A 3 µl aliquot of each sample solution was injected onto the GLC column using the autojector. The amount of METHANE SULFONYL CHLORIDE collected in the second bubbler in series was negligible or below the limits of detection, therefore concentration data are calculated from the figures for the first trap only. The concentration of METHANE SULFONYL CHLORIDE in the sample solutions was evaluated from the expression:

$$C_x = \frac{A_x}{A_s}$$

Where C_x = concentration of METHANE SULFONYL CHLORIDE in aliquot (mg/ml)

A_x = peak area due to METHANE SULFONYL CHLORIDE

A_s = response factor (area/unit concentration) for METHANE SULFONYL CHLORIDE

4.3. Standardisation

Approximately 50 mg of METHANE SULFONYL CHLORIDE was accurately weighed into a 50 ml volumetric flask, dissolved in hexane and diluted to volume with hexane. The solution was diluted to obtain standard solutions containing METHANE SULFONYL CHLORIDE at concentrations within the range of 0.1 and 0.5 mg/ml. Aliquots of the standard solutions were injected and the mean peak areas for METHANE SULFONYL CHLORIDE were calculated for each standard concentration. The mean response factor (A_s) for METHANE SULFONYL CHLORIDE was calculated from the mean peak areas by regression analysis.

Macroscopic pathology

Group	Rat	Region/organ affected	Observation
1♂ (Control)	51		No abnormalities detected (NAD)
	52		NAD
	53		NAD
	54		NAD
	55		NAD
1♀ (Control)	56		NAD
	57		NAD
	58		NAD
	59	Lungs	Right anterior and right mid-lobes congested
	60		NAD
2♂ (95.5 mg/m ³)	61		NAD
	62		NAD
	63x	External appearance	Minimal brown staining around snout and jaws
		Lungs	Congested
	64		NAD
2♀ (95.5 mg/m ³)	65		NAD
	66		NAD
	67		NAD
	68		NAD
	69		NAD
3♂ (251.6 mg/m ³)	70		NAD
	71x	External appearance	Wet around the mouth
		Eyes	Opaque
		Lungs	Right anterior lobe congested
	72x	Lungs	Congested
	73x	External appearance	Clear fluid discharge from nostrils
		Eyes	Opaque
	Lungs	Congested; pale red fluid in trachea	
74x	External appearance	Dark red fluid in nostrils	
	Lungs	Congested; a few red depressed areas (up to 3 x 4 mm)	
75x	Lungs	Congested; red fluid in trachea	

x Decedents

APPENDIX 2

PWT/45

(Macroscopic pathology - continued)

Group	Rat	Region/organ affected	Observation
3 [♀] (251.6 mg/m ³)	76x	External appearance	Clear discharge from snout
		Eyes	Opaque
		Stomach	Distended with gas
	77x	Lungs	Congested
		External appearance	Clear discharge from snout
	78x	Stomach	Distended with gas
		Lungs	Congested
	79x	Eyes	Opaque
		Lungs	Congested
	80x	Stomach	Gas-filled
Lungs		Congested	
4 [♂] (130.6 mg/m ³)	81	Eyes	Opaque
		Lungs	Congested
	82x	External appearance	Pale; area of hepatisation (5 x 4 mm) on right posterior lobe
		Stomach	Red staining around snout
	83x	Lungs	Distended with gas
		External appearance	Congested
	84x	Stomach	Red staining around jaws
Lungs		Gas-filled	
85x	External appearance	Congested; pale red fluid in trachea	
	Stomach	Gas-filled	
85x	Lungs	Congested; pale red fluid in trachea	
	External appearance	Red staining around snout	
	Eyes	Surface of right eye appeared rough	
		Stomach	Distended with gas
		Lungs	Congested

x Decedents

(Macroscopic pathology - continued)

Group	Rat	Region/organ affected	Observation
4 σ (130.6 mg/m ³)	86x	External appearance Eyes Lungs	Clear fluid in nostrils Right eye opaque Congested
	87x	Eyes Lungs	Opaque Congested
	88x	Lungs Stomach	Congested; pale red fluid in trachea Gas-filled
	89x	External appearance Lungs	Red staining around snout Congested
	90x	Eyes Lungs	Surface of right eye appeared rough Congested

x Decedents

Filed: MN 1256-85 (Microfilming)

TR 89-310

Case 124-63-0 KW

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S1573

3818 HJ Amersfoort - Regentesselaan 2 - Tel. 033 - 17245	LAB. RAPPORT	86.0628-A
betreft: Pennwalt Holland B.V. Rotterdam.-	datum :	88.03.01
	lid/order :	0752.00.6717
	blz/van :	1/1
	bemonsterd :	
	ontvangen :	86.05.21

Sample MN 1256 S-1573
 Methanesulfonylchloride (MSC, assay 99,5 %).
 Pennwalt Holland B.V., commercial grade, code nr. 6210.00.

Toxicity to fish

Determination of the acute lethal toxicity (LC50).
 Static method; temperature 20 °C; pH 7,0.
 Fish = *Lebistes reticulatus* (gup).

Test-substance concentration mg/l	number of fish	surviving after			
		24h	48h	72h	96h
1210 X	4	4	4	4	4
1210	4	4	4	4	4

X = during the whole test of 96 h no aeration.

Conclusion

The LC50 96 h at pH 7,0 to fish (*Lebistes reticulatus*) is:
 >1200 mg/l.

J. de Boer
 J. de Boer
 The Laboratory Chief

PENNWALT CORPORATION
 Technical Division

JUN 12 1989

Technical Records Center

PENNWALT HOLLAND BV
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CAS 124-63-0

37333 S-2539

IMPERIAL CHEMICAL INDUSTRIES PLC
CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD CHESHIRE UK

CATEGORY B REPORT (CONFIDENTIAL)
Not to be Copied Except by a
Reports Centre

Division: Organics
Division Ref: ORG/105/82 ✓
CTL Ref: Y02538/001/001
Study No: HM0464
Work Done: October 1982 -
January 1983
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REPORT NO: CTL/T/2059

SUMMARY

METHANE SULPHONYL CHLORIDE: ASSESSMENT
OF RESPIRATORY IRRITANCY IN MICE

by

L Pinto

THIS DOCUMENT CONTAINS INFORMATION CONFIDENTIAL AND TRADE SECRET TO ICI
The toxicological information contained in this report is derived from studies which fulfil the requirements of good laboratory practice. The protocol and report format may not meet the requirements of all Regulatory Authorities and, therefore, advice should be sought from the Toxicity Section, Central Toxicology Laboratory, before the report is presented to a Regulatory Authority or to persons outside the Company.

Approved by: J E Doe
Study Director

Date: *J. E. Doe*
23 May 83

Work carried out by,
or under the
supervision of: L Pinto
Study Investigator

Date: *Luciano Pinto*
20th May 83

1. INTRODUCTION

- 1.1 This study was carried out to determine the effect of methane sulphonyl chloride (MSC) on the respiration rate of mice. The depression of respiration rate caused by a chemical is considered to be a good model for sensory irritation in humans (Pinto 1983, Alarie 1981), and as such can be used as a guide in predicting acceptable exposure levels for humans.

All raw data obtained from this study are held in the Archives and the report in the Reports Centre, both at Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK.

2. METHODS

- 2.1 Species: Five male Alpk/AP mice per group in a 22-25g weight range.
- 2.2 Atmosphere generation: Alderley Park sintered bubbler and/or syringe atomiser.
- 2.3 Atmosphere Analysis: The test atmosphere was drawn through a glass tube containing Tenax GC, in order to trap the MSC, which was then thermally desorbed into a gas chromatograph equipped with a flame ionisation detector. The areas of the peaks due to MSC were then used to calculate the atmospheric concentrations.
- 2.4 Exposure period: 15 minutes
- 2.5 Rate monitoring: Pressure plethysmography.

3. RESULTS

Rate depression (RD_{50}) was calculated from the formula:

$$\left[1 - \left(\frac{\text{lowest respiration rate during exposure period}}{\text{mean respiration rate during control period}} \right) \right] \times 100$$

The dose response is shown graphically in figure 1. Respiratory rate depression occurred immediately on exposure to MSC, the fall in the rate being maintained during the exposure period. On cessation of exposure there was an immediate return to the control respiration rate.

The RD_{50} was calculated by least squares regression to be 0.84 (95.1% confidence limits 0.650, 1.095)ppm.

3. COMMENT

The dose response produced by MSC is one of a classical sensory irritant.

Alarie (Alarie 1981) recommends a hygiene standard for sensory irritants of 1/30 of the RD_{50} , which suggests an exposure limit to prevent irritation, of 0.03ppm. Any hygiene or handling standards made for MSC should take into account its sensory irritation properties.

It should be remembered that this test is specifically for sensory irritation and does not take into account toxicity.

A full report will be issued on request.

LP/CH (241)
15/05/83

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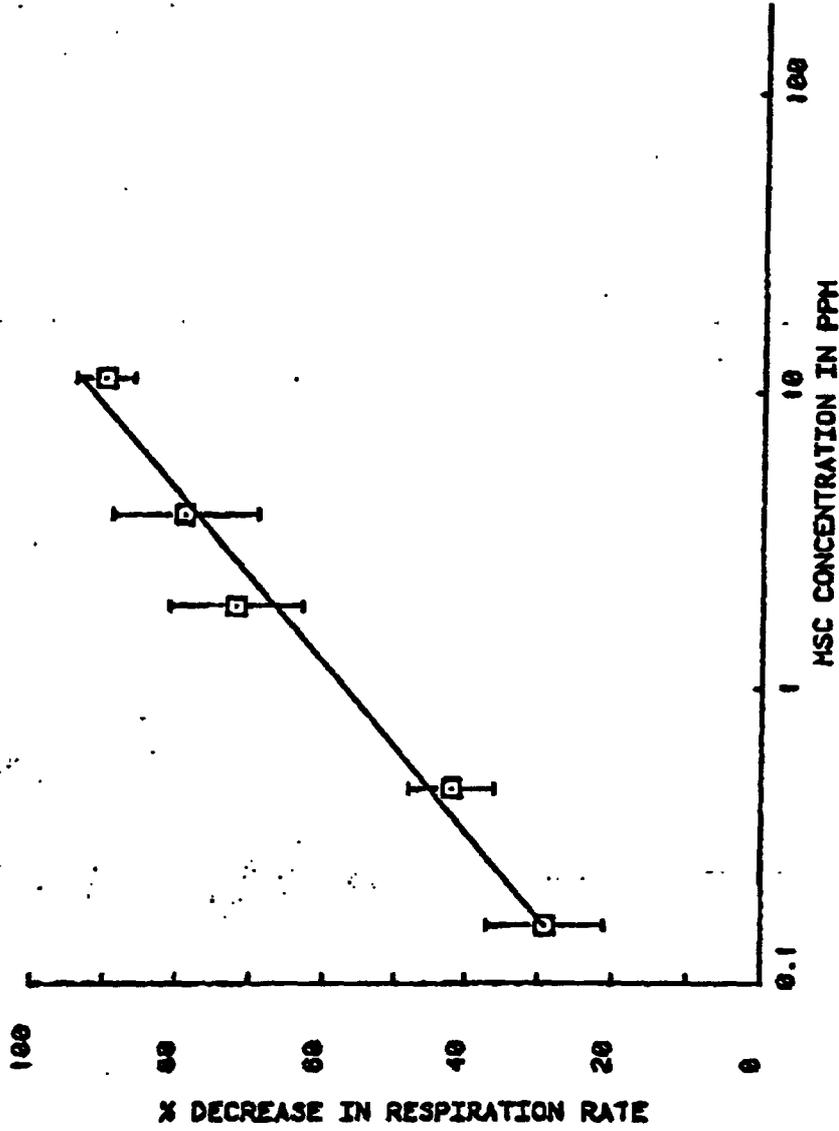
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Alarie Y, Toxicological Evaluation of Airborne Chemical Irritants and Allergens Using Respiratory Reflex Reactions.

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METHANE SULPHONYL CHLORIDE: ASSESSMENT OF RESPIRATORY IRRITANCY IN MICE
FIG 1 EFFECT OF METHANE SULPHONYL CHLORIDE ON RESPIRATORY RATE



585

Final Report

**Methane sulfonyl chloride: Reverse Mutation in two
Histidine-requiring strains of *Salmonella typhimurium***

Report for: Elf Atochem
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ENGLAND

Report No: 514/48-1052

Report Issue: March 1998

Page No: 1 of 48

**STUDY DIRECTOR AUTHENTICATION
AND GLP COMPLIANCE STATEMENT**

**Methane sulfonyl chloride: Reverse Mutation in two Histidine-requiring
strains of *Salmonella typhimurium***

I, the undersigned, hereby declare that the work described in this report* was performed under my supervision, as Study Director, and that the report provides a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol, unless otherwise stated, and the study objectives were achieved. The study was also performed in accordance with Covance Laboratories Limited Standard Operating Procedures and the principles of the following codes of Good Laboratory Practice:

United Kingdom Good Laboratory Practice Regulations 1997

OECD
Good Laboratory Practice in the Testing of Chemicals
Final Report ISBN 92-64-12367-9
Paris 1982

United States Environmental Protection Agency, Title 40 Code of Federal Regulations Part 160, Federal Insecticide, Fungicide and Rodenticide Act, Good Laboratory Practice Standards, Issued 29 November 1983 Federal Register, Plus subsequent amendments.



**N Dawkes BSc
Study Director**

Date: 25 March 1998

* Excluding Appendix 6 which is the responsibility of the Sponsor

REVIEWING SCIENTIST'S STATEMENT

Methane sulfonyl chloride: Reverse Mutation in two Histidine-requiring strains of *Salmonella typhimurium*

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Director and that the interpretation and presentation of the data in the report are consistent with the results obtained.



M Ballantyne BSc
Scientist

Date: 25-3-98

RESPONSIBLE PERSONNEL

**Vice President,
Consultancy and Regulatory Services** : D Kirkland BSc MIBiol PhD
Head of Molecular Toxicology : J Clements BSc PhD
Head of Quality Assurance : S White HNC
Study Director : N Dawkes BSc
Investigator : K Young HNC

Sponsor's Monitor : Dr J-F Regnier

Date of initiation of study 2 October 1997

Date of Sponsor's approval of study: 6 October 1997

Date of start of experimental work: 14 October 1997

Date of completion of experimental work: 4 November 1997

Date of study completion 25 March 1998

**QUALITY ASSURANCE RECORD
AND AUTHENTICATION STATEMENT**

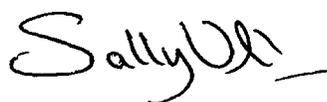
**Methane sulfonyl chloride: Reverse Mutation in two Histidine-requiring
strains of *Salmonella typhimurium***

The study described in this report* was subject to audit by the independent Quality Assurance Department as indicated below. The findings of each audit were reported to the Study Director and management as prescribed by Standard Operating Procedures.

The report audit was designed to confirm that as far as can be reasonably established the methods described and results incorporated in the report accurately reflect the raw data produced during the study.

Inspection programme	Inspection date	Report date
Procedure inspection	14 October 1997	14 October 1997
Data review	March 1998	16 March 1998
Draft study report	March 1998	16 March 1998

* Excluding Appendix 6 which is the responsibility of the Sponsor



S White
Head of Quality Assurance

Date: 27 March 1998

ARCHIVE STATEMENT

All primary data, or authenticated copies thereof, specimens and the final report will be retained in the Covance Laboratories Limited archives for three years after the submission of the final report. At this time the Sponsor will be contacted to determine whether data should be returned, retained or destroyed on their behalf.

Specimens requiring storage deep frozen are specifically excluded from the above. These will be retained for as long as the quality of the material permits evaluation but for no longer than three months after submission of the final report. The study Sponsor will be notified before specimens are destroyed on their behalf.

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1 SUMMARY

Methane sulfonyl chloride was assayed for mutation in two histidine-requiring strains (TA100 and TA1535) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9), in two separate experiments.

Experiment 1 treatments were carried out in both strains, using approximate final concentrations of Methane sulfonyl chloride at 1625, 3400, 5025, 6650, 8425 and 10050 $\mu\text{g}/\text{plate}$, plus negative (solvent) and positive controls. Following these treatments evidence of toxicity, in the form of diminution of the background bacterial lawn was observed on all plates treated at the higher test concentrations, both in the absence and presence of S-9. These results contained insufficient treatment doses that could be evaluated for mutation. These data were not therefore considered acceptable for comprehensive mutagenicity assessment.

Experiment 1 treatments with both test strains were therefore repeated using revised dose-ranges (740-3400 $\mu\text{g}/\text{plate}$ for treatments with both strains in the absence of S-9, 740-5025 $\mu\text{g}/\text{plate}$ for treatments with TA100 in the presence of S-9 and 740-6650 $\mu\text{g}/\text{plate}$ for treatments with TA1535 in the presence of S-9). The maximum treatment concentrations for all these treatments were reduced to estimates of the lower limit of the toxic range. Following these Experiment 1 repeat treatments, evidence of toxicity was observed on all plates treated at the two highest treatment concentrations. In all cases sufficient test doses remained scorable to allow valid and comprehensive mutagenicity assessment.

Experiment 2 treatments retained the same dose range as employed in the Experiment 1 repeat treatments, in order to investigate the doses of Methane sulfonyl chloride previously demonstrating a mutagenic response. No modification of the metabolic activation conditions (i.e. pre-incubation) was employed for the treatments in Experiment 2. Following Experiment 2 treatments, once again, evidence of toxicity was observed with both the test strains at the higher treatment concentrations.

Negative (solvent) and positive control treatments were included for all strains in both experiments. The mean numbers of revertant colonies on negative control plates all fell within acceptable ranges, and were significantly elevated by positive control treatments.

Methane sulfonyl chloride treatments of strain TA1535 both in the absence and in the presence of S-9, resulted in statistically significant and reproducible increases in revertant numbers (when data were analysed at the 1% level using Dunnett's test), that were considered to be indicative of a weak mutagenic effect.

It was concluded that Methane sulfonyl chloride exhibited evidence of weak mutagenic activity in *Salmonella typhimurium* strain TA1535, both in the absence and presence of a rat liver metabolic activation system (S-9), when tested under the conditions employed for this study, which included treatments at concentrations extending into the toxic range.

2 INTRODUCTION

The Ames test is a rapid, reliable and economical method of evaluating the mutagenic potential of a test article by measuring genetic activity in one or more histidine-requiring strains of *Salmonella typhimurium* in the absence and presence of a liver metabolising system (1). A large data base has been accumulated with this assay, confirming its ability to detect genetically active compounds of most chemical classes with around 80-90% sensitivity and specificity (2). The following bacterial strains were used in this study:

Strain	Type of mutation in the histidine gene
TA100	base-pair substitution
TA1535	base-pair substitution

These strains require biotin as well as histidine for growth. Test strain TA100 is a pKM101 plasmid derivative and has increased sensitivity to certain mutagens as the pKM101 plasmid codes for an error-prone DNA repair system (3).

When exposed to a mutagen, some of the bacteria in the treated population undergo genetic changes which revert them to a non-histidine-requiring state, and they can then grow without exogenous histidine. Different tester strains are used because each is mutated by particular chemical classes of compound. A compound that is mutagenic in one strain need not be so in another (4).

The objective of this study was therefore to evaluate the mutagenic activity of Methane sulfonyl chloride by examining its ability to revert two strains of *Salmonella typhimurium* in the absence and presence of a rat liver metabolising system (S-9).

This study was performed according to the protocol and three amendments.

3 MATERIALS

3.1 Test article

Methane sulfonyl chloride (MSC), batch number G16E-1T, was a clear colourless liquid, which was received on 30 September 1997. It was stored at 1-10°C desiccated in the dark. Purity was stated as 99.8%. The expiry date of the test article was not stated. The Sponsor's test article description is presented in Appendix 6. Determinations of the stability and characteristics of the test article were the responsibility of the Sponsor.

A 'neat' Methane sulfonyl chloride solution was supplied by the Sponsor for treatments in this study. Based on the test article information supplied by the Sponsor, with regard to the density of the supplied solution, treatment volumes/dose levels were calculated. An aliquot of this solution was filter-sterilized (Gelman Acrodisc 13 CR filter, 0.2 μm pore size) prior to each experiment. No dilutions of the supplied test article were performed. The test article solutions (retained in subdued lighting) were added to the test system within approximately 1½ hours of removal from the storage conditions stated above.

Pre-determined volumes of the supplied 'neat' test article solution were used per plate. For each treatment, if necessary, an additional volume of sterile purified water was added to provide consistency in treatment volume. The following additions were made for each treatment and the dose ranges overleaf were tested:

Experiment	Volume of 'neat' MSC (μ L)	Volume of water (μ L)	Final concentration (μ g/plate)*
Experiment 1	0.0	6.8	0
	1.1	5.7	1625
	2.3	4.5	3400
	3.4	3.4	5025
	4.5	2.3	6650
	5.7	1.1	8425
	6.8	0.0	10050
Experiment 1 repeat and Experiment 2 (TA100 and TA1535 -S-9)	0.0	2.3	0
	0.5	1.8	740
	0.75	1.55	1100
	1.0	1.3	1480
	1.5	0.8	2220
	2.3	0	3400
	Experiment 1 repeat and Experiment 2 (TA100 +S-9)	0.0	3.4
0.50		2.9	740
0.75		2.65	1100
1.0		2.4	1480
1.5		1.9	2220
2.3		1.1	3400
3.4		0	5025
Experiment 1 repeat and Experiment 2 (TA1535 +S-9)	0.0	4.5	0
	0.50	4.0	740
	0.75	3.75	1100
	1.0	3.5	1480
	1.5	3.0	2220
	2.3	2.2	3400
	3.4	1.1	5025
	4.5	0	6650

* Due to the size of the volume additions, the final concentrations stated are approximations.

3.2 Controls

Negative control treatments were performed using the same addition volumes per plate as the test article treatments (see section 3.1), comprising treatments with sterile purified water. Positive controls comprised treatments with the appropriate stock positive control solution (addition volume 0.1mL). The positive control chemicals were supplied and used as tabulated below:

Chemical	Source	Stock* concentration ($\mu\text{g}/\text{mL}$)	Final concentration ($\mu\text{g}/\text{plate}$)	Use	
				Strain(s)	S-9
Sodium azide (NaN_3)	Sigma Chemical Co, Poole, UK	20	2.0	TA100	-
				TA1535	-
2-aminoanthracene (AAN)	Sigma Chemical Co, Poole, UK	50	5.0	TA100	+
				TA1535	

* Stock solution AAN was prepared in sterile anhydrous analytical grade dimethyl sulphoxide (DMSO). Stock solution NaN_3 was prepared in sterile purified water. Both stock solutions were stored in aliquots at 1-10°C in the dark.

3.3 Metabolic activation system

The mammalian liver post-mitochondrial fraction (S-9) used for metabolic activation was prepared from male Sprague Dawley rats induced with Aroclor 1254 and obtained from Molecular Toxicology Incorporated, USA. Batches of MolTox™ S-9 were stored frozen at -80°C, and thawed just prior to incorporation into the top agar (5). Each batch was checked by the manufacturer for sterility, protein content, ability to convert ethidium bromide and cyclophosphamide to bacterial mutagens, and cytochrome P-450-catalysed enzyme activities (alkoxyresorufin-O-dealkylase activities). The quality control statement, relating to the batch of S-9 preparation used, is included in Appendix 5 of this report.

3.3.1 Preparation of 10% S-9 mix and buffer solutions

Quantities were prepared in the following ratios per 100 mL:

Ingredient	Concentration	Quantity (mL)	
		10% S-9 mix	Buffer solution
Sodium phosphate buffer pH 7.4	500 mM	20	20
Glucose-6-phosphate (disodium)	180 mg/mL	0.845	-
NADP (disodium)	25 mg/mL	12.6	-
Magnesium chloride	250 mM	3.2	-
Potassium chloride	150 mM	22	-
L-histidine HCl (in 250 mM MgCl ₂)	1 mg/mL	4	4
d-biotin	1 mg/mL	4.88	4.88
S-9	as detailed above	10	-
Water	-	to volume	to volume

3.4 Bacteria

Two bacterial strains of *Salmonella typhimurium* (TA100 and TA1535) were used in this study. Both tester strains were originally obtained from the UK NCTC. For all assays, bacteria were cultured for 10 hours at 37°C in nutrient broth (containing ampicillin for strain TA100). Incubation was carried out in a shaking incubator. Bacteria were taken from vials of frozen cultures, which had been checked for strain characteristics of histidine dependence, *rfa* character and resistance to ampicillin (TA100). Checks were carried out according to Maron and Ames (3) and De Serres and Shelby (6). All experimentation commenced within 2 hours of the end of the incubation period.

4 METHODS

4.1 Mutation experiments

Methane sulfonyl chloride was tested for mutation in two strains of *Salmonella typhimurium* (TA100 and TA1535), in two separate experiments, at the concentrations detailed in 3.1 using triplicate plates without and with S-9. Negative (solvent) controls were included in each assay, in quintuplicate without and with S-9. In each experiment, bacterial strains were treated with diagnostic mutagens in triplicate in the absence of S-9 as detailed in 3.2. The activity of the S-9 mix used in each experiment was confirmed by AAN treatments (again in triplicate) of both strains in the presence of S-9 (see Appendices 1, 2 and 3 for details of actual strains tested). These platings were achieved by the following sequence of additions to 2.5 mL molten agar at 46°C:

0.1 mL bacterial culture

Volumes of MSC and/or water (see section 3.1) or

0.1 mL positive control solution

0.5 mL 10% S-9 mix or buffer solution

followed by rapid mixing and pouring on to Minimal Davis agar plates. When set, the plates were inverted and incubated at 37°C in the dark for 3 days. Following incubation, these plates were examined for evidence of toxicity to the background lawn, and where possible revertant colonies were counted (4.2).

Following initial Experiment 1 treatments of both the test strains performed in the absence and presence of S-9, a majority of the test doses were affected in some way by toxicity. The data from these treatments were not therefore considered to have been able to give a comprehensive determination of the test article mutagenic activity. Repeat treatments in the absence and in the presence of metabolic activation were therefore performed. The results of these treatments are those presented as the Experiment 1 repeat mutagenicity data.

As both the initial Experiment 1 and the Experiment 1 repeat experiment produced increases in revertant numbers sufficient to be considered as indicative of mutagenic activity, no modification of the metabolic activation conditions were considered necessary (i.e. incorporation of a pre-incubation step) for the Experiment 2 treatments.

4.2 Colony counting

Colonies were counted electronically using a Seescan Colony Counter (Seescan plc), or manually, where physical effects (e.g split agar) interfered with the accuracy of the automated counter. The background bacterial lawn of each test plate was inspected for signs of toxicity.

4.3 Analysis of results

4.3.1 Treatment of data

Individual plate counts from all experiments were recorded separately and the mean and standard deviation of the plate counts for each treatment were determined.

The accepted normal ranges for mean numbers of spontaneous revertants on solvent control plates for this laboratory are presented in Appendix 4. The ranges quoted are based on a large volume of historical control data accumulated from experiments where the responses of the strains to positive control compounds were considered satisfactory. Data for this laboratory are consistent with ranges of spontaneous revertants per plate considered acceptable elsewhere (6).

For evaluation of data there are many statistical methods in use, and several are acceptable (7,8). The m-statistic was calculated to check that the data were Poisson-distributed (8), and Dunnett's test was used to compare the counts of each dose with the control. The presence or otherwise of a dose response was checked by linear regression analysis (8).

4.3.2 Acceptance criteria

The assay was considered valid if the following criteria were met:

- 1) the mean negative control counts fell within the normal ranges as defined in Appendix 4
- 2) the positive control chemicals induced clear increases in revertant numbers confirming discrimination between different strains, and an active S-9 preparation

- 3) no more than 5% of the plates were lost through contamination or some other unforeseen event.

4.3.3 Evaluation criteria

The test article was considered to be mutagenic if:

- 1) the assay was valid (see 4.3.2)
 - 2) Dunnett's test gave a significant response ($p < 0.01$), and the data set(s) showed a significant dose-correlation
 - 3) the positive responses described in 2) were reproducible.
-

5 RESULTS

5.1 Toxicity, solubility and dose selection

Details of all treatment solution concentrations and final Methane sulfonyl chloride doses are provided in section 3.1.

Methane sulfonyl chloride was assayed for mutation in two histidine-requiring strains (TA100 and TA1535) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9), in two separate experiments.

Experiment 1 treatments were carried out in both strains, using approximate final concentrations of Methane sulfonyl chloride at 1625, 3400, 5025, 6650, 8425 and 10050 $\mu\text{g}/\text{plate}$, plus negative (solvent) and positive controls. The maximum test dose of approximately 10050 $\mu\text{g}/\text{plate}$ was selected since this was the maximum treatment concentration employed for the previous study performed with a different Methane sulfonyl chloride formulation (CLE Study Number 514/29). Following these treatments evidence of toxicity, in the form of diminution of the background bacterial lawn, was observed on all plates treated at the higher test concentrations, both in the absence and presence of S-9. These results contained insufficient treatment doses that could be evaluated for mutation, and these data were not therefore considered acceptable for comprehensive mutagenicity assessment.

Experiment 1 treatments with both test strains were therefore repeated using revised dose-ranges with the maximum treatment concentrations reduced to estimates of the lower limit of the toxic range. Following these Experiment 1 repeat treatments evidence of toxicity was observed on all plates treated at the two highest treatment concentrations. In all cases sufficient test doses remained scorable to allow valid and comprehensive mutagenicity assessment.

Experiment 2 treatments retained the same dose range as employed in the Experiment 1 repeat treatments, in order to investigate the doses of Methane sulfonyl chloride previously demonstrating a mutagenic response. No modification of the metabolic activation conditions (i.e. pre-incubation) was employed for the treatments in Experiment 2. Following Experiment 2 treatments, once again, evidence of toxicity was observed with both the test strains at the higher treatment concentrations.

The test article was completely soluble in the aqueous assay system at all concentrations treated, in each of the experiments performed.

5.2 Mutation

The individual plate counts were averaged to give mean values which are presented in Appendices 1, 2 and 3. From the data it can be seen that mean solvent control counts fell within the normal historical ranges (Appendix 4), that the positive control chemicals all induced large increases in revertant numbers in the appropriate strains, and that less than 5% of plates were lost, leaving adequate numbers of plates at all treatments. The study was accepted as valid.

The mutation data for the individual strains were evaluated as follows:

Salmonella typhimurium strain TA1535

Methane sulfonyl chloride treatments of strain TA1535 both in the absence and in the presence of S-9 resulted in statistically significant and reproducible increases in revertant numbers (when data were analysed at the 1% level using Dunnett's test), with at least some evidence of a dose-relationship. These increases in revertant numbers were considered to be evidence of a weak mutagenic effect (Appendices 1, 2 and 3, Tables 3, 4, 7, 8, 11 and 12).

Salmonella typhimurium strain TA100

Methane sulfonyl chloride treatments of strain TA100 in the absence of S-9 resulted in very small, reproducible, statistically significant (when data were analysed at the 1% level using Dunnett's test) increases in revertant numbers (Appendices 1, 2 and 3, Tables 1, 5 and 9). Methane sulfonyl chloride treatments of strain TA100 in the presence of S-9 also produced very small increases in revertant numbers. However, only following Experiment 2 treatments with strain TA100 in the presence of S-9 was the increase in revertant numbers of sufficient magnitude to reach the threshold of statistical significance (Appendix 3, Table 10). Due to the magnitude of the increases in revertant numbers observed with strain TA100 and the lack of a dose-relationship, it was considered that these increases in revertant numbers were such that their biological significance was equivocal.

The results obtained in this study are comparable with those observed in the previous Bacterial Mutation Study performed with a different formulation of this test article (CLE Study Number 514/29).

6 CONCLUSION

It was concluded that Methane sulfonyl chloride exhibited evidence of weak mutagenic activity in *Salmonella typhimurium* strain TA1535, both in the absence and presence of a rat liver metabolic activation system (S-9), when tested under the conditions employed for this study, which included treatments at concentrations extending into the toxic range.

7 REFERENCES

- 1 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.
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APPENDIX 1

**Methane sulfonyl chloride: raw plate counts and calculated mutagenicity data
Experiment 1**

Methane sulfonyl chloride: summary of mean revertant colonies (-S-9) - Experiment 1

Substance	Dose Level $\mu\text{g}/\text{plate}$	TA100	TA1535
		Mean \pm SD	Mean \pm SD
WATER	6.8 μl	111 \pm 11	18 \pm 3
Methane sulfonyl chloride	1625	144 \pm 4	38 \pm 11
	3400	137 \pm 8 (S)	21 \pm 5 (S)
	5025	77 \pm 3 (V)	4 \pm 3 (V+M)
	6650	11 \pm 4 (V+M)	1 \pm 1 (A+M)
	8425	11 (T)	0 (T)
	10050	- (T)	- (T)
Positive controls	Compound	NaN3	NaN3
	Dose Level	2 μg	2 μg
	Mean \pm SD	749 \pm 54	440 \pm 43

SD Standard deviation

NaN3 Sodium azide

S : Slight thinning of b/g lawn
T : Toxic, no revertant colonies
V : Very thin background lawn
A : Absence of background lawn
M : Plate counted manually

Methane sulfonyl chloride: summary of mean revertant colonies (+S-9) - Experiment 1

Substance	Dose Level $\mu\text{g}/\text{plate}$	TA100	TA1535
		Mean \pm SD	Mean \pm SD
WATER	6.8 μl	138 \pm 6	16 \pm 6
Methane sulfonyl chloride	1625	171 \pm 5	55 \pm 3
	3400	172 \pm 8	56 \pm 7
	5025	125 \pm 3 (S)	42 \pm 5
	6650	115 \pm 24 (S)	30 \pm 6 (S)
	8425	78 \pm 43 (V+M)	11 \pm 3 (V+M)
	10050	31 \pm 12 (A+M)	20 \pm 16 (S)
Positive controls	Compound	AAN	AAN
	Dose Level	5 μg	5 μg
	Mean \pm SD	1854 \pm 89	180 \pm 26

SD Standard deviation

AAN 2-Aminoanthracene

S : Slight thinning of b/g lawn
V : Very thin background lawn
A : Absence of background lawn
M : Plate counted manually

Methane sulfonyl chloride Experiment 1

Table 1

Test strain: TA100 -S-9

Treatment ($\mu\text{g}/\text{plate}$)	Revertant numbers/plate				
	Solvent	125	118	100	99
1625	141	149	142		
3400	129 s	144 s	139 s		
5025	77 v	80 s	75 v+M		
6650	11 v+M	15 v+M	8 v+M		
8425	11 v+M	T	T		
10050	T	T	T		
Positive	689	795	762		

Treatment ($\mu\text{g}/\text{plate}$)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Solvent	111.20	5		11.39			
1625	144.00	3	1.29 [^]	4.36	0.89 ***	0.02	4.87 ***
3400	137.33	3	1.24	7.64	0.71 **	0.01	3.93 ***
5025	77.33	3	0.70	2.52	0.35 NS	-0.00	-5.78 NS
6650	11.33	3	0.10	3.51	0.73 NS	-0.01	-23.90 NS
8425	11.00	1	0.10		0.78	-0.01	
Positive	748.67	3	6.73	54.24			
M Statistic = 0.677							

Key to significance:

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.005$ NS not significant

Key to postfixes:

[^] maximum increase above control

S : Slight thinning of b/g lawn
T : Toxic, no revertant colonies
V : Very thin background lawn
M : Plate counted manually

Methane sulfonyl chloride Experiment 1

Table 2

Test strain: TA100 +S-9

Treatment ($\mu\text{g}/\text{plate}$)	Revertant numbers/plate				
Solvent	140	136	129	142	143
1625	169	177	168		
3400	165	181	169		
5025	126 s	122 s	128 s		
6650	88 s	121 s	135 s		
8425	44 V+M	65 V+M	126 s		
10050	28 A+M	20 A+M	44 A+M		
Positive	1951	1777	1833		

Treatment ($\mu\text{g}/\text{plate}$)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Solvent	138.00	5		5.70			
1625	171.33	3	1.24	4.93	0.96 ***	0.02	1.80 NS
3400	171.67	3	1.24^	8.33	0.85 ***	0.01	1.82 NS
5025	125.33	3	0.91	3.06	0.07 NS	-0.00	-0.74 NS
6650	114.67	3	0.83	24.13	0.42 NS	-0.00	-1.45 NS
8425	78.33	3	0.57	42.59	0.64 NS	-0.01	-4.17 NS
10050	30.67	3	0.22	12.22	0.79 NS	-0.01	-8.43 NS
Positive	1853.67	3	13.43	88.82			
M Statistic = 4.275							

Key to significance:

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.005$ NS not significant

Key to postfixes:

^ maximum increase above control

S : Slight thinning of b/g lawn
V : Very thin background lawn
A : Absence of background lawn
M : Plate counted manually

Methane sulfonyl chloride Experiment 1

Table 3

Test strain: TA1535 -S-9

Treatment ($\mu\text{g}/\text{plate}$)	Revertant numbers/plate				
	Solvent	22	15	16	18
1625	31	51	32		
3400	17 s	21 s	26 s		
5025	1 V+M	7 V+M	3 V+M		
6650	2 A+M	1 A+M	1 A+M		
8425	0 A+M	T	T		
10050	T	T	T		
Positive	448	393	478		

Treatment ($\mu\text{g}/\text{plate}$)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Solvent	18.40	5		3.05			
1625	38.00	3	2.07 [^]	11.27	0.84 ***	0.01	4.36 ***
3400	21.33	3	1.16	4.51	0.21 NS	0.00	0.77 NS
5025	3.67	3	0.20	3.06	0.43 NS	-0.00	-5.88 NS
6650	1.33	3	0.07	0.58	0.64 NS	-0.00	-7.43 NS
8425	0.00	1	0.00		0.67	-0.00	
Positive	439.67	3	23.89	43.11			
M Statistic = 1.350							

Key to significance:

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.005$ NS not significant

Key to postfixes:

[^] maximum increase above control

S : Slight thinning of b/g lawn
T : Toxic, no revertant colonies
V : Very thin background lawn
A : Absence of background lawn
M : Plate counted manually

Methane sulfonyl chloride Experiment 1

Table 4

Test strain: TA1535 +S-9

Treatment ($\mu\text{g}/\text{plate}$)	Revertant numbers/plate				
	Solvent	25	16	17	15
1625	58	52	54		
3400	60	59	48		
5025	38	40	47		
6650	32 s	35 s	24 s		
8425	13 V+M	7 V+M	12 V+M		
10050	38 s	12 s	10 V+M		
Positive	186	152	203		

Treatment ($\mu\text{g}/\text{plate}$)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Solvent	16.20	5		6.06			
1625	54.67	3	3.37	3.06	0.97 ***	0.02	6.02 ***
3400	55.67	3	3.44^	6.66	0.87 ***	0.01	6.13 ***
5025	41.67	3	2.57	4.73	0.63 **	0.01	4.36 ***
6650	30.33	3	1.87	5.69	0.31 NS	0.00	2.68 *
8425	10.67	3	0.66	3.21	0.14 NS	-0.00	-1.28 NS
10050	20.00	3	1.23	15.62	0.25 NS	-0.00	0.52 NS
Positive	180.33	3	11.13	25.97			
M Statistic = 2.534							

Key to significance:

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.005$ NS not significant

Key to postfixes:

^ maximum increase above control

S : Slight thinning of b/g lawn
V : Very thin background lawn
M : Plate counted manually

APPENDIX 2

**Methane sulfonyl chloride: raw plate counts and calculated mutagenicity data
Experiment 1 repeat**

**Methane sulfonyl chloride: summary of mean revertant colonies (-S-9)
Experiment 1 repeat**

Substance	Dose Level $\mu\text{g}/\text{plate}$	TA100	TA1535
		Mean \pm SD	Mean \pm SD
WATER	2.3 μl	130 \pm 11	19 \pm 5
Methane sulfonyl chloride	740	156 \pm 9	33 \pm 10
	1100	148 \pm 9	33 \pm 5
	1480	154 \pm 7	40 \pm 4
	2220	168 \pm 5 (S)	34 \pm 7 (S)
	3400	132 \pm 7 (S)	18 \pm 2 (S)
Positive controls	Compound	NaN ₃	NaN ₃
	Dose Level	2 μg	2 μg
	Mean \pm SD	659 \pm 8	532 \pm 58

SD Standard deviation

NaN₃ Sodium azide

S : Slight thinning of b/g lawn

**Methane sulfonyl chloride: summary of mean revertant colonies (+S-9)
Experiment 1 repeat**

Substance	Dose Level $\mu\text{g}/\text{plate}$	TA100	TA1535
		Mean \pm SD	Mean \pm SD
WATER	3.4/4.5 μl^*	146 \pm 8	21 \pm 4
Methane sulfonyl chloride	740	169 \pm 3	43 \pm 12
	1100	177 \pm 23	42 \pm 9
	1480	173 \pm 6	44 \pm 5
	2220	175 \pm 8	39 \pm 2
	3400	164 \pm 19 (S)	42 \pm 3
	5025	130 \pm 12 (S)	37 \pm 22 (S)
	6650		22 \pm 3 (S)
Positive controls	Compound	AAN	AAN
	Dose Level	5 μg	5 μg
	Mean \pm SD	1673 \pm 128	180 \pm 20

SD Standard deviation

AAN 2-Aminoanthracene

S : Slight thinning of b/g lawn

* Volume of water for negative control treatments with strains TA100 and TA1535 were 3.4 and 4.5 μL respectively.

Methane sulfonyl chloride Experiment 1 repeat

Table 5

Test strain: TA100 -S-9

Treatment ($\mu\text{g}/\text{plate}$)	Revertant numbers/plate				
Solvent	127	114	136	142	130
740	163	146	159		
1100	141	158	146		
1480	147	154	161		
2220	166 s	164 s	174 s		
3400	126 s	129 s	140 s		
Positive	666	659	651		

Treatment ($\mu\text{g}/\text{plate}$)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Solvent	129.80	5		10.55			
740	156.00	3	1.20	8.89	0.83 **	0.04	4.17 ***
1100	148.33	3	1.14	8.74	0.70 **	0.02	2.99 *
1480	154.00	3	1.19	7.00	0.71 ***	0.02	3.87 ***
2220	168.00	3	1.29^	5.29	0.81 ***	0.02	5.96 ***
3400	131.67	3	1.01	7.37	0.18 NS	0.00	0.33 NS
Positive	658.67	3	5.07	7.51			
M Statistic = 0.519							

Key to significance:

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.005$ NS not significant

Key to postfixes:

^ maximum increase above control

S : Slight thinning of b/g lawn

Methane sulfonyl chloride Experiment 1 repeat

Table 6

Test strain: TA100 +S-9

Treatment (µg/plate)	Revertant numbers/plate				
Solvent	151	142	140	138	157
740	167	167	173		
1100	192	150	189		
1480	166	178	174		
2220	181	166	178		
3400	161 s	147 s	184 s		
5025	116 s	137 s	138 s		
Positive	1621	1819	1580		

Treatment (µg/plate)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Solvent	145.60	5		8.08			
740	169.00	3	1.16	3.46	0.88 ***	0.03	2.55 *
1100	177.00	3	1.22^	23.43	0.78 ***	0.03	3.32 *
1480	172.67	3	1.19	6.11	0.74 ***	0.02	2.93 *
2220	175.00	3	1.20	7.94	0.68 ***	0.01	3.17 *
3400	164.00	3	1.13	18.68	0.38 *	0.01	1.99 NS
5025	130.33	3	0.90	12.42	0.27 NS	-0.00	-1.78 NS
Positive	1673.33	3	11.49	127.81			
M Statistic = 0.995							

Key to significance:

* p < 0.05 ** p < 0.01 *** p < 0.005 NS not significant

Key to postfixes:

^ maximum increase above control

S : Slight thinning of b/g lawn

Methane sulfonyl chloride Experiment 1 repeat

Table 7

Test strain: TA1535 -S-9

Treatment ($\mu\text{g}/\text{plate}$)	Revertant numbers/plate				
	Solvent	18	19	14	16
740	43	32	23		
1100	34	28	38		
1480	35	42	43		
2220	31 s	42 s	30 s		
3400	17 s	17 s	20 s		
Positive	563	567	465		

Treatment ($\mu\text{g}/\text{plate}$)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Solvent	19.00	5		5.39			
740	32.67	3	1.72	10.02	0.72 *	0.02	3.33 **
1100	33.33	3	1.75	5.03	0.75 ***	0.01	3.56 **
1480	40.00	3	2.11^	4.36	0.82 ***	0.01	4.94 ***
2220	34.33	3	1.81	6.66	0.67 ***	0.01	3.76 ***
3400	18.00	3	0.95	1.73	0.04 NS	0.00	-0.22 NS
Positive	531.67	3	27.98	57.77			
M Statistic = 1.260							

Key to significance:

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.005$ NS not significant

Key to postfixes:

^ maximum increase above control

S : Slight thinning of b/g lawn

Methane sulfonyl chloride Experiment 1 repeat

Table 8

Test strain: TA1535 +S-9

Treatment (µg/plate)	Revertant numbers/plate				
Solvent	15	21	21	26	24
740	44	31	54		
1100	52	38	36		
1480	43	49	40		
2220	37	40	41		
3400	40	45	41		
5025	18 s	32 s	62 s		
6650	21 M+s	20 M+s	26 M+s		
Positive	165	172	203		

Treatment (µg/plate)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Solvent	21.40	5		4.16			
740	43.00	3	2.01	11.53	0.85 ***	0.03	3.40 **
1100	42.00	3	1.96	8.72	0.80 ***	0.02	3.30 *
1480	44.00	3	2.06^	4.58	0.79 ***	0.02	3.60 **
2220	39.33	3	1.84	2.08	0.63 ***	0.01	2.96 *
3400	42.00	3	1.96	2.65	0.55 **	0.01	3.33 **
5025	37.33	3	1.74	22.48	0.30 NS	0.00	2.35 NS
6650	22.33	3	1.04	3.21	0.08 NS	-0.00	0.20 NS
Positive	180.00	3	8.41	20.22			
M Statistic = 2.364							

Key to significance:

* p ≤ 0.05 ** p ≤ 0.01 *** p ≤ 0.005 NS not significant

Key to postfixes:

^ maximum increase above control

S : Slight thinning of b/g lawn

M : Plate counted manually

APPENDIX 3

**Methane sulfonyl chloride: raw plate counts and calculated mutagenicity data
Experiment 2**

**Methane sulfonyl chloride: summary of mean revertant colonies (-S-9)
Experiment 2**

Substance	Dose Level $\mu\text{g}/\text{plate}$	TA100	TA1535
		Mean \pm SD	Mean \pm SD
WATER	2.3 μl	133 \pm 9	20 \pm 5
Methane sulfonyl chloride	740	169 \pm 16	31 \pm 6
	1100	178 \pm 21	37 \pm 7
	1480	176 \pm 24	45 \pm 3
	2220	170 \pm 30	42 \pm 9
	3400	177 \pm 2 (S)	46 \pm 15 (S)
Positive controls	Compound	NaN3	NaN3
	Dose Level	2 μg	2 μg
	Mean \pm SD	633 \pm 24	561 \pm 29

SD Standard deviation

NaN3 Sodium azide

S : Slight thinning of b/g lawn

**Methane sulfonyl chloride: summary of mean revertant colonies (+S-9)
Experiment 2**

Substance	Dose Level $\mu\text{g}/\text{plate}$	TA100	TA1535
		Mean \pm SD	Mean \pm SD
WATER	3.4/4.5 μl *	151 \pm 8	28 \pm 3
Methane sulfonyl chloride	740	172 \pm 16	38 \pm 6
	1100	195 \pm 12	51 \pm 9
	1480	214 \pm 50	57 \pm 8
	2220	195 \pm 35	58 \pm 8
	3400	189 \pm 21 (S)	58 \pm 10
	5025	154 \pm 17 (S)	49 \pm 8 (S)
	6650		32 \pm 5 (S)
Positive controls	Compound	AAN	AAN
	Dose Level	5 μg	5 μg
	Mean \pm SD	1891 \pm 122	190 \pm 18

SD Standard deviation

AAN 2-Aminoanthracene

S : Slight thinning of b/g lawn

* Volume of water for negative control treatments with strains TA100 and TA1535 were 3.4 and 4.5 μL respectively

Methane sulfonyl chloride Experiment 2

Table 9

Test strain: TA100 -S-9

Treatment (µg/plate)	Revertant numbers/plate				
Solvent	128	130	137	125	147
740	181	150	175		
1100	159	201	173		
1480	177	152	199		
2220	139	198	172		
3400	179 s	176 s	177 s		
Positive	657	634	609		

Treatment (µg/plate)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Solvent	133.40	5		8.79			
740	168.67	3	1.26	16.44	0.86 ***	0.05	2.80 *
1100	177.67	3	1.33^	21.39	0.84 ***	0.04	3.46 **
1480	176.00	3	1.32	23.52	0.77 ***	0.03	3.32 **
2220	169.67	3	1.27	29.57	0.58 **	0.02	2.83 *
3400	177.33	3	1.33	1.53	0.55 **	0.01	3.46 **
Positive	633.33	3	4.75	24.01			
M Statistic = 1.949							

Key to significance:

* p ≤ 0.05 ** p ≤ 0.01 *** p ≤ 0.005 NS not significant

Key to postfixes:

^ maximum increase above control

S : Slight thinning of b/g lawn

Methane sulfonyl chloride Experiment 2

Table 10

Test strain: TA100 +S-9

Treatment (µg/plate)	Revertant numbers/plate				
Solvent	159	148	139	155	156
740	189	169	158		
1100	208	194	184		
1480	173	270	198		
2220	155	216	215		
3400	213 s	176 s	177 s		
5025	156 s	170 s	137 s		
Positive	2028	1794	1851		

Treatment (µg/plate)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Solvent	151.40	5		8.02			
740	172.00	3	1.14	15.72	0.72 *	0.03	1.24 NS
1100	195.33	3	1.29	12.06	0.86 ***	0.04	2.58 *
1480	213.67	3	1.41^	50.36	0.75 ***	0.04	3.47 **
2220	195.33	3	1.29	34.93	0.61 **	0.02	2.52 *
3400	188.67	3	1.25	21.08	0.44 *	0.01	2.19 NS
5025	154.33	3	1.02	16.56	0.03 NS	0.00	0.17 NS
Positive	1891.00	3	12.49	122.02			
M Statistic = 3.160							

Key to significance:

* p ≤ 0.05 ** p ≤ 0.01 *** p ≤ 0.005 NS not significant

Key to postfixes:

^ maximum increase above control

S : Slight thinning of b/g lawn

Methane sulfonyl chloride Experiment 2

Table 11

Test strain: TA1535 -S-9

Treatment ($\mu\text{g}/\text{plate}$)	Revertant numbers/plate				
Solvent	27	15	15	21	20
740	25 M	36	32		
1100	42	39	29		
1480	48	43	45		
2220	53	39	35		
3400	60	48 s	30 s		
Positive	558	534	591		

Treatment ($\mu\text{g}/\text{plate}$)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnnett's t value
Solvent	19.60	5		4.98			
740	31.00	3	1.58	5.57	0.78 *	0.02	2.39 NS
1100	36.67	3	1.87	6.81	0.84 ***	0.02	3.39 **
1480	45.33	3	2.31	2.52	0.91 ***	0.02	4.82 ***
2220	42.33	3	2.16	9.45	0.82 ***	0.01	4.31 ***
3400	46.00	3	2.35^	15.10	0.73 ***	0.01	4.80 ***
Positive	561.00	3	28.62	28.62			
M Statistic = 1.714							

Key to significance:

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.005$ NS not significant

Key to postfixes:

^ maximum increase above control

S : Slight thinning of b/g lawn

M : Plate counted manually

Methane sulfonyl chloride Experiment 2

Table 12

Test strain: TA1535 +S-9

Treatment ($\mu\text{g}/\text{plate}$)	Revertant numbers/plate				
Solvent	25	28	32	30	25
740	42	32	41		
1100	61	44	48		
1480	52	52	66		
2220	50	59	65		
3400	61	46	66		
5025	50 s	40 s	56 s		
6650	29 M+s	38 s	30 M+s		
Positive	201	199	169		

Treatment ($\mu\text{g}/\text{plate}$)	Mean (of)	N	Fold Increase	Standard Deviation	Correlation Coefficient	Slope of best fit	Dunnett's t value
Solvent	28.00	5		3.08			
740	38.33	3	1.37	5.51	0.82 **	0.01	2.43 NS
1100	51.00	3	1.82	8.89	0.87 ***	0.02	4.98 ***
1480	56.67	3	2.02	8.08	0.90 ***	0.02	6.04 ***
2220	58.00	3	2.07^	7.55	0.87 ***	0.02	6.29 ***
3400	57.67	3	2.06	10.41	0.77 ***	0.01	6.20 ***
5025	48.67	3	1.74	8.08	0.54 ***	0.00	4.54 ***
6650	32.33	3	1.15	4.93	0.11 NS	0.00	1.06 NS
Positive	189.67	3	6.77	17.93			
M Statistic = 1.014							

Key to significance:

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.005$ NS not significant

Key to postfixes:

^ maximum increase above control

S : Slight thinning of b/g lawn

M : Plate counted manually

APPENDIX 4

Historical negative (solvent) control values for *Salmonella* strains

Strain	- or + S-9	Mean N ^a of spontaneous revertants	SD	Range*	
				lower	upper
TA98	-	29	7.3	10	48
	+	34	7.3	15	53
TA100	-	112	18.3	65	160
	+	130	16.0	89	171
TA1535	-	17	5.1	4	30
	+	21	5.3	7	34
TA1537	-	13	5.8	1	28
	+	14	6.7	1	32
TA102	-	320	46.1	201	439
	+	374	68.1	198	549

* 99% confidence limits about the mean

The above are pooled data from at least 20 consecutive experiments over the period 7.7.97 to 11.8.97

APPENDIX 5

Quality control statement for S-9

MOLTOX™ POST MITOCHONDRIAL SUPERNATANT (S-9)
QUALITY CONTROL & PRODUCTION CERTIFICATE

LOT NO.: <u>0769</u>	SPECIES: <u>Rat</u>	PREPARATION DATE: <u>23 July 1997</u>
PART NO.: <u>11-101</u>	STRAIN: <u>Sprague Dawley</u>	EXPIRATION DATE: <u>23 July 1999</u>
VOLUME: <u>5 ml</u>	SEX: <u>Male</u>	BUFFER: <u>0.154 M KCl</u>
	TISSUE: <u>Liver</u>	INDUCING AGENT(s): <u>Aroclor 1254</u>
REFERENCE: <u>Maron, D. & Ames, B. Mutat. Res. 113:173, 1983</u>		<u>(Monsanto KL615) 500mg/kg i.p.</u>

BIOCHEMISTRY:

- PROTEIN

16.98 mg/ml

Assayed according to the method of Lowry et al.,
JBC 193:265, 1951 using bovine serum albumin as the standard

- ALKOXYRESORUFIN-O-DEALKYLASE ACTIVITIES

	Activity	Fold -
	PA50	Induction
EROD	1A1, 1A2	283.8
PROD	2B1	294.8
BROD	2B1	34.8
MROD	1A2	26.9

Assays for ethoxyresorufin-O-deethylase (EROD), pentoxy-, benzyl- and methoxyresorufin-O-dealkylases (PROD, BROD, MROD) were conducted using a modification of the methods of Burke, et al., Biochem Pharm 34:3337, 1985. Fold-inductions were calculated as the ratio of the sample vs. uninduced specific activities (SA's). Control SA's (pmoles/min/mg protein) were 1.9, 0.2, 10.6, & 1.1 for EROD, PROD, BROD and MROD, respectively.

BIOASSAY:

- STERILITY TEST

Samples of S-9 were assayed for the presence of contaminating microflora by plating 1.0ml volumes on Nutrient Agar and Minimal Glucose (Vogel-Bonner E, supplemented with 0.05mM L-histidine and D-biotin) media. Triplicate plates were read after 48 or 72h incubation at 37C. No evidence of contamination was observed.

- PROMUTAGEN ACTIVATION

No. His+ Revertants	
EtBr/ CPA/	
TA98 TA1535	
7772 704	

The ability of the sample to activate ethidium (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lecca, et al., Mutation Res 129:299, 1984. Data were expressed as revertants per ug EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.2 - 10% in S9 mix, were tested for their ability to activate benzo(a)pyrene (BP) and 2-aminoanthracene (2-AA) to intermediates mutagenic to TA100. Assays were conducted using duplicate plates as described by Maron & Ames, Mutat. Res. 113:173, 1983.).

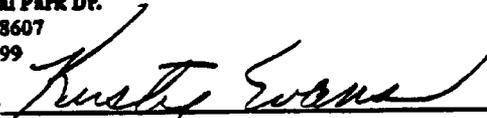
ul S9 per plate/number his+ revertants per plate

Promutagens	0	1	5	10	20	50
BP (5ug)	188	347	588	672	997	1130
2-AA (2.5ug)	234	495	4377	2008	1584	304

MOLECULAR TOXICOLOGY, INC.

157 Industrial Park Dr.
Boone, NC 28607
(704) 264-9099

Approved by



APPENDIX 6

Sponsor's test article description

elf atochem**ATO**Direction Sécurité Environnement Produit
Département de Toxicologie Industrielle

TEST ARTICLE DESCRIPTION

CONFIDENTIAL

CHLORURE DE METHANESULFONYLE

STRUCTURAL FORMULA

 $\text{CH}_3\text{-SO}_2\text{Cl}$

IDENTITY

Test article name	: G16E-1T
Chemical name	: METHANESULFONYL CHLORIDE
CAS number	: 124-63-0
EINECS number	: 204-706-1
Molecular formula	: $\text{CH}_3\text{SO}_2\text{Cl}$
Molecular weight	: 114,55
Purity	: 99,8%
Origin and batch	: G16E

585

Final *Report*

Methane sulphonyl chloride: Induction of
Chromosome Aberrations in Cultured Human
Peripheral Blood Lymphocytes

Prepared for: Elf Atochem

Report number: 514/30-1052
Issue date: March 1997

NOW

COVANCE

THE DEVELOPMENT SERVICES COMPANY

FINAL REPORT

**Methane sulphonyl chloride: Induction of
Chromosome Aberrations
in Cultured Human Peripheral
Blood Lymphocytes**

Report for: Elf Atochem
Direction Sécurité Environnement Produit
Département Toxicologie Industrielle
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ENGLAND

Report number: 514/30-1052

Report issue: March 1997

Page number: 1 of 42

**STUDY DIRECTOR AUTHENTICATION
AND GLP COMPLIANCE STATEMENT**

**Methane sulphonyl chloride: Induction of
Chromosome Aberrations
in Cultured Human Peripheral
Blood Lymphocytes**

I, the undersigned, hereby declare that the work described in this report* was performed under my supervision, as Study Director, and that the report provides a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol, unless otherwise stated, and the study objectives were achieved. The study was also performed in accordance with Covance Laboratories Standard Operating Procedures and the principles of the following codes of Good Laboratory Practice:

UK Principles of Good Laboratory Practice
The UK Compliance Programme
Department of Health
London 1989

OECD
Good Laboratory Practice in the Testing of Chemicals
Final Report ISBN 92-64-12367-9
Paris 1982



R Marshall BSc PhD
Study Director

Date: 26.3.97

* Excluding Appendix 8 which is the responsibility of the Sponsor

REVIEWING SCIENTIST'S STATEMENT

**Methane sulphonyl chloride: Induction of
Chromosome Aberrations
in Cultured Human Peripheral
Blood Lymphocytes**

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Director and that the interpretation and presentation of the data in the report are consistent with the results obtained.

S E Riley

S Riley BSc
Scientist

Date: 24-3-97

RESPONSIBLE PERSONNEL

Director of Toxicology : D Kirkland BSc MIBiol PhD
Head of Molecular Toxicology : J Clements BSc PhD
Head of Quality Assurance : S White HNC
Study Director : R Marshall BSc PhD
Investigator : S Robinson
Slide Analysts : C Svennevik BSc
: Y Stoddart BSc
: C Trott BSc DPhil
: D Crellin ONC

Sponsor's Monitor : B Molinier

Date of initiation of study 22 August 1996

Date of Sponsor's approval of study 22 August 1996

Date of start of experimental work 28 August 1996

Date of completion of experimental work 23 November 1996

Date of study completion 24 March 1997

**QUALITY ASSURANCE RECORD
AND AUTHENTICATION STATEMENT****Methane sulphonyl chloride: Induction of
Chromosome Abberations
in Cultured Human Peripheral
Blood Lymphocytes**

The study described in this report was subject to audit by the independent Quality Assurance Department as indicated below. Studies of this type which involve frequent repetition of similar or identical procedures are inspected in accordance with a "batch process" programme, as described in Quality Assurance Department Standard Operating Procedures.

Critical procedures relevant to this study type were selected without bias from at least one study on the batch process programme. The inspection dates indicated below are of those inspections conducted closest to the time of study conduct.

The findings of each audit were reported to the Study Director and management as prescribed by Standard Operating Procedures.

The report audit was designed to confirm that as far as can be reasonably established the methods described and results incorporated in the report accurately reflect the raw data produced during the study.

Inspection programme	Inspection date	Report date
Protocol review	29 August 1996	29 August 1996
Procedure inspection (2)	17 September 1996	17 September 1996
Procedure inspection	6 November 1996	6 November 1996
Procedure inspection	8 November 1996	8 November 1996
Data review	January 1997	22 January 1997
Draft study report	January 1997	22 January 1997

PA Smith
P Smith
Section Head Quality Assurance

Date: 26 March 1997

ARCHIVE STATEMENT

All primary data, or authenticated copies thereof, specimens and the final report will be retained in the Covance Laboratories archives for ten years after the submission of the final report. At this time the Sponsor will be contacted to determine whether data should be returned, retained or destroyed on their behalf.

Specimens requiring storage deep frozen are specifically excluded from the above. These will be retained for as long as the quality of the material permits evaluation but for no longer than three months after submission of the final report. The study Sponsor will be notified before specimens are destroyed on their behalf.

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1 SUMMARY

Methane sulphonyl chloride was tested in an *in vitro* cytogenetics assay using duplicate human lymphocyte cultures from a male human donor. Treatments covering a broad range of doses, separated by narrow intervals, were performed both in the absence and presence of metabolic activation by a rat liver post-mitochondrial fraction (S-9) from Aroclor 1254 induced animals. The test article was dissolved in acetone and the highest dose level used, 1150 $\mu\text{g}/\text{mL}$, was equivalent to a concentration of 10 mM.

Treatment in the absence of S-9 was continuous for 20 or 44 hours (20+0, 44+0). Treatment in the presence of S-9 was for 3 hours only followed by a 17 or 41 hour recovery period prior to harvest (3+17, 3+41). The test article dose levels for chromosome analysis were selected by evaluating the effect of methane sulphonyl chloride on mitotic index. Chromosome aberrations were analysed in cells receiving 20+0 hour treatments in the absence of S-9 and 3+17 hour treatments in its presence at three consecutive dose levels. The highest concentration chosen for analysis was 193.3 $\mu\text{g}/\text{mL}$, which induced approximately 52% and 66% mitotic inhibition following treatment in the absence or presence of S-9 respectively. The effects of this single concentration only, (without and with S-9) were investigated at the delayed (44+0, 3+41) sampling time.

Appropriate negative (solvent and untreated) control cultures were included in the test system under each treatment condition. The proportion of cells with structural aberrations in solvent control cultures fell within historical solvent control ranges. Aberration frequencies in untreated control cultures were not analysed. 4-Nitroquinoline 1-oxide and cyclophosphamide were employed as positive control chemicals in the absence and presence of liver S-9 respectively. Cells receiving these were sampled 20 hours after the start of treatment; both compounds induced statistically significant increases in the proportion of cells with structural aberrations.

Treatment of cultures with methane sulphonyl chloride in both the absence and presence of S-9 resulted in frequencies of cells with aberrations which were higher than those seen in concurrent solvent controls. With the exception of continuous treatment for 44 hours in the absence of S-9, increases were statistically significant under all treatment conditions. The numbers of aberrant cells in most treated cultures exceeded historical negative control ranges.

It is concluded that methane sulphonyl chloride induced chromosome aberrations in cultured human peripheral blood lymphocytes. The effect was seen following treatment in both the absence and presence of S-9.

2 INTRODUCTION

Chromosome defects are recognised as being the basis of a number of human genetic diseases (1). Although there are a number of assays which detect genotoxic chemicals, relatively few of these detect chemicals which either interfere with the process of mitosis or cause chromosome aberrations. No one assay has been extensively evaluated on the same compounds in several laboratories but there is a large database on the use of chromosomal assays for screening purposes (2). The use of human lymphocytes is recommended because the cells are only used in short-term culture and maintain a stable karyotype (3). Experiments with these cells can also be performed in conjunction with a rat liver metabolising system since, for short incubation periods at least, no toxicity is induced by the liver homogenate itself.

As a number of chemicals have been reported as only exerting positive effects after considerable delay (4,5,6), provision was made for a delayed sampling time. The first sampling time was performed 20 hours after the start of treatment. This is equivalent to approximately one and a half times the average generation time of cultured lymphocytes from the panel of donors used in this laboratory. The second sampling time was carried out 24 hours (or approximately two cell cycles) later.

The objective of this study was to evaluate the clastogenic potential of methane sulphonyl chloride by examining its effects on the chromosomes of the lymphocytes of human donors, cultured *in vitro* and treated in the absence and presence of a rat liver metabolising system (S-9). The methodology in this study complies with OECD Test Guideline 473 (1983).

With the exception of the minor deviations detailed in Appendix 9, none of which in any way prejudice the validity of the study, this study was performed according to the protocol and two amendments.

3 MATERIALS

3.1 Test article

Methane sulfonyl chloride, batch number 96000478, was a brown liquid, which was received on 9 July 1996. It was stored desiccated, under nitrogen, at 1-10°C in the dark. A certificate of analysis, provided by the sponsor, is presented in Appendix 8. The expiry date of the test article was not stated. Determinations of the stability and characteristics of the test article were the responsibility of the Sponsor.

Test article solutions were prepared by diluting methane sulfonyl chloride (supplied as a 3004.5 mg/mL solution in acetone) in nitrogen purged analytical grade acetone, immediately prior to assay to give 115 mg/mL such that 100-fold dilution into culture medium would give a final concentration equivalent to 10 mM. The stock solutions were not filter-sterilized. Dilutions were made using sterile acetone. The test article solutions were then used within one hour of initial formulation as follows:

Experiment 1 Concentration of treatment solution (mg/mL)	Final concentration (µg/mL)	Hours treatment + hours recovery	
		20+0 -S-9	3+17 +S-9
2.274	22.74	✓	✓
3.248	32.48	✓	✓
4.641	46.41	✓	✓
6.630	66.30	✓	✓
9.471	94.71	✓	✓
13.53	135.3	✓	✓
19.33	193.3	✓	✓
27.61	276.1	✓	✓
39.45	394.5	✓	✓
56.35	563.5	✓	✓
80.50	805.0	✓	✓
115.0	1150	✓	✓

✓ Indicates concentration tested

Measurements on post-treatment media without and with S-9 indicated that the test article at its highest concentration had no effect on pH. Osmolality was not measured because the known toxic effects of this compound made this impractical in the laboratory (Appendix 9).

3.2 Controls

Acetone was added to cultures designated as solvent controls as described in 4.1. Untreated controls received culture medium alone. The positive control chemicals were dissolved in sterile anhydrous analytical grade dimethyl sulphoxide immediately prior to use, as follows:

Chemical	Supplier	Concentration of treatment solution (mg/mL)	Final concentration ($\mu\text{g/mL}$)	S-9
4-nitroquinoline 1-oxide (NQO)	Fluka Chemicals Ltd, Gillingham, UK	0.125	1.25	-
		0.250	2.50	-
		0.500	5.00	-
Cyclophosphamide (CPA)	Sigma Chemical Co, Poole, UK	1.25	12.5	+
		2.50	25.0	+

Cells treated with 2.5 μg NQO/mL and 25 μg CPA/mL, gave satisfactory responses in terms of quality and quantity of mitoses and extent of chromosomal damage and were selected for analysis.

3.3 Metabolic activation system

The mammalian liver post-mitochondrial fraction (S-9) used for metabolic activation was prepared from male Sprague Dawley rats induced with Aroclor 1254 and obtained from Molecular Toxicology Incorporated, Annapolis, Maryland, USA. The batches of MolTox™ S-9 were stored frozen in aliquots at -80°C and thawed just prior to use. Each batch was checked by the manufacturer for sterility, protein content (minimum 32 mg/mL), ability to convert known promutagens to bacterial mutagens and cytochrome P-450-catalyzed enzyme activities (alkoxyresorufin-O-dealkylase activities). The quality control statement, relating to the batch of S-9 preparation used, is included in Appendix 7 of this report.

3.3.1 Preparation of S-9 mix

Glucose-6-phosphate (180 mg/mL), NADP (25 mg/mL), 150 mM KCl and rat liver S-9 (3.3) were mixed in the ratio 1:1:1:2. An aliquot of the resulting S-9 mix was added to each cell culture designated for treatment in the presence of S-9 to achieve the required final concentration in a total of 10 mL. The final concentration of liver homogenate in the test system was 2%. Cultures treated in the absence of S-9 received an equal volume of 150 mM KCl.

3.4 Blood cultures

A healthy, non-smoking volunteer (male) was used in this study. The donor was not suspected of any virus infection nor had been exposed to high levels of radiation or hazardous chemicals. An appropriate volume of whole blood was drawn from the peripheral circulation one day prior to assay. Blood was stored refrigerated until use. Whole blood cultures were established in sterile disposable centrifuge tubes by placing 0.4 mL heparinised blood into 9.0 mL Hepes-buffered RPMI medium containing 20% (v/v) foetal calf serum and 50 $\mu\text{g}/\text{mL}$ gentamycin. Phytohaemagglutinin (PHA, reagent grade) was included at a concentration of approximately 10 $\mu\text{g}/\text{mL}$ of culture to stimulate the lymphocytes to divide. Blood cultures were incubated for approximately 48 hours at 37°C and rocked continuously.

4 METHODS

4.1 Treatment

S-9 mix or KCl (0.5 mL) were added appropriately as detailed in 3.3.1. One set of quadruplicate cultures (A, B, C and D) for each of the treatment regimes was then treated with the solvent and one set of duplicate cultures with the test article as detailed in 3.1 (0.1 mL per culture). Additional duplicate cultures received culture medium alone to act as untreated controls. Further duplicate cultures for 20+0 hour treatments in the absence of S-9 and 3+17 hour treatments in its presence, were treated with 0.1 mL of the positive control chemicals as detailed in 3.2. This scheme is illustrated below:

Treatment	S-9	Number of cultures			
		20+0*	3+17*	44+0*	3+41*
Negative control	-	4		4	
	+		4		4
Untreated control	-	2		2	
	+		2		2
Test article (all doses)	-	2		2	
	+		2		2
Positive controls (all doses)	-	2			
	+		2		

* Hours treatment + hours recovery

Treatment media remained on cultures receiving continuous treatments (- S-9) until sampling, that is, 20 hours and 44 hours after the beginning of treatment. Cultures were treated in the presence of S-9 for 3 hours only. They were then pelleted (200 x 'g', 5 minutes), washed twice with sterile saline, and resuspended in fresh medium containing foetal calf serum and gentamycin. Cultures were incubated for a further 17 or 41 hours before harvesting. The delayed sample was adopted for test and negative control cultures only, not for positive controls.

Summary of treatment conditions

Treatment	S-9	Duration of treatment (hours)	Harvest time (hours after start of treatment)
Continuous	20+0	-	20
	44+0	-	44
Pulse	3+17	+	20*
	3+41	+	44

* Includes positive control treatments

4.2 Harvesting

Approximately 1½ hours prior to harvest, colchicine was added to give a final concentration of approximately 1 µg/mL to arrest dividing cells in metaphase. Cultures were then centrifuged at 200 x 'g' for 5 minutes, the supernatant was carefully removed and cells were resuspended in 4 mL hypotonic (0.075 M) KCl and incubated at 37°C for 15 minutes to allow cell swelling to occur. Cells were then fixed by dropping the KCl suspension into an equal volume of fresh, ice-cold methanol/glacial acetic acid (3:1, v/v). The fixative was changed by centrifugation (200 x 'g', 5 minutes) and resuspension. This procedure was repeated several times (centrifuging at 1250 x 'g', 2-3 minutes) until the supernatants were clean.

4.3 Preparation of metaphase spreads

Lymphocytes were kept in fixative in the refrigerator before slides were prepared but slides were not made on the day of harvest to ensure cells were adequately fixed. Cells were pelleted and resuspended in a minimal amount of fresh fixative so as to give a milky suspension. Several drops of 45% (v/v) aqueous acetic acid were added to each suspension to enhance chromosome spreading, and several drops of suspension were transferred to clean microscope slides. Slides were flamed to improve metaphase spreading.

After the slides had dried the cells were stained for 5 minutes in 4% (v/v) filtered Giemsa stain in pH 6.8 buffer. The slides were rinsed, dried and mounted with coverslips.

4.4 Selection of doses for cytogenetic analysis

Slides were examined, uncoded, for mitotic index (MI) or percentage of cells in mitosis. Slides from enough dose levels from each treatment regime were scored to determine if chemically induced mitotic inhibition had occurred. This is defined as a clear decrease in mitotic index compared with negative controls (based on at least 1000 cells counted), preferably dose-related.

4.4.1 Rationale for dose selection

The top dose for chromosome analysis from 20+0 hour, -S-9 and 3+17 hour, +S-9 treatments, was to be one at which a 50-70% reduction in MI occurred or was to be the highest dose tested as discussed in 3.1. Slides from this dose and the next two lower doses were to be taken for microscopic analysis.

A single dose from the delayed harvest was to be scored. The dose level selected was to correspond to the top dose scored following the 20+0 hour treatment -S-9 or the 3+17 hour treatment +S-9, unless mitotic inhibition was too severe in which case the highest scorable dose was to be selected.

For each treatment regime, two solvent control cultures were initially to be analysed for chromosome aberrations. Slides from the remaining solvent control cultures were only to be analysed if considered necessary, for example, to help resolve an equivocal result. A single positive control dose level, which gave satisfactory responses in terms of quality and quantity of mitoses and extent of chromosomal damage, was to be analysed (3.2). Untreated controls were only to be analysed if the aberration frequency (excluding gaps) in the solvent control cultures exceeded the normal range. Mitotic index data and the results of dose selection are presented in 5.1.

4.5 Scoring of aberrations

At least, 25 cells from each of the selected NQO and CPA positive control cultures (section 3.2) were analysed to ensure that the system was operating satisfactorily.

Slides from the selected treatments and from solvent controls were coded using randomly generated letters by a person not connected with the scoring of the slides. Labels bearing only the study reference number, the sex of the donor and the code were used to cover treatment details on the slides.

One hundred metaphases from each code were analysed for chromosome aberrations. Only cells with 44-46 chromosomes were considered acceptable for analysis of structural aberrations. Any cell with more than 46 chromosomes, that is polyploid, endoreduplicated and hyperdiploid cells, observed during this search was noted and recorded separately. Classification of structural aberrations was based on the scheme described by ISCN (7) and is detailed in Appendix 2. Observations (summarised in Appendices 3 and 4) were recorded on raw data sheets with the microscope stage coordinates of any aberrant cell.

4.6 Analysis of results

4.6.1 Treatment of data

After completion of microscopic analysis, data were decoded. The aberrant cells in each culture were categorised as follows:

- 1) cells with structural aberrations including gaps
- 2) cells with structural aberrations excluding gaps
- 3) polyploid, endoreduplicated or hyperdiploid cells.

The totals for category 2 in negative control cultures were used to determine whether the assay was acceptable or not (section 4.6.2). The proportions of aberrant cells in each replicate were used to establish acceptable heterogeneity between replicates by means of a binomial dispersion test (8).

The proportion of cells in category 2 for each test treatment condition, was compared with the proportion in concurrent negative controls using Fisher's exact test (8). Probability values of $p \leq 0.05$ were accepted as significant. The proportions of cells in categories 1, 2 and 3 were examined in relation to historical negative control (normal) ranges.

4.6.2 Acceptance criteria

The human lymphocyte assay was to be considered valid if the following criteria were met:

- 1) the binomial dispersion test demonstrated acceptable heterogeneity between replicate cultures, and
- 2) the proportion of cells with structural aberrations (excluding gaps) in negative control cultures fell within the normal range, and
- 3) at least 160 cells out of an intended 200 were analysable at each dose level, and
- 4) the positive control chemicals induced statistically significant increases in the number of cells with structural aberrations.

4.6.3 Evaluation criteria

The test article was to be considered as positive in this assay if:

- 1) a statistically significant increase in the proportion of cells with structural aberrations (excluding gaps) occurred at one or more concentrations, and
- 2) the proportion of cells with structural aberrations at such doses exceeded the normal range.

A positive result only at the delayed harvest was to be taken as evidence of clastogenicity provided criteria 1 and 2 were met. Increases in numbers of cells with gaps or increases in the proportions of cells with structural aberrations, not exceeding the normal range or occurring only at very high or very toxic concentrations, were likely to be concluded as "equivocal". Full assessment of the biological importance of such increases is likely only to be possible with reference to data from other test systems. Cells with exchange aberrations or cells with greater than one aberration were to be considered of particular biological significance.

5 RESULTS

5.1 Selection of doses for cytogenetic analysis

The results of mitotic index determinations for the treatments without and with S-9 sampled at 20 and 44 hours were as follows:

Treatment ($\mu\text{g/mL}$)	Mitotic index (%)							
	20+0 -S-9		3+17 +S-9		44+0 -S-9		3+41 +S-9	
	A	B	A	B	A	B	A	B
Untreated	5.2	3.5	4.5	5.1	4.3	4.4	4.2	4.4
Solvent	2.6	1.8	4.4	4.1	2.6*	3.2	5.3	4.6
22.74	NS	NS	NS	NS	NS	NS	NS	NS
32.48	NS	NS	NS	NS	NS	NS	NS	NS
46.41	NS	NS	NS	NS	NS	NS	NS	NS
66.3	NS	NS	NS	NS	NS	NS	NS	NS
94.71	2.2	1.8	3.4	3.0	NS	NS	NS	NS
135.3	1.4	1.6	2.8	2.3	NS	NS	NS	NS
193.3	0.8	1.3	1.5	1.4	0.8	1.7	1.8	2.1
276.1	0.2	0.5	0.2	0.6	0.9	0.5	1.3	1.2
394.5	0.3	0	0.1	0.1	0.5	0.6	0.4	0.1
563.5	0.4	0	0	0.3	0.9	0.1	0	0.3
805	0.3	0.2	0.1	0	0	0	0	0
1150	0	0.1	0.2	0.1	0	0	0	0

NS = not scored * Culture C analysed
(Except where noted, slides from solvent control cultures C and D not scored)

The following doses were selected for analysis according to the criteria discussed in 4.4:

	Mitotic inhibition†
20+0 hours, -S-9: 94.71, 135.3, 193.3 $\mu\text{g/mL}$	52%
3+17 hours, +S-9: 94.71, 135.3, 193.3 $\mu\text{g/mL}$	66%

44+0 hours, -S-9: 193.3 $\mu\text{g}/\text{mL}$	57%
3+41 hours, -S-9: 193.3 $\mu\text{g}/\text{mL}$	61%

† At (highest) analysed dose

5.2 Chromosome aberration analysis

5.2.1 Raw data

The raw data for the observations on the test article plus positive and negative controls are retained by Covance Laboratories. A summary of the number of cells containing structural aberrations is given in Appendix 1, Tables 1-4 for each of the different treatment regimes in Experiment 1 and 2. The numbers and types of structural aberrations seen per cell are given in Appendix 3. Frequencies of cells with numerical aberrations observed are given in Appendix 4.

5.2.2 Validity of study

Acceptance criteria for this assay are listed in section 4.6.2. The data in Appendix 1, Tables 1-4 and Appendices 5 and 6 indicate that:

- 1) In most cases, no evidence of significant heterogeneity between replicate cultures was obtained in the binomial dispersion test (Appendix 5). An exception to this was seen for cultures treated in the presence of S-9 and sampled at 20 hours where marked heterogeneity was seen (Appendix 5a). Although the reason for this observation is not clear, heterogeneity is known to frequently occur between replicate cultures when positive effects are apparent (8).
- 2) The proportion of cells with structural aberrations (excluding gaps) in negative control cultures fell within the normal range (Appendix 6).
- 3) At least 160 cells out of an intended 200 were analysed at each dose level (Appendix 1, Tables 1-4).

- 4) The positive control chemicals NQO and CPA induced statistically significant increases in the number of cells with structural aberrations (Appendix 1, Tables 1 and 2, Appendix 5).

5.2.3 Analysis of data

Structural aberrations

Treatment of cultures with methane sulphonyl chloride in both the absence and presence of S-9 resulted in frequencies of cells with aberrations which were higher than those seen in concurrent solvent controls (Appendix 1, Tables 1-4). With the exception of continuous treatment for 44 hours in the absence of S-9, increases were statistically significant under all treatment conditions (Appendix 5). The numbers of aberrant cells in most treated cultures exceeded historical negative control ranges (Appendix 6).

Heterogeneity in response between replicate cultures was apparent at some concentrations following treatment in the presence of S-9 at the 20 hour sampling time. The effect was most pronounced at 135.3 $\mu\text{g}/\text{mL}$. The reason for this is not immediately clear insofar as the two replicates suffered similar mitotic inhibition. Heterogeneity in response is, however, known to frequently occur between replicate cultures when positive effects are observed (8).

Numerical aberrations

Increased frequencies of cells with numerical aberrations were apparent at the 44 hour sampling time (Appendices 4c and 4d), but the increase was only statistically significant following treatment in the presence of S-9. Frequencies of cells with numerical aberrations, outside the historical control range, in negative control cultures treated in the absence of S-9 may have been attributable to prolonged treatment with the solvent (acetone).

6 CONCLUSION

It is concluded that methane sulphonyl chloride induced chromosome aberrations in cultured human peripheral blood lymphocytes. The effect was seen following treatment in both the absence and presence of S-9.

7 REFERENCES

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APPENDIX 1

Methane sulphonyl chloride: cells with structural aberrations

TABLE 1

20 hour treatment -S-9, 0 hour recovery (20+0)

Donor sex: male

Treatment ($\mu\text{g/mL}$)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	5	4		2.6
	B	100	2	1		1.8
	Totals	200	7	5		(2.2)
94.71	A	100	18	12		2.2
	B	100	16	13		1.8
	Totals	200	34	23	$p \leq 0.001$	(2.0)
135.3	A	100	30	27		1.4
	B	100	21	15		1.6
	Totals	200	51	42	$p \leq 0.001$	(1.5)
193.3	A	100	23	19		0.8
	B	100	15	13		1.3
	Totals	200	38	30	$p \leq 0.001$	(1.1)
NQO, 2.5	A	25	13	11		
	B	25	5	5		
	Totals	50	18	16	$p \leq 0.001$	

§ Statistical significance (Appendix 5a)

NS = not significant

Numbers highlighted exceed historical negative control range (Appendix 6)

APPENDIX 1

Methane sulphonyl chloride: cells with structural aberrations

TABLE 2
3 hour treatment +S-9, 17 hour recovery (3+17)
Donor sex: male

Treatment ($\mu\text{g/mL}$)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	5	2		4.4
	B	100	1	0		4.1
	Totals	200	6	2		(4.3)
94.71	A	100	7	4		3.4
	B	100	7	6		3.0
	Totals	200	14	10	$p \leq 0.05$	(3.2)
135.3	A	100	7	2		2.8
	B	100	24	23		2.3
	Totals	200	31	25	$p \leq 0.001$	(2.6)
193.3	A	100	6	4		1.5
	B	100	16	10		1.4
	Totals	200	22	14	$p \leq 0.001$	(1.5)
CPA, 25	A	75	16	15		
	B	26	7	3		
	Totals	101	23	18	$p \leq 0.001$	

§ Statistical significance (Appendix 5a)

NS = not significant

Numbers highlighted exceed historical negative control range (Appendix 6)

APPENDIX 1

Methane sulphonyl chloride: cells with structural aberrations

TABLE 3

44 hour treatment -S-9, 0 hour recovery (44+0)

Donor sex: male

Treatment ($\mu\text{g/mL}$)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	6	2		3.2
	B	100	8	5		2.6
	Totals	200	14	7		(2.9)
193.3	A	100	9	7		0.8
	B	100	8	3		1.7
	Totals	200	17	10	NS	(1.3)

TABLE 4

3 hour treatment +S-9, 41 hour recovery (3+41)

Donor sex: male

Treatment ($\mu\text{g/mL}$)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	3	3		5.3
	B	100	1	1		4.6
	Totals	200	4	4		(5.0)
193.3	A	100	10	6		1.8
	B	100	12	10		2.1
	Totals	200	22	16	$p < 0.01$	(2.0)

§ Statistical significance (Appendix 5b)

NS = not significant

Numbers highlighted exceed historical negative control range (Appendix 6)

APPENDIX 2

Abbreviations and classification of observations

abs	=	aberrations
rep	=	replicate
tot	=	total
Gaps (g)		
csg	=	chromosome gap
ctg	=	chromatid gap
Chromosome deletions (Chr del)		
del	=	chromosome deletion
d min	=	double minute
f	=	isolocus fragment
Chromosome exchanges (Chr exch)		
t	=	interchange between chromosomes (eg reciprocal translocation)
inv	=	chromosome intrachange (eg pericentric inversion)
dic	=	dicentric
dic+f	=	dicentric with accompanying fragment
acr	=	acentric ring
r+f	=	centric ring with accompanying fragment
r	=	centric ring
Chromatid deletions (Ctd del)		
del	=	chromatid deletion
su	=	isochromatid deletion with sister union of broken ends
nud	=	isochromatid deletion with non-union of broken ends distally
nup	=	isochromatid deletion with non-union of broken ends proximally
min	=	single minute
Chromatid exchanges (Ctd exch)		
qr	=	interchange between chromatids of different chromosomes (eg quadriradial)
cx	=	obligate complex interchange
e	=	chromatid intrachange
tr/tr+f	=	isochromatid/chromatid interchange (triradial)/with accompanying fragment
Other structural aberrations		
pvz	=	pulverised cell
mabs	=	multiple aberrations (greater than seven aberrations per cell or too many aberrations to permit accurate analysis)
Numerical aberrations (num abs)		
E	=	endoreduplicated
H	=	hyperdiploid (47-68 chromosomes)
P	=	polyploid (greater than 68 chromosomes)

APPENDIX 3a

Methane sulphonyl chloride: summary of the numbers and types
of structural aberrations observed

20+0 hours, -S-9

Treatment ($\mu\text{g}/\text{mL}$)	Rep	Cells *	g	Chr del	Chr exch	Ctd del	Ctd exch	Other	Abs +g	Abs -g
Solvent	A	100	2	1	0	3	0	0	6	4
	B	100	1	0	0	1	0	0	2	1
	A+B	200	3	1	0	4	0	0	8	5
94.71	A	100	9	3	0	13	0	0	25	16
	B	100	8	4	0	8	0	0	20	12
	A+B	200	17	7	0	21	0	0	45	28
135.3	A	100	12	2	0	26	5	0	45	33
	B	100	13	5	0	17	1	0	36	23
	A+B	200	25	7	0	43	6	0	81	56
193.3	A	100	5	4	0	24	4	0	37	32
	B	100	9	2	0	8	3	0	22	13
	A+B	200	14	6	0	32	7	0	59	45
NQO, 2.5	A	25	3	2	0	8	2	1	16	13
	B	25	0	1	0	2	1	1	5	5
	A+B	50	3	3	0	10	3	2	21	18

* = Total cells examined for structural aberrations

Totals given for each culture may differ from values given in Appendix 1, Tables 1-4 if cells are observed which have more than one aberration

APPENDIX 3b

Methane sulphonyl chloride: summary of the numbers and types
of structural aberrations observed

3+17 hours, +S-9

Treatment ($\mu\text{g/mL}$)	Rep	Cells *	g	Chr del	Chr exch	Ctd del	Ctd exch	Other	Abs +g	Abs -g
Solvent	A	100	3	0	0	1	1	0	5	2
	B	100	1	0	0	0	0	0	1	0
	A+B	200	4	0	0	1	1	0	6	2
94.71	A	100	3	1	0	3	0	0	7	4
	B	100	2	1	0	4	1	0	8	6
	A+B	200	5	2	0	7	1	0	15	10
135.3	A	100	7	1	0	1	1	0	10	3
	B	100	4	8	0	23	0	0	34	30
	A+B	200	11	9	0	24	1	0	45	34
193.3	A	100	4	1	0	6	0	0	11	7
	B	100	7	1	1	8	0	0	17	10
	A+B	200	11	2	1	14	0	0	28	17
CPA, 25	A	75	11	4	0	18	4	0	37	26
	B	26	4	3	0	2	1	0	10	6
	A+B	101	15	7	0	20	5	0	47	32

* = Total cells examined for structural aberrations

Totals given for each culture may differ from values given in Appendix 1, Tables 1-4 if cells are observed which have more than one aberration

APPENDIX 3c

Methane sulphonyl chloride: summary of the numbers and types
of structural aberrations observed

44+0 hours, -S-9

Treatment ($\mu\text{g/mL}$)	Rep	Cells *	g	Chr del	Chr exch	Ctd del	Ctd exch	Other	Abs +g	Abs -g
Solvent	A	100	4	0	0	2	0	0	6	2
	B	100	3	0	0	5	0	0	8	5
	A+B	200	7	0	0	7	0	0	14	7
193.3	A	100	4	4	0	5	1	1	15	11
	B	100	6	1	0	2	0	0	9	3
	A+B	200	10	5	0	7	1	1	24	14

3+41 hours, +S-9

Treatment ($\mu\text{g/mL}$)	Rep	Cells *	g	Chr del	Chr exch	Ctd del	Ctd exch	Other	Abs +g	Abs -g
Solvent	A	100	1	1	0	2	0	0	4	3
	B	100	0	1	0	0	0	0	1	1
	A+B	200	1	2	0	2	0	0	5	4
193.3	A	100	5	3	0	4	0	0	12	7
	B	100	2	3	0	8	1	0	14	12
	A+B	200	7	6	0	12	1	0	26	19

* = Total cells examined for structural aberrations

Totals given for each culture may differ from values given in Appendix 1, Tables 1-4 if cells are observed which have more than one aberration

APPENDIX 4a

Methane sulphonyl chloride: summary of the numbers and types
of numerical aberrations observed

20+0 hours, -S-9

Donor sex: male

Treatment ($\mu\text{g}/\text{mL}$)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	101	0	0	1	1	1.0
	B	103	0	0	3	3	2.9
	A+B	204	0	0	4	4	2.0
94.71	A	101	0	0	1	1	1.0
	B	100	0	0	0	0	0
	A+B	201	0	0	1	1	0.5
135.3	A	102	0	0	2	2	2.0
	B	100	0	0	0	0	0
	A+B	202	0	0	2	2	1.0
193.3	A	100	0	0	0	0	0
	B	100	0	0	0	0	0
	A+B	200	0	0	0	0	0
NQO, 2.5	A	25	0	0	0	0	0
	B	25	0	0	0	0	0
	A+B	50	0	0	0	0	0

** = Total cells examined for numerical aberrations

APPENDIX 4b

Methane sulphonyl chloride: summary of the numbers and types
of numerical aberrations observed

3+17 hours, +S-9

Donor sex: male

Treatment ($\mu\text{g/mL}$)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	100	0	0	0	0	0
	B	102	0	2	0	2	2.0
	A+B	202	0	2	0	2	1.0
94.71	A	101	0	0	1	1	1.0
	B	101	0	0	1	1	1.0
	A+B	202	0	0	2	2	1.0
135.3	A	102	0	0	2	2	2.0
	B	101	0	0	1	1	1.0
	A+B	203	0	0	3	3	1.5
193.3	A	100	0	0	0	0	0
	B	101	0	0	1	1	1.0
	A+B	201	0	0	1	1	0.5
CPA, 25	A	75	0	0	0	0	0
	B	26	0	0	0	0	0
	A+B	101	0	0	0	0	0

** = Total cells examined for numerical aberrations

APPENDIX 4c

Methane sulphonyl chloride: summary of the numbers and types of numerical aberrations observed

44+0 hours, -S-9

Donor sex: male

Treatment ($\mu\text{g/mL}$)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	105	0	0	5	5	4.8
	B	107	0	0	7	7	6.5
	A+B	212	0	0	12	12	5.7
193.3	A	112	1	0	11	12	10.7
	B	110	0	0	10	10	9.1
	A+B	222	1	0	21	22	9.9, NS

3+41 hours, +S-9

Donor sex: male

Treatment ($\mu\text{g/mL}$)	Rep	Cells **	H	E	P	Tot abs	% with num abs
Solvent	A	103	0	0	3	3	2.9
	B	101	1	0	0	1	1.0
	A+B	204	1	0	3	4	2.0
193.3	A	107	1	2	4	7	6.5
	B	110	2	1	7	10	9.1
	A+B	217	3	3	11	17	7.8, $p < 0.01$

** = Total cells examined for numerical aberrations

Numbers highlighted exceed historical negative control range (Appendix 6)

NS = not significant

APPENDIX 5a

Methane sulphonyl chloride: statistical analysis of test article data

Cells with structural aberrations excluding gaps

20+0 hours, -S-9

Binomial Dispersion Test $\chi^2 = 8.75$	DF = 4
Significance NS	

Treatment ($\mu\text{g/mL}$)	Cells	Aberrant cells	Proportion	Fisher's exact test	Significance
Solvent	200	5	0.0250		
94.71	200	23	0.1150	0.000	$p \leq 0.001$
135.3	200	42	0.2100	0.000	$p \leq 0.001$
193.3	200	30	0.1500	0.000	$p \leq 0.001$
NQO, 2.5	50	16	0.3200	0.000	$p \leq 0.001$

3+17 hours, +S-9

Binomial Dispersion Test $\chi^2 = 25.37$	DF = 4
Significance $p \leq 0.001$	

Treatment ($\mu\text{g/mL}$)	Cells	Aberrant cells	Proportion	Fisher's exact test	Significance
Solvent	200	2	0.0100		
94.71	200	10	0.0500	0.010	$p \leq 0.05$
135.3	200	25	0.1250	0.000	$p \leq 0.001$
193.3	200	14	0.0700	0.001	$p \leq 0.001$
CPA, 25	101	18	0.1782	0.000	$p \leq 0.001$

NS = not significant

DF = degrees of freedom

APPENDIX 5b

Methane sulphonyl chloride: statistical analysis of test article data

Cells with structural aberrations excluding gaps

44+0 hours, -S-9

Binomial Dispersion Test $\chi^2 = 3.02$	DF = 2
Significance NS	

Treatment ($\mu\text{g/mL}$)	Cells	Aberrant cells	Proportion	Fisher's exact test	Significance
Solvent	200	7	0.0350		
193.3	200	10	0.0500	0.236	NS

3+41 hours, +S-9

Binomial Dispersion Test $\chi^2 = 2.11$	DF = 2
Significance NS	

Treatment ($\mu\text{g/mL}$)	Cells	Aberrant cells	Proportion	Fisher's exact test	Significance
Solvent	200	4	0.0200		
193.3	200	16	0.0800	0.003	$p \leq 0.01$

NS = not significant

DF = degrees of freedom

APPENDIX 6

Historical ranges for solvent controls: male donors

Sampling time (hours) and S-9 treatment	Category	Total number of cells scored	Aberrant cells scored per 100 cells	
			Mean	Calculated normal range
20 -S-9	Structural aberrations including gaps	8400	2.05	0-7
	Structural aberrations excluding gaps	8400	1.17	0-5
	Polyploid cells	8439	0.29	0-2
	Numerical aberrations	8439	0.46	0-3

Sampling time (hours) and S-9 treatment	Category	Total number of cells scored	Aberrant cells scored per 100 cells	
			Mean	Calculated normal range
3+17 +S-9	Structural aberrations including gaps	8372	1.66	0-6
	Structural aberrations excluding gaps	8372	0.96	0-4
	Polyploid cells	8412	0.22	0-2
	Numerical aberrations	8412	0.47	0-3

Calculated in October 1996

APPENDIX 6

Historical ranges for solvent controls: male donors

Sampling time (hours) and S-9 treatment	Category	Total number of cells scored	Aberrant cells scored per 100 cells	
			Mean	Calculated normal range
44 -S-9	Structural aberrations including gaps	3200	2.72	0-9
	Structural aberrations excluding gaps	3200	1.16	0-5
	Polyploid cells	3217	0.40	0-3
	Numerical aberrations	3217	0.52	0-3

Sampling time (hours) and S-9 treatment	Category	Total number of cells scored	Aberrant cells scored per 100 cells	
			Mean	Calculated normal range
44 +S-9	Structural aberrations including gaps	3200	1.44	0-6
	Structural aberrations excluding gaps	3200	0.66	0-3
	Polyploid cells	3209	0.12	0-1
	Numerical aberrations	3209	0.28	0-2

Calculated in October 1996

APPENDIX 7

Quality control statement for S-9

MOLTOX™ POST MITOCHONDRIAL SUPERNATANT (S-9)
PRODUCTION & QUALITY CONTROL CERTIFICATE

LOT NO.: <u>0679</u>	SPECIES: <u>Rat</u>	PREPARATION DATE: <u>08 July 1996</u>
PART NO.: <u>11-101</u>	STRAIN: <u>Sprague Dawley</u>	EXPIRATION DATE: <u>08 July 1998</u>
VOLUME: <u>5 ml</u>	SEX: <u>Male</u>	BUFFER: <u>0.154M KCl</u>
	TISSUE: <u>Liver</u>	INDUCING AGENT(s): <u>Aroclor 1254</u>
REFERENCE: <u>Maron, D & Ames, B. Mutat. Res. 113:173, 1983</u>		<u>Monsanto Lot No. KL615 - 500mg/kg</u>

BIOCHEMISTRY:

- PROTEIN

41.9 mg/ml

Assayed according to the method of Lowry et al., JBC 193:265, 1951 using bovine serum albumin as the standard.

- ALKOXYRESORUFIN-O-DEALKYLASE ACTIVITIES

Activity	P450	Fold - Induction
EROD	IA1, IA2	234.8
PROD	2B1	63.5
BROD	2B1	49.3
MROD	1A2	128.4

Assays for ethoxyresorufin-O-deethylase (EROD), pentoxy-, benzyl- and methoxyresorufin-O-dealkylases (PROD: BROD; MROD) were conducted using a modification of the methods of Burke, et al., Biochem Pharm 34:3337, 1985. Fold-inductions were calculated as the ratio of the sample vs. uninduced specific activities (SA's). Control SA's (pmoles/min/mg protein) were 9.91, 3.82, 49.3, & 5.7 for EROD, PROD, BROD and MROD, respectively.

BIOASSAY:

- STERILITY TEST

Samples of S-9 were assayed for the presence of contaminating microflora by plating 1.0ml volumes on Trypticase Soy and Minimal Glucose (Vogel-Bonner E, supplemented with 0.05mM L-histidine and D-biotin) media. Triplicate plates were read after 48 or 72h incubation at 37C. No evidence of contamination was observed.

- PROMUTAGEN ACTIVATION

No. His+ Revertants	
EtBr/	CPA/
TA98	TA1535
1048	1027

The ability of the sample to activate ethidium bromide (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lesca, et al., Mutation Res 129:299, 1984. Data were expressed as revertants per ug EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.2 - 10% in S9 mix, were tested for their ability to activate benzo(a)pyrene (BP) and 2-aminoanthracene (2-AA) to intermediates mutagenic to TA100. Assays were conducted using duplicate plates as described by Maron & Ames (Mutat. Res.113:173, 1983.)

ul S9 per plate/number his⁺ revertants per plate

Promutagen	0	1	5	10	20	50
BP (5ug)	99.5	181	350	555.5	935	1497.5
2-AA (2.5ug)	102	768	2457	2666.5	2727	2331.5

MOLECULAR TOXICOLOGY, INC.
111 Gibraltar St.
Annapolis, MD 21401
(410) 268 7232

APPENDIX 8

Sponsor's certificate of analysis

elf aquitaine production 

adresse postale :
BP 22 84170 Lacq
téléphone - 33 - 59 92 22 22
télécopie - 33 - 59 92 22 22

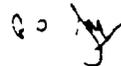
direction exploration production france

Ref: 96000478

ANALYSIS for METHANE SULFONYL CHLORIDE (MSC)

CHARACTERISTICS		ANALYSIS
Purity	(%Weight)	99.85
Water	(ppm)	< 10
Colour	APHA	17

Lacq June 25th 1996


P. AUCLAIR

Elf Aquitaine Production
société anonyme au capital de 1 055 000 000 F
siège social : tour Elf 2 place de la Coupole
La Défense 8 Courbevoie (Hauts-de-Seine)
rcs Pau b 632 022 711

APPENDIX 9

Minor deviations from protocol

Protocol section	Subject	Deviation
3.1	pH and osmolality	pH was measured at the highest concentration at the request of the Sponsor. Osmolality was not measured because of the health and safety risk known to be posed by this chemical (data provided by the Sponsor)
4.1, 4.2	Centrifugation	Centrifugation at 200 x 'g' was carried out for 5 rather than 10 minutes. This has been shown to be of sufficient duration for this procedure.
4.4	Selection of doses for analysis	Cultures harvested at 44 hours were analysed in spite of a positive effect being seen at the 20 hour harvest. Error in protocol.
8	Responsible Personnel	With effect from 1 September 1996, the Head of Molecular Toxicology changed from S Dean to J Clements.
	Test facility	The Company trading name has changed from Corning Hazleton (CHE) to Covance Laboratories Ltd (CLE). For the purposes of the study and reporting these can be regarded as equivalent.

Final *Report*

Methane Sulfonyl Chloride: Reverse Mutation in
five Histidine-requiring strains of *Salmonella*
typhimurium

Prepared for: ELF Atochem

Report number: 514/29-1052
Issue date: January 1997

CORNING Hazleton

FINAL REPORT

Methane Sulfonyl Chloride: Reverse Mutation in five Histidine-requiring strains of *Salmonella typhimurium*

Report for: ELF Atochem
Direction Sécurité Environnement Produit
Département Toxicologie Industrielle
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Report number: 514/29-1052

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Page number: 1 of 56

**STUDY DIRECTOR AUTHENTICATION
AND GLP COMPLIANCE STATEMENT**

**Methane Sulfonyl Chloride: Reverse Mutation in five
Histidine-requiring strains of *Salmonella typhimurium***

I, the undersigned, hereby declare that the work described in this report* was performed under my supervision, as Study Director, and that the report provides a true and accurate record of the results obtained.

The study was performed in accordance with the agreed protocol, unless otherwise stated, and the study objectives were achieved. The study was also performed in accordance with Corning Hazleton (Europe) Standard Operating Procedures and the principles of the following codes of Good Laboratory Practice:

UK Principles of Good Laboratory Practice
The UK Compliance Programme
Department of Health
London 1989

OECD
Good Laboratory Practice in the Testing of Chemicals
Final Report ISBN 92-64-12367-9
Paris 1982


N Dawkes BSc
Study Director

Date: 16-1-97

* Excluding Appendix 7 which is the responsibility of the Sponsor

REVIEWING SCIENTIST'S STATEMENT

**Methane Sulfonyl Chloride: Reverse Mutation in five
Histidine-requiring strains of *Salmonella typhimurium***

I, the undersigned, hereby declare that I have reviewed this report in conjunction with the Study Director and that the interpretation and presentation of the data in the report are consistent with the results obtained.



M Ballantyne BSc
Scientist

Date: 16-1-97

RESPONSIBLE PERSONNEL

Director of Toxicology : D Kirkland BSc MIBiol PhD
Head of Molecular Toxicology : J Clements BSc PhD (Appendix 8)
Head of Quality Assurance : S White HNC
Study Director : N Dawkes BSc
Investigator : M Hayes MSc

Sponsor's Monitor : B Molinier

Date of initiation of study 19 August 1996

Date of Sponsor's approval of study: 28 August 1996

Date of start of experimental work: 3 October 1996

Date of completion of experimental work: 10 November 1996

Date of study completion 16 January 1997

**QUALITY ASSURANCE RECORD
AND AUTHENTICATION STATEMENT**

**Methane Sulfonyl Chloride: Reverse Mutation in five
Histidine-requiring strains of *Salmonella typhimurium***

The study described in this report was subject to audit by the independent Quality Assurance Unit as indicated below. Studies of this type which involve frequent repetition of similar or identical procedures are inspected in accordance with a "batch process" programme, as described in Quality Assurance Unit Standard Operating Procedures.

Critical procedures relevant to this study type were selected without bias from at least one study on the batch process programme. The inspection dates indicated below are of those inspections conducted closest to the time of study conduct.

The findings of each audit were reported to the Study Director and management as prescribed by Standard Operating Procedures.

The report audit was designed to confirm that as far as can be reasonably established the methods described and results incorporated in the report accurately reflect the raw data produced during the study.

Inspection programme	Inspection date	Report date
Protocol review	22 August 1996	22 August 1996
Procedure inspection (4)	6 September 1996	6 September 1996
Procedure inspection	8 November 1996	8 November 1996
Procedure inspection	20 November 1996	20 November 1996
Data review	January 1997	15 January 1997
Draft study report	January 1997	15 January 1997

Gaul Wood

**G Wood
Section Head Quality Assurance**

Date: 17 January 1997

ARCHIVE STATEMENT

All primary data, or authenticated copies thereof, specimens and the final report will be retained in the Corning Hazleton (Europe) archives for ten years after the submission of the final report. At this time the Sponsor will be contacted to determine whether data should be returned, retained or destroyed on their behalf.

Specimens requiring storage deep frozen are specifically excluded from the above. These will be retained for as long as the quality of the material permits evaluation but for no longer than three months after submission of the final report. The study Sponsor will be notified before specimens are destroyed on their behalf.

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1 SUMMARY

Methane Sulfonyl Chloride was assayed for mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium*, both in the absence and in the presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9), in two separate experiments.

An initial toxicity range-finder experiment was carried out in strain TA100 only, using final concentrations of Methane Sulfonyl Chloride at 8, 40, 200, 1000 and 5000 $\mu\text{g}/\text{plate}$, plus solvent and positive controls. Following these treatments, and those in Experiment 1 performed using the same dose range, no clear evidence of toxicity was observed, as would normally be indicated by a thinning of the background bacterial lawn.

In order to investigate small increases in revertant numbers observed following Experiment 1 treatments, the maximum treatment concentration for Experiment 2 was increased to 10000 $\mu\text{g}/\text{plate}$. Experiment 2 treatments utilised a narrowed dose range in order to more closely investigate those doses of Methane Sulfonyl Chloride previously demonstrating a mutagenic response. Treatments in the presence of S-9 initially included a pre-incubation step, in order to increase the range of mutagenic chemicals that could be detected using this assay system. Following these initial Experiment 2 treatments performed using pre-incubation methodology, a majority of the test doses were affected in some way by toxicity. The data from these treatments were not therefore considered to have been able to give a valid determination of the test article mutagenic activity. Repeat treatments of these strains in the presence of S-9 were therefore performed using standard plate-incorporation methodology. Following this additional experimentation signs of toxicity were observed with strain TA102 at the two highest treatment concentrations. Experiment 2 treatments in the absence of S-9 also gave rise to signs of toxicity in a majority of the strains at the higher treatment concentrations.

Negative (solvent) and positive control treatments were included for all strains in both experiments. The mean numbers of revertant colonies on negative control plates all fell within acceptable ranges, and were significantly elevated by positive control treatments.

Methane Sulfonyl Chloride treatments of strain TA1535 both in the absence and in the presence of S-9, resulted in statistically significant and reproducible increases in revertant numbers (when data were analysed at the 1% level using Dunnett's test), that were considered to be clear evidence of a mutagenic effect. Methane Sulfonyl Chloride

treatments of strain TA100 in the absence and presence of S-9 also gave rise to statistically significant and reproducible increases in revertant numbers. Although the magnitude of these increases was markedly smaller than those observed with strain TA1535, it was considered that these increases in revertant numbers provided further evidence of Methane Sulfonyl Chloride mutagenic activity.

No treatment of strains TA98, TA1537 and TA102 either in the absence or presence of metabolic activation resulted in a reproducible increase in revertant numbers sufficient to be considered as evidence of mutagenic activity.

It was concluded that Methane Sulfonyl Chloride did induce mutation in *Salmonella typhimurium* strain TA1535, and exhibited evidence of weak mutagenic activity in *Salmonella typhimurium* strain TA100, both in the absence and presence of a rat liver metabolic activation system (S-9), when tested under the conditions employed for this study, which included treatments at concentrations up to 10000 $\mu\text{g}/\text{plate}$.

2 INTRODUCTION

The Ames test is a rapid, reliable and economical method of evaluating the mutagenic potential of a test article by measuring genetic activity in one or more histidine-requiring strains of *Salmonella typhimurium* in the absence and presence of a liver metabolising system (1). A large data base has been accumulated with this assay, confirming its ability to detect genetically active compounds of most chemical classes with around 80-90% sensitivity and specificity (2). The following bacterial strains were used in this study:

Strain	Type of mutation in the histidine gene
TA98	frame-shift
TA100	base-pair substitution
TA1535	base-pair substitution
TA1537	frame-shift
TA102	base-pair substitution

With the exception of strain TA102, these strains require biotin as well as histidine for growth. In strain TA102 the critical mutation in the histidine gene is located on a multicopy plasmid pAQ1. This strain is particularly sensitive to the activities of oxidative and cross-linking mutagens. The pKM101 plasmid derivatives (TA98, TA100 and TA102) have increased sensitivity to certain mutagens as the pKM101 plasmid codes for an error-prone DNA repair system (3).

When exposed to a mutagen, some of the bacteria in the treated population undergo genetic changes which revert them to a non-histidine-requiring state, and they can then grow without exogenous histidine. Different tester strains are used because each is mutated by particular chemical classes of compound. A compound that is mutagenic in one strain need not be so in another (4).

The objective of this study was therefore to evaluate the mutagenic activity of Methane Sulfonyl Chloride by examining its ability to revert five strains of *Salmonella typhimurium* in the absence and presence of a rat liver metabolising system (S-9). The procedures used in this study were in accordance with OECD Test Guideline 471 (1983), EEC Annex V Test B14 (1993) and UKEMS Guidelines (1990).

This study was performed according to the protocol and two amendments, with the exception of the minor deviations detailed in Appendix 8, none of which in any way prejudiced the validity of this study.

3 MATERIALS

3.1 Test article

Methane Sulfonyl Chloride, batch number 96000478, was a brown liquid, which was received on 9 July 1996. It was stored desiccated, under nitrogen, at 1-10 °C in the dark. A certificate of analysis, provided by the sponsor, is presented in Appendix 7. The expiry date of the test article was not stated. Determinations of the stability and characteristics of the test article were the responsibility of the Sponsor.

Test article solutions were prepared by diluting Methane Sulfonyl Chloride (supplied as a 3004.5 mg/mL solution in acetone) in nitrogen purged analytical grade acetone, immediately prior to assay to give the maximum required treatment solution concentration. This solution was not filter-sterilized and further dilutions were made using nitrogen purged acetone. The test article solutions were protected from light and used within approximately 5 hours of the initial formulation of the test article. Solutions were used as follows:

Experiment	S-9	Concentration of treatment solution (mg/mL)	Final concentration (µg/plate)
Range-finder Experiment and Mutation Experiment 1	- and +	0.40	8
		2.00	40
		10.00	200
		50.00	1000
		250.00	5000
Mutation Experiment 2	- and +	50.00	1000
		100.0	2000
		150.0	3000
		200.0	4000
		250.0	5000
		375.0	7500
		500.0	10000

Treatments were performed using 0.02 mL addition volumes per plate.

3.2 Controls

Control treatments were performed using the same addition volumes per plate as the test article treatments (0.02 mL). Negative controls comprised treatments with the solvent nitrogen purged acetone. The positive control chemicals were supplied and used as tabulated below:

Chemical	Source	Stock* concentra- tion ($\mu\text{g/mL}$)	Final concentra- tion ($\mu\text{g/plate}$)	Use	
				Strain(s)	S-9
2-nitrofluorene (2NF)	Koch-Light Haverhill, UK	250	5.0	TA98	-
Sodium azide (NaN ₃)	Sigma Chemical Co, Poole, UK	100	2.0	TA100 TA1535	- -
9-aminoacridine (AAC)	Koch-Light, Haverhill, UK	2500	50.0	TA1537	-
Glutaraldehyde (GLU)	Sigma Chemical Co, Poole, UK	1250	25.0	TA102	-
2-aminoanthracene (AAN)	Sigma Chemical Co, Poole, UK	250	5.0	At least one strain	+

* With the exception of NaN₃ and GLU, which were prepared in water, all stock solutions were prepared in sterile anhydrous analytical grade dimethyl sulphoxide (DMSO). NaN₃, 2NF, AAC, GLU and AAN were stored in aliquots at 1-10°C in the dark.

3.3 Metabolic activation system

The mammalian liver post-mitochondrial fraction (S-9) used for metabolic activation was prepared from male Sprague Dawley rats induced with Aroclor 1254 and obtained from Molecular Toxicology Incorporated, Annapolis, Maryland, USA. Batches of MolTox™ S-9 were stored frozen at -80°C, and thawed just prior to incorporation into the top agar (5). Each batch was checked by the manufacturer for sterility, protein content, ability to convert ethidium bromide and cyclophosphamide to bacterial mutagens, and cytochrome P-450-catalysed enzyme activities (alkoxyresorufin-O-dealkylase activities). The quality control statements, relating to the batches of S-9 preparation used, are included in Appendix 6 of this report.

3.3.1 Preparation of 10% S-9 mix and buffer solutions

Quantities were prepared in the following ratios per 100 mL:

Ingredient	Concentration	Quantity (mL)	
		10% S-9 mix	Buffer solution
Sodium phosphate buffer pH 7.4	500 mM	20	20
Glucose-6-phosphate (disodium)	180 mg/mL	0.845	-
NADP (disodium)	25 mg/mL	12.6	-
Magnesium chloride	250 mM	3.2	-
Potassium chloride	150 mM	22	-
L-histidine HCl (in 250 mM MgCl ₂)	1 mg/mL	4	4
d-biotin	1 mg/mL	4.88	4.88
S-9	as section 3.3	10	-
Water	-	to volume	to volume

3.4 Bacteria

Five bacterial strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) were used in this study. All the tester strains, with the exception of strain TA102, were originally obtained from the UK NCTC. Strain TA102 was originally obtained from Glaxo Group Research Limited. For all assays, bacteria were cultured for 10 hours at 37°C in nutrient broth (containing ampicillin for strains TA98 and TA100 and ampicillin and tetracycline for strain TA102). Bacteria were taken from vials of frozen cultures, which had been checked for strain characteristics of histidine dependence, *rfa* character and resistance to ampicillin (TA98 and TA100) or ampicillin plus tetracycline (TA102). Checks were carried out according to Maron and Ames (3) and De Serres and Shelby (6). All experimentation commenced within 2 hours of the end of the incubation period.

4 METHODS

4.1 Range-finder experiment

Methane Sulfonyl Chloride was tested for toxicity in strain TA100, at the concentrations detailed in 3.1. Triplicate plates without and with S-9 mix were used. Negative (solvent) and positive controls were included in quintuplicate and triplicate respectively without and with S-9 mix. These platings were achieved by the following sequence of additions to 2.5 mL molten agar at 46°C:

0.1 mL bacterial culture

0.02 mL test article solution or control

0.5 mL 10% S-9 mix or buffer solution

followed by rapid mixing and pouring on to Minimal Davis agar plates. When set, the plates were inverted and incubated at 37°C in the dark for 3 days. Following incubation, these plates were examined for evidence of toxicity to the background lawn, and where possible revertant colonies were counted (4.3).

4.2 Mutation experiments

Methane Sulfonyl Chloride was tested for mutation in five strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102), in two separate experiments, at the concentrations detailed in 3.1 using triplicate plates without and with S-9. Negative (solvent) controls were included in each assay, in quintuplicate without and with S-9. In each experiment, bacterial strains were treated with diagnostic mutagens in triplicate in the absence of S-9 as detailed in 3.2. The activity of the S-9 mix used in each experiment was confirmed by AAN treatments (again in triplicate) of at least one strain in the presence of S-9 (see Appendices 2, 3 and 4 for details of actual strains tested). Platings were achieved as described above.

As the results of the first experiment were equivocal, treatments in the presence of S-9 in Experiment 2 initially included a pre-incubation step, where the quantities of test article or control solution, bacteria and S-9 mix detailed in 4.1, were mixed together and incubated for 1 hour at 37°C, before the addition of 2.5 mL molten agar at 46°C. Plating of these treatments then proceeded as for the normal plate-incorporation procedure. In this way, it was hoped to increase the range of

mutagenic chemicals that could be detected in the assay. To further investigate an increase in revertant numbers observed following Experiment 1 treatments of strain TA1535, Experiment 2 treatments of this strain in the presence of S-9 were also performed using the plate-incorporation methodology. Due to the fact no positive control treatments were performed with these plate-incorporation treatments in the presence of S-9, the activity of the S-9 mix using this method could not be confirmed. These treatments were therefore repeated, alongside the other test strains (see below), and the data presented as Experiment 2 mutagenicity data.

Following initial Experiment 2 treatments of all the test strains performed in the presence of S-9 using pre-incubation methodology, a majority of the test doses were affected in some way by toxicity. The data from these treatments were not therefore considered to have been able to give a valid determination of the test article mutagenic activity. Repeat treatments of these strains in the presence of metabolic activation were therefore performed, using standard plate-incorporation methodology. The results of these treatments are those presented as the Experiment 2 mutagenicity data, but diagnostic control treatments in the absence of S-9 performed in parallel with these treatments are not presented.

4.3 Colony counting

Colonies were counted electronically using a Seescan Colony Counter (Seescan plc) and the background lawn inspected for signs of toxicity.

4.4 Analysis of results

4.4.1 Treatment of data

Individual plate counts from both experiments were recorded separately and the mean and standard deviation of the plate counts for each treatment were determined.

The accepted normal ranges for mean numbers of spontaneous revertants on solvent control plates for this laboratory are presented in Appendix 5. The ranges quoted are based on a large volume of historical control data accumulated from experiments where the responses of the strains to positive control compounds were considered satisfactory. Data for this laboratory are

consistent with ranges of spontaneous revertants per plate considered acceptable elsewhere (6).

For evaluation of data there are many statistical methods in use, and several are acceptable (7,8). The m-statistic was calculated to check that the data were Poisson-distributed (8), and Dunnett's test was used to compare the counts of each dose with the control. The presence or otherwise of a dose response was checked by linear regression analysis (8).

4.4.2 Acceptance criteria

The assay was considered valid if the following criteria were met:

- 1) the mean negative control counts fell within the normal ranges as defined in Appendix 5
- 2) the positive control chemicals induced clear increases in revertant numbers confirming discrimination between different strains, and an active S-9 preparation
- 3) no more than 5% of the plates were lost through contamination or some other unforeseen event.

4.4.3 Evaluation criteria

The test article was considered to be mutagenic if:

- 1) the assay was valid (see 4.4.2)
- 2) Dunnett's test gave a significant response ($p \leq 0.01$), and the data set showed a significant dose-correlation
- 3) the positive responses described in 2) were reproducible.

5 RESULTS

5.1 Toxicity, solubility and dose selection

Details of all treatment solution concentrations and final Methane Sulfonyl Chloride doses are provided in section 3.1.

An initial toxicity range-finder experiment was carried out in strain TA100 only, using final concentrations of Methane Sulfonyl Chloride at 8, 40, 200, 1000 and 5000 $\mu\text{g}/\text{plate}$ plus negative and positive controls. Following these treatments, no evidence of toxicity (as would normally have been indicated by a thinning of the background bacterial lawn and/or a marked reduction in revertant numbers) was observed. In order to assess the reproducibility of significant increases in revertant numbers observed following the range-finder experiment (Appendix 2, Tables 1RF and 2RF), strain TA100 was treated alongside the other tester strains in Experiment 1.

Experiment 1 treatments were performed using the same dose range as that employed in the range-finder experiment. Following these treatments once again no clear evidence of toxicity was observed.

In order to investigate small increases in revertant numbers observed following Experiment 1 treatments, the maximum treatment concentration for Experiment 2 was increased to 10000 $\mu\text{g}/\text{plate}$. Experiment 2 treatments utilised a narrowed dose range in order to more closely investigate those doses of Methane Sulfonyl Chloride previously demonstrating a mutagenic response. Treatments in the presence of S-9 initially included a pre-incubation step, in order to increase the range of mutagenic chemicals that could be detected using this assay system. Following these initial Experiment 2 treatments performed using pre-incubation methodology, a majority of the test doses were affected in some way by toxicity. It was considered that the solvent (acetone) was at least partly responsible for the toxic effects observed using this methodology. The data from these treatments were not therefore considered to have been able to give a valid determination of the test article mutagenic activity. Due to the fact pre-incubation methodology was not considered appropriate, repeat treatments of these strains in the presence of S-9 were therefore performed using standard plate-incorporation methodology. Following this additional experimentation signs of toxicity were observed with strain TA102 at the two highest treatment

concentrations. Experiment 2 treatments in the absence of S-9 also gave rise to signs of toxicity at the maximum treatment concentration in strains TA98 and TA1535, and at the two highest test doses in strains TA1537 and TA102.

No precipitation of the test article was observed on any of the test plates in any of the experiments performed.

5.2 Mutation

The individual plate counts were averaged to give mean values which are presented in Appendices 2 and 3. From the data it can be seen that mean solvent control counts fell within the normal historical ranges (Appendix 5), that the positive control chemicals all induced large increases in revertant numbers in the appropriate strains, and that less than 5% of plates were lost, leaving adequate numbers of plates at all treatments. The study was accepted as valid.

The mutation data for the individual strains were evaluated as follows:

Salmonella typhimurium strain TA1535

Methane Sulfonyl Chloride treatments of strain TA1535 both in the absence and in the presence of S-9 resulted in dose-related, statistically significant and reproducible increases in revertant numbers (when data were analysed at the 1% level using Dunnett's test) that were considered to be clear evidence of a mutagenic effect (Appendices 3 and 4, Tables 3, 8, 13 and 18).

Salmonella typhimurium strain TA100

Methane Sulfonyl Chloride treatments of strain TA100 both in the absence and in the presence of S-9 resulted in dose-related, statistically significant (when data were analysed at the 1% level using Dunnett's test) and reproducible increases in revertant numbers (Appendices 2, 3 and 4, Tables 1RF, 2RF, 2, 12 and 17). Although the magnitude of these increases was markedly smaller than those observed with genetically related strain TA1535, it was considered that these increases in revertant numbers provided further evidence of Methane Sulfonyl Chloride mutagenic activity.

Salmonella typhimurium strains TA98, TA1537 and TA102

Methane Sulfonyl Chloride treatments of strain TA98 in Experiment 1 and strain TA102 in Experiment 2, both in the absence of S-9, resulted in statistically significant (when data were analysed at the 1% level using Dunnett's test) increases in revertant numbers (Appendices 3 and 4, Tables 1 and 15). However, since there was no evidence of any reproducibility of these increases they were not regarded to have been the result of Methane Sulfonyl Chloride mutagenic activity, but were considered more likely to have been chance occurrences. No treatment of strains TA98 or TA102 in the presence of S-9, or strain TA1537 (either with or without S-9), resulted in an increase in revertant numbers sufficient to be considered as evidence of mutagenic activity.

6 CONCLUSION

It was concluded that Methane Sulfonyl Chloride did induce mutation in *Salmonella typhimurium* strain TA1535, and exhibited evidence of weak mutagenic activity in *Salmonella typhimurium* strain TA100, both in the absence and presence of a rat liver metabolic activation system (S-9), when tested under the conditions employed for this study, which included treatments at concentrations up to 10000 $\mu\text{g}/\text{plate}$.

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APPENDIX 1

Table of symbols

P	=	precipitation of test article observed
C	=	contaminated plate
W	=	wet plate
M	=	plate counted manually
L	=	plate lost (plate damage or technical error)
S	=	slight toxicity (some thinning of background lawn and/or presence of microcolonies)
V	=	very thin background lawn
A	=	absence of background lawn
T	=	toxic, no revertant colonies observed
-ve	=	negative (solvent) controls
+ve	=	positive controls
N/A	=	not applicable

APPENDIX 2

**Methane Sulfonyl Chloride: raw plate counts and calculated mutagenicity data
Range-finder Experiment**

```

=====
Table Number      :1RF
-----
CHE Study Number  :514/29          Experiment Number :Range-finder
Positive Control  :NaN3           S-9 Present      :NO
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA100
-----
Point            -ve      2      3      4      5      6              +ve
-----
Replicates       5        3        3        3        3        3              3
Dose ug/plate    0         8        40       200      1000     5000          2
-----
Revertants       81        93        95        82        73        136           L
                  90        91        95        87        81        122           470
                  91        101       70        85        82        131           493
                  80
                  91
-----
Mean             86.6     95.0     86.7     84.7     78.7     129.7         481.5
-----
Increase over    1.1      1.0      1.0      0.9      1.5^
Control
-----
Standard Deviation 5.6     5.3     14.4     2.5     4.9     7.1           16.3
-----
R-Coefficient           0.08    0.24    0.48    0.84
Gradient               -0.04   -0.02   -0.01   0.01
Degrees of freedom      9       12     15     18
Significance            NS      NS      NS     ***
-----
Dunnett's t value     1.54   -0.05  -0.35  -1.51   7.24
Significance           NS      NS      NS      NS     ***
-----
M Statistic          0.60
=====

```

Key NS represents Not Significant
* represents $p \leq 0.05$
** represents $p \leq 0.01$
*** represents $p \leq 0.001$
^ represents maximum increase over control
For Dunnett's t value *** represents $p < 0.01$

Point	-ve	2	3	4	5	6	+ve
Replicates	5	3	3	3	3	3	3
Dose ug/plate	0	8	40	200	1000	5000	5
Revertants	102	127	133	103	108	163	1265
	97	112	107	118	77	166	1076
	93	93	100	102	107	168	1077
	119						
	109						
Mean	104.0	110.7	113.3	107.7	97.3	165.7	1139.3
Increase over Control		1.1	1.1	1.0	0.9	1.6 [^]	11.0
Standard Deviation	10.3	17.0	17.4	9.0	17.6	2.5	108.8
R-Coefficient			0.28	0.03	0.31	0.82	
Gradient			0.21	0.00	-0.01	0.01	
Degrees of freedom			9	12	15	18	
Significance			NS	NS	NS	***	
Dunnnett's t value		0.67	0.94	0.39	-0.75	5.75	
Significance		NS	NS	NS	NS	***	
M Statistic		1.61					

Key NS represents Not Significant
 * represents p <= 0.05
 ** represents p <= 0.01
 *** represents p <= 0.001
 ^ represents maximum increase over control
 For Dunnnett's t value *** represents p < 0.01

APPENDIX 3

**Methane Sulfonyl Chloride: raw plate counts and calculated mutagenicity data
Experiment 1**

```

=====
Table Number      :1
-----
CHE Study Number  :514/29          Experiment Number :1
Positive Control  :2NF             S-9 Present      :NO
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA98
-----
Point            -ve      2      3      4      5      6      +ve
-----
Replicates       5       3       3       3       3       3       3
Dose ug/plate    0       8      40     200    1000   5000    5
-----
Revertants       18      18      11      22      27      29      309
                  24      18      25      18      20      34      370
                  12      23      18      27      24      41      300
                  20
                  22
-----
Mean             19.2    19.7    18.0    22.3    23.7    34.7    326.3
-----
Increase over    1.0     0.9     1.2     1.2     1.8^    17.0
Control
-----
Standard         4.6     2.9     7.0     4.5     3.5     6.0     38.1
Deviation
-----
R-Coefficient           0.13    0.28    0.38    0.78
Gradient               -0.03    0.02    0.00    0.00
Degrees of freedom           9      12      15      18
Significance              NS      NS      NS      ***
-----
Dunnett's t value    0.19   -0.42    0.90    1.27    3.85
Significance          NS      NS      NS      NS      ***
-----
M Statistic         1.12
=====

```

Key NS represents Not Significant
* represents $p \leq 0.05$
** represents $p \leq 0.01$
*** represents $p \leq 0.001$
^ represents maximum increase over control
For Dunnett's t value *** represents $p < 0.01$

```

=====
Table Number      :2
-----
CHE Study Number  :514/29          Experiment Number :1
Positive Control  :NaN3           S-9 Present       :NO
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA100
-----
Point            -ve      2      3      4      5      6              +ve
-----
Replicates       5       3       3       3       3       3              3
Dose ug/plate    0       8      40     200    1000   5000           2
-----
Revertants       120    119    128    117    120    153            754
                  109    124    129    119    140    148            798
                  118    106    123    124    127    177            740
                  109
                  129
-----
Mean             117.0  116.3  126.7  120.0  129.0  159.3          764.0
-----
Increase over    1.0    1.1    1.0    1.1    1.4^         6.5
Control
-----
Standard         8.4    9.3    3.2    3.6    10.1   15.5           30.3
Deviation
-----
R-Coefficient                    0.54   0.13   0.45   0.86
Gradient                      0.25   0.01   0.01   0.01
Degrees of freedom                9     12     15     18
Significance                      NS     NS     NS     ***
-----
Dunnett's t value  -0.11  1.53  0.49  1.87  6.22
Significance          NS     NS     NS     NS     ***
-----
M Statistic       0.63
=====

```

Key NS represents Not Significant
* represents $p \leq 0.05$
** represents $p \leq 0.01$
*** represents $p \leq 0.001$
^ represents maximum increase over control
For Dunnett's t value *** represents $p < 0.01$

Point	-ve	2	3	4	5	6	+ve
Replicates	5	3	3	3	3	3	3
Dose ug/plate	0	8	40	200	1000	5000	2
Revertants	26	24	31	33	34	48	352
	22	24	35	24	27	42	544
	29	26	20	20	39	54	524
	30						
	21						
Mean	25.6	24.7	28.7	25.7	33.3	48.0	473.3
Increase over Control		1.0	1.1	1.0	1.3	1.9 [^]	18.5
Standard Deviation	4.0	1.2	7.8	6.7	6.0	6.0	105.6
R-Coefficient Gradient			0.32	0.01	0.50	0.85	
Degrees of freedom			9	12	15	18	
Significance			NS	NS	*	***	
Dunnett's t value	-0.22	0.74	-0.02	1.92	5.07		
Significance	NS	NS	NS	NS	***		
M Statistic	1.00						

Key NS represents Not Significant
 * represents $p \leq 0.05$
 ** represents $p \leq 0.01$
 *** represents $p \leq 0.001$
 ^ represents maximum increase over control
 For Dunnett's t value *** represents $p < 0.01$

```

=====
Table Number      :4
-----
CHE Study Number  :514/29          Experiment Number :1
Positive Control  :AAC             S-9 Present      :NO
Compound Name     :Methane Sulfonyl Chloride
Strain Number     :TA1537
-----
Point             -ve      2      3      4      5      6      +ve
-----
Replicates        5       3       3       3       3       3       3
Dose ug/plate     0       8      40     200    1000   5000   50
-----
Revertants        17      15      19      17      19      16      242
                  11      11      19      12      13      28      157
                  11      12      20      13      16      16      320
                  10
                  18
-----
Mean              13.4    12.7    19.3    14.0    16.0    20.0    239.7
Increase over
Control           0.9     1.4     1.0     1.2     1.5^    17.9
Standard
Deviation         3.8     2.1     0.6     2.6     3.0     6.9     81.5
-----
R-Coefficient
Gradient          0.71    0.03    0.16    0.45
                  0.16    0.00    0.00    0.00
Degrees of freedom 9       12      15      18
Significance      *       NS      NS      *
-----
Dunnett's t value -0.25   2.32   0.30   1.08   2.42
Significance      NS      NS      NS      NS      NS
-----
M Statistic      0.85
=====

```

Key NS represents Not Significant
 * represents $p \leq 0.05$
 ** represents $p \leq 0.01$
 *** represents $p \leq 0.001$
 ^ represents maximum increase over control
 For Dunnett's t value *** represents $p < 0.01$

```

=====
Table Number      :5
-----
CHE Study Number  :514/29          Experiment Number :1
Positive Control  :GLU             S-9 Present      :NO
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA102
-----
Point            -ve    2    3    4    5    6            +ve
-----
Replicates       5     3     3     3     3     3            3
Dose ug/plate    0     8    40   200  1000  5000         25
-----
Revertants       278   238   254   180   323   275          437
                  277   232   259   211   292   280          437
                  296   282   239   237   297   273          429
                  274
                  277
-----
Mean              280.4  250.7  250.7  209.3  304.0  276.0        434.3
-----
Increase over    0.9    0.9    0.7    1.1^    1.0            1.5
Control
-----
Standard         8.8    27.3   10.4   28.5   16.6    3.6          4.6
Deviation
-----
R-Coefficient           0.52   0.78   0.44   0.24
Gradient               -0.63  -0.31   0.04   0.00
Degrees of freedom      9     12    15    18
Significance            NS     NS     NS     NS
-----
Dunnett's t value  -2.27  -2.24  -5.63   1.68  -0.32
Significance          NS     NS     NS     NS     NS
-----
M Statistic        1.26
=====

```

Key NS represents Not Significant
 * represents p <= 0.05
 ** represents p <= 0.01
 *** represents p <= 0.001
 ^ represents maximum increase over control
 For Dunnnett's t value *** represents p < 0.01

```

=====
Table Number      :6
-----
CHE Study Number  :514/29          Experiment Number :1
Positive Control  :AAN             S-9 Present       :YES
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA98
-----
Point            -ve    2      3      4      5      6              +ve
-----
Replicates       5      3      3      3      3      3              3
Dose ug/plate   0      8     40    200   1000  5000          5
-----
Revertants       25     28    16    24    28    23             491
                 23     25    23    17    27    23             567
                 30     18    28    23    26    25             450
                 29
                 24
-----
Mean             26.2   23.7   22.3   21.3   27.0   23.7          502.7
-----
Increase over    0.9    0.9    0.8    1.0^    0.9          19.2
Control
-----
Standard         3.1    5.1    6.0    3.8    1.0    1.2           59.4
Deviation
-----
R-Coefficient           0.35   0.36   0.23   0.02
Gradient               -0.09  -0.02   0.00  -0.00
Degrees of freedom      9      12     15     18
Significance            NS      NS      NS      NS
-----
Dunnett's t value   -0.92  -1.43  -1.75   0.29  -0.85
Significance         NS      NS      NS      NS      NS
-----
M Statistic        0.61
=====

```

Key NS represents Not Significant
* represents p <= 0.05
** represents p <= 0.01
*** represents p <= 0.001
^ represents maximum increase over control
For Dunnett's t value *** represents p < 0.01

```

=====
Table Number      :7
-----
CHE Study Number  :514/29          Experiment Number :1
Positive Control  :AAN             S-9 Present      :YES
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA100
-----
Point            -ve      2      3      4      5      6      +ve
Replicates       5       3      3      3      3      3      3
Dose ug/plate    0       8     40     200    1000   5000   5
-----
Revertants       125    108    118    131    146    167    954
                  114    138    126    110    120    145    961
                  123    162    126    137    146    136    784
                  117
                  141
-----
Mean             124.0  136.0  123.3  126.0  137.3  149.3  899.7
Increase over
Control          1.1    1.0    1.0    1.1    1.2^    7.3
Standard
Deviation        10.5   27.1   4.6    14.2   15.0   15.9   100.2
-----
R-Coefficient
Gradient          0.09   0.05   0.27   0.50
                  -0.08  -0.01  0.01   0.00
Degrees of freedom 9      12     15     18
Significance      NS     NS     NS     *
-----
Dunnett's t value 1.02  -0.05  0.17   1.19   2.22
Significance      NS     NS     NS     NS     NS
-----
M Statistic      1.75
=====

```

Key NS represents Not Significant
 * represents $p \leq 0.05$
 ** represents $p \leq 0.01$
 *** represents $p \leq 0.001$
 ^ represents maximum increase over control
 For Dunnett's t value *** represents $p < 0.01$

```

=====
Table Number      :8
-----
CHE Study Number  :514/29          Experiment Number :1
Positive Control  :N/A            S-9 Present       :YES
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA1535
-----
Point            -ve    2    3    4    5    6                +ve
-----
Replicates       5     3    3    3    3    3                0
Dose ug/plate    0     8   40  200 1000 5000             0
-----
Revertants       20    21   29   17   29   46
                  21    19   27   26   36   65
                  27    27   21   34   40   60
                  28
                  29
-----
Mean             25.0  22.3  25.7  25.7  35.0  57.0
-----
Increase over    0.9   1.0   1.0   1.4   2.3^
Control
-----
Standard         4.2   4.2   4.2   8.5   5.6   9.8
Deviation
-----
R-Coefficient           0.13  0.12  0.65  0.90
Gradient                0.03  0.01  0.01  0.01
Degrees of freedom      9     12   15   18
Significance            NS    NS   **   ***
-----
Dunnett's t value  -0.68  0.17  0.08  2.28  6.31
Significance          NS    NS   NS   NS   ***
-----
M Statistic        1.18
=====

```

Key NS represents Not Significant
 * represents p <= 0.05
 ** represents p <= 0.01
 *** represents p <= 0.001
 ^ represents maximum increase over control
 For Dunnett's t value *** represents p < 0.01

```

=====
Table Number      :9
-----
CHE Study Number  :514/29          Experiment Number :1
Positive Control  :N/A            S-9 Present       :YES
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA1537
-----
Point            -ve      2      3      4      5      6      +ve
-----
Replicates       5       3       3       3       3       3       0
Dose ug/plate    0       8      40     200    1000   5000   0
-----
Revertants       26      15      15      14      14      10
                  17      15      21      19      20      10
                  13      17       9       8       15      14
                  17
                  13
-----
Mean             17.2    15.7    15.0    13.7    16.3    11.3
-----
Increase over    0.9     0.9     0.8     0.9     0.7
Control
-----
Standard         5.3     1.2     6.0     5.5     3.2     2.3
Deviation
-----
R-Coefficient                    0.20    0.26    0.01    0.37
Gradient                      -0.05  -0.01    0.00  -0.00
Degrees of freedom                9      12      15      18
Significance                      NS     NS     NS     NS
-----
Dunnett's t value  -0.37  -0.71  -1.13  -0.20  -1.83
Significance                NS     NS     NS     NS
-----
M Statistic      1.30
=====

```

Key NS represents Not Significant
* represents p <= 0.05
** represents p <= 0.01
*** represents p <= 0.001
^ represents maximum increase over control
For Dunnett's t value *** represents p < 0.01

```

=====
Table Number      :10
-----
CHE Study Number  :514/29          Experiment Number :1
Positive Control  :N/A            S-9 Present      :YES
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA102
-----
Point            -ve      2      3      4      5      6      +ve
-----
Replicates       5       3       3       3       3       3       0
Dose ug/plate    0       8       40      200     1000    5000    0
-----
Revertants       321     353     295     322     362     393
                 285     310     299     317     382     315
                 267     295     302     323     310     322
                 329
                 244
-----
Mean             289.2   319.3   298.7   320.7   351.3   343.3
-----
Increase over    1.1     1.0     1.1     1.2^    1.2
Control
-----
Standard Deviation 35.9   30.1   3.5     3.2     37.2   43.2
-----
R-Coefficient           0.06   0.33   0.59   0.41
Gradient               0.10   0.11   0.05   0.01
Degrees of freedom      9     12     15     18
Significance             NS     NS     *     NS
-----
Dunnett's t value     1.38   0.48   1.46   2.75   2.41
Significance           NS     NS     NS     *     NS
-----
M Statistic          3.02
=====

```

Key NS represents Not Significant
 * represents p <= 0.05
 ** represents p <= 0.01
 *** represents p <= 0.001
 ^ represents maximum increase over control
 For Dunnett's t value *** represents p < 0.01

APPENDIX 4

**Methane Sulfonyl Chloride: raw plate counts and calculated mutagenicity data
Experiment 2**

```

=====
Table Number      :11
-----
CHE Study Number  :514/29          Experiment Number :2
Positive Control  :2NF             S-9 Present      :NO
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA98
-----
Point            -ve      2      3      4      5      6      7      8      +ve
Replicates       5        3        3        3        3        3        3        3        3
Dose ug/plate    0      1000    2000    3000    4000    5000    7500    10000   5
-----
Revertants       31        14        27        40        38        23        14        20S     215
                  24        29        29        29        32        27        27        23S     263
                  L        17        28        40        27        16        17        20S     213
                  24
                  29
-----
Mean             27.0     20.0     28.0     36.3     32.3     22.0     19.3     21.0     230.3
Increase over
Control          0.7      1.0      1.3^     1.2      0.8      0.7      0.8      8.5
Standard
Deviation        3.6      7.9      1.0      6.4      5.5      5.6      6.8      1.7      28.3
-----
R-Coefficient
Gradient         0.03     0.52     0.53     0.14     0.20     0.31
Degrees of freedom 8        11       14       17       20       23
Significance      NS        NS        *        NS        NS        NS
-----
Dunnett's t value -1.89    0.25    2.02    1.19    -1.28    -2.04    -1.49
Significance      NS        NS        NS        NS        NS        NS        NS
-----
M Statistic      1.16
=====

```

Key NS represents Not Significant
 * represents p <= 0.05
 ** represents p <= 0.01
 *** represents p <= 0.001
 ^ represents maximum increase over control
 For Dunnett's t value *** represents p < 0.01

```

=====
Table Number      :12
-----
CHE Study Number  :514/29          Experiment Number :2
Positive Control  :NaN3           S-9 Present      :NO
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA100
-----
Point            -ve      2      3      4      5      6      7      8      +ve
-----
Replicates       5       3       3       3       3       3       3       3       3
Dose ug/plate    0      1000   2000   3000   4000   5000   7500  10000   2
-----
Revertants       118    137    134    132    155    132    186    157    596
                  98     127    127    90     149    153    156    161    553
                  110    138    132    128    141    150    191    127    503
                  111
                  98
-----
Mean             107.0  134.0  131.0  116.7  148.3  145.0  177.7  148.3  550.7
-----
Increase over    1.3    1.2    1.1    1.4    1.4    1.7^   1.4    5.1
Control
-----
Standard Deviation  8.8    6.1    3.6    23.2    7.0    11.4    18.9    18.6    46.5
-----
R-Coefficient          0.77   0.31   0.60   0.66   0.81   0.68
Gradient              0.01   0.00   0.01   0.01   0.01   0.01
Degrees of freedom     9     12    15     18     21     24
Significance            **    NS     *     **    ***    ***
-----
Dunnnett's t value    2.88   2.58   0.99   4.28   3.95   6.93   4.24
Significance           *     *     NS    ***    ***    ***    ***
-----
M Statistic          1.33
=====

```

```

Key      NS represents Not Significant
         * represents p <= 0.05
         ** represents p <= 0.01
         *** represents p <= 0.001
         ^ represents maximum increase over control
         For Dunnnett's t value *** represents p < 0.01

```

```

=====
Table Number      :13
-----
CHE Study Number  :514/29          Experiment Number :2
Positive Control  :NaN3           S-9 Present       :NO
Compound Name     :Methane Sulfonyl Chloride
Strain Number     :TA1535
-----
Point             -ve      2      3      4      5      6      7      8      +ve
Replicates        5        3      3      3      3      3      3      3      3
Dose ug/plate     0      1000  2000  3000  4000  5000  7500  10000  2
-----
Revertants        17      12      32      34      45      34      75      50S    445
                  19      18      28      36      30      50      61      70S    492
                  9       22      26      33      55      59      70      58S    470
                  21
                  23
-----
Mean              17.8    17.3    28.7    34.3    43.3    47.7    68.7    59.3    469.0
Increase over
Control           1.0     1.6     1.9     2.4     2.7     3.9^    3.3     26.3
Standard
Deviation         5.4     5.0     3.1     1.5     12.6    12.7    7.1     10.1    23.5
-----
R-Coefficient
Gradient           0.65    0.83    0.85    0.86    0.93    0.88
Degrees of freedom 9       12     15     18     21     24
Significance       *       ***    ***    ***    ***    ***
-----
Dunnett's t value -0.08   2.44   3.50   4.90   5.57   8.51   7.28
Significance       NS     NS     **    ***    ***    ***    ***
-----
M Statistic       1.62
=====

```

Key NS represents Not Significant
* represents $p \leq 0.05$
** represents $p \leq 0.01$
*** represents $p \leq 0.001$
^ represents maximum increase over control
For Dunnett's t value *** represents $p < 0.01$

Point	-ve	2	3	4	5	6	7	8	+ve
Table Number	:14								
CHE Study Number	:514/29				Experiment Number :2				
Positive Control	:AAC				S-9 Present :NO				
Compound Name	:Methane Sulfonyl Chloride								
Strain Number	:TA1537								
Replicates	5	3	3	3	3	3	3	3	3
Dose ug/plate	0	1000	2000	3000	4000	5000	7500	10000	50
Revertants	13	11	15	10	7	11	12S	15S	299
	8	17	17	13	8	11	23S	16S	542
	11	17	13	13	16	20	14S	12S	538
	11								
	11								
Mean	10.8	15.0	15.0	12.0	10.3	14.0	16.3	14.3	459.7
Increase over Control		1.4	1.4	1.1	1.0	1.3	1.5^	1.3	42.6
Standard Deviation	1.8	3.5	2.0	1.7	4.9	5.2	5.9	2.1	139.2
R-Coefficient Gradient			0.65	0.26	0.07	0.06	0.27	0.27	
Degrees of freedom			9	12	15	18	21	24	
Significance			*	NS	NS	NS	NS	NS	
Dunnett's t value		1.65	1.69	0.52	-0.34	1.22	2.07	1.44	
Significance		NS	NS	NS	NS	NS	NS	NS	
M Statistic	0.96								

Key NS represents Not Significant
 * represents p <= 0.05
 ** represents p <= 0.01
 *** represents p <= 0.001
 ^ represents maximum increase over control
 For Dunnett's t value *** represents p < 0.01

```

=====
Table Number      :15
-----
CHE Study Number  :514/29          Experiment Number :2
Positive Control  :GLU             S-9 Present      :NO
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA102
-----
Point            -ve    2    3    4    5    6    7    8    +ve
Replicates       5      3    3    3    3    3    3    3    3
Dose ug/plate    0    1000 2000 3000 4000 5000 7500 10000 25
-----
Revertants       269  294  381  377  346  403  308S 273S 528
                  332  340  382  377  330  339  284S 242S 567
                  322  332  366  403  380  344  276S 258S 473
                  317
                  277
-----
Mean              303.4 322.0 376.3 385.7 352.0 362.0 289.3 257.7 522.7
Increase over
Control           1.1   1.2   1.3^  1.2   1.2   1.0   0.8   1.7
Standard
Deviation         28.4  24.6   9.0  15.0  25.5  35.6  16.7  15.5  47.2
-----
R-Coefficient
Gradient          0.80  0.86  0.67  0.60  0.01  0.39
Degrees of freedom 9    12    15    18    21    24
Significance      **   ***   **   **   NS   NS
-----
Dunnett's t value 1.12  4.20  4.70  2.84  3.38 -0.84 -2.85
Significance      NS   ***   ***   *    **   NS   NS
-----
M Statistic      1.69
=====

```

Key NS represents Not Significant
 * represents p <= 0.05
 ** represents p <= 0.01
 *** represents p <= 0.001
 ^ represents maximum increase over control
 For Dunnett's t value *** represents p < 0.01

```

=====
Table Number      :16
-----
CHE Study Number  :514/29          Experiment Number :2
Positive Control  :AAN             S-9 Present      :YES
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA98
-----
Point            -ve      2      3      4      5      6      7      8      +ve
Replicates       5        3        3        3        3        3        3        3        3
Dose ug/plate    0      1000    2000    3000    4000    5000    7500    10000   5
-----
Revertants       18       33       21       25       27       20       27       31      1007
                 26       33       23       24       25       24       43       26      934
                 26       23       20       25       23       30       25       22      976
                 26
                 20
-----
Mean              23.2    29.7    21.3    24.7    25.0    24.7    31.7    26.3    972.3
Increase over
Control           1.3     0.9     1.1     1.1     1.1     1.1     1.4^    1.1     41.9
Standard
Deviation         3.9     5.8     1.5     0.6     2.0     5.0     9.9     4.5     36.6
-----
R-Coefficient                    0.07    0.03    0.01    0.01    0.34    0.27
Gradient                      -0.00   -0.00    0.00    0.00    0.00    0.00
Degrees of freedom                9      12      15      18      21      24
Significance                       NS      NS      NS      NS      NS      NS
-----
Dunnett's t value    1.88   -0.56   0.49   0.58   0.44   2.34   0.95
Significance          NS      NS      NS      NS      NS      NS      NS
-----
M Statistic          0.84
=====

```

Key NS represents Not Significant
 * represents p <= 0.05
 ** represents p <= 0.01
 *** represents p <= 0.001
 ^ represents maximum increase over control
 For Dunnett's t value *** represents p < 0.01

```

=====
Table Number      :17
-----
CHE Study Number  :514/29          Experiment Number :2
Positive Control  :AAN             S-9 Present       :YES
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA100
-----
Point            -ve      2      3      4      5      6      7      8      +ve
-----
Replicates       5       3       3       3       3       3       3       3       3
Dose ug/plate    0      1000    2000    3000    4000    5000    7500    10000   5
-----
Revertants       137    159    164    151    154    154    181    180    1320
                  136    156    171    160    165    154    173    154    1609
                  115    166    144    139    174    167    178    193    1645
                  127
                  128
-----
Mean              128.6  160.3  159.7  150.0  164.3  158.3  177.3  175.7  1524.7
-----
Increase over    1.2    1.2    1.2    1.3    1.2    1.4^    1.4    11.9
Control
-----
Standard Deviation  8.8    5.1    14.0    10.5    10.0    7.5    4.0    19.9  178.2
-----
R-Coefficient
Gradient          0.79   0.56   0.64   0.60   0.73   0.71
Degrees of freedom  9     12     15     18     21     24
Significance       **     *      **     **     ***    ***
-----
Dunnett's t value  4.19   4.09   2.87   4.68   3.94   6.26   6.02
Significance       ***    ***    *      ***    ***    ***    ***
-----
M Statistic      0.74
=====

```

Key NS represents Not Significant
 * represents p <= 0.05
 ** represents p <= 0.01
 *** represents p <= 0.001
 ^ represents maximum increase over control
 For Dunnett's t value *** represents p < 0.01

```

=====
Table Number      :18
-----
CHE Study Number  :514/29          Experiment Number :2
Positive Control  :N/A            S-9 Present       :YES
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA1535
-----
Point            -ve      2      3      4      5      6      7      8      +ve
-----
Replicates       5        3        3        3        3        3        3        3        0
Dose ug/plate   0      1000    2000    3000    4000    5000    7500    10000   0
-----
Revertants      23        23        21        44        42        37        44        47
                17        21        28        29        44        39        44        49
                19        18        34        37        31        45        30        39
                14
                21
-----
Mean            18.8     20.7     27.7     36.7     39.0     40.3     39.3     45.0
-----
Increase over   1.1      1.5      2.0      2.1      2.1      2.1      2.1      2.4^
Control
-----
Standard Deviation 3.5      2.5      6.5      7.5      7.0      4.2      8.1      5.3
-----
R-Coefficient   0.68     0.82     0.86     0.87     0.79     0.80
Gradient        0.00     0.01     0.01     0.00     0.00     0.00
Degrees of freedom 9        12        15        18        21        24
Significance     *        ***      ***      ***      ***      ***
-----
Dunnett's t value 0.60     2.51     4.70     5.23     5.56     5.29     6.53
Significance     NS        NS        ***      ***      ***      ***      ***
-----
M Statistic     0.96
=====

```

Key NS represents Not Significant
 * represents p <= 0.05
 ** represents p <= 0.01
 *** represents p <= 0.001
 ^ represents maximum increase over control
 For Dunnett's t value *** represents p < 0.01

```

=====
Table Number      :19
-----
CHE Study Number  :514/29          Experiment Number :2
Positive Control  :N/A            S-9 Present      :YES
Compound Name    :Methane Sulfonyl Chloride
Strain Number    :TA1537
-----
Point            -ve      2      3      4      5      6      7      8      +ve
-----
Replicates       5        3      3      3      3      3      3      3      0
Dose ug/plate    0      1000  2000  3000  4000  5000  7500  10000  0
-----
Revertants       8        13     10     11     13     7      9      10
                  14        10     6      9      12     12     13     17
                  6        14     14     8      9      10     4      10
                  9
                  12
-----
Mean              9.8     12.3  10.0   9.3    11.3   9.7    8.7    12.3
-----
Increase over    1.3^    1.0    1.0    1.2    1.0    0.9    1.3
Control
-----
Standard         3.2     2.1    4.0    1.5    2.1    2.5    4.5    4.0
Deviation
-----
R-Coefficient                0.08   0.09   0.04   0.04   0.17   0.06
Gradient                    0.00  -0.00   0.00  -0.00  -0.00   0.00
Degrees of freedom              9     12     15     18     21     24
Significance                    NS     NS     NS     NS     NS     NS
-----
Dunnett's t value    1.09   0.06  -0.13   0.70  -0.02  -0.61   1.04
Significance          NS     NS     NS     NS     NS     NS     NS
-----
M Statistic         1.00
=====

```

Key NS represents Not Significant
* represents p <= 0.05
** represents p <= 0.01
*** represents p <= 0.001
^ represents maximum increase over control
For Dunnett's t value *** represents p < 0.01

```

=====
Table Number      :20
-----
CHE Study Number  :514/29          Experiment Number :2
Positive Control  :N/A            S-9 Present       :YES
Compound Name     :Methane Sulfonyl Chloride
Strain Number     :TA102
-----
Point             -ve    2    3    4    5    6    7    8    +ve
Replicates        5     3    3    3    3    3    3    3    0
Dose ug/plate     0   1000 2000 3000 4000 5000 7500 10000 0
-----
Revertants        303   247  289  316  230  235  160S  151S
                  268   336  304  266  254  229  179S  159S
                  267   285  285  287  285  226  156S  155S
                  229
                  259
-----
Mean              265.2 289.3 292.7 289.7 256.3 230.0 165.0 155.0
Increase over
Control           1.1  1.1^  1.1  1.0  0.9  0.6  0.6
Standard
Deviation         26.4  44.7  10.0  25.1  27.6  4.6  12.3  4.0
-----
R-Coefficient          0.43  0.37  0.01  0.36  0.72  0.83
Gradient              0.01  0.01 -0.00 -0.01 -0.01 -0.01
Degrees of freedom      9    12    15    18    21    24
Significance            NS    NS    NS    NS    NS    NS
-----
Dunnett's t value     1.34  1.59  1.41 -0.52 -2.10 -6.52 -7.26
Significance           NS    NS    NS    NS    NS    NS
-----
M Statistic          2.08
=====

```

Key NS represents Not Significant
* represents $p \leq 0.05$
** represents $p \leq 0.01$
*** represents $p \leq 0.001$
^ represents maximum increase over control
For Dunnett's t value *** represents $p < 0.01$

APPENDIX 5

Historical negative (solvent) control values for *Salmonella* strains

Strain	- or + S-9	Mean N ^a of spontaneous revertants	SD	Range*	
				lower	upper
TA98	-	15	4.4	4	27
	+	22	6.5	6	39
TA100	-	90	13.6	55	125
	+	115	15.6	75	155
TA1535	-	13	4.7	1	26
	+	19	5.5	4	33
TA1537	-	8	3.5	1	17
	+	11	4.3	1	22
TA102	-	253	35.1	162	343
	+	314	45.6	197	432

* 99% confidence limits about the mean

The above are pooled data from at least 27 consecutive experiments over the period 21.3.95 to 7.9.95

APPENDIX 6

Quality control statements for S-9

MOLTOX™ POST MITOCHONDRIAL SUPERNATANT (S-9)
PRODUCTION & QUALITY CONTROL CERTIFICATE

LOT NO.: <u>0679</u>	SPECIES: <u>Rat</u>	PREPARATION DATE: <u>08 July 1996</u>
PART NO.: <u>11-101</u>	STRAIN: <u>Sprague Dawley</u>	EXPIRATION DATE: <u>08 July 1998</u>
VOLUME: <u>5 ml</u>	SEX: <u>Male</u>	BUFFER: <u>0.154M KCl</u>
	TISSUE: <u>Liver</u>	INDUCING AGENT(s): <u>Aroclor 1254</u>
REFERENCE: <u>Maron, D & Ames, B. Mutat. Res. 113:173, 1983</u>		<u>Monsanto Lot No. KL615 - 500mg/kg</u>

BIOCHEMISTRY:

- PROTEIN

41.9 mg/ml

Assayed according to the method of Lowry et al., JBC 193:265, 1951 using bovine serum albumin as the standard.

- ALKOXYRESORUFIN-O-DEALKYLASE ACTIVITIES

Activity	P450	Fold - Induction
EROD	IA1, IA2	234.8
PROD	2B1	63.5
BROD	2B1	49.3
MROD	1A2	128.4

Assays for ethoxyresorufin-O-deethylase (EROD), pentoxy-, benzyl- and methoxyresorufin-O-dealkylases (PROD, BROD, MROD) were conducted using a modification of the methods of Burke, et al., Biochem Pharm 34:3337, 1985. Fold-inductions were calculated as the ratio of the sample vs. uninduced specific activities (SA's). Control SA's (pmoles/min/mg protein) were 9.91, 3.82, 49.3, & 5.7 for EROD, PROD, BROD and MROD, respectively.

BIOASSAY:

- STERILITY TEST

Samples of S-9 were assayed for the presence of contaminating microflora by plating 1.0 ml volumes on Trypticase Soy and Minimal Glucose (Vogel-Bonner E, supplemented with 0.05 mM L-histidine and D-biotin) media. Triplicate plates were read after 48 or 72 h incubation at 37°C. No evidence of contamination was observed.

- PROMUTAGEN ACTIVATION

No. His+ Revertants	
EtBr/	CPA/
TA98	TA1535
1048	1027

The ability of the sample to activate ethidium bromide (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lesca, et al., Mutation Res 129:299, 1984. Data were expressed as revertants per ug EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.2 - 10% in S9 mix, were tested for their ability to activate benzo(a)pyrene (BP) and 2-aminoanthracene (2-AA) to intermediates mutagenic to TA100. Assays were conducted using duplicate plates as described by Maron & Ames (Mutat. Res. 113:173, 1983.)

µl S9 per plate/number his⁺ revertants per plate

Promutagen	0	1	2	10	20	50
BP (5ug)	99.5	181	350	555.5	935	1497.5
2-AA (2.5ug)	102	768	2457	2666.5	2727	2331.5

MOLECULAR TOXICOLOGY, INC.
111 Gibraltar St.
Annapolis, MD 21401
(410) 268 7232

**MOLTOX™ POST MITOCHONDRIAL SUPERNATANT (S-9)
PRODUCTION & QUALITY CONTROL CERTIFICATE**

LOT NO.: <u>0701</u>	SPECIES: <u>Rat</u>	PREPARATION DATE: <u>24 Sept. 1996</u>
PART NO.: <u>11-101</u>	STRAIN: <u>Sprague Dawley</u>	EXPIRATION DATE: <u>24 Sept. 1998</u>
VOLUME: <u>5 ml</u>	SEX: <u>Male</u>	BUFFER: <u>0.154M KCl</u>
	TISSUE: <u>Liver</u>	INDUCING AGENT(s): <u>Aroclor 1254</u>
REFERENCE: <u>Maron, D. & Ames, B. Mutat. Res. 113:173, 1983</u>		<u>Monsanto Lot No. KL615 - 500mg/kg</u>

BIOCHEMISTRY:**- PROTEIN**44.8 mg/ml

Assayed according to the method of Lowry et al., JBC 193:265, 1951 using bovine serum albumin as the standard.

- ALKOXYRESORUFIN-O-DEALKYLASE ACTIVITIES

Activity	P450	Fold - Induction
EROD	IA1, IA2	104.6
PROD	2B1	17.3
BROD	2B1	51.9
MROD	1A2	34.1

Assays for ethoxyresorufin-O-deethylase (EROD), pentoxy-, benzyl- and methoxyresorufin-O-dealkylases (PROD; BROD; MROD) were conducted using a modification of the methods of Burke, et al., Biochem Pharm 34:3337, 1985. Fold-inductions were calculated as the ratio of the sample vs. uninduced specific activities (SA's). Control SA's (pmoles/min/mg protein) were 4.4, 1.3, 9.19, & 2.99 for EROD, PROD, BROD and MROD, respectively.

BIOASSAY:**- STERILITY TEST**

Samples of S-9 were assayed for the presence of contaminating microflora by plating 1.0ml volumes on Trypticase Soy and Minimal Glucose (Vogel-Bonner E, supplemented with 0.05mM L-histidine and D-biotin) media. Triplicate plates were read after 48 or 72h incubation at 37C. No evidence of contamination was observed.

- PROMUTAGEN ACTIVATION

No. His+ Revertants	EtBr/ CPA/
TA98	TA1535
726.8	1290

The ability of the sample to activate ethidium bromide (EtBr) and cyclophosphamide (CPA) to intermediates mutagenic to TA98 and TA1535, respectively, was determined according to Lesca, et al., Mutation Res 129:299, 1984. Data were expressed as revertants per ug EtBr or per mg CPA.

Dilutions of the sample S9, ranging from 0.2 - 10% in S9 mix, were tested for their ability to activate benzo(a)pyrene (BP) and 2-aminoanthracene (2-AA) to intermediates mutagenic to TA100. Assays were conducted using duplicate plates as described by Maron & Ames (Mutat. Res. 113:173, 1983.).

ul S9 per plate/number his+ revertants per plate

Promutagen	0	1	5	10	20	50
BP (5ug)	55	212	361	501	819	1034
2-AA (2.5ug)	61	143	542	1183	2023	2103

MOLECULAR TOXICOLOGY, INC.
157 Industrial Park Dr.
Boone, NC 28607
(704) 264-9099

APPENDIX 7

Sponsor's certificate of analysis and test article description

elf aquitaine production 

adresse postale
BP 22 64170 Lacq
téléphone : 33 - 59 92 22 22
télécopie : 33 - 59 92 22 22

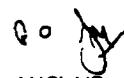
direction exploration production france

Ref: 96000478

ANALYSIS for METHANE SULFONYL CHLORIDE (MSC)

CHARACTERISTICS		ANALYSIS
Purity	(%Weight)	99.85
Water	(ppm)	< 10
Colour	APHA	17

Lacq June 25th 1996


P. AUCLAIR

Elf Aquitaine Production
société anonyme au capital de 1 055 000 000 F
siège social : tour Elf 2 place de la Coupole
La Défense 6 Courbevoie (Hauts-de-Seine)
rcs Pau b 632 022 711

x x

elf atochem
ATO

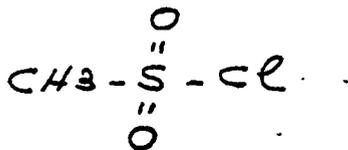
Direction Sécurité Environnement Produit
Département de Toxicologie Industrielle

TEST ARTICLE DESCRIPTION

CONFIDENTIAL

METHANE SULFONYL CHLORIDE

STRUCTURAL FORMULA



IDENTITY

Test article name	: MSC
Chemical name	: METHANE SULFONYL CHLORIDE
CAS number	: 124-63-0
EINECS number	: 2047061
Molecular formula	: CH ₃ .SO ₂ -Cl
Molecular weight	: 114.59
Purity	: ≥ 99.5%
Origin and batch	:

elf atochem**ATO**Direction Sécurité Environnement Produit
Département de Toxicologie Industrielle**PHYSICAL AND CHEMICAL PROPERTIES**

Appearance	: LIGHT YELLOW LIQUID
Viscosity	: 1.33 (centistokes 25°C)
Specific gravity	: 1.475 - 1.480
Particle size	:
Melting point	: -33°C
Boiling point	: 164°C (760 mm Hg)
Vapor pressure	: 2,8 mbar (20°C)
Flash point	: < 10°C
Solubility	: in acetone

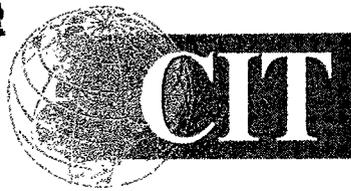
USE SAFETYVERY TOXIC BY INHALATION
REFER TO THE SAFETY DATA SHEET**STORAGE AND DISPOSAL**

Storage	: REFER TO THE
Expiry date	: SAFETY DATA SHEET
Disposal	:

APPENDIX 8

Minor deviations from protocol

Protocol section	Subject	Deviation
3.4	Bacteria	Bacterial cultures were incubated for 10 hours at 37°C on a shaking platform in an anhydric incubator, rather than using a shaking waterbath as indicated in the protocol. Both incubation methods are considered to provide equivalent and satisfactory viable cell densities in the resulting bacterial cultures, so either method is equally acceptable.
8	Responsible personnel	As of 1 September 1996 the Head of Molecular Toxicology changed from Dr S Dean to Dr J Clements.



CONFIDENTIAL

SPONSOR

ATOFINA

Département Toxicologie et Environnement
4-8 Cours Michelet
La Défense 10
92091 Paris-la-Défense
France

TEST ITEM

Methane Sulfonyl Chloride

STUDY TITLE

BONE MARROW MICRONUCLEUS TEST
BY INTRAPERITONEAL ROUTE IN MICE

STUDY DIRECTOR

Hasnaà Haddouk

DATE OF ISSUE

07 November 2003

TEST FACILITY

CIT

BP 563 - 27005 Evreux - France

LABORATORY STUDY NUMBER

25873 MAS

Safety & Health Research Laboratories

IFM Recherche

S.N.C. au Capital de 846.092 €
788 060 465 R.C.S. Evreux
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STATEMENT OF THE STUDY DIRECTOR AND CIT SCIENTIFIC MANAGEMENT

The study was performed in compliance with the following Principles of Good Laboratory Practice Regulations:

- OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
- Commission Directive 1999/11/EC of 8 March 1999 adapting to technical progress the Principles of Good Laboratory Practice as specified in Council Directive 87/18/EEC on the harmonisation of laws, regulations and administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L77 of 23.3.1999).
- Décret N° 98-1312 du 31 Décembre 1998 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 1er janvier 1999), Ministère de l'Economie, des Finances et de l'Industrie.

The study was conducted in compliance with the following Animal Health regulation:

- Council Directive No. 86/609/EEC of 24th November 1986 on the harmonization of laws, regulations or administrative provisions relating to the protection of animals used for experimental or other scientific purposes.

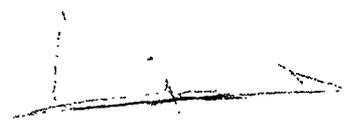
This study was performed at CIT (BP 563, 27005 Evreux, France), except for:

- analysis of the slides which was performed at Microptic cytogenetic services (2 Langland Close Mumbles, Swansea SA3 4LY, UK).

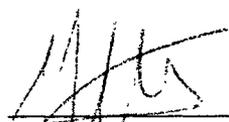
The corresponding GLP certificate is presented in appendix 1.

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

Mutagenicity



H. Haddouk Study completion date: 07 November 2003
Study Director
Doctor of Applied Biochemistry
Head of Genetic Toxicology



S. de Louffrey Date: 07 November 2003
Doctor of Veterinary Medicine
Scientific Management

OTHER SCIENTIST INVOLVED IN THIS STUDY

For slide analysis: Dr. N. Danford
Microptic cytogenetic services
Swansea, UK.

STATEMENT OF QUALITY ASSURANCE UNIT**Inspections performed at CIT:**

Type of inspection	Dates		
	Inspection	Reported to Study Director (*)	Reported to Management (*)
Study Plan	6 May 2003	6 May 2003	9 May 2003
Study	3 June 2003	4 June 2003	12 June 2003
Report	16 September 2003	22 September 2003	7 October 2003

In addition to the above-mentioned inspections, at about the same time as this study described in the present report, process-based and routine facility inspections of critical procedures relevant to this study type were also made by the Quality Assurance Unit.

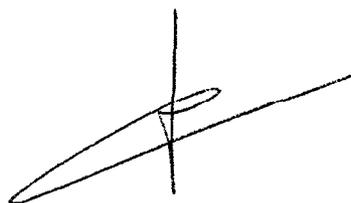
The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and Principles of Good Laboratory Practice.

Inspections performed at PI test site:

The inspections performed at the PI test site are presented in appendix 1.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.



C. Galli-Kar Date: 07 Nov. 2003
 Ing. Biol.
 CIT Head of Quality Assurance Unit

(*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

SUMMARY

The objective of this study was to evaluate the potential of the test item Methane Sulfonyl Chloride (batch No. RW 1491 F 10 CD1, purity: 99.9%) to induce structural or numerical damage in bone marrow cells of mice.

The study was performed according to the international guidelines (OECD 474, Commission Directive No. B12) and in compliance with the Principles of Good Laboratory Practice Regulations.

Methods

A preliminary toxicity test was performed to define the dose-levels to be used for the cytogenetic study.

In the main study, three groups of five male and five female Swiss Ico: OF1 (IOPS Caw) mice were given intraperitoneal administrations of Methane Sulfonyl Chloride at dose-levels of 7.5, 15 and 30 mg/kg/day, over a 2-day period.

One group of five males and five females received the vehicle (paraffin oil) under the same experimental conditions, and acted as control group.

One group of five males and five females received the positive control test item (cyclophosphamide) once by oral route at the dose-level of 50 mg/kg.

The animals of the treated and vehicle control groups were killed 24 hours after the last treatment and the animals of the positive control group were killed 24 hours after the single treatment. Bone marrow smears were then prepared.

For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes. The polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE + NE).

Results

The top dose-level for the cytogenetic test was selected according to the criteria specified in the international guidelines; since toxic effects were observed in the preliminary test, the choice of the top dose-level was based on the level of toxicity, such that a higher dose-level was expected to induce lethality.

Consequently, 30 mg/kg/day was selected as the top dose-level for the main test. The two other selected dose-levels were 15 and 7.5 mg/kg/day.

For both males and females, the mean values of MPE as well as the PE/NE ratio in the groups treated with the test item, were equivalent to those of the vehicle group.

Cyclophosphamide induced a highly significant increase in the frequency of MPE, indicating the sensitivity of the test system under our experimental conditions. The study was therefore considered valid.

Conclusion

Under our experimental conditions, the test item Methane Sulfonyl Chloride (batch No. RW 1491 F 10 CD1, purity: 99.9%) did not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after two intraperitoneal administrations, with a 24-hour interval, at the dose-levels of 7.5, 15 and 30 mg/kg/day.

RESUME

L'objectif de cette étude était d'évaluer le potentiel du produit d'étude Methane Sulfonyl Chloride (lot n° RW 1491 F 10 CD1, pureté : 99,9 %) à induire des aberrations de structures ou numériques au niveau des cellules de la moelle osseuse de souris.

L'étude a été réalisée conformément aux lignes directrices internationales (OCDE 474, Commission Directive n° B12) et selon les règles de Bonnes Pratiques de Laboratoire.

Méthodes

Un essai préliminaire a été réalisé pour définir les niveaux des doses qui devront être utilisés dans l'étude cytogénétique.

Dans l'essai principal, 3 groupes de 5 souris mâles et 5 souris femelles Swiss Ico: OF1 (IOPS Caw) ont reçu 2 traitements par voie intrapéritonéale avec le produit Methane Sulfonyl Chloride aux doses de 7,5 ; 15 et 30 mg/kg/jour, à 24 heures d'intervalle.

Un groupe de 5 mâles et 5 femelles a reçu le véhicule (huile de paraffine) dans les mêmes conditions expérimentales, et a servi de groupe témoin.

Un groupe de 5 mâles et 5 femelles a reçu le témoin positif (cyclophosphamide), une seule fois par voie orale à la dose de 50 mg/kg.

Les animaux des groupes traités avec le produit et ceux du groupe témoin véhicule ont été sacrifiés 24 heures après la dernière administration et les animaux du groupe témoin positif ont été sacrifiés 24 heures après l'administration unique. Les frottis de moelle osseuse ont été ensuite préparés.

Pour chaque animal, le nombre d'érythrocytes polychromatophiles micronucléés (MPE) a été compté dans 2000 érythrocytes polychromatophiles. Le ratio des érythrocytes polychromatophiles (PE) sur érythrocytes normochromatophiles (NE) a été établi en dénombrant un total de 1000 érythrocytes (PE + NE).

Résultats

La forte dose utilisée pour le test cytogénétique est choisie selon les recommandations des lignes directrices internationales ; étant donné que le produit d'étude a montré des effets toxiques dans l'essai préliminaire, le choix de la forte dose est basé sur le niveau de toxicité, de telle sorte qu'une dose supérieure est susceptible d'induire une mortalité.

Par conséquent, 30 mg/kg/jour sont choisis comme forte dose pour l'essai principal. Les 2 autres doses sont 15 et 7,5 mg/kg/jour.

Pour les mâles et les femelles, les valeurs moyennes de MPE ainsi que du ratio PE/NE dans les groupes traités avec le produit d'étude, sont équivalentes à celles de leurs groupes témoins véhicule respectifs.

Le cyclophosphamide induit une augmentation très significative de la fréquence de MPE, indiquant la sensibilité du système d'étude dans nos conditions expérimentales. L'étude est par conséquent considérée valide.

Conclusion

Dans nos conditions expérimentales, le produit Methane Sulfonyl Chloride (lot n° RW 1491 F 10 CD1 pureté : 99,9 %) n'induit pas de dommages aux chromosomes ou à l'appareil mitotique au niveau de la moelle osseuse de souris, après 2 traitements par voie intrapéritonéale, à 24 heures d'intervalle, aux doses de 7,5 ; 15 et 30 mg/kg/jour.

1. INTRODUCTION

This study was performed at the request of ATOFINA, Paris-la-Défense, France.

The objective of this study was to evaluate the potential of the test item to induce damage to the chromosomes or the mitotic apparatus in bone marrow cells of mice.

Apart from detecting chromosome breakage events (clastogenesis), the micronucleus test ^(a) is capable of detecting chemicals which induce whole chromosome loss (aneuploidy) in the absence of clastogenic activity.

The basis of this test is the increase in the number of micronucleated polychromatic erythrocytes in the bone marrow of mice exposed to a chemical which induces cytogenetic damage. Chromosomal fragments or entire chromosomes which are left behind at cell division are not incorporated into the nuclei of daughter cells. Most of these fragments condense and form one or more micronuclei in the cytoplasm. The visualization of micronuclei is facilitated in erythrocytes because their nucleus is extruded during erythropoiesis.

Substances which inhibit either proliferation or maturation of erythroblasts and those which are toxic for nucleated cells, decrease the proportion of polychromatic erythrocytes (PE) when compared to normochromatic erythrocytes (NE). Thus, the cytotoxicity of a substance can be determined by a decrease in the PE/NE ratio.

This test was performed according to the method described by Schmid (1975) ^(b) and modified by Salamone and coll. (1980) ^(c).

This study was designed in accordance with the following guidelines:

- . OECD guideline No. 474, adopted on 21st July 1997,
- . Commission Directive No. 2000/32/EC, B12, 8 June 2000.

2. MATERIALS AND METHODS

2.1 TEST AND CONTROL ITEMS

2.1.1 Identification

2.1.1.1 Test item

- . supplier: ATOFINA
- . name:
 - Study plan: Methane Sulfonyl Chloride
 - labeling: Chlorure de methanesulfonyle

The test item was supplied by the Sponsor as preparations at 200.25 mg/mL and 20.03 mg/mL in paraffin oil. Only the latter preparation was used for this study.

- . batch number:
 - Study plan: RW 1491 F 10 CD1
 - labeling: none
- . Sponsor's filing number: 0079/02
- . description of the preparation supplied by the Sponsor at receipt: colorless liquid
- . description of the active item on the test article description: light yellow liquid
- . container: one glass flask
- . date of receipt: 6 May 2003
- . storage conditions: at room temperature and protected from humidity
- . purity: 99.9%.

Confirmation of test item identity is the responsibility of the Sponsor.

The batch number "RW 1491 F 10 CD1", which was absent from the label on the container was confirmed by the Study Monitor on the test article description (Riverview batch: 1491F10CD1) and in an e-mail dated 7 May 2003.

Data relating to the characterisation of the test item are documented in an analytical certificate and a test article description (presented in appendix 2 provided by the Sponsor).

2.1.1.2 Vehicle

The vehicle was paraffin oil, supplied by the Sponsor.

2.1.2 Formulation procedure

The test item was supplied by the Sponsor as a preparation in paraffin oil at 200.25 mg/mL and 20.03 mg/mL. The latter was used directly for treatment or diluted in the vehicle immediately before use, depending on the target dose-level.

2.1.3 Positive controls

The positive control was cyclophosphamide (CPA), batch No. OD203A (Laboratoire Asta Médica, Mérignac, France) dissolved in distilled water at a concentration of 5 mg/mL.

The preparation was stored at -20°C and thawed immediately before use.

2.2 TEST SYSTEM

2.2.1 Animals

Preliminary toxicity test: 9 male and 9 female mice were used,

Main cytogenetic test: 56 mice, 28 males and 28 females were used,

Strain: Swiss Ico: OF1 (IOPS Caw).

Reason for this choice: rodent species generally accepted by regulatory authorities for this type of study.

Breeder: Charles River Laboratories France, l'Arbresle, France.

Age: on the day of treatment, the animals were approximately 6 weeks old.

Veterinary care at CIT: upon their arrival at CIT, the animals were given a complete examination to ensure that they were in good clinical conditions.

Acclimation: at least 5 days before the day of treatment.

Constitution of groups: upon arrival, the animals were randomly allocated to the groups by sex.

Subsequently, each group was assigned to a different treatment group.

Identification: individual tail marking upon treatment.

2.2.2 Environmental conditions

Upon their arrival at CIT, the animals were housed in an animal room, with the following environmental conditions:

- . temperature: $22 \pm 2^\circ\text{C}$,
- . relative humidity: 30 to 70%,
- . light/dark cycle: 12 h/12 h (07:00 - 19:00),
- . ventilation: at least 12 cycles/hour of filtered non-recycled fresh air.

The temperature and relative humidity were under continuous control and recording. The housing conditions (temperature, relative humidity and ventilation) and corresponding instrumentation and equipment were verified and calibrated at regular intervals.

The animals were housed by groups in polycarbonate cages. Each cage contained autoclaved sawdust (SICSA, Alfortville, France).

Sawdust is analyzed by the supplier for composition and contaminant levels.

2.2.3 Food and water

All animals had free access to A04 C pelleted maintenance diet (SAFE, Villemoisson-sur-Orge, France).

Each batch of food is analysed by the supplier for composition and contaminant levels.

Drinking water filtered by a FG Millipore membrane (0.22 micron) was provided *ad libitum*.

Bacteriological and chemical analysis of water are performed regularly by external laboratories. These analyses include the detection of possible contaminants (pesticides, heavy metals and nitrosamines).

No contaminants were known to have been present in the diet, drinking water or bedding material at levels which may be expected to interfere with or prejudice the outcome of the study.

2.3 EXPERIMENTAL DESIGN

2.3.1 Preliminary toxicity test

In order to determine the highest dose-level, several preliminary tests were performed on groups of six animals (three males and three females). Clinical signs and any mortality were recorded for a period of 48 hours. At the end of this period, the animals were killed by CO₂ inhalation in excess.

2.3.2 Cytogenetic study

2.3.2.1 Groups and sampling time

Treatment	Animals per group	Target dose-level mg/kg/day	Sampling time after the last treatment
Vehicle	Principal 5 males 5 females	0	24 h
Low dose-level	Principal 5 males 5 females	7.5	24 h
Intermediate dose-level	Principal 5 males 5 females	15	24 h
High dose-level	Principal 8 males (1) 8 females (1)	30	24 h
Positive control CPA	Principal 5 males 5 females	50	24 h

(1) Since no mortality occurred, only the five surviving animals of each sex were subjected to bone marrow analysis. The supplementary animals were humanely killed and bone marrow smears were not prepared.

2.3.2.2 Administration

- . Route for the vehicle and the test item: at the request of the Sponsor, the intraperitoneal route was used,
- . Frequency: two treatments separated by 24 hours,
- . Volume: 10 mL/kg,
- . CPA: oral route, one treatment.

The quantity of each item administered to each animal was adjusted according to the most recently recorded body weight.

2.3.2.3 Preparation of the bone marrow smears

At the time of sacrifice, all the animals were killed by CO₂ inhalation in excess. The femurs of the animals were removed and the bone marrow was flushed out using fetal calf serum. After centrifugation, the supernatant was removed and the cells in the sediment were resuspended by shaking. A drop of this cell suspension was placed and spread on a slide. The slides were air-dried and stained with Giemsa. The slides were coded so that the scorer is unaware of the treatment group of the slide under evaluation ("blind" scoring).

2.3.2.4 Microscopic examination of the slides

For each animal, the number of the micronucleated polychromatic erythrocytes (MPE) was counted in 2000 polychromatic erythrocytes; the polychromatic (PE) and normochromatic (NE) erythrocyte ratio was established by scoring a total of 1000 erythrocytes (PE + NE).

The analysis of the slides was performed at Microptic, cytogenetic services (2 Langland Close Mumbles, Swansea SA3 4LY, UK), in compliance with GLP, and the Principal Investigator was Natalie Danford. Details concerning the method used, the format of the data, communication, quality assurance and archiving are indicated in a PIDS (Principal Investigator Data Sheet, appendix 3).

2.4 EVALUATION OF THE RESULTS

Treatment of results

All the individual data are presented in tabular form. The number of MPE/2000 PE and the PE/NE ratio are given for each animal.

The means and the standard deviations of the frequency of MPE/1000 PE and the PE/NE ratio are given for each experimental group.

Statistical analysis

When there was no significant within-group heterogeneity, using the heterogeneity chi-square test value (Lovell *et al.*, 1989)^(d), the frequencies of MPE in each treated group was compared with those in the concurrent vehicle control groups by using a 2 x 2 contingency table to determine the χ^2 value (Lovell *et al.*, 1989)^(d).

When there was significant within-group heterogeneity, then that group was compared with the control group using a non-parametric analysis, the Mann-Whitney test (Schwartz, 1969)^(e).

The student "t" test was used for the PE/NE ratio comparison.

Probability values of $p \leq 0.05$ was considered as significant.

Evaluation criteria

For a result to be considered positive, a statistically significant increase in the frequency of MPE must be demonstrated when compared to the concurrent vehicle control group. Reference to historical data, or other considerations of biological relevance was also taken into account in the evaluation of data obtained.

2.5 STUDY PLAN ADHERENCE

The study was performed in accordance with the Study plan No. 25873 MAS and subsequent amendments. There were no deviations from the agreed Study plan.

2.6 ARCHIVING

The study documentation and specimens generated during the course of the study are archived at CIT, BP 563, 27005 Evreux, France, for 10 years after the end of the experimental phase of the study.

The archived study materials include:

- . Study plan and possible amendments,
- . raw data,
- . PIDS and PI contributions to the final report,
- . slides,
- . correspondence,
- . final report and possible amendments,
- . a sample of the test item.

On completion of this period, the archived study materials will be returned to the Sponsor, or may be archived by CIT for a further period (at additional cost). The total duration of archiving (depending on regulations) will be the responsibility of the Sponsor.

In addition, raw data not specific to the study including, but not limited to, certificates of analyses for food, water and bedding (if applicable) and records of environmental data and equipment calibration, are also archived by CIT for at least 30 years.

2.7 CHRONOLOGY OF THE STUDY

The chronology of the study is summarized as follows:

Procedure	Date
<u>Study plan approved by:</u>	
. Study Director	7 May 2003
. Study Monitor	20 May 2003
<u>Experimental starting date:</u>	
. Preliminary toxicity test: first day of treatment	21 May 2003
<u>Cytogenetic test:</u>	
. First day of treatment	3 June 2003
. Sacrifice of the animals and preparation of bone marrow smears	5 June 2003
<u>Experimental completion date:</u>	
. Analysis of the last slide	21 June 2003

3. RESULTS

3.1 PRELIMINARY TOXICITY TEST

In order to select the top dose-level for the cytogenetic study, 200.3, 50 or 30 mg/kg/day were administered twice, to three males and three females. The interval between each administration was 24 hours.

At 200.3 mg/kg/day, 3/3 males and 2/3 females dead in the 24 hours following the first treatment.

At 50 mg/kg/day, hypoactivity and piloerection were noted in all treated animals. At the end of the observation period no mortality was noted (table 1). However, at the time of sacrifice of the animals 24 hours later, 3/6 treated animals were found dead showing the high toxicity of this dose (documented in raw data but not reported on table 1).

At 30 mg/kg/day, hypoactivity and piloerection were noted in all treated animals but no mortality was observed.

The top dose-level for the cytogenetic test was selected according to the criteria specified in the international guidelines; since toxic effects were observed, the choice of the top dose-level was based on the level of toxicity, such that a higher dose-level was expected to induce lethality.

Consequently, 30 mg/kg/day was selected as the top dose-level for the main test. The two other selected dose-levels were 15 and 7.5 mg/kg/day.

3.2 CYTOGENETIC TEST

No clinical signs and no mortality were observed in the animals of both sexes given 7.5 or 15 mg/kg/day. Hypoactivity and piloerection were noted in all treated animals at 30 mg/kg/day (table 2).

For both males and females, the mean values of MPE as well as the PE/NE ratio in the groups treated with the test item, were equivalent to those of the vehicle group.

Cyclophosphamide induced a highly significant increase ($p < 0.001$) in the frequency of MPE, indicating the sensitivity of the test system under our experimental conditions. The study was therefore considered valid.

4. CONCLUSION

Under our experimental conditions, the test item Methane Sulfonyl Chloride (batch No. RW 1491 F 10 CD1) did not induce damage to the chromosomes or the mitotic apparatus of mice bone marrow cells after two intraperitoneal administrations, at a 24-hour interval, at the dose-levels of 7.5, 15 and 30 mg/kg/day.

5. REFERENCES

- (a) Heddle J A (1973). A rapid *in vivo* test for chromosomal damage. *Mutation Research*, 18, 187-190.
- (b) Schmid W (1975). The micronucleus test. *Mutation Research*, 31, 9-15.
- (c) Salamone M, Heddle J, Stuart E and Katz M (1980). Toward an improved micronucleus test. Studies on 3 model agents, mitomycin C, CPA and dimethylbenzanthracene. *Mutation Research*, 74, 347-356.
- (d) Lovell DP, Anderson D, Albanese R, Amphlett GE, Clare G, Ferguson R, Richold M, Papworth DG and Savage JRK (1989). Statistical analysis of *in vivo* cytogenetic assays. In "Statistical Evaluation of Mutagenicity Test Data" (UKEMS Guidelines Sub-Committee Report, Part III). Ed. D.J. Kirkland. Cambridge University Press, pp 184-232.
- (e) Schwartz D (1969). Les tests non paramétriques. But et principaux tests In ".Méthodes statistiques à l'usage des médecins et des biologistes". 3^{ème} édition. Ed. Flammarion Médecine Sciences, pp 245-257.

Table 1: Results of the preliminary toxicity test

Doses mg/kg/day	Time	Animal number		Clinical signs
		Males	Females	
200.3*	2 h	01-02-03	04-05-06	Hypoactivity, piloerection
	6 h	01		Death
		02-03		Sedation, piloerection
24 h	02-03	04-05-06 05-06 04	Piloerection, hypoactivity Death Sedation, piloerection	
50 *	2 h	07-08-09	10-11-12	None
	6 h	07-08-09	10-11-12	Piloerection
	24 h	07-08-09	10-11-12	Hypoactivity, piloerection
50 **	2 h - 4 h - 24 h	07-08-09	10-11-12	Hypoactivity, piloerection
30 *	2 h - 6 h - 24 h	13-14-15	16-17-18	Piloerection
30 **	2 h - 6 h	13-14-15	16-17-18	Piloerection, hypoactivity
	24 h	13-14-15	16-17-18	None

* : first treatment

** : second treatment

h : hour

Table 2: Results of the cytogenetic test: clinical signs

Doses mg/kg/day	Time	Animal number		Clinical signs
		Males	Females	
0 *	2 h - 24 h	01-02-03-04-05	06-07-08-09-10	None
0 **	2 h - 24 h	01-02-03-04-05	06-07-08-09-10	None
7.5 *	2 h - 24 h	11-12-13-14-15	16-17-18-19-20	None
7.5 **	2 h - 24 h	11-12-13-14-15	16-17-18-19-20	None
15 *	2 h - 24 h	21-22-23-24-25	26-27-28-29-30	None
15 **	2 h - 24 h	21-22-23-24-25	26-27-28-29-30	None
30 *	2 h	31-32-33-34-35	36-37-38-39-40	None
	24 h	31-32-33-34-35	36-37-38-39-40	Piloerection
30 **	2 h - 24 h	31-32-33-34-35	36-37-38-39-40	Hypoactivity, piloerection
30 * ⁽¹⁾	2 h	51-52-53	54-55-56	None
	24 h	51-52-53	54-55-56	Piloerection
30 ** ⁽¹⁾	2 h - 24 h	51-52-53	54-55-56	Hypoactivity, piloerection
CPA	2 h - 24 h	41-42-43-44-45	46-47-48-49-50	None

0 : vehicle control

* : first treatment

** : second treatment

⁽¹⁾ : supplementary animals

h : hour

CPA : cyclophosphamide

Table 3: Results of the cytogenetic test: data summary

Group	Doses (mg/kg/day)	MPE/1000PE		PE/NE ratio		Time of sacrifice after the last administration
		mean	(sd)	mean	(sd)	
Males						
Vehicle	-	1.7	(0.6)	0.3	(0.1)	
	7.5	1.4	(1.0)	0.4	(0.1)	24 h
	15	1.1	(0.7)	0.5	(0.2)	
	30	0.9	(0.4)	0.3	(0.1)	
Cyclophosphamide	50 mg/kg	18.6	(7.1) ***	0.5	(0.1)	
Females						
Vehicle	-	0.9	(0.5)	0.6	(0.2)	
	7.5	0.9	(1.1)	0.8	(0.2)	24 h
	15	1.5	(0.6)	0.4	(0.1)	
	30	0.6	(0.8)	0.5	(0.1)	
Cyclophosphamide	50 mg/kg	17.1	(5.4) ***	0.6	(0.1)	

Five animals per group

Route: intraperitoneal

- vehicle and test item: two administrations separated by a 24-hour interval

- cyclophosphamide: one administration

Vehicle: paraffine oil

MPE: Micronucleated Polychromatic Erythrocytes

PE: Polychromatic Erythrocytes

NE: Normochromatic Erythrocytes

sd: Standard Deviation

Statistical tests used : *** p < 0.001

The 2 x 2 contingency table for MPE

Student's "t" test for PE/NE ratio

Table 4: Results of the cytogenetic test: individual values

Vehicle					
sex	slide	MPE/2000PE	PE	NE	Ratio
Male	10	3	295	705	0.42
	37	4	159	841	0.19
	21	5	241	759	0.32
	43	2	216	784	0.28
	22	3	247	753	0.33
mean		3.40			0.31
sd		1.14			0.08
Female	44	2	411	589	0.70
	24	2	467	533	0.88
	09	0	258	742	0.35
	30	3	313	687	0.46
	31	2	403	597	0.68
mean		1.80			0.61
sd		1.10			0.21

Test item (15 mg/kg/day)					
sex	slide	MPE/2000PE	PE	NE	Ratio
Male	28	2	333	667	0.50
	11	0	425	575	0.74
	42	3	296	704	0.42
	18	4	313	687	0.46
	06	2	200	800	0.25
mean		2.20			0.47
sd		1.48			0.18
Female	07	4	276	724	0.38
	33	3	199	801	0.25
	32	1	295	705	0.42
	16	3	326	674	0.48
	46	4	367	633	0.58
mean		3.00			0.42
sd		1.22			0.12

Cyclophosphamide (50 mg/kg)					
sex	slide	MPE/2000PE	PE	NE	Ratio
Male	14	27	326	674	0.48
	04	25	269	731	0.37
	48	56	355	645	0.55
	26	29	293	707	0.41
	03	49	369	631	0.58
mean		37.20			0.48
sd		14.25			0.09
Female	50	23	353	647	0.55
	25	46	315	685	0.46
	39	43	411	589	0.70
	15	36	447	553	0.81
	49	23	352	648	0.54
mean		34.20			0.61
sd		10.85			0.14

Test item (7.5 mg/kg/day)					
sex	slide	MPE/2000PE	PE	NE	Ratio
Male	20	2	280	720	0.39
	05	2	195	805	0.24
	36	6	299	701	0.43
	23	3	323	677	0.48
	02	1	289	711	0.41
mean		2.80			0.39
sd		1.92			0.09
Female	17	0	483	517	0.93
	08	1	479	521	0.92
	29	3	369	631	0.58
	41	0	463	537	0.86
	45	5	384	616	0.62
mean		1.80			0.78
sd		2.17			0.17

Test item (30 mg/kg/day)					
sex	slide	MPE/2000PE	PE	NE	Ratio
Male	19	3	183	817	0.22
	01	1	269	731	0.37
	27	1	168	832	0.20
	13	2	263	737	0.36
	35	2	239	761	0.31
mean		1.80			0.29
sd		0.84			0.08
Female	34	1	357	643	0.56
	12	0	333	667	0.50
	40	4	251	749	0.34
	47	0	351	649	0.54
	38	1	354	646	0.55
mean		1.20			0.50
sd		1.64			0.09

Route: intraperitoneal
Vehicle: paraffine oil

MPE: Micronucleated Polychromatic Erythrocytes
PE: Polychromatic Erythrocytes
NE: Normochromatic Erythrocytes
sd: standard deviation

APPENDICES

1. GLP certificate and Quality assurance statement of Microptic Cytogenetic Services

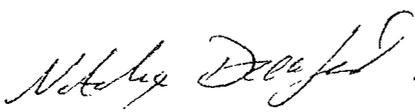
microptic2 LANGLAND CLOSE
MUMBLES, SWANSEA, UK, SA3 4LY

cytogenetic services

Natalie Danford, BSc, MPH, PhD

MICROPTIC CYTOGENETICS	Client No./Ref.: 10/190
GOOD LABORATORY PRACTICE	Study No.: 25873 MAS
COMPLIANCE STATEMENT	Date: 4 July 2003

I hereby confirm that the work conducted at Microptic Cytogenetics, in respect of this study, was in compliance with Good Laboratory Practice (GLP) as required by the United Kingdom GLP Compliance Regulations 1999 (SI 1999 No. 3106) and which are in accordance with the OECD Principles of GLP 1997 (ENV/MC/CHEM(98) 17).



NATALIE DANFORD, BSc, MPH, PhD
PRINCIPAL INVESTIGATOR

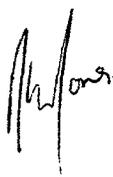
FORM No.F20.2

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FAX: 01792 366714MOBILE: 07802 934350
Email: natalie.danford@microptic.com

microptic2 LANGLAND CLOSE
MUMBLES, SWANSEA, UK, SA3 4LY

cytogenetic services

Natalie Danford, BSc, MPH, PhD

<p>MICROPTIC CYTOGENETICS QUALITY ASSURANCE STATEMENT</p>	<p>Client No./Ref: 10/190 Study No.: 25873 MAS Date: 4 July 2003</p>
<p>At the time this work was conducted the Quality Co-ordinator for Microptic Cytogenetics was conducting regular scheduled inspections in accordance with the current <i>Standard Operating Procedure (SOP-07)</i> for monitoring inspections and audits.</p> <p>The last inspection/audit of Head Office management systems was conducted on the 16 May 2003 and no significant deficiencies were found.</p> <p></p> <p>N. JONES QUALITY CO-ORDINATOR</p>	

FORM No.F20.2

TEL: 01792 366714
FAX: 01792 366714MOBILE: 07802 934350
Email: natalie.danford@microptic.com

2. Analytical certificate and test article description

Ship From:
RIVERVIEW, MI - PLANT

Ship-To:
ATOFINA
ELECTRONIC GRADE
PLATEFORME SOBEGI
F-64150 MOURENX

Customer Number: 33891
Customer PO: SAMPLE
Ship Date: 02.07.2002
Delivery Number: 80712861
Order Number: 110035485

Additional Copy/Fax to:

Material: 2292 METHANE SULFONYL CHLORIDE 55 GAL OP DRUM

Characteristic	Atofina Specification Limits	Batch Analysis
Container Number: DET 027 353248 6		
Batch : 1491E07CD1 Quantity: 1,0 DRM		
MSC, %	>=99,7	99,9
Color, Visual	Pass	Pass
Water, ppm	<=250	214
Batch : 1491F10CD1 Quantity: 1,0 DRM		
MSC, %	>=99,7	99,9
Color, Visual	Pass	Pass
Water, ppm	<=250	199
Batch : 1491F17CD1 Quantity: 1,0 DRM		
MSC, %	>=99,7	99,9
Color, Visual	Pass	Pass
Water, ppm	<=250	175
Batch : 1491F25CD1 Quantity: 1,0 DRM		
MSC, %	>=99,7	99,9
Color, Visual	Pass	Pass
Water, ppm	<=250	220

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TOXICOLOGY & ENVIRONMENT DEPARTMENT

CONFIDENTIAL

La défense 10, Cours Michelet
92091 Paris-la-Défense cedex, France**TEST ARTICLE DESCRIPTION****Methane Sulfonyl Chloride****IDENTITY**

Test article name : Methane Sulfonyl Chloride
Chemical name : Methane Sulfonyl Chloride
CAS number : 124-63-0
EINECS number : 204-706-1
Molecular formula : CH₃ClO₂S
Molecular weight : 114.55 g
Purity : 99.9%
Origin and batch : Atofina , Riverview, Batch:1491F10CD1
Atofina filing number : 0079/02

PHYSICAL AND CHEMICAL PROPERTIES

Appearance : Light yellow liquid
Viscosity : 1.97 mPas (Cp)
Specific gravity : 1.48
Melting point : -33°C
Boiling point : 161°C
Vapor pressure : 2.79 mBar (20°C)
Flash point : >110°C
Solubility : Insoluble in water (20°C)
Soluble in : Acetone, Ethyl ether

TOXICOLOGICAL INFORMATION AND USE SAFETY

See FDS

STORAGE AND DISPOSAL

Storage : in dark and at room temperature
Expiry date : November 2003
Disposal : incineration

3. Principal investigator data sheet

CIT/Study No. 25873 MAS

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Principal Investigator Data Sheet	
Details regarding the conduct of delegated phase(s) of a study Application of OECD Principles of GLP to the Organization and Management of Multi-site studies – 7 June 2002	
Microptic Cytogenetic Services PIDS No. : 1	
Laboratory	ATOFINA
Test item	Methane Sulfonyl Chloride
Study Title	Bone marrow micronucleus test by intraperitoneal route in mice
Study Identification Numbers	CIT/ Study No.: 25873 MAS Sponsor Reference No. (if applicable):
Test Facility	
. CIT Test Site Name of Study Director (SD)	Name: Hasnaà Haddouk Address: CIT, BP 563, 27005 Evreux, France E-mail: Hasnaa.Haddouk@citox.com Phone No.: (33) 232292626 Fax No.: (33) 232678705
. PI Test Site Name of Principal Investigator (PI)	Name: Natalie Danford Address: Microptic Cytogenetic Services, 2 Langland Close, Mumbles, Swansea, SA3 4LY, UK E-mail: natalie.danford@microptic.com Phone No.: (44) 01792366714 / Mobile No.: 07802834350 Fax No.: (44) 1792366714
Reference of the general contract	General contract between Microptic and CIT dated on 31 March 2003
Lead QA/Responsible Person	Name: C. Galli-Kar Address: BP 563 27005 Evreux Cedex France E-mail: celine.galli@citox.com Phone No.: (33) 232292626 Fax No.: (33) 232678705

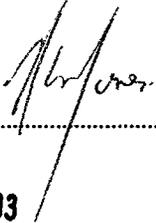
<p>Phase(s) conducted at the PI Test Site</p>	<p>Description of the phase(s) and summary of the methods: <i>All analyses of slides are performed "blind" since slides are coded and code number are kept by the Study Director and not supplied to the PI.</i> Magnification: x 1000 For each animal (i.e. for each code), 2000 polychromatic erythrocytes (PE) should be analysed for the presence of micronucleus (MPE) For each animal (i.e. for each code), a total of 1000 cells (polychromatic and normochromatic cells: PE + NE) should be scored to establish the ratio PE/NE CIT forms should be used for the collection of data</p> <p>In addition, SOP-08 version 2, SOP-03 version 5 and SOP-07 version 3 are followed.</p> <p>Quality Assurance of the Principal Investigator test site should review sections of the study plan relating to operations to be conducted at their site. They should maintain a copy of the approved study plan and relevant amendments. Quality Assurance test site should inspect study related work at their site according to their own SOPs.</p> <p>The Principal Investigator should report promptly in writing to the Study Director, CIT management and lead Quality Assurance, any inspection results regarding the delegated phase of the study. The Study Director will then send to the Principal Investigator his/her comments regarding the possible impact of these results on the study. The dispatch date of the inspection results will be reported in the test site Quality Assurance statement.</p>
<p>Proposed time schedule for the delegated phase</p>	<p>The PI will be informed (either by mail, e-mail or fax) of the date of receipt of the relevant material to conduct the delegated phase. At reception of slides, the PI should acknowledge receipt by faxing the "acknowledgment of receipt". Proposed time schedule for analysis of all slides generated in the study:</p> <ul style="list-style-type: none"> • Experimental starting date : 12 June 2003 • Experimental completion date : 27 June 2003 <p>Unless otherwise specified, slides, raw data and original document of PIDS should be returned to CIT at the latest 2 weeks after the end of slide analysis.</p>
<p>Quality Compliance of delegated phase(s) conducted at the PI Test Site</p>	<p>A GLP Compliance Statement for the PI test site is available</p> <p>References of the GLP regulatory compliance followed: UK GLP Compliance Regulations 1999 (SI 1999 No. 3106) in accordance with the OECD Principles of GLP 1997 (ENV/MC/CHEM(98) 17).</p> <p>QA unit of the PI test site : Name of responsible person: Neville W. Jones Phone No.: (44) 0 1558 822820 Fax No.: (44) 0 1558 822820 E-mail: nevjones@talkgas.net</p> <p>Study related work which will be audited: No specific study delegated phase inspections are performed. At the time of performing the delegated phase, regular scheduled inspections are conducted in accordance with current SOP-07</p>

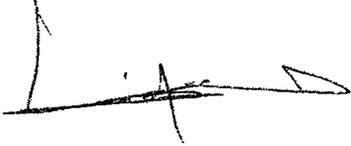
<p>Reporting of delegated phase(s) conducted at the PI test site</p>	<p>How will the PI reports his/her results to the SD: <input type="checkbox"/> under a report format <input checked="" type="checkbox"/> raw-data only</p> <p>The PI report/results will be audited by the PI QA</p> <p>A QA Statement be included in the PI report/results</p> <p>The PI report/results will include a Statement of Compliance with GLP signed by the PI</p>
<p>Communication</p>	<ul style="list-style-type: none"> . Together with the approved study plan, a PIDS will be sent by the Study Director to the Principal Investigator. It should be completed and signed by the Principal Investigator, Principal Investigator test site management and Quality Assurance and returned, upon receipt (prior to slide analysis) to the Study Director. . Relevant amendments to the study plan (the ones which could have an impact on the delegated phases(s)) will be sent to the Principal Investigator by the Study Director. . It is the responsibility of the Principal Investigator to distribute all documents (study plans, PIDS, amendments, as well as the comments of the Study Director concerning the impact of the inspection results; see above) to the Principal Investigator test site management and Quality Assurance. . The Study Director should be kept informed by the Principal Investigator, in due time, of the progress of the delegated phase(s) of the study and any relevant information concerning this(these) phase(s). . The lead Quality Assurance will liaise with the Principal Investigator test site Quality Assurance in order to ensure adequate quality inspection coverage throughout the study. The information requested will include but will not be limited to: <ul style="list-style-type: none"> - GLP regulatory compliance followed availability of GLP compliance statement for the Principal Investigator test site, - foreseen inspected study-related work (to be documented in the PIDS), - ways of reporting inspection results. <p>In order to document communication between the SD and the PI, all relevant exchanges (e-mails, letters, hard copy of telephone conversations...) will be kept in the study archives. The PI should report promptly in writing to the SD, CIT management and lead QA, any inspection results regarding the delegated phase(s) of the study. The SD will then send to the PI his/her comments regarding the possible impact of these results on the study.</p> <ul style="list-style-type: none"> . The Study Director will inform the Sponsor of any deviation concerning the delegated phase(s) of the study for which he/she considers that it could impact the integrity of the study.
<p>Archiving of specific data for the study generated by the PI test site</p>	<p>Name of archiving site: CIT Address: BPP 563 27005 EVREUX cedex FRANCE</p> <p>Duration of archiving: as specified in the study plan</p>
<p>Archiving of data not specific to the study generated by the PI test site</p>	<p>Name of archiving site: Address:</p> <p>Duration of archiving :</p>

CIT/Study No. 25873 MAS

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..... N. DANFORD
Date 13 JUN 2003 Name and signature of test site Management
and of Principal Investigator


.....
Date 13 JUN 2003 Signature of PI test site QA


.....
Date 19 JUN 2003 Signature of Study Director



CONFIDENTIAL

SPONSOR

ATOFINA

Département de Toxicologie Industrielle
4-8 Cours Michelet
La Défense 10
92091 Paris-la-Défense
France

TEST ITEM

Methanesulfonyl chloride

STUDY TITLE

**SKIN SENSITIZATION TEST
IN GUINEA PIGS**

(Maximization method of Magnusson and Kligman)

STUDY DIRECTOR

Xavier Manciaux

DATE OF ISSUE

02 December 2003.

TEST FACILITY

CIT

BP 563 - 27005 Evreux - France

LABORATORY STUDY NUMBER

25982 TSG

Safety & Health Research Laboratories

IFM Recherche

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STATEMENT OF THE STUDY DIRECTOR

The study was performed in compliance with the principles of Good Laboratory Practice as described in:

- . OECD Principles on Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM (98) 17.
- . Commission Directive 1999/11/EC of 8 March 1999 adapting to technical progress the Principles of Good Laboratory Practice as specified in Council Directive 87/18/EEC on the harmonization of laws, regulations and administrative provisions relating to the application of the Principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (OJ No. L 77 of 23.3.1999).
- . Décret N° 98-1312 du 31 décembre 1998 concernant les Bonnes Pratiques de Laboratoire (Journal Officiel du 1er janvier 1999), Ministère de l'Economie, des Finances et de l'Industrie.

The study was also conducted in compliance with the following Animal Health regulations:

- . Council Directive 86/609/EEC of 24th November 1986 on the harmonization of laws, regulations or administrative provisions relating to the protection of animals used for experimental or other scientific purposes.
- . Guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation, OECD Environmental Health and Safety Publications, No. 19.

I declare that this report constitutes a true and faithful record of the procedures undertaken and the results obtained during the performance of the study.

This study was performed at CIT, BP 563, 27005 Evreux, France.

Toxicology



X. Manciaux Study completion date: 2 Dec 2003
Study Director
Doctor of Pharmacy

OTHER SCIENTIST INVOLVED IN THIS STUDY

For Pharmacy: X. Manciaux
 Doctor of Pharmacy

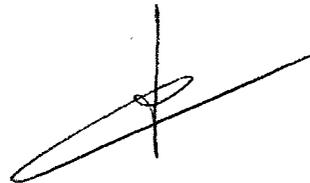
STATEMENT OF QUALITY ASSURANCE UNIT

Type of inspections	Dates		
	Inspections	Reported to Study Director (*)	Reported to Management (*)
Study plan	19 June 2003	23 June 2003	23 June 2003
Report	25 September 2003	4 November 2003	17 November 2003

In addition to the above-mentioned inspections, at about the same time as the study described in the present report, "process-based" and routine facility inspections of critical procedures relevant to this study type were also made by the Quality Assurance Unit. The findings of these inspections were reported to the Study Director and to CIT Management.

The inspections were performed in compliance with CIT Quality Assurance Unit procedures and Principles of Good Laboratory Practice.

The reported methods and procedures were found to describe those used and the results to constitute an accurate and complete reflection of the study raw data.



C. Galli-Kar Date: 02 Dec. 2003
Ing. Biol.
Head of Quality Assurance Unit

(*) The dates indicated correspond to the dates of signature of audit reports by Study Director and Management.

SUMMARY

At the request of ATOFINA, Paris-la Défense, France, the potential of the test item Methanesulfonyl chloride (200.25 mg/mL solution of batch 1491F10CD1 in paraffin oil) to induce delayed contact hypersensitivity was evaluated in guinea pigs according to the maximization method of Magnusson and Kligman and to OECD (No. 406, 17th July 1992) and EC (96/54/EEC, B.6, 30 July 1996) guidelines.

The study was conducted in compliance with the principles of Good Laboratory Practice Regulations.

Methods

The test item used in the study was Methanesulfonyl chloride, which was provided as a 200.25 mg/mL solution in paraffin oil for ease of manipulation.

Thirty guinea pigs were allocated to two groups: a control group of five males and five females and a treated group of ten males and ten females.

On day 1, three pairs of intradermal injections were performed in the interscapular region of all animals:

- . Freund's complete adjuvant (FCA) diluted to 50% with 0.9% NaCl (both groups),
- . test item at the concentration of 2 mg/mL (i.e. solution at 1%) in paraffin oil (treated group) or vehicle alone (control group),
- . test item at the concentration of 2 mg/mL (i.e. solution at 1%) in a mixture FCA/0.9% NaCl (50/50) (treated group) or vehicle at the concentration of 50% in a mixture FCA/0.9% NaCl (50/50) (control group).

On day 8, the animals of the treated group received a topical application of the test item at the concentration of 200 mg/mL in paraffin oil (i.e. undiluted solution) to the same test site, which was then covered by an occlusive dressing for 48 hours. The animals of the control group received an application of vehicle under the same experimental conditions.

On day 22, all animals of both groups were challenged by a cutaneous application of the test item at the concentration of 200 mg/mL in paraffin oil (i.e. undiluted solution) to the right flank. The test item was maintained under an occlusive dressing for 24 hours. The vehicle was applied to the left flank under the same experimental conditions.

Skin reactions were evaluated approximately 24 and 48 hours after removal of the dressing.

As equivocal cutaneous reactions were noted after the first challenge, a second challenge application was performed on day 40. For this second challenge application, a control group of ten naive animals (five males and five females) was included in the study. The test item in paraffin oil was applied at the concentration of 50 mg/mL (i.e. solution at 25% (w/w)) to the left flank and at the concentration of 20 mg/mL (i.e. solution at 10% (w/w)) to the right flank of animals of all groups, under the same experimental conditions as for the first challenge application.

At the end of the study, animals were killed without examination of internal organs. No skin samples were taken from the challenge application sites.

Results

No systemic clinical signs and no deaths related to treatment were noted during the study.

After the first challenge application, a discrete or moderate erythema was observed in 9/10 animals of the control group.

In the treated group, a moderate or intense erythema was noted in all animals. Dryness of the skin, crusts (which have sometimes masked the evaluation of erythema) and oedema were also recorded.

After the second challenge application, no cutaneous reactions were observed in the animals of the control group 3.

In the control group 1, a discrete erythema was observed on the left flank of 2/10 animals and a discrete or moderate erythema was noted on the right flank of 9/10 animals at the 24 and 48-hour readings.

In the treated group, a discrete or moderate erythema was recorded on the left flank of 13/18 animals at the 24-hour reading and 11/18 animals at the 48-hour reading. On the right flank, a discrete to intense erythema was observed in all animals at the 24-hour reading; it was masked by crusts in most of them at the 48-hour reading. Oedema and dryness of the skin were noted in almost all animals.

These results confirmed those of the first challenge application. The cutaneous reactions observed in all the animals of the treated group after the first challenge application, which were of higher severity than those recorded in the animals of the control group, were therefore attributed to delayed contact hypersensitivity.

Conclusion

Under our experimental conditions and according to the maximization method of Magnusson and Kligman, the test item Methanesulfonyl chloride (200.25 mg/mL solution of batch 1491F10CD1 in paraffin oil) induces delayed contact hypersensitivity in 20/20 (100%) guinea pigs.

RESUME

A la demande de ATOFINA, Paris-la-Défense, France, le potentiel du produit Methanesulfonyl chloride (solution à 200,25 mg/mL du lot 1491F10CD1 dans l'huile de paraffine) à induire une hypersensibilisation cutanée retardée est évalué chez le Cobaye selon la méthode de maximisation de Magnusson et Kligman et conformément aux lignes directrices de l'OCDE (n° 406, 17 juillet 1992) et de la CEE (96/54/CEE, B.6, 30 juillet 1996). L'étude est réalisée conformément aux règles de Bonnes Pratiques de Laboratoire.

Méthodes

Le produit utilisé dans cette étude est le Methanesulfonyl chloride, qui a été fourni en solution à 200,25 mg/mL dans l'huile de paraffine pour en faciliter la manipulation.

Trente cobayes sont répartis en 2 groupes : un groupe témoin de 5 mâles et 5 femelles et un groupe traité de 10 mâles et 10 femelles.

Au jour 1, 3 paires d'injections intradermiques sont effectuées au niveau de la région interscapulaire de tous les animaux :

- . adjuvant complet de Freund (FCA) dilué à 50 % dans du NaCl à 0,9 % (groupe traité et groupe témoin),
- . produit à tester à la concentration de 2 mg/mL (i.e. solution à 1 %) dans l'huile de paraffine (groupe traité) ou véhicule seul (groupe témoin),
- . produit à tester à la concentration de 2 mg/mL (i.e. solution à 1 %) dans un mélange FCA/NaCl à 0,9 % (50/50) (groupe traité) ou véhicule à la concentration de 50 % dans un mélange FCA/NaCl à 0,9 % (50/50) (groupe témoin).

Au jour 8, les animaux du groupe traité reçoivent une application topique du produit à la concentration de 200 mg/mL dans l'huile de paraffine (i.e. solution telle quelle) sur le même site, qui est ensuite recouvert d'un pansement occlusif pendant 48 heures. Les animaux du groupe témoin reçoivent une application du véhicule dans les mêmes conditions expérimentales.

Au jour 22, les animaux des 2 groupes reçoivent une application cutanée déclenchante de produit à la concentration de 200 mg/mL dans l'huile de paraffine (i.e. solution telle quelle) sur le flanc droit. Le produit est maintenu sous pansement occlusif pendant 24 heures. Le véhicule est appliqué sur le flanc gauche dans les mêmes conditions expérimentales.

L'évaluation des réactions cutanées est effectuée environ 24 et 48 heures après l'enlèvement du pansement.

Des réactions équivoques étant observées, une seconde application déclenchante est effectuée au jour 40. Pour cette seconde application déclenchante, un nouveau groupe témoin de 10 animaux naîfs (5 mâles et 5 femelles) est inclu dans l'étude. Le produit dans l'huile de paraffine est appliqué à la concentration de 50 mg/mL (i.e. solution à 25 % (p/p)) sur le flanc gauche et à la concentration de 20 mg/mL (i.e. solution à 10 % (p/p)) sur le flanc droit des animaux des 3 groupes, dans les mêmes conditions expérimentales que pour la première application déclenchante.

A la fin de l'étude, les animaux sont sacrifiés sans examen des organes internes.

Aucun prélèvement cutané n'est effectué au niveau des sites d'application déclenchante.

Résultats

Aucun signe clinique systémique ni aucune mortalité liés au traitement ne sont notés pendant l'étude.

Après la première application déclenchante, un érythème discret ou modéré est observé chez 9/10 animaux du groupe témoin.

Dans le groupe traité, un érythème modéré ou intense est noté chez tous les animaux. Une sécheresse cutanée, des croûtes (qui masquent parfois l'évaluation de l'érythème) et de l'œdème sont également enregistrés.

Après la seconde application déclenchante, aucune réaction cutanée n'est observée chez les animaux du groupe témoin 3.

Dans le groupe témoin 1, un érythème discret est observé sur le flanc gauche de 2/10 animaux et un érythème discret ou modéré est noté sur le flanc droit de 9/10 animaux aux lectures 24 et 48 heures.

Dans le groupe traité, un érythème discret ou modéré est observé sur le flanc gauche de 13/18 animaux à la lecture 24 heures et de 11/18 animaux à la lecture 48 heures. Sur le flanc droit, un érythème discret à intense est observé chez tous les animaux à la lecture 24 heures ; il est masqué par une croûte chez la plupart d'entre eux à la lecture 48 heures. Un œdème et une sécheresse cutanée sont notés chez la plupart des animaux.

Ces résultats confirment ceux de la première application déclenchante. Les réactions cutanées observées chez tous les animaux du groupe traité après la première application déclenchante, qui sont de sévérité plus importante que celles enregistrées chez les animaux du groupe témoin, sont donc attribuées à une hypersensibilisation cutanée retardée.

Conclusion

Dans nos conditions expérimentales et selon la méthode de maximisation de Magnusson et Kligman, le produit Methanesulfonyl chloride (solution à 200,25 mg/mL du lot 1491F10CD1 dans l'huile de paraffine) induit des réactions cutanées attribuables à une hypersensibilisation cutanée retardée chez 20/20 (100%) Cobayes.

1. INTRODUCTION

The objective of this study, performed according to the maximization method of Magnusson and Kligman (1), was to evaluate the potential of the test item Methanesulfonyl chloride to induce delayed contact hypersensitivity in guinea pigs.

The test item used in the study was Methanesulfonyl chloride, which was provided as a 200.25 mg/mL solution in paraffin oil for ease of manipulation.

The results of the study are of value in predicting the contact sensitization potential of the test material in humans.

The study was conducted in compliance with:

- . OECD guideline No. 406, 17th July 1992,
- . EC Directive No. 96/54/EEC, B.6, 30 July 1996.

2. MATERIALS AND METHODS

2.1 TEST MATERIALS

2.1.1 Test item

- . name:
 - on the Study plan: Methanesulfonyl chloride
 - labeling: Chlorure de Méthane Sulfonyle

Both names correspond to the same test item

- . batch number: none (solution in paraffin oil)
 - on the analytical certificate: 1491F10CD1
- . Sponsor's filing number: CAS 124-63-0
- . description: colorless liquid
- . container: one glass flask
- . date of receipt: 6 May 2003
- . storage conditions: at room temperature and protected from humidity
- . purity: the test item received at CIT is a 200.25 mg/mL solution of Methanesulfonyl chloride, batch No. 1491F10CD1, in paraffin oil.

Data relating to the characterisation of the original test item are documented in an analytical certificate (presented in appendix 1) provided by the Sponsor.

2.1.2 Vehicle

The vehicle used in the study was paraffin oil, as requested by the Sponsor.

Paraffin oil was supplied by the Sponsor (colorless liquid received on 6 May 2003) or was purchased by CIT (batch No. 28060, Gifrer, Decines, France).

2.1.3 Dosage form preparations

All dosage form preparations were made freshly on the morning of administration and any unused material was discarded that same day.

(1) Magnusson B. and Kligman A.M. The identification of contact allergens by animal assay. The guinea pig maximization test. *J. Invest. Derm.*, 52: 268-276 (1969).

2.1.4 Other materials

The other materials used were sterile isotonic saline solution (0.9% NaCl), batch No. DVF12A (Laboratoire Fresenius, Sèvres, France) and Freund's complete adjuvant, batch No. 062K8933 (Sigma, Saint-Quentin-Fallavier, France).

2.2 TEST SYSTEM

2.2.1 Animals

Species and sex: male and nulliparous and non-pregnant female guinea pigs.

Strain and sanitary status: Hartley CrI: (HA) BR, *Caesarian obtained, Barrier sustained - Virus Antibody Free (COBS - VAF®)*.

Reason for this choice: species generally accepted by regulatory authorities for this type of study. The strain used has been shown to produce a satisfactory sensitization response using known sensitizers.

Breeder: Charles River Laboratories France, L'Arbresle, France.

Number: . two males and two females for the preliminary test,
. 40 animals (20 males and 20 females) for the main test.

Allocation to the groups: on day -1, the animals were weighed and randomly allocated to two groups: a control group of ten animals (five males and five females) and a treated group of 20 animals (ten males and ten females). A new control group of ten animals was included in the study for the second challenge application.

Age/weight: on day 1, the animals of the main test were 1-2 months old and had a mean body weight \pm standard deviation of 458 ± 19 g for the males and 383 ± 16 g for the females.

Acclimation: at least 5 days before the beginning of the study.

Identification: by individual ear-tattoo.

2.2.2 Environmental conditions

The conditions in the animal room were set as follows:

- . temperature: $22 \pm 2^\circ\text{C}$
- . relative humidity: 30 to 70%
- . light/dark cycle: 12 h/12 h
- . ventilation: approximately 12 cycles/hour of filtered, non-recycled air.

The temperature and relative humidity were under continuous control and recording. The records were checked daily and filed. In addition to these daily checks, the housing conditions and corresponding instrumentation and equipment are verified and calibrated at regular intervals.

During the acclimation period and throughout the study, the animals were housed individually in polycarbonate cages with stainless steel lid (48 cm x 27 cm x 20 cm) equipped with a polypropylene bottle.

Each cage contained autoclaved sawdust (SICSA, Alfortville, France).

Sawdust is analysed by the supplier for composition and contaminant levels.

2.2.3 Food and water

During the study, the animals had free access to 106 pelleted diet (SAFE, Villemoisson, Epinay-sur-Orge, France).

Food is analysed regularly by the supplier for composition and contaminant levels.

The diet formula is presented in appendix 2.

Drinking water filtered by a FG Millipore membrane (0.22 micron) was provided *ad libitum*.

Bacteriological and chemical analyses of water are performed regularly by external laboratories. These analyses include the detection of possible contaminants (pesticides, heavy metals and nitrosamines).

No contaminants were known to have been present in the diet, drinking water or bedding material at levels which may be expected to have interfered with or prejudiced the outcome of the study.

2.3 TREATMENT

2.3.1 Preparation of the animals

For both the preliminary test and the main test, the application sites of all animals were:

- . clipped before intradermal injections (interscapular region 4 cm x 3 cm),
- . clipped before topical applications of the induction phase (same region),
- . clipped and shaved before topical applications of the challenge phases (each flank 3 cm x 3 cm),
- . shaved before the 48-hour reading of the challenge phases and before the 24-hour reading of the induction phase (preliminary test).

2.3.2 Preliminary test

A preliminary test was conducted in order to determine the concentrations to be tested in the main study.

By intradermal route (tested concentrations: 2 mg/mL and 0.2 mg/mL, i.e. solution at 1% and 0.1% (w/w)):

- . intradermal injections of the dosage form preparations (0.1 mL) were performed in the interscapular region,
- . local reactions were evaluated approximately 24, 48 hours and 6 days after the injections.

By cutaneous route

Under the conditions of the induction phase (tested concentration: 200 mg/mL, i.e. solution at 100%):

- . a filter paper (approximately 8 cm²) was fully-loaded with a dosage form preparation and was then applied to the clipped area of the skin. The filter paper was held in place by means of an occlusive dressing for 48 hours,
- . cutaneous reactions were evaluated 24 and 48 hours after removal of the dressing.

Under the conditions of the challenge phase (tested concentrations: 200 mg/mL and 100 mg/mL, i.e. solution at 100% and 50% (w/w)):

- . the filter paper of a chamber (Finn Chamber[®]) was fully-loaded with a dosage form preparation. The chamber was then applied to the clipped area of the skin (one concentration per flank). The chamber was held in place by means of an occlusive dressing for 24 hours,
- . cutaneous reactions were evaluated 24 and 48 hours after removal of the dressings.

Criteria for selection of concentrations

The following criteria were used:

- . the concentrations should be well-tolerated systemically and locally,
- . intradermal injections should cause moderate irritant effects (no necrosis or ulceration of the skin),
- . cutaneous application for the induction should cause at most weak or moderate skin reactions or be the maximal practicable concentration,
- . cutaneous application for the challenge phase should be the highest concentration which does not cause irritant effects.

2.3.3 Main test

2.3.3.1 Induction phase by intradermal and cutaneous routes

2.3.3.1.1 Intradermal route

On day 1, six injections were made deep into the dermis of a 4 cm x 2 cm clipped interscapular area, using a needle (diameter: 0.50 x 16 mm) mounted on a 1 mL plastic syringe (0.01 mL graduations).

Three injections of 0.1 mL were made into each side of this interscapular region (i.e. three pairs of sites), as follows:

Injection	Site	Treated group	Control group
1	Anterior	FCA at 50% (v/v) in 0.9% NaCl	FCA at 50% (v/v) in 0.9% NaCl
2	Middle	test item at 2 mg/mL (i.e. solution at 1% (w/w)) in paraffin oil	paraffin oil
3	Posterior*	test item at 2 mg/mL (i.e. solution at 1% (w/w)) in the mixture FCA/0.9% NaCl (50/50)	vehicle at 50% (v/w) in a mixture FCA/0.9% NaCl (50/50)

FCA: Freund's complete adjuvant

* : The test item was suspended in FCA prior to be combined with the aqueous phase. The final concentration of the test item was equal to that used in injection 2.

The anterior and middle pairs of injections were performed close to each other and nearest the head, while the posterior pair was performed towards the caudal part of the test area.

2.3.3.1.2 Cutaneous route

As the test item was shown to be irritant during the preliminary test, a topical application of sodium lauryl sulfate was not necessary on day 7.

On day 8, a pad of filter paper (approximately 8 cm²) was fully-loaded with the test item at the concentration of 200 mg/mL in paraffin oil (i.e. undiluted solution) and was then applied to the interscapular region of the animals of the treated group.

The animals of the control group received an application of the vehicle alone under the same experimental conditions.

The pad was held in place for 48 hours by means of an adhesive hypoallergenic dressing and an adhesive anallergenic waterproof plaster.

On removal of the dressing (day 10), no residual test item was observed.

A local irritation was recorded in all the animals of the control and treated groups.

2.3.3.2 Challenge phase

First challenge application

On day 22, the animals of treated and control groups received an application of the test item and vehicle. The filter paper of a chamber (Finn Chamber[®]) was fully-loaded with the test item at the concentration of 200 mg/mL in paraffin oil (i.e. undiluted solution) and was then applied to a clipped area of the skin of the posterior right flank of all animals.

The vehicle was applied under the same experimental conditions to the skin of the posterior left flank.

The chambers were held in contact with the skin for 24 hours by means of an adhesive anallergenic waterproof plaster.

As equivocal cutaneous reactions were noted, a second challenge application was performed after a rest period of 17 days.

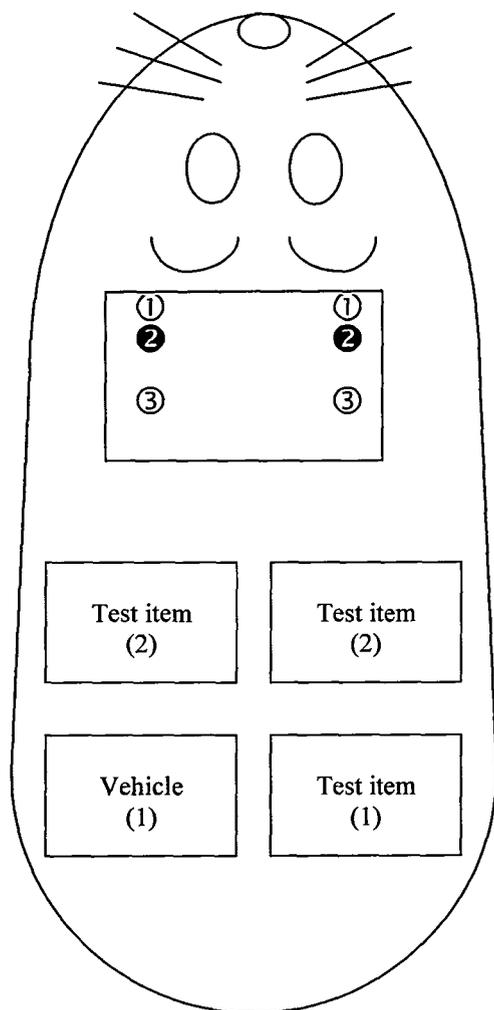
Second challenge application

A new control group of naive animals (control group 3) was included in the study for the second challenge application.

On day 40, the test item was applied at the concentration of 50 mg/mL (i.e. solution at 25% (w/w)) to the median left flank and at the concentration of 20 mg/mL (i.e. solution at 10% (w/w)) to the median right flank of animals of all groups, under the same experimental conditions as for the first challenge application.

2.4 SUMMARY DIAGRAM

Figure 1: Treatment sites



Induction site

Intradermal injections (day 1)*

Cutaneous application (day 8):
vehicle (control group)
or test item at the chosen
concentration (treated group)

Challenge application sites

Cutaneous application

(1) First challenge application
(day 22)

(2) Second challenge application
(day 40)

* Intradermal injections:

- ① 50% Freund's complete adjuvant in 0.9% NaCl
- ② vehicle (control group) or test item at the chosen concentration in the vehicle (treated group)
- ③ vehicle at 50% (control group) or test item at the chosen concentration (treated group) in the mixture Freund's complete adjuvant/0.9% NaCl (50/50)

2.5 SCORING OF CUTANEOUS REACTIONS

Before the second challenge application and 24 and 48 hours after removal of the dressing of each challenge application, both flanks of the treated and control animals were observed in order to evaluate cutaneous reactions, according to the following scale:

. no visible change.....	0
. discrete or patchy erythema	1
. moderate and confluent erythema	2
. intense erythema.....	3

Any observed oedema was recorded.

Any other lesions were noted.

2.6 CLINICAL EXAMINATIONS

The animals were observed at least once a day during the study in order to check for clinical signs and mortality.

2.7 BODY WEIGHT

The animals were weighed individually on the day of allocation into the groups (day -1 for groups 1 and 2, day 39 for group 3), on the first day of the study (day 1, groups 1 and 2), on day 25 (groups 1 and 2) and on the last day of the study (day 43, groups 1, 2 and 3).

2.8 PATHOLOGY

2.8.1 Sacrifice

At the end of the study, all the surviving animals were killed by carbon dioxide asphyxiation. No necropsy was performed.

2.8.2 Skin samples

No skin samples were taken.

2.9 DETERMINATION OF THE ALLERGENICITY LEVEL

The animals of the treated group show a positive reaction if macroscopic cutaneous reactions are clearly visible (score ≥ 1) and are of greater intensity and/or duration of response than the maximum reaction seen in control animals, or if macroscopic reactions are confirmed at microscopic examination as being due to the sensitization process.

The allergenicity level of the test item is determined according to the number of animals of the treated group showing a positive skin reaction, as follows:

% of animals showing a reaction	Allergenicity level	Classification
0 - 8	I	weak
9 - 28	II	mild
29 - 64	III	moderate
65 - 80	IV	strong
81 - 100	V	extreme

The sensitivity of the experimental technique is regularly assessed using a known moderate sensitizer, MERCAPTObENZOTHIAZOLE. In a recent study performed under CIT experimental conditions, the strain of guinea pigs used showed a satisfactory sensitization response in 80% animals (see appendix 4).

2.10 ARCHIVING

The following study materials are archived by CIT, 27005 Evreux, France, for 10 years after the end of the *in vivo* phase of the study:

- . Study plan and possible amendments,
- . raw data,
- . correspondence,
- . final report and possible amendments.

On completion of this period, the archived study materials will be returned to the Sponsor, or may be archived by CIT for a further period (at additional cost). The total duration of archiving (depending on regulations) will be the responsibility of the Sponsor.

In addition, raw data not specific to the study including, but not limited to, certificates of analyses for food, water and bedding (if applicable) and records of environmental data and equipment calibration, are also archived by CIT for at least 30 years.

2.11 CHRONOLOGY OF THE STUDY

The chronology of the study is summarized as follows:

Procedure	Date	Day
Experimental starting date (beginning of the preliminary test)	12 June 2003	
Arrival of the animals of the main test	19 June 2003	- 8
First treatment (induction)	27 June 2003	1
Experimental completion date (sacrifice of the animals)	8 August 2003	43

2.12 STUDY PLAN ADHERENCE

The study was performed in accordance with Study plan No. 25982 TSG and subsequent amendments, with the following deviation from the agreed Study plan:

- . the relative humidity recorded in the animal room was sometimes outside of the target ranges specified in the Study plan.

This minor deviation was not considered to have compromised the validity or integrity of the study.

3. RESULTS

3.1 CHOICE OF THE VEHICLE

The vehicle used was paraffin oil: a homogeneous dosage form preparation was obtained whatever the proportion.

The dosage form preparation at the concentration of 2 mg/mL (i.e. solution at 1% (w/w)) passed freely through a needle and into the dermis.

3.2 PRELIMINARY STUDY

3.2.1 Administration by intradermal route

Results were as follows:

Animal number	Concentration of the test item solution % (w/w)	Scoring after treatment		
		24 hours	48 hours	6 days
male 301	1 + FCA	I	I	I
	1	I	LI	LI
	0.1 + FCA	I	I	I
	0.1	I	LI	LI
female 302	1 + FCA	I	I	I
	1	I	LI	LI
	0.1 + FCA	I	I	I
	0.1	I	LI	LI

FCA : mixture Freund's Complete Adjuvant/0.9% NaCl (50/50, v/v)

I : irritation

LI : slight irritation

In order to respect the criteria for the selection of concentrations (the concentration should be well-tolerated systemically and locally, intradermal injections should cause moderate irritant effect but no necrosis or ulceration of the skin), concentration chosen for the main study was 2 mg/mL (i.e. solution at 1% (w/w)).

3.2.2 Application by cutaneous route

Results were as follows:

Under the conditions of the induction phase

Animal number	Concentration of the test item solution %	Scoring after removal of the dressing	
		24 hours	48 hours
male 303	100	1	1
female 304	100	1	0

Under the conditions of the challenge phase

Animal number	Concentration of the test item solution %		Scoring after removal of the dressing	
			24 hours	48 hours
male 301	100	RF	0	0
	50 (w/w)	LF	0	0
female 302	100	RF	0	0
	50 (w/w)	LF	0	0

RF : right flank

LF : left flank

On removal of the dressing, no residual test item was observed.

In order to respect the criteria for the selection of concentrations (the concentrations should be well-tolerated systemically and locally, cutaneous application for the induction should cause at most weak or moderate skin reactions or be the maximal practicable concentration, cutaneous application for the challenge phase should be the highest concentration which does not cause irritant effect), concentration chosen for the topical application of the induction phase (day 8) and for the challenge application (day 22) was 200 mg/mL (i.e. undiluted solution).

3.3 MAIN STUDY

3.3.1 Clinical examinations

Marked local reactions at the intradermal injection sites were noted in all animals of the treated group 2, from day 7. Two of these animals (male No. 39 and female No. 60) were killed on day 28 for ethical reasons.

No systemic clinical signs and no spontaneous deaths were observed during the study.

3.3.2 Body weight

The body weight gain of the treated animals was similar to that of controls (appendix 3).

3.3.3 Challenge phase - Scoring of cutaneous reactions

On removal of the dressing, no residual test item was observed.

3.3.3.1 First challenge application

Scoring of skin reactions was as follows:

		Control group 1					
Sex	Animal number	24 hours		48 hours			
		LF	RF	LF	RF		
Male	31	0	0	0	0		
	32	0	1	0	2		
	33	0	1	0	1		
	34	0	1	0	1		
	35	0	1	0	1		
Female	46	0	1/S	0	1/S		
	47	1	1	0	1/S		
	48	0	1	0	1		
	49	0	0	0	1		
	50	0	1	0	1		
		Treated group 2					
Sex	Animal number	24 hours		48 hours			
		LF	RF	LF	RF		
Male	36	0	3/S/A/Oe	0	3/S/A/Oe		
	37	0	LA/S/Oe	0	LA/Oe		
	38	0	2	0	2/S/Oe		
	39	0	2/S/A/Oe	0	3/S/A/Oe		
	40	0	2/S/A	0	2/S/A/Oe		
	41	0	2/S/A	0	3/S/A/Oe		
	42	0	2/S	0	2/S/Oe		
	43	0	2/S/A/Oe	0	3/S/A/Oe		
	44	0	2/A	0	2/S/A/Oe		
	45	0	3/S/A	0	3/S/A/Oe		
	Female	51	0	2/S	0	2/S/A/Oe	
		52	0	3/S	0	3/S/Oe	
		53	1	3/S	1	3/S/Oe	
		54	0	2/S/A	0	3/S/A/Oe	
		55	0	LA/S	0	LA/S/Oe	
56		0	2/S	0	LS/A/Oe		
57		0	2/S	0	2/S		
58		0	2	0	2/S		
59		0	2/S/A	0	LA/Oe		
60		0	2/A	0	2/S/A/Oe		

LF : left flank (vehicle)

RF : right flank (test item at the concentration of 200 mg/mL in paraffin oil (i.e. undiluted solution))

S : dryness of the skin

A : crusts

Oe : oedema

LA : scoring masked by crusts

LS : scoring masked by dryness of the skin

A discrete or moderate erythema (grade 1 or 2) was observed in 9/10 animals of the control group.

In the treated group, a moderate or intense erythema (grade 2 or 3) was noted in all animals. Dryness of the skin, crusts (which have sometimes masked the evaluation of erythema) and oedema were also recorded.

In order to determine whether the observed cutaneous reactions are attributable to delayed contact hypersensitivity or to an irritant effect of the test item, a second challenge application was performed at lower concentrations.

3.3.3.2 Second challenge application

Scoring of skin reactions was as follows:

		Control group 1					
Sex	Animal number	before treatment		24 hours		48 hours	
		LF	RF	LF	RF	LF	RF
Male	31	0	0	0	1	0	1
	32	0	0	0	1	0	1
	33	0	0	0	1	0	1/S
	34	0	0	1	2	1/S	2/S
	35	0	0	1	1	1/S	2/S
Female	46	0	0	0	0	0	0/S
	47	0	0	0	1	0	1
	48	0	0	0	1	0	1/S
	49	0	0	0	1	0	2/S
	50	0	0	0	1	0	2
		Control group 3					
Sex	Animal number	before treatment		24 hours		48 hours	
		LF	RF	LF	RF	LF	RF
Male	76	0	0	0	0	0	0
	77	0	0	0	0	0	0
	78	0	0	0	0	0	0
	79	0	0	0	0	0	0
	80	0	0	0	0	0	0
Female	81	0	0	0	0	0	0
	82	0	0	0	0	0	0
	83	0	0	0	0	0	0
	84	0	0	0	0	0	0
	85	0	0	0	0	0	0

LF : left flank (test item at 50 mg/mL in paraffin oil (i.e. solution at 25%))

RF : right flank (test item at the concentration of 20 mg/mL in paraffin oil (i.e. solution at 10%))

S : dryness of the skin

Sex	Animal number	Treated group 2					
		before treatment		24 hours		48 hours	
		LF	RF	LF	RF	LF	RF
Male	36	0	0	2	2/N/A/Oe	2/S/Oe	3/S/Oe/AR
	37	0	0	1	2/A	1/S	LA
	38	0	0	1	2	1	2/S/Oe
	39	-	-	-	-	-	-
	40	0	0	1	2/A/Oe/ZB	1	LA
	41	0	0	1	2/A/Oe/ZB	1/S	LA
	42	0	0	1	2/Oe	1	LA
	43	0	0	1	1	0/S	1/S
	44	0	0	0	2/A/Oe	0	LA
	45	0	0	1	2/A/Oe/ZBI	1/S	LA
Female	51	0	0	1	2/A/S/Oe	1/S	LA
	52	0	0	1	2	0	LS
	53	0	0	0	2/S/Oe	0	LA
	54	0	0	0	3/S/Oe/ZBI	0	LA
	55	0	0	1	LA	1/S	LA
	56	0	0	1	2/S/A/Oe	1	LA/S
	57	0	0	1	2/A/Oe	1	2/A/Oe
	58	0	0	0	3/S/Oe	0	LA
	59	0	0	0	2/S/Oe	0	LA
	60	-	-	-	-	-	-

LF : left flank (test item at 50 mg/mL in paraffin oil (i.e. solution at 25%))

RF : right flank (test item at the concentration of 20 mg/mL in paraffin oil (i.e. solution at 10%))

- : dead animal

S : dryness of the skin

A : crusts

Oe : oedema

LA : scoring masked by crusts

LS : scoring masked by dryness of the skin

ZB : brownish area

ZBI : whitish area

AR : crusts pulled out

N : necrosis

No cutaneous reactions were observed in the animals of the control group 3.

In the control group 1, a discrete erythema (grade 1) was observed on the left flank of 2/10 animals and a discrete or moderate erythema (grade 1 or 2) was noted on the right flank of 9/10 animals at the 24 and 48-hour readings.

In the treated group, a discrete or moderate erythema (grade 1 or 2) was recorded on the left flank of 13/18 animals at the 24-hour reading and 11/18 animals at the 48-hour reading. On the right flank, a discrete to intense erythema (grades 1 to 3) was observed in all animals at the 24-hour reading; it was masked by crusts in most of them at the 48-hour reading. Oedema and dryness of the skin were noted in almost all animals.

These results confirmed those of the first challenge application.

The cutaneous reactions observed in all the animals of the treated group after the first challenge application, which were of higher severity than those recorded in the animals of the control group, were therefore attributed to delayed contact hypersensitivity.

4. CONCLUSION

Under our experimental conditions and according to the maximization method of Magnusson and Kligman, the test item Methanesulfonyl chloride (200.25 mg/mL solution of batch 1491F10CD1 in paraffin oil) induces delayed contact hypersensitivity in 20/20 (100%) guinea pigs.

APPENDICES

1. Analytical certificate

ATOFINA Chemicals, Inc.

CERTIFICATE OF ANALYSIS

Ship From:
RIVERVIEW, MI - PLANT

Ship-To:
ATOFINA
ELECTRONIC GRADE
PLATEFORME SOBEGI
F-64150 MOURENX

Customer Number: 33891
Customer PO: SAMPLE
Ship Date: 02.07.2002
Delivery Number: 80712861
Order Number: 110035485

Additional Copy/Fax to:

Material: 2292 METHANE SULFONYL CHLORIDE 55 GAL OP DRUM

Characteristic	Atofina Specification Limits	Batch Analysis
Container Number: DET 027 353248 6		
Batch : 1491E07CD1 Quantity: 1,0 DRM		
MSC, %	>=99,7	99,9
Color, Visual	Pass	Pass
Water, ppm	<=250	214
Batch : 1491F10CD1 Quantity: 1,0 DRM		
MSC, %	>=99,7	99,9
Color, Visual	Pass	Pass
Water, ppm	<=250	199
Batch : 1491F17CD1 Quantity: 1,0 DRM		
MSC, %	>=99,7	99,9
Color, Visual	Pass	Pass
Water, ppm	<=250	175
Batch : 1491F25CD1 Quantity: 1,0 DRM		
MSC, %	>=99,7	99,9
Color, Visual	Pass	Pass
Water, ppm	<=250	220

TECHNICAL INFORMATION AND DATA REGARDING THE COMPOSITION, PROPERTIES OR USE OF THE PRODUCTS DESCRIBED HEREIN IS BELIEVED RELIABLE HOWEVER NO REPRESENTATION OR WARRANTY IS MADE WITH RESPECT THERETO EXCEPT AS MADE BY ATOFINA CHEMICALS, INC. IN WRITING AT TIME OF SALE ATOFINA CHEMICALS INC. CANNOT ASSUME RESPONSIBILITY FOR ANY PATENT LIABILITY WHICH MAY ARISE FROM THE USE OF ANY PRODUCT IN A PROCESS, MANNER OR FORMULA NOT DESIGNED BY ATOFINA CHEMICALS, INC

CONTROLLED COMPUTER PRINTOUT - VALID WITHOUT A SIGNATURE

2. Diet formula

Ref: 106

**COMPLETE DIET
GUINEA-PIG MAINTENANCE DIET**

Appearance: 4.5 mm diameter granules

Conditioning: bags of 20 kgs

Daily portion: Guinea-pigs 35-50 g, water *ad libitum*.

FORMULA %

Cereals	52.4
Grain biproducts and legumes..	36
Vegetable protein (soya bean meal, yeast)	9
Vitamin and mineral mixture ...	2.6

AVERAGE ANALYSIS %

Calorific value (Kcal/kg).....	2600
Moisture.....	10.5
Proteins.....	16
Lipids.....	3.5
Carbohydrates (N.F.E.)	50.5
Fibre.....	13.5
Minerals (ash).....	6

AMINO ACID VALUES
(calculated in mg/kg)

Arginine.....	8500
Cystine.....	2500
Lysine	7200
Methionine.....	2100
Tryptophan	2000
Glycine	6000

FATTY ACID VALUES
(calculated in mg/kg)

Palmitic acid.....	3600
Palmitoleic acid	0
Stearic acid	700
Oleic acid.....	5900
Linoleic acid	11200
Linolenic acid	3000

MINERALS (calculated in mg/kg)

	Nat. val.	CMV val.	Total
P	7400	1400	8800
Ca	5400	5600	11000
K	12000	0	12000
Na	1300	1950	3250
Mg.....	3270	130	3400
Mn	60	40	100
Fe	170	150	320
Cu	10	15	25
Zn	40	45	85
Co	0.1	1.5	1.6
I	0	0	0
Cl	0	0	0

VITAMINS (calculated per kg)

	Nat. val.	CMV val.	Total
Vitamin A	3500 IU	7500 IU	11000 IU
Vitamin D3	30 IU	2000 IU	2030 IU
Vitamin B1	6 mg	6.4 mg	12.4 mg
Vitamin B2	5 mg	6.4 mg	11.4 mg
Vitamin B3	22 mg	26 mg	48 mg
Vitamin B6	0.7 mg	2.7 mg	3.4 mg
Vitamin B12	0.003 mg	0.012 mg	0.015 mg
Vitamin C	0 mg	400 mg	400 mg
Vitamin E	15 mg	60 mg	75 mg
Vitamin K3	5 mg	12.6 mg	17.6 mg
Vitamin PP	97 mg	14.5 mg	111.5 mg
Folic acid	2.2 mg	1.3 mg	3.5 mg
P.A.B. acid	0 mg	2.5 mg	2.5 mg
Biotin	0.02 mg	0.06 mg	0.08 mg
Choline	1010 mg	60 mg	1070 mg
Meso-Inositol	0 mg	62.5 mg	62.5 mg

This food is supplemented with stabilized coated vitamin C, avoiding the need of other food substances (greenery, ascorbic acid) if used within 4 months of date of manufacture.

SAFE, 7 rue Galliéni, Villemoisson, 91360 Epinay-sur-Orge
Tel: 01.69.04.03.57 - Fax : 01.69.04.81.97
(Ref. Doc. UAR: 2000)

3. Individual body weight values

INDIVIDUAL BODY WEIGHT VALUES (g)

Groups	Sex	Animals	Days					
			-1	1	(1)	25	(1)	43
Control 1	Male	31	446	451	130	581	99	680
		32	434	435	141	576	88	664
		33	452	452	203	655	118	773
		34	461	474	166	640	102	742
		35	434	431	128	559	96	655
		M	445	449	154	602	101	703
		SD	12	17	31	42	11	52
	Female	46	381	397	115	512	82	594
		47	397	405	166	571	103	674
		48	396	399	86	485	65	550
		49	384	376	138	514	87	601
		50	367	371	124	495	107	602
		M	385	390	126	515	89	604
		SD	12	15	29	33	17	45
Treated 2	Male	36	446	446	108	554	70	624
		37	468	467	172	639	113	752
		38	451	460	152	612	111	723
		39	423	425	52	477		
		40	486	493	150	643	118	761
		41	462	456	158	614	87	701
		42	472	476	68	544	133	677
		43	455	461	129	590	80	670
		44	453	461	122	583	111	694
	45	491	480	159	639	113	752	
	M	461	463	127	590	104	706	
	SD	20	19	40	53	20	46	
	Female	51	357	360	89	449	95	544
		52	359	355	137	492	90	582
		53	368	373	134	507	101	608
		54	377	385	136	521	69	590
		55	378	382	71	453	76	529
		56	374	373	91	464	108	572
57		397	390	139	529	97	626	
58		399	410	148	558	71	629	
59		367	372	93	465	87	552	
60	395	398	59	457				
M	377	380	110	490	88	581		
SD	15	17	32	38	14	36		

(1) = Body weight gain

M = Mean

SD = Standard Deviation

Animals sacrificed during the study not mentioned

INDIVIDUAL BODY WEIGHT VALUES (g)

Groups	Sex	Animals	Days		
			39	(1)	43
Control 3	Male	76	382	10	392
		77	375	-59	316
		78	344	11	355
		79	340	7	347
		80	338	25	363
		M	356	-1	355
	SD	21	33	27	
	Female	81	309	10	319
		82	386	-29	357
		83	331	-2	329
		84	322	18	340
		85	317	13	330
		M	333	2	335
SD		31	19	14	

(1) = Body weight gain

M = Mean

SD = Standard Deviation

4. Positive control

Sensitization – Control of the sensitivity of guinea pigs to a reference item

Method : Magnusson and Kligman

CIT Study - Date : CIT/Study No. 24767 TSG - December 2002

Reference item : Mercaptobenzothiazole

Concentration : induction phase 1% (w/w) on day 1 (intra dermal route)
20% (w/w) on day 8 (cutaneous route)
challenge phase 20% (w/w) on day 22 (cutaneous route)

Vehicle : corn oil

Animals

Species, strain : Guinea pigs, Hartley Crl: (HA) BR

Breeder : Charles River France

Number : five control animals and ten treated animals

Conclusion

Under our experimental conditions and according to the Magnusson and Kligman method, the test item Mercaptobenzothiazole at the concentration of 20% (w/w) induced positive skin sensitization reactions in 80% (8/10) guinea pigs.

**CHALLENGE PHASE
Individual reactions**

Groups	Sex	Animals	24-hour		48-hour		Conclusion
			LF	RF	LF	RF	
Control	Female	96	0	1	0	1/S	-
		97	0	1	0	1	-
		98	0	1	0	1	-
		99	0	1	0	1	-
		100	0	1	0	1	-
Treated	Female	101	0	2	0	2/Oe/S	+
		102	0	2	0	1/S	-
		103	0	3/Oe/A	0	3/Oe/S/A	+
		104	0	1	0	2/S	+
		105	0	1	0	1	-
		106	0	2/Oe	0	2/Oe/S	+
		107	0	2/Oe	0	2/Oe/S	+
		108	0	3/Oe	0	2/Oe/S	+
		109	0	2/Oe	0	2/Oe/S	+
		110	0	2/Oe	0	2/Oe/S	+

LF : left flank (vehicle)

RF : right flank (test item at the concentration of 20% (w/w))

S : dryness of the skin

Oe : oedema

A : crusts

- : negative

+ : positive