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Chemical Category ETHYLENE OXIDE (75-21-8)		

CONTAINS NO CBI

Shell Oil Company



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SUBJECT: TSCA SECTION 8(D), HEALTH AND SAFETY DATA REPORTING

In accordance with the Toxic Substances Control Act, Section 8(d), Shell Oil Company is submitting copies of health and safety studies as outlined in 40 CFR § 716. Three reports are enclosed (Report Numbers 477 through 479). Each study is accompanied by a cover page as requested under § 716.30 (b). An index is also enclosed which includes the study title, date, specific chemical substance, CAS number, and Shell's report reference number for ease of future identification.

Sincerely,

G. A. Van Gelder, Manager
Product Safety and Compliance
Oil & Chemical Products, HSE

JWH/ljb

Enclosures

CG9109202.WP - 0001.0.0

REPORT NO. 478

SUBMISSION OF COPIES OF HEALTH AND SAFETY STUDIES - TSCA SECTION 8(D)

CHEMICAL NAMES OF LISTED SUBSTANCES

CAS NUMBER(S)

ETHYLENE OXIDE

000075-21-8

STUDY TITLE: SHELL BRAKE FLUID DOT 4 SUPER: ACUTE ORAL AND DERMAL TOXICITY, SKIN AND EYE
IRRITANCY AND SKIN SENSITISATION POTENTIAL

STUDY REFERENCE: SBGR.90.189

CONFIDENTIAL {Y/N}: N

86910000772



86910000772

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INDEX OF HEALTH AND SAFETY STUDIES SUBMITTED TO EPA
SUBMITTED APRIL, 1991

SUB. NO.	TITLE	RPT. DATE	CHEMICAL	CAS NUMBER
477	AIR TOXIC "HOT SPOTS" - AB 2588 HEALTH RISK ASSESSMENT - SHELL OIL COMPANY MARTINEZ REFINERY (VOL. I & II)	910100	TOLUENE ETHYLENE DICHLORIDE XYLENES O-CRESOL M-CRESOL P-CRESOL NAPHTHALENE	000108-88-3 000107-06-2 001330-20-7 000095-48-7 000108-39-4 000106-44-5 000091-20-3

INDEX OF HEALTH AND SAFETY STUDIES SUBMITTED TO EPA
SUBMITTED APRIL, 1991

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03/25/91

SUB. NO.	TITLE	RPT. DATE	CHEMICAL	CAS NUMBER
478	SHELL BRAKE FLUID DOT 4 SUPER: ACUTE ORAL AND DERMAL TOXICITY, SKIN AND EYE IRRITANCY AND SKIN SENSITISATION POTENTIAL	900800	ETHYLENE OXIDE	000075-21-8

INDEX OF HEALTH AND SAFETY STUDIES SUBMITTED TO EPA
SUBMITTED APRIL, 1991

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03/25/91

SUB. NO.	TITLE	RPT. DATE	CHEMICAL	CAS NUMBER
479	BIODEGRADATION OF BENZENE AND TOLUENE VAPORS IN UNSATURATED SOIL COLUMNS	900200	TOLUENE	000108-88-3

INDEX OF HEALTH AND SAFETY STUDIES SUBMITTED TO EPA
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CONTAINS NO CBI



yes

E & EA REPORTS
FILE COPY

GROUP RESEARCH REPORT

SBGR.90.189

500 70 539

IP 3901

Shell Brake Fluid Dot 4 Super: Acute oral
and dermal toxicity, skin and
eye irritancy and skin
sensitisation potential

J.R. Gardner

S1CC, CTMB

SHELL RESEARCH LIMITED, LONDON

SITTINGBOURNE RESEARCH CENTRE

SECURITY CLASS:

DOCUMENT TYPE: GROUP RESEARCH REPORT

E & EA REPORTS
FILE COPY

DOCUMENT NUMBER: SBGR.90.189

TITLE: Shell Brake Fluid Dot 4 Super: Acute oral and dermal toxicity, skin and eye irritancy and skin sensitisation potential

AUTHOR(S): Gardner JR SEP/1

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PARTICIPANT(S): Barry MP SEP/1
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PROJECT NUMBER: SRC38490

SUB PROJECT: 4608

PROJECT TITLE: Toxicology/Ecotoxicology of ethylene oxide and derivatives

SPONSOR: SICC, CTMB

BUDGET CODE: 500 70 539

SOURCE: Shell Research Limited, Sittingbourne Research Centre.

ORIGINATING DEPT: Environmental and Product Safety Department

DATE: August, 1990

SMT/506

CONTAINS NO CBI

REPORT NO. 478

SUBMISSION OF COPIES OF HEALTH AND SAFETY STUDIES - TSCA SECTION 8(D)

CHEMICAL NAMES OF LISTED SUBSTANCES

CAS NUMBER(S)

ETHYLENE OXIDE

000075-21-8

STUDY TITLE: SHELL BRAKE FLUID DOT 4 SUPER: ACUTE ORAL AND DERMAL TOXICITY, SKIN AND EYE IRRITANCY AND SKIN SENSITISATION POTENTIAL

STUDY REFERENCE: SBGR.90.189

CONFIDENTIAL {Y/N}: N

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Study Title

Shell Brake Fluid Dot 4 Super: Acute oral and dermal toxicity,
skin and eye irritancy and skin sensitisation potential

Regulatory Data Requirement

OECD guidelines for testing of chemicals (OECD, 1981)

Author

J.R. Gardner

Study Completed On

5 December 1990

Performing Laboratory

Sittingbourne Research Centre,
Sittingbourne, Kent, ME9 8AG, England

Laboratory Project Identity

Experiment No. 4608

Report No. SBGR.90.189

(Total number of pages in the report: 36)

CHRONOLOGY OF STUDY

Approval of Protocol

16 February 1990

Duration of study

Commencement

Completion

Acute oral toxicity

20 February 1990

14 March 1990

Acute dermal toxicity

20 February 1990

14 March 1990

Skin irritation study

26 February 1990

6 March 1990

Eye irritation study

26 February 1990

13 March 1990

Skin sensitisation

6 March 1990

6 April 1990

LOCATION of Raw Data, Specimens and Final Report:

Sittingbourne Research Centre
Sittingbourne ME9 8AG
England

Shell Brake Fluid Dot 4 Super: Acute oral and dermal toxicity, skin
and eye irritancy and skin sensitisation potential
(Experiment Number 4608)

SUMMARY:

1. The acute oral LD₅₀ of Shell Brake Fluid Dot 4 Super in fasted rats was shown to be greater than 5000 mg/kg. The undiluted test material was administered by oral gavage.
2. The acute dermal LD₅₀ of the undiluted test material in rats was shown to be greater than 2000 mg/kg. An area of the dorsum of each rat was clipped, subject to topical application of the test material and covered by an impervious dressing for approximately 24 hours.
3. Semi-occluded application of the undiluted test material to the clipped dorsum of six rabbits for 4 hours caused no irritation reactions or other dermal changes.
4. Single ocular instillation of the undiluted test material (0.1 ml) resulted in conjunctival irritation reactions not exceeding a crimson-red appearance, chemosis sufficient to cause partial eversion of the eye lids and an ocular discharge. These resolved within 48 hours of treatment. The cornea and iris were not visibly affected by the test material.

Upon administration of the test material there was a moderate initial pain response.

5. In the guinea-pig maximisation test of Magnusson and Kligman none of the twenty test animals showed a positive response 24 or 48 hours after removal of the challenge patches.

H.C. Volger

H.C. Volger, Ph.D., Manager & Director Research,
Shell Research Limited,
Sittingbourne Research Centre,
Sittingbourne, Kent, ME9 8AG, England.

Date: 7th December 1990

TEXT:

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PROFESSIONAL AND SUPERVISORY PERSONNEL INVOLVED IN STUDY

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STUDY DIRECTOR: J.R. Gardner, B.Sc., C.Biol., M.I. Biol.



(Signature)

5 December 1990
(Date)

INTRODUCTION

The work reported here was undertaken to provide suitable data on which to base an assessment of any acute hazards associated with the use of Shell Brake Fluid Dot 4 Super.

This study was not intended for regulatory submission.

MATERIALS

Sample

Details of the identification and characterisation of the sample and the formulations used in toxicological testing are presented in Appendix A.

Rats

Fischer 344 rats aged 8-9 weeks were obtained from Charles River (U.K.) Ltd. On arrival they were housed in single sex groups of up to 10 rats to a cage. The animals were quarantined for a minimum of four days in a non-barriered animal room with access restricted to essential personnel. At least five days before dosing the animals were rehoused (as single sex groups of up to three rats) in cages with stainless steel wire-mesh walls, floors and tops. Each cage measured 33 cm x 20 cm x 15 cm. Paper-lined trays for excreta were placed beneath each cage and changed three times weekly. A pelleted diet (PRD, Special Diet Services Ltd.) and water from the public supply were provided ad libitum. There were no excursions of animal room environmental conditions beyond target values of 19° to 23°C and 30% to 70% R.H. that were considered to have influenced the outcome of the experiment. Lighting (fluorescent tubes) was automatically controlled to provide a 12 hour day and 12 hour night. Animals assigned to the study were identified by cage-labels displaying the animal numbers, experiment number, sex and dose-level and by ear-notches denoting the animal number.

Rabbits

New Zealand White rabbits were obtained from a commercial supplier (Froxfield Farms (U.K.) Ltd). Their bodyweights were between 2.94 and 3.65 kg and their ages 3 to 5 months at the time of receipt. They were allowed to become accustomed to the laboratory environment for at least two weeks before any experimentation commenced. The animals were singly housed in hanging, stainless steel cages with perforated dimple or wire-mesh flooring. Sawdust-filled undertrays for excreta were placed beneath each cage and changed twice weekly. Each cage measured 67 cm x 43 cm x 45 cm. A pelleted diet (Standard Rabbit Diet, S.Q.C., Special Diet Services Ltd.) and water from the public supply were provided ad libitum. There were no excursions of animal room environmental conditions beyond target values of 15° to 20°C and 30% to 70% R.H. that were considered to have influenced the outcome of the study. Lighting (fluorescent tube) was automatically controlled to provide a 12 hour day and 12 hour night. Animals assigned to the study were identified by cage-labels displaying the animal number, experiment number and treatment regime and by uniquely numbered ear-tags.

Guinea-pigs

Guinea-pigs (Dunkin-Hartley strain) were obtained from Harlan Porcellus. Their bodyweights were between 247 and 335 g and their ages 5 to 9 weeks at the time of receipt. They were allowed to acclimatise to the laboratory environment for at least two weeks before any experimentation commenced. The animals were housed initially in single sex groups of ten animals. In the two day period before the study began they were re-allocated to cages accommodating two or three animals. Hanging galvanised steel cages with

wire-mesh floors were used, each measuring 54 cm x 31 cm x 36 cm. Sawdust-filled trays for excreta were placed beneath each cage and changed twice weekly. Pelleted diet (SG1 with vitamin C supplement, Grain Harvesters Ltd.) and water from the public supply were provided ad libitum. There were no excursions of animal room environmental conditions beyond target values of 19° to 23°C and 30% to 70% R.H. that were considered to have influenced the outcome of the study. Lighting (fluorescent tube) was automatically controlled to provide a 12 hour day and 12 hour night. Animals assigned to the study were identified by cage-labels displaying animal numbers, experiment number, sex and treatment regime and, within the cage, by coloured dye-marking of the fur.

METHODS

Acute oral toxicity (OECD Test Guideline No. 401)

Five male and five female rats were fasted overnight, weighed and given a single dose of the undiluted test material by gavage, using a ball pointed cannula and syringe. Approximately three hours after dosing on Day 1 the animals were allowed food again ad libitum.

A careful clinical examination was made five times on the day of dosing and twice daily thereafter for the remainder of the 14 day observation period. The initial (Day 1), Day 8 and Day 15 bodyweights were recorded, and changes in bodyweight calculated.

All animals were subject to necropsy. Animals were killed by intraperitoneal injection of sodium pentobarbitone. The cranial, thoracic and abdominal cavities and viscera were examined and any gross pathological changes recorded.

Acute dermal toxicity (OECD Test Guideline No. 402)

Five male and five female rats were used. On the day before dosing the dorsal fur was removed from the animals using electric clippers. Any rat showing signs of damage or irritation of the dorsum was replaced. On Day 1 the animals were weighed and a single dose of the undiluted test material was applied to the skin. The test material was held in place with a lint dressing (approx 6 x 8 cm) covered with waterproof adhesive tape. The rats were then individually housed. Following a 24 hour exposure the dressings were removed, the skin washed with warm dilute detergent solution, dried, and the animals returned to group housing.

A careful clinical examination was made five times on the day of dosing and twice daily thereafter for the remainder of the 14 day observation period. The initial (Day 1), Day 8 and Day 15 bodyweights were recorded, and changes in bodyweight calculated.

All animals were subject to necropsy. Animals were killed by an intraperitoneal injection of sodium pentobarbitone. The cranial, thoracic and abdominal cavities and viscera were examined and any gross pathological changes recorded.

Acute dermal irritation (OECD Test Guideline No. 404)

Six rabbits were used. On the day before dosing the dorsal fur was removed using electric clippers. Any rabbit with signs of damage or irritation of the dorsum was replaced. The undiluted test material (0.5 ml) was applied to the skin on a 6 cm² lint patch, covered with gauze and held in place by a semi-occlusive elastic adhesive bandage. After a 4 hour exposure the dressings were removed, the skin washed with water and dried.

After treatment the animals were examined for erythema, oedema and other lesions. Erythema and oedema were each scored on a five point scale

(Attachment A). The mean scores at each time point and group mean scores at 24, 48 and 72 hours were calculated.

Acute eye irritation (OECD Test Guideline No. 405)

Six rabbits with eyes free from irritation, ocular defects or corneal injury were used. The undiluted test material (0.1 ml) was placed into the lower conjunctival sac of one eye of each animal. The treated eye was gently held closed for a few seconds to prevent loss of the test material. The eyes were not irrigated.

The immediate reactions of the rabbits were scored as an initial pain response using a six point scale (Attachment A). Other ocular reactions to treatment were noted and scored using standard grades (Attachment A). The mean scores at each time point and the group mean scores at 24, 48 and 72 hours were calculated.

Skin sensitisation (OECD Test Guideline No. 406)

The skin sensitisation potential of the test material was assessed using the maximisation method of Magnusson and Kligman (1969) and Magnusson et al. (1979).

Range finding tests

The purpose of the range finding test was to determine the concentrations of test material to be used for intradermal induction, topical induction and topical challenge in the main study.

Two male and two female guinea-pigs were closely shorn in the shoulder region using electric clippers followed by an electric razor. 0.1 ml doses of several dilutions of the test material were injected intradermally on each side of the mid-line. The animals were examined on the following day to determine the maximum concentration that could be used in the main test without causing untoward toxicity.

The flank of each animal in further groups of two male and two female guinea-pigs, was closely shorn. 0.3 ml doses of several dilutions of the test material were absorbed onto 16 cm² Whatman No. 3 filter paper patches. The patches were applied to skin on the shorn flanks, covered by occlusive tape, and retained by an elastic adhesive bandage for 24 hours. After removal of the patches and bandages the dermal test sites were examined for signs of irritation which were scored using a four point scale (Attachment A). The concentration selected for topical induction in the main test was that which just caused irritation and the concentration chosen for topical challenge was that which was just non-irritant.

Main test

The main test was conducted using a group of ten male and ten female guinea-pigs together with a control group of five males and five females. Individual bodyweights were recorded at the beginning of the main study and before challenge. The test procedure was divided into two stages:

a) Induction

The animals were closely shorn in the shoulder region using electric clippers followed by an electric razor; two rows of intradermal injections were made, one on either side of the mid-line, as follows:

Test animals	Anterior sites	0.1 ml of Freund's Complete Adjuvant (FCA)
	Middle sites	0.1 ml of test material in vehicle
	Posterior sites	0.1 ml of test material in 50:50 FCA/vehicle
Control animals	Anterior sites	0.1 ml of FCA
	Middle sites	0.1 ml of vehicle
	Posterior sites	0.1 ml of 50:50 FCA/vehicle

Freund's Complete Adjuvant was prepared for use as a 50% v/v aqueous emulsion. One week after induction by intradermal injection, the same area of dorsal skin was shaven using electric clippers only. A 16 cm² patch of Whatman No. 3 filter paper was moistened with 0.3 ml of the undiluted test material and placed over the sites of intradermal injections. The patches were covered with occlusive tape and held in place by elastic adhesive bandage for 48 hours. Similar patches of filter paper moistened with the vehicle alone were applied to the control group guinea-pigs. Any abnormal reactions to the induction procedure were recorded.

b) Challenge

Challenge was carried out three weeks after the intradermal phase of induction. Hair was removed from one flank of all test and control animals by clipping and shaving. A 4 cm² patch of Whatman No. 3 filter paper, moistened with 0.1 ml of the appropriate dilution of test material, was placed on the shaven area, covered by occlusive tape and held in position by elastic adhesive bandage. Control group animals were treated with the same formulation of test material that was applied to test group animals. After 24 hours the patches and bandages were removed and the challenge sites examined for any response. The response was scored using a four point scale (Attachment A).

The result of the test is expressed as the number of positive responses shown by the test animals at 24 and/or 48 hours after removal of the challenge patches. The frequency of positive responses rather than their intensity is regarded as the important statistic in this test.

RESULTS

Acute oral toxicity (Tables 1 to 4)

A preliminary test utilising groups of one male and one female rat treated at 2000 and 5000 mg/kg indicated that the acute median lethal oral dose (LD₅₀) was greater than 5000 mg/kg.

Five male and five female rats received a single oral dose of Shell Brake Fluid Dot 4 Super at 5000 mg/kg. The test material was administered in the undiluted state at a dose volume of 4.70 ml/kg.

None of the rats died. The acute oral LD₅₀ of Shell Brake Fluid Dot 4 Super was therefore greater than 5000 mg/kg.

The sole common sign of reaction to treatment was a hunched posture apparent from within one hour of dosing. Other isolated clinical signs were lachrymation, abasia, lethargy, unkempt appearance and encrustation of the periorbital zone. Recovery, as judged by external appearance and behaviour, was complete by Day 3.

All rats had gained weight relative to their Day 1 bodyweights by the end of the 14 day observation period.

Necropsy on Day 15 revealed no macroscopic abnormalities.

Acute dermal toxicity (Tables 5 to 8)

A preliminary test utilising one male and one female rat treated at 2000 mg/kg indicated that the acute median lethal dermal dose (LD₅₀) was greater than this dose-level.

Five male and five female rats received a single dermal application of Shell Brake Fluid Dot 4 Super at 2000 mg/kg.

The test material was administered in the undiluted state at a dose volume of 1.88 ml/kg.

None of the rats died. The acute dermal LD₅₀ of Shell Brake Fluid Dot 4 Super was therefore greater than 2000 mg/kg.

There were no signs of systemic reaction to treatment. Sites of application of the test material showed no irritation or other dermal changes.

All rats had gained weight relative to their Day 1 bodyweights by the end of the 14 day observation period.

Necropsy on Day 15 revealed no macroscopic changes of the principal organs and tissues.

Acute dermal irritation (Table 9)

Application of 0.5 ml undiluted Shell Brake Fluid Dot 4 Super to the clipped dorsal skin of six New Zealand White rabbits elicited no irritation reaction or other dermal change.

Acute eye irritation (Table 10)

Instillation of 0.1 ml undiluted Shell Brake Fluid Dot 4 Super into one eye of each of six rabbits resulted in moderate initial pain responses.

All rabbits developed an ocular discharge, slight chemosis and either injection of the conjunctival blood vasculature or a crimson-red appearance of the conjunctivae within one hour of treatment. Two rabbits showed chemosis causing partial eversion of the eyelids four hours after treatment. Subsequent examinations determined that resolution of conjunctival irritation was advanced on the following day and complete within 48 hours of treatment.

The cornea and iris were not visibly affected by ocular instillation of Shell Brake Fluid Dot 4 Super.

Skin sensitisation (Tables 11 and 12)

Range finding tests were conducted to determine the concentration of the test material to be used for intradermal induction, topical induction and topical challenge in the main test.

Based on the range finding tests, the following concentrations of test material were selected for the main study:

Intradermal induction:	0.6% (m/v) in water/FCA
Topical induction:	undiluted Shell Brake Fluid Dot 4 Super
Topical challenge:	60% (m/v) in water

None of the twenty test animals showed any positive response at either 24 or 48 hours after removal of the challenge patches. It may be concluded, therefore, that the test material is not a skin sensitiser in guinea pigs.

REFERENCES:

Magnusson, B. and Kligman, A. M. (1969).
The identification of contact allergens by animal assay. The guinea-pig
maximisation test.

J. Invest. Dermat., 52, 268-276.

Magnusson, B., Fregert, S. and Wahlberg, J. (1979).
Determination of skin sensitisation potential of chemicals. Predictive
testing in guinea pigs.

Arbetar och Hälsa, 1979:26 (E).

OECD (1981).

OECD Guidelines for testing of chemicals.

OECD Publications Office.

2, rue Andre-Pascal, 75772.

Paris Cedex 16, France.

Table 1 - Mortality following acute oral administration of Shell Brake Fluid Dot 4 Super

Dose (mg/kg)	Sex	Daily mortality (Day No.)														Cumulative mortality		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Male	Female
5000	M																0/5	
	F																	0/5

0 0 2 7

Table 2 - Bodyweight and bodyweight change following acute oral administration of Shell Brake Fluid Dot 4 Super

Dose (mg/kg)	Males				Females			
	Animal number & sex	Body wt (g)	Body wt change (g)		Animal number & sex	Body wt (g)	Body wt change (g)	
		Day 1*	Day 8 ⁺	Day 15 ⁺		Day 1*	Day 8 ⁺	Day 15 ⁺
5000	853 M	181	32	40	853 F	133	19	24
	854 M	193	24	34	854 F	137	24	26
	855 M	183	33	44	855 F	136	26	30
	856 M	175	34	49	856 F	130	22	28
	857 M	187	28	40	857 F	134	20	27

* Weight in brackets is weight of cadaver

+ Bodyweight gain relative to bodyweight on Day 1; a - (minus) sign before the bodyweight change indicates a bodyweight loss

Table 3 - Clinical observations following acute oral administration of Shell Brake Fluid Dot 4 Super

Dose: 5000 mg/kg

Clinical sign	Animal number and sex					Time of first observation	Time of recovery amongst rats surviving treatment
	853 M	854 M	855 M	856 M	857 M		
Lachrymation	-	-	-	-	-		
Hunched back	+	+	-	-	-	1 h	Day 3
Lethargy	-	-	-	-	-		
Abasia	-	-	-	-	-		
Periorbital zone - encrustation	+	-	-	-	-	0.5 h	Day 2
Unkempt appearance	+	+	-	-	-	Day 2	Day 3

Key: + Sign of reaction present
 - Sign of reaction absent
 S Sign of reaction confined to rat(s) that died
 h Hour(s) after dosing

Table 3 - Continued

Dose: 5000 mg/kg

Clinical sign	Animal number and sex					Time of first observation	Time of recovery amongst rats surviving treatment
	853 F	854 F	855 F	856 F	857 F		
Lachrymation	-	-	-	-	+	2.7 h	4 h
Hunched back	+	+	+	+	+	1 h	Day 2
Lethargy	-	-	+	-	+	1 h	5 h
Abasia	-	-	-	-	+	0.5 h	1 h
Periorbital zone - encrustation	-	-	-	-	-		
Unkempt appearance	-	-	-	-	-		

Key: + Sign of reaction present
 - Sign of reaction absent
 S Sign of reaction confined to rat(s) that died
 h Hour(s) after dosing

Table 4 - Necropsy findings in male and female Fischer 344 rats following a single oral dose of Shell Brake Fluid Dot 4 Super

	<u>Dose (mg/kg)</u>	
	5000	
	<u>Animal Numbers</u>	
	M	F
<u>Animal Dosed:</u>	853	853
	854	854
	855	855
	856	856
	857	857
 <u>Decedents:</u>	-	-
 <u>No Abnormalities Detected:</u>	853	853
	854	854
	855	855
	856	856
	857	857
 <u>External Findings:</u>	-	-
 <u>Internal Findings:</u>		
Stomach - food present	853	853
	854	854
	855	855
	856	856
	857	857

Table 6 - Bodyweight and bodyweight change following acute dermal administration of Shell Brake Fluid Dot 4 Super

Dose (mg/kg)	Males					Females			
	Animal number & sex	Body wt	Body wt change		Animal number & sex	Body wt	Body wt change		
		(g)	(g)	(g)		(g)	(g)	(g)	(g)
		Day 1*	Day 8 ⁺	Day 15 ⁺		Day 1*	Day 8 ⁺	Day 15 ⁺	
2000	858 M	206	1	15	858 F	142	6	16	
	859 M	213	8	19	859 F	150	4	16	
	860 M	221	3	22	860 F	141	5	18	
	861 M	219	3	16	861 F	151	6	17	
	862 M	208	7	20	862 F	153	6	16	

* Weight in brackets is weight of cadaver

+ Bodyweight gain relative to bodyweight on Day 1; a - (minus) sign before the bodyweight change indicates a bodyweight loss

Table 7 - Clinical observations following acute dermal administration of Shell Brake Fluid Dot 4 Super

Dose: 2000 mg/kg

Clinical sign	Animal number and sex	Time of first observation	Time of recovery amongst rats surviving treatment
	858 M 859 M 860 M 861 M 862 M		

No signs

Key: + Sign of reaction present
- Sign of reaction absent
S Sign of reaction confined to rat(s) that died
h Hour(s) after dosing

Table 7 - Continued

Dose: 2000 mg/kg

Clinical sign	Animal number and sex					Time of first observation	Time of recovery amongst rats surviving treatment
	-----	-----	-----	-----	-----		
	858 F	859 F	860 F	861 F	862 F		

No signs

Key: + Sign of reaction present
 - Sign of reaction absent
 S Sign of reaction confined to rat(s) that died
 h Hour(s) after dosing

Table 8 - Necropsy findings in male and female Fischer 344 rats following a single dermal dose of Shell Brake Fluid Dot 4 Super

	Dose (mg/kg)	
	2000	
	Animal Numbers	
	M	F
<u>Animal Dosed:</u>	858	858
	859	859
	860	860
	861	861
	862	862
<u>Decedents:</u>	-	-
<u>No Abnormalities Detected:</u>	858	858
	859	859
	860	860
	861	861
	862	862
<u>External Findings:</u>	-	-
<u>Internal Findings:</u>	-	-

Table 9 - Dermal irritation following a 4 hour application to rabbit skin of Shell Brake Fluid Dot 4 Super

Animal number, sex and initial bodyweight	Dermal responses	Time after patch removed				
		0.5 h	24 h	48 h	72 h	7 d
925 M (4.30 kg)	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0
926 M (4.64 kg)	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0
930 M (4.05 kg)	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0
957 F (5.14 kg)	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0
958 F (4.96 kg)	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0
959 F (4.70 kg)	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0
Mean Erythema score		0.0	0.0	0.0	0.0	0.0
Mean Oedema score		0.0	0.0	0.0	0.0	0.0

Group mean 24, 48 and 72 hour scores

Erythema 0.0

Oedema 0.0

Table 10 - Eye irritation following the instillation into rabbits eyes of Shell Brake Fluid Dot 4 Super

Animal number, sex and initial bodyweight	Initial effect		Time after instillation					
			1 h	4 h	24 h	48 h	72 h	7 d
927 M (4.09 kg)	Moderate	Redness	1	1	1	0	0	0
		Chemosis	1a	1a	0	0	0	0
		Opacity	0	0	0	0	0	0
		Iris	0	0	0	0	0	0
928 M (3.94 kg)	Moderate	Redness	1	1	1	0	0	0
		Chemosis	1a	1a	1	0	0	0
		Opacity	0	0	0	0	0	0
		Iris	0	0	0	0	0	0
929 M (3.80 kg)	Moderate	Redness	1	1	1	0	0	0
		Chemosis	1a	1a	1	0	0	0
		Opacity	0	0	0	0	0	0
		Iris	0	0	0	0	0	0
969 F (4.67 kg)	Moderate	Redness	1	1	1	0	0	0
		Chemosis	1a	1a	1	0	0	0
		Opacity	0	0	0	0	0	0
		Iris	0	0	0	0	0	0
970 F (4.35 kg)	Moderate	Redness	2	2	1	0	0	0
		Chemosis	1a	2a	1	0	0	0
		Opacity	0	0	0	0	0	0
		Iris	0	0	0	0	0	0
971 F (5.29 kg)	Moderate	Redness	1	2	1	0	0	0
		Chemosis	1a	2a	1	0	0	0
		Opacity	0	0	0	0	0	0
		Iris	0	0	0	0	0	0
Mean Score		Redness	1.2	1.3	1.0	0.0	0.0	0.0
		Chemosis	1.0	1.3	0.8	0.0	0.0	0.0
		Opacity	0.0	0.0	0.0	0.0	0.0	0.0
		Iris	0.0	0.0	0.0	0.0	0.0	0.0

^a Ocular discharge

Group mean 24, 48 and 72 hour scores	
Redness	0.3
Chemosis	0.3
Corneal opacity	0.0
Iridial effects	0.0

Table 11 - Results of range finding tests with Shell Brake Fluid Dot 4 Super (Magnusson and Kligman test)

Intradermal injection [% (m/v) in water]

Animal number and sex	Percentage conc.			
	0.06	0.2	0.6	2.0
	Response on day after administration			
321 M	1	1	1	2
322 M	1	1	1n	2n
301 F	1	1	1	2n
303 F	1	1	1n	2n

n - slight necrosis at injection site

Topical applications [% (m/v) in water]

Conc.		Conc.	
Animal number and sex	10%	Animal number and sex	25%
	Response on day after administration to left flank		Response on day after administration to right flank
323 M	0	323 M	0
324 M	0	324 M	0
304 F	0	304 F	0
305 F	0	305 F	0

Conc.		Conc.	
Animal number and sex	60%	Animal number and sex	Undiluted
	Response on day after administration to left flank		Response on day after administration to right flank
325 M	0	325 M	0
326 M	0	326 M	1
307 F	0	307 F	0
308 F	0	308 F	1

Table 12 - Skin sensitisation following occluded topical challenge with Shell Brake Fluid Dot 4 Super in guinea pigs (Magnusson and Kligman test)

Animal number and sex	Bodyweight (g) at		Response to challenge			
			Time after challenge			
			Start	End	0 h	24 h
Test animals	371 M	521	678	0	0	0
	372 M	540	684	0	0	0
	373 M	508	665	0	0	0
	374 M	520	657	0	0	0
	375 M	510	720	0	0	0
	354 F	412	562	0	0	0
	355 F	498	507	0	0	0
	356 F	412	615	0	0	0
	357 F	430	560	0	0	0
	358 F	480	575	0	0	0
	376 M	473	715	0	0	0
	377 M	539	703	0	0	0
	378 M	540	670	0	0	0
	379 M	545	720	0	0	0
	380 M	560	740	0	0	0
	381 F	378	516	0	0	0
	382 F	412	560	0	0	0
	383 F	400	541	0	0	0
384 F	452	533	0	0	0	
385 F	412	605	0	0	0	
Control animals	391 M	565	795	0	0	0
	392 M	618	812	0	0	0
	393 M	548	765	0	0	0
	394 M	535	740	0	0	0
	395 M	536	720	0	0	0
	386 F	418	652	0	0	0
	387 F	418	580	0	0	0
	388 F	418	586	0	0	0
	389 F	469	600	0	0	0
	390 F	427	601	0	0	0

ATTACHMENT A

Criteria for scoring of skin and eye irritancy and skin sensitisation studies.

ATTACHMENT A

Standard scores for erythema and oedema in the 4 hour semi-occluded skin irritancy test in rabbits

Reaction	Description	Score
Erythema:	No erythema	0
	Very slight erythema (perceptible) area not defined	1
	Well defined erythema (pale red, area well defined)	2
	Moderate to severe erythema (bright red, area well defined)	3
	Severe erythema (beet or crimson red)	4
Oedema:	No oedema	0
	Very slight oedema (perceptible, edges of area not defined)	1
	Slight oedema (edges of area defined by raising)	2
	Moderate oedema (area well defined, raised 1 mm)	3
	Severe oedema (area raised more than 1 mm, extends beyond exposure area)	4

ATTACHMENT A

Standard scores for pain response immediately following instillation
of the test material into rabbit eye

Grade	Descriptive rating
1	No initial pain
2	Practically no initial pain
3	Slight initial pain
4	Moderate initial pain
5	Severe initial pain
6	Very severe initial pain

ATTACHMENT A

Standard scores for irritancy in cornea, iris and conjunctivae following instillation of the test material into rabbit eye

Cornea

Opacity	No ulceration or opacity	0
	Scattered or diffuse opaque areas, details of iris clearly visible	1
	Easily discernible opaque areas, details of iris slightly obscured	2
	Opaque areas, no details of iris visible, size of pupil barely discernible	3
	Complete corneal opacity, iris not visible	4

Iris

Normal	0
Markedly deepened folds, congestion, swelling moderate circumcorneal injection, iris still reacting to light	1
No reaction to light, haemorrhage, gross destruction (any or all of these)	2

Conjunctivae (palpebral and bulbar)

Redness:	Vessels normal	0
	Some vessels definitely injected	1
	Diffuse, crimson red, individual vessels not easily discernible	2
	Diffuse, beefy red	3
Chemosis:	No swelling	0
	Any swelling above normal (includes nictitating membrane)	1
	Obvious swelling with partial eversion of lids	2
	Swelling with lids about half closed	3
	Swelling with lids more than half closed	4

1 1 4 4

ATTACHMENT A

Standard scores for erythema following intradermal injection or occluded topical application of the test material in the guinea pig maximisation test

Description	Score
No difference from surrounding skin	0
Slight redness, edges not defined	1
Pink/red area with defined edges	2
Beet red area with well defined edges	3

Title of main report: Shell Brake Fluid Dot 4 Super :
Acute oral and dermal toxicity, skin and eye
irritancy, and skin sensitisation potential.

Experiment number: 4608

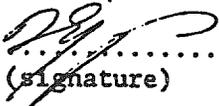
APPENDIX A

Title of appendix: Compound Control and Formulation Chemistry Report

Summary: Details of the test substance and its formulations
released for use in this study are reported.

Participants: A.E.Box, D.D.Lad

Compound Controller &
Formulation Chemist :

..... 
(signature) 13th August 1990
(date)

Scientific Reviewer:

..... Michael K. Boyd
(signature) 14th August 1990
(date)

1. TEST SUBSTANCE

1.1 Identity of the test substance

The data for the test substance released for use in this experiment are tabulated below.

NAME Shell Brake Fluid Dot 4 Super
BATCH (& OTHER) NUMBERS 1; KSLA Ref. 7842/89 (0.0039)
TOXICOLOGY REF. NUMBER ST90/023
SOURCE Kininklijke/Shell Laboratorium, Amsterdam
DATE RECEIVED 23rd January 1990
APPEARANCE Clear colourless liquid
CHARACTERIZATION
ERBP = 281 °C
WERBP = 183 °C
Viscosity at -40 °C = 1110 mm²/s
Water = 0.16 % m/m
Colour Pt/Co = 50
DATE RELEASED 15th February 1990

1.2 Storage of this test substance

Following its arrival in Compound Control this test substance was stored in the dark at ambient temperature.

1.3 Stability of this test substance.

I consider that this test substance was stable for the duration of this study under the storage conditions employed.

2. FORMULATION OF THE TEST SUBSTANCE

The test substance was supplied undiluted, or mixed with water, or a 1:1 (v/v) mixture of water and a Freund's Complete Adjuvant/water emulsion. Details are given below.

	<u>Concentrations supplied (mg/ml)</u>	<u>Carrier (*)</u>
Acute oral test	200 Undiluted	water none
Acute dermal test	Undiluted	none
Skin irritancy test	Undiluted	none
Eye irritancy test	Undiluted	none
Skin sensitisation test :		
Range finding	Undiluted	none
Intradermal induction	600, 250, 100, 20, 6, 2, 0.6 6, 0 6, 0	water water water:
Topical induction	0 Undiluted	FCA emulsion FCA emulsion none
Topical challenge	0 600	water water

(*) FCA (Freund's Complete Adjuvant) was supplied by Difco Laboratories, USA. FCA emulsion was a 50% v/v mixture of FCA in AnalaR water, mixed using a Silverson mixer. AnalaR water was supplied by BDH Ltd.

3. STABILITY OF FORMULATIONS OF THE TEST SUBSTANCE

It is very unlikely that the test substance would react with water at ambient temperature in the time scale of the storage of the solutions - less than 7.5 hours. The adjuvant is mainly paraffin oil and a surfactant and no reaction would be anticipated with these ingredients or the water used to prepare the emulsion. I therefore consider that the formulations of the test substance in these media used in this study were stable for their period of use.

SBGR.90.189

Shell Brake Fluid Dot 4 Super: Acute oral and dermal toxicity, skin and eye irritancy and skin sensitisation potential

DISTRIBUTION

SIPC (ODLC/731)	3
SICC (CHSEL/2)	5
SICC (CMRT)	1
SICC (CTMDF/221)	1
SICM (CMF/063)	5
Shell Dev. Co. (SDWR)	12
ORC (RSOK) (via Calgary)	1
SIPM (HSE/51)	2
CRCSL (GAI)	1
KSLA (IDC/122)	2

FURTHER DETAILS FOR DATA BASE ENTRY

INDEX TERMS: 15. TOXICOLOGY
 10. CHEMICALS

KEYWORDS: Acute oral toxicity, Acute dermal toxicity,
 Rat, Skin irritancy, Eye irritancy, Rabbit, Skin
 sensitisation, Guinea pig, Shell Brake Fluid Dot 4
 Super.

CERTIFICATE OF AUTHENTICITY

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