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June 2001

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

t-Amyl methyl ether (TAME)

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Director
Chemicals Notification and Assessment

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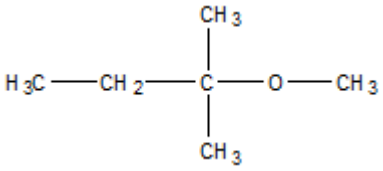
1. APPLICANT

Caltex Refineries (Qld) Ltd of South St Lytton QLD 4178 (ACN 008 425 581) has submitted a standard notification statement in support of their application for an assessment certificate for *t*-amyl methyl ether. Exempt status was not sought for any information.

2. IDENTITY OF THE CHEMICAL

Chemical Name:	butane, 2-methoxy-2-methyl-
Chemical Abstracts Service (CAS) Registry No.:	994-05-8
Other Names:	*t*-amyl methyl ether tertiary amyl methyl ether 1,1-dimethylpropyl methyl ether methyl *tert*-pentyl ether
Marketing Name:	TAME
Molecular Formula:	$C_6H_{14}O$
Structural Formula:	
Molecular Weight:	102.18
Method of Detection and Determination:	may be detected in water by gas chromatography (GC) method EPA 524.2 (United States Environmental Protection Agency, 1992) and in gasoline by GC method ASTM D 4815 94a (American Society for Testing and Materials, 1994) or infrared spectroscopy (IR) method ASTM D 5845 95 (American Society for Testing and Materials, 1995); characterised by IR and mass spectroscopy (MS)

Spectral Data:	Aldrich Library of FT-IR Spectra: 2(1), 305B (Aldrich Chemical Co, 1997) Aldrich FT-IR Library (Vapour Phase) 3268A (Pouchert, 1989) IR: 2981, 2941, 2834, 1470, 1370, 1245, 1193, 1095, 844 cm^{-1} (ManTech Environmental Technology, 1993) MS: m/e 101, 87, 85, 75, 73, 71, 67, 59, 55, 45, 43, 38 (ManTech Environmental Technology, 1993)

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C & 101.3 kPa:	colourless liquid
Boiling Point:	86.3°C
Specific Gravity:	0.7703
Vapour Pressure:	9.0 kPa at 20°C
Water Solubility:	10.71 g/L at 20°C
Henry's law Constant:	90 $Pa.m^3/mol$
Partition Co-efficient (n-octanol/water):	log P_{ow} = 1.55
Hydrolysis as a Function of pH:	not determined
Adsorption/Desorption:	log K_{oc} = 1.82 (calculated)
Dissociation Constant:	no dissociable groups are present
Henry's Law Constant:	90 $Pa.m^3/mol$
Flash Point:	-11°C
Flammability Limits:	Upper Explosive Limit = 7.1 % Lower Explosive Limit = 1.0 %
Autoignition Temperature:	430°C
Explosive Properties:	not spontaneously explosive; explosive vapour/air mixtures may be formed; spontaneous combustion of clothing or rags soaked in TAME may occur
Reactivity/Stability:	stable under normal environmental conditions; reacts with strong oxidising agents

3.1 Comments on Physico-Chemical Properties

The values for the physico-chemical properties of vapour pressure, water solubility, Henry's Law constant, partition co-efficient and soil adsorption/desorption were derived from a literature paper on the environmental hazards of TAME (Huttenen et al., 1997).

Vapour pressure was measured using Grabner apparatus using the ASTM D 5191 method. Water solubility was measured using the shake flask method based on OECD TG 105 with GPC detection. Partition co-efficient was determined using a shake flask method based on OECD TG 117, detected by GPC. Soil adsorption was estimated from solubility and K_{ow} using literature values (Kenaga & Goring, 1980).

The low log P_{ow} indicates that the chemical should remain in the aquatic compartment and should not become associated with organics. Likewise the low log K_{oc} suggests that the chemical will not become adsorbed to soils or sediments. The calculated Henry's law constant of 90 $Pa.m^3/mol$ indicates that it is readily volatile from aqueous solutions and that any spilt material will quickly evaporate to the atmosphere.

The notified chemical contains no functional groups likely to hydrolyse in the environmental pH range 4-9. It does not contain any groups likely to dissociate.

The notified chemical is a Class 3 dangerous good (flammable liquid), with a flash point of -11ºC. It is classified with the risk phrase R11: Highly flammable.

4. PURITY OF THE CHEMICAL

Degree of Purity: > 90 %

Hazardous Impurities: the notified chemical may contain the following hazardous impurities as a result of the manufacture process; concentrations of individual impurities were not provided; unreacted isoamylenes and methanol starting materials are expected to be major impurities (Järvelin, 2000)

Chemical Name	*Synonyms*	*CAS No.*	*Hazardous Properties* (NOHSC, 1999b)
methanol		67-56-1	R23/25: toxic by inhalation and if swallowed
2-methyl-1-butene	isoamylene	563-46-2	
2-methyl-2-butene	isoamylene	513-35-9	
diisoamylene		—	
1,1-dimethyl-1-propanol	*t*-amyl alcohol	75-85-4	R20: harmful if swallowed
dimethyl ether		115-10-6	

Additives/Adjuvants:

Chemical name:	gasoline
CAS No.:	8006-61-9
Weight percentage:	94.55 %

5. USE, VOLUME AND FORMULATION

The present notification covers a one-off importation of a single cargo of unleaded petrol containing the notified chemical for sale on the Queensland market. The cargo comprises 49000 tonnes of unleaded petrol, containing 5.45 % TAME. The imported quantity would be expected to serve the Queensland market for several weeks. The notified chemical is used in gasoline as an octane enhancer, and also as a fuel oxygenate, intended to lower the tailpipe emissions of unburnt hydrocarbons and carbon monoxide.

6. OCCUPATIONAL EXPOSURE

The unleaded petrol containing the notified chemical is transferred from the ship to the terminal storage tanks by closed pipework, and, where unloaded at refinery terminals, transferred to storage sites by closed fixed pipes. At the terminal it is stored in tanks then transferred by closed fixed pipes to road tankers for delivery to minor depots or directly to service station storage tanks.

The notifier estimated that 20 workers at the terminal sites, over 100 service station managers and over 300 road tanker drivers would have occupational contact with the unleaded petrol containing the notified chemical. No estimates of exposure times or frequencies were provided.

At the terminal and depot sites, the workers involved include terminal supervisors, whose role is supervision of tanker discharge into tanks, sampling and analysis of product; laboratory personnel; and fitters who connect and disconnect pipework and maintain equipment. Tanker drivers would have exposure to the notified chemical during loading and unloading, and in underground tank dipping. Service station managers would be exposed during underground tank dipping and during the cleanup of spills.

Exposure to the notified chemical may arise from dermal contact with the product containing the notified chemical, and also by inhalation. Inhalation exposure would be widespread due to the volatility of the notified chemical. A Finnish study indicated that a geometric mean concentration of 0.98 mg/m^3 TAME (range 0.22 – 6.9 mg/m^3) over a 30 min exposure time was measured in the breathing zone for 20 tanker drivers involved in unloading petrol, approximately 69 % of which contained 5.95 % TAME (Vainiotalo et al., 1998). The mean air temperature was 14.1ºC. Most blood and urine samples taken as part of the study showed levels of TAME and its metabolite, *t*-amyl alcohol, below the quantification limits of 7 nmol/L and 100 nmol/L respectively. For the TAME analogue, methyl *t*-butyl ether (MTBE), which was present at higher concentrations in the gasoline used, the levels in blood and urine and in the breathing zone were correlated. The authors therefore concluded that inhalation

exposure was likely to be mainly responsible for the measured blood and urine levels.

Two additional studies by the same group were located by NICNAS. An occupational study involving tanker drivers gave geometric mean 30 minute time weighted average breathing zone concentrations of TAME during loading and unloading gasoline of 0.30 to 1.1 mg/m^3 (Vainiotalo & Ruonakangas, 1999). The fuel loaded and unloaded contained up to 9.0 % notified chemical. A study of consumer exposure during refuelling gave a geometric mean exposure to TAME of 1.9 mg/m^3 over a 1 minute period, and also indicated that the ambient air in two service stations (at the middle of the pump island) had a TAME concentration of 0.031 mg/m^3 (Vainiotalo et al., 1999). The majority (75 %) of the fuel supplied at the service stations in this study contained an average of 8.5 % notified chemical, while the remainder contained no notified chemical. The mean air temperatures during the measurements were 22.7^{o}C and 18.9^{o}C, and wind speeds 1.7 and 1.0 m/s respectively.

Dermal exposure to the notified chemical could occur where drips and spills of the unleaded petrol are present, including during pipe connection and disconnection, cleanup of spillages, and sampling and analysis.

The notifier indicated that, standardly, long sleeves, long pants, safety glasses and chemical resistant gloves are worn during these activities, and that respiratory protection is available.

7. PUBLIC EXPOSURE

The petrol containing the chemical is transferred from ship to storage containers and then to road tankers by closed pipework. Public exposure could potentially occur following an accidental spill, however the primary spill hazards are those associated with petrol spillage, and management of these should be the primary aim.

The product, petrol containing 5.45 % of the notified chemical, will be sold to the public at service stations. Member of the public will be exposed to the chemical at the bowser, and during transfer of fuel from personal storage containers, e.g. jerry cans. Public contact will consist of dermal contact, after a spill, or inhalation of fumes from the volatile product. Inhalation of the notified chemical following combustion (i.e. in the exhaust) is not considered likely, as it would be expected to be consumed in the combustion process.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

After importation by sea the petrol containing the notified chemical is transferred to product storage tanks at the sites at Gladstone, Lytton and Kurnell. Any large spillages would be collected in underground spill tanks before being reprocessed or reblended into product. Small spills would be immediately soaked up using absorbent material and presumably incinerated or disposed of to landfill. The notifier has not provided any estimates for the actual volume of release that would be expected but it is likely that, barring major accidents, any release to the environment should be very small.

The petrol is then pumped into the road tanker trucks to be transported to fuel stations and

rural depots in Queensland and NSW. At service stations, the road tankers will be emptied *via* their own 2-2.5 metre flexible hose into underground storage tanks. Potential release would mainly be through accidental spills. The MSDS details procedures to protect the environment in these cases. The notifier has not indicated the losses that may be expected during this procedure but the amount of petrol lost due to spills and leaks is likely to be less than 5 L per load, which is < 300 g of the notified chemical.

The notifier has also not provided any data that takes into account the frequent minor spills (< 1 L) that would occur at petrol bowsers as customers fill their vehicles with fuel. However, given the low percentage in fuel, the loss of notified chemical in these spills would be expected to have been low.

8.2 Fate

If the notified chemical were released to the aquatic environment as result of either a spill or leak from a storage tank, it would be expected to be dispersed and diluted rapidly and also to evaporate. The calculated Henry's Law constant, 90 $Pa.m^3/mol$, indicates that it is readily volatile from aqueous solutions. If released into soil, the chemical is expected to evaporate or penetrate the soil and eventually reach ground water, due to the low log Pow (1.55) and low calculated log Koc (1.82).

The notifier has not supplied any biodegradability test reports. Literature provided (Huttenen et al., 1997) indicates that the chemical has a half-life of 2.1 days in reaction with atmospheric hydroxyl radicals but is not readily biodegradable in the aquatic environment (< 5.0 % using a closed bottle method based on OECD TG 301D). It is therefore likely to be persistent in the environment but is not expected to bioaccumulate due to its low log P_{ow} (Connell, 1990).

A study (ManTech Environmental Technology, 1993) was performed on the atmospheric behaviour of TAME and its photochemical decomposition products. TAME was found to be removed in the atmosphere solely by reaction with OH and from this study the tropospheric lifetime of 2.1 days was calculated. In the presence of NO_x ten products were formed; the major ones being *t*-amyl formate, methyl acetate, acetaldehyde and formaldehyde.

The notifier provided no data concerning the effect of the notified chemical on exhaust emissions and if imports of this chemical were to be continued then secondary notification including this data would be required.

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

The test substance used in the dermal toxicity and eye irritation studies was identified as MRD-85-548. This substance was identified by the notifier as an olefin stream containing 94.5 % notified chemical.

Summary of the acute toxicity of *t*-amyl methyl ether (TAME)

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} < 5000 mg/kg	(Amoco, 1991c)
acute dermal toxicity	rabbit	LD_{50} > 3160 mg/kg	(Exxon, 1985a)
acute inhalation toxicity	rat	LC_{50} > 5.4 mg/L	(Amoco, 1991b)
skin irritation	rabbit	moderate irritant	(Amoco, 1991a)
eye irritation	rabbit	slight to moderate irritant	(Exxon, 1985b)
skin sensitisation	guinea pig	non-sensitiser	(American Petroleum Institute, 1995a)

9.1.1 Oral Toxicity (Amoco, 1991c)

Species/strain:	rat/Sprague-Dawley
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	gavage; test material used as received; dose 5 g/kg
Test method:	limit test; protocol not stated
Mortality:	5 males and 3 females died within 24 hours of dosing; the remaining 2 females survived to the end of the study
Clinical observations:	most animals showed hypoactivity, ataxia, salivation and wet inguinal fur; other clinical signs seen in 25 – 50 % of the animals included coma, lacrimation, clear nasal discharge, rales and discoloured inguinal fur; there was also scattered incidence of redness around the eyes and nose, chromodacryorrhoea, dyspnea, hyperactivity, red discoloured ingenuinal fur and discoloured paws small body weight gains over the course of the study were observed in the surviving animals
Morphological findings:	no gross abnormalities were seen in the animals which survived until the study end; in the animals which died during the study, red lungs, stomachs distended with gas or fluids and red-yellow, fluid filled small intestines were observed; one male also showed urinary calculi
Comment:	the study authors considered that the acute oral toxicity of

	the notified chemical was sufficiently established in the present study in light of a reported LD_{50} value for the analogue MTBE of 3.87 g/kg
LD_{50}:	< 5000 mg/kg
Result:	the acute oral toxicity of notified chemical was not completely characterised in this study; a further indication of the acute oral toxicity was given from a preliminary study for the 28-day oral repeat dose study (no experimental details provided); this stated that the acute oral LD_{50} was approximately 2100 mg/kg

9.1.2 Dermal Toxicity (Exxon, 1985a)

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	3/sex
Observation period:	14 days
Method of administration:	occlusive patch; 24 hour exposure; test material used as received; dose level 3160 mg/kg
Test method:	limit test; protocol not stated
Mortality:	there were no premature decedents during the study
Dermal observations:	very slight to severe erythema and slight to very slight oedema were observed in all animals (tabulated below); desquamation was seen in all animals on days 10 and 14; eschar was seen in five animals and atonia in three animals; one animal showed blanching on day 3

Draize scores:

Time after treatment (days)	• *Animal #*					
	1	***2***	***3***	***4***	***5***	***6***
Erythema	[i]					
1	1	2	2	2	2	2
3	1	2	1	2	2	1
7	4	1	1	0	4	0
10	4	1	4	4	4	4
14	4	1	4	1	4	1
Oedema						
1	2	0	1	1	2	1
3	2	0	1	0	2	1
7	2	0	0	0	2	0
10	0	0	1	1	1	0
14	0	0	1	1	1	0

[i] see Attachment 1 for Draize scales

Clinical observations: the most frequent observations were nasal discharge, abdominal staining, anogenital staining and unthrifty coat; one female lost weight during the first week of the study

Morphological findings: at necropsy, desquamation was noted in two animals and another was considered to be slightly emaciated

LD_{50}*:* > 3160 mg/kg

Result: the notified chemical was of low dermal toxicity in rats; it was irritating to the skin of rats

9.1.3 Inhalation Toxicity (Amoco, 1991b)

Species/strain: rat/Sprague-Dawley Crl:CDBR

Number/sex of animals: 5/sex

Observation period: 14 days

Method of administration: whole body; 4 hour exposure; notified chemical present as vapour at 5.4 mg/L (measured by infrared absorption)

Test method: not stated

Mortality:	there were no premature decedents during the study
Clinical observations:	all animals showed rales, persisting for 2 hours following the end of the exposure period in seven animals; redness around the nose was observed in seven animals; salivation and wet paws were each observed in one animal
Morphological findings:	seven animals showed external haemorrhagic lung foci, with one female having numerous foci (> 10); one male had a diffuse red area on the lungs; six animals showed enlarged mandibular lymph nodes
Comment:	the study authors indicated that the observed lung foci were in most cases of a type and number commonly seen in control animals of this strain
LC_{50}:	> 5.4 mg/L
Result:	the notified chemical was of very low acute inhalational toxicity in rats

9.1.4 Skin Irritation (Amoco, 1991a)

In addition to the dermal toxicity and irritancy study discussed in Section 9.1.2 above, the notifier provided a single page summary of a study where the notified chemical was applied at a dose of 0.5 mL for 4 hours under occlusive conditions to the skin of 3 rabbits. Irritation scores were reported to have ranged from 4.3/8.0 at 30 – 60 minutes after unwrapping to 5.0/8.0 at 24 hours. A Primary Dermal Irritation Score of 4.7 was quoted. No signs of corrosivity were observed.

9.1.5 Eye Irritation (Exxon, 1985b)

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	3/sex
Observation period:	14 days
Method of administration:	0.1 mL notified chemical as received was instilled in the conjunctival sac of the right eye of each animal; the left eye remained untreated and served as control; treated eyes were not washed
Test method:	not stated, similar to OECD TG 405

Draize scores of unirrigated eyes:

	Time after instillation									
Animal	***1 hour***		***4 hours***		***1 day***		***2 days***		***3 days***	
Cornea	***o***	***a***	***o***	***a***	***o***	***a***	***o***	***a***	***o***	***a***
1♀	[1]0	0	0	0	0	0	0	0	0	0
2♀	0	0	0	0	0	0	0	0	0	0
3♀	0	0	0	0	0	0	0	0	0	0
4♂	*	2	*	2	u,s	0	u,s	0	0	0
5♂	0	0	0	0	0	0	0	0	0	0
6♂	0	0	0	0	0	0	0	0	0	0
Iris										
1♀	0		0		0		0		0	
2♀	0		0		0		0		0	
3♀	1		0		0		0		0	
4♂	1		1		0		0		0	
5♂	0		0		0		0		0	
6♂	0		0		0		0		0	
Conjunctiva	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***
1♀	3	1	3	2	1	1	1	0	0	1	0	0	0	0	0
2♀	3	2	2	3	1	1	1	0	0	1	0	0	1	0	0
3♀	3	1	2	2	1	1	1	0	0	1	0	0	1	0	0
4♂	3	3	3	3	3	3	3	2	2	3	1	0	3	1	0
5♂	3	1	1	2	1	1	1	1	0	1	0	0	1	0	0
6♂	3	1	2	3	1	1	1	0	0	1	0	0	1	0	0

	Time after instillation											
Animal	***4 days***			***7 days***			***10 days***			***14 days***		
Cornea	all Draize scores were zero											
Iris	all Draize scores were zero											
Conjunctiva	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***
1♀	0	0	0	0	0	0	0	0	0	0	0	0
2♀	0	0	0	1	0	0	1	0	0	0	0	0
3♀	0	0	0	1	0	0	0	0	0	0	0	0
4♂	1	1	0	1	0	0	0	0	0	0	0	0
5♂	0	0	0	0	0	0	0	0	0	0	0	0
6♂	0	0	0	0	0	0	0	0	0	0	0	0

[1] see Attachment 1 for Draize scales

o = opacity a = area r = redness c = chemosis d = discharge u = ulceration s = stippling

Comment: for animal 4, slight dulling of normal lustre was observed at 1 and 4 hours, indicated by *; ulceration (> 25 % of cornea at 24 hours) and stippling were observed; white discharge was observed in five animals, persisting to 24 hours in one animal

the eye irritation scores were not of sufficient magnitude or persistence for the notified chemical to be classified as an eye irritant

Result: the notified chemical was a slight to moderate irritant to the eyes of rabbits

9.1.6 Skin Sensitisation (American Petroleum Institute, 1995a)

Species/strain: guinea pig/Dunkin Hartley

Number of animals: test group: 10/sex
control group: 5/sex

Induction procedure:

test group:
day 1, 8, 15 neat notified chemical (approximately 0.3 mL) was applied to a clipped area on the back for 6 hr, using an occlusive chamber; excess material was wiped off at the conclusion of each exposure

control group:
day 1, 8, 15 mineral oil was applied in place of the notified chemical under similar conditions

Challenge procedure:

test and control groups:
day 29 neat test material was applied to a clipped area on the back which had not previously been exposed for 6 hr, using an occlusive chamber; a vehicle control (mineral oil) was also used; a further previously untreated group of 5/sex was used as irritation control

Positive control: 1-chloro-2,4-dinitrobenzene (DNCB)

Test method: TSCA TG 798.4100 (Buehler method)

Challenge outcome:

Challenge concentration	• *Test animals* • *Control animals*			
	• *24 hours**	• *48 hours**	• *24 hours*	• *48 hours*
100 %	**0/20	0/20	0/10	0/10

* time after patch removal
** number of animals exhibiting positive response

Comment: one female was exposed for to the notified chemical for 48 hours at the second induction time

one test group male lost weight during the study, and was found dead one week later

Result: the notified chemical was non-sensitising to the skin of guinea pigs

9.2 Repeated Dose Toxicity

9.2.1 28 day Repeated Dose Inhalation Toxicity (Amoco, 1992; White et al., 1995)

Species/strain: rat/Sprague-Dawley

Number/sex of animals: 14/sex/dose group

Method of administration: whole body exposure to notified chemical vapour

Dose/Study duration: target concentrations 0, 500, 2000 and 4000 ppm; actual TWA concentrations 0, 505 (range 453 – 553), 2030 (range 1840 – 2190), 3930 (range 3480 – 4470) ppm (measured by infrared absorption); 6 hours per day, 5 days per week for 4 weeks

Test method: not stated

Mortality:
Three out of 14 males and 4 out of 14 females in the 4000 ppm group died during the study, 3 animals died during the first week, 2 during the second week and 2 during the third week.

Clinical observations:
The 2000 ppm and 4000 ppm groups showed signs of central nervous system (CNS) depression including coma, sedation, ataxia, coldness to touch, ptosis, hyperirritability, hypoactivity and posture effects including lying on side, hunching and flattening. These showed dose related increases in incidence. In addition, signs of toxicity including lacrimation, chromodacryorrhoea (coloured tears), dyspnea, rales, diarrhoea, piloerection and wet or discoloured inguinal fur were observed. Redness around the eyes, mouth or

face, salivation and discolouration around the mouth and of the paws were also observed. Other observations were considered to be incidental.

For the 500 ppm group, the general appearance was similar to the controls, but redness around the eyes, mouth or face, ptosis and lacrimation were observed, although at lower incidence than for the higher dose groups.

Neurological observations:
For 4 animals/sex/group, designated as satellite animals, functional observation battery (FOB) tests were performed immediately after exposure; for the remaining animals these tests were performed 18 hours after exposure. FOB tests were performed prior to exposure, and after 1, 5 and 20 exposures.

The satellite animals in the 2000 and 4000 ppm groups showed signs of CNS depression such as lying on side, ataxia, ptosis, reduced temperature and hypoactivity when tested. These signs were not observed in the main group animals 18 hours after exposure. Only scattered cases of ptosis were observed in the 500 ppm animals. The satellite animals, particularly the 4000 ppm animals, displayed some reductions in tail pinch response, righting reflex and negative geotaxis, along with reduced body temperatures, impaired rotorod performance and increased hindlimb splay. Significant neurological changes were not seen in animals tested 18 hours after exposure.

Body Weights
Significant decreases in body weight gain were observed in the 4000 ppm males resulting in significantly reduced mean body weights during weeks 2 - 4. No other significant effects on body weight were reported.

Clinical chemistry/Haematology
Alanine aminotransferase was increased in the 4000 ppm males; one male was reported to show a very high value for this parameter; this animal also showed multifocal hepatocellular necrosis which was considered to be associated with the increase; excepting this animal, the value for the 4000 ppm males was considered normal.

Aspartate aminotransferase was decreased in the 2000 ppm females; cholesterol was increased in the 2000 ppm males and 4000 ppm males and females, and triglycerides were decreased in the 4000 ppm males.

Platelets were significantly increased in the 2000 ppm and 4000 ppm males; no other significant haematological changes were observed.

Gross Pathology:
Absolute brain weights were significantly decreased for the 4000 ppm males and absolute liver weights were significantly increased for the 2000 ppm males and 4000 ppm females. Increased relative adrenal and lung weights were observed in the 4000 ppm females, and increased relative liver weight was observed in the 4000 ppm and 2000 ppm males and females. Many relative organ weights were increased for the 4000 ppm males due to the reduced body weights of these animals.

Low incidences of red areas or foci on the lungs and of enlarged and/or red mandibular lymph nodes were observed in both test and control animals. Animals which died during

the study showed enlarged livers.

Histopathology:
These observations were performed on the main group animals (10/sex/dose) only. No microscopic changes in nasal turbinates or tracheas were observed. In animals which died during the study, lung lesions including diffuse or multifocal haemorrhage or congestion were observed; no similar observations were made for animals which survived to the study termination. In these animals, the most common lesions were lymphoid and plasma cell hyperplasia in the mandibular lymph nodes and lymphoid hyperplasia and haemorrhage in the respiratory lymph nodes. These lesions were observed at similar incidence in control animals.

Comment:
The enlarged livers in the animals which died were considered to be due to pooling of blood after death. The deaths were considered to be due to severe CNS depression. The increase in cholesterol was considered to be a direct or indirect response to the observed CNS effects.

Result:
A No Observed Effect Level (NOEL) was not determined in this study due to scattered clinical observations at 500 ppm. A No Observed Adverse Effect Level (NOAEL) of 500 ppm was determined.

9.2.2 28 day Repeated Dose Oral Toxicity (Daughtrey & Bird, 1995)

Species/strain:	rat/Sprague-Dawley
Number/sex of animals:	5/sex/dose
Method of administration:	gavage; vehicle corn oil; dose volume 2 mL/kg
Dose/Study duration:	0, 125, 500, 1000 mg/kg daily for 29 days
Test method:	not stated

Mortality:
Two males and 2 females of the 1000 mg/kg/day group died between days 6 and 9 of the study; two of the deaths were attributed to dosing accidents, while the other two were presumed to be treatment related although the cause of death was not exactly determined.

Clinical observations:
Rales and anogenital staining were observed at low frequency in the 1000 mg/kg/day groups. The majority of animals showed no clinical signs of toxicity.

Mean body weights of the 1000 mg/kg/day males were significantly reduced compared to controls on days 7, 21 and 28. Females of the 1000 mg/kg/day group showed a decrease (not statistically significant) in body weight gain. Food consumption was decreased in the 1000 mg/kg/day males in weeks 1 and 2, and in the 1000 mg/kg/day females during week 1.

Clinical chemistry/Haematology
A small increase in activated partial thromboplastin time was observed in the 1000 mg/kg/day males; this group also showed a decrease in mean serum glucose. Similar observations were not made in the high dose females.

Gross Pathology:
Significant increases in absolute and relative adrenal weight and in relative kidney weight were observed in the 500 and 1000 mg/kg/day males but not females. No gross lesions were reported.

Histopathology:
No treatment related histopathological changes, including in organs where weight changes were observed, were reported. All observed lesions were present at similar frequencies in all groups including controls.

Comment:
The study report states that preliminary test data indicated an acute oral LD_{50} of ca. 2100 mg/kg for the notified chemical.

Result:
Based on organ weight changes at 500 mg/kg/day and mortalities at 1000 mg/kg/day, a NOEL of 125 mg/kg/day and a NOAEL of 500 mg/kg/day were established in this study.

9.2.3 13 Week Repeated Dose Inhalation Toxicity (American Petroleum Institute, 1997a)

Species/strain:	rat/Fischer 344 mouse/CD-1
Number/sex of animals:	rats: 51/sex (control and high dose) 41/sex (low and mid dose) mice: 46/sex (control and high dose; two groups each) 36/sex (low and mid dose)
Method of administration:	whole body exposure to notified chemical vapour; animals were rotated through exposure chamber positions; no aerosol formation occurred
Dose/Study duration:	target concentrations 0, 250, 1500 and 3500 ppm; a new high dose group of mice at 2500 ppm and corresponding control group were established due to high mortality at 3500 ppm actual TWA concentrations 0, 251, 1500, 2506, 3519 ppm (measured by infrared absorption) exposure 6 hours per day, generally 5 days per week for 13 weeks (minimum 65 exposures); groups of 10/sex at 0 ppm

and the highest dose (3500 ppm for rats and 2500 ppm for mice) were allowed a 4 week recovery period

Test method: TSCA TG 798.2450
US EPA TG 40 CFR Part 798 Subpart G

Data for rats and mice are presented separately below.

Rats:

Mortality:
One male and one female of the 3500 ppm group were found dead (days 36 and 33 respectively). These deaths were considered likely to be treatment related. The causes of death were not determined during postmortem examinations. Several other animals (2 control females, one 250 ppm female and one 3500 ppm female) died during the study due to collection of blood.

Body Weights:
Body weights and body weight gain were significantly decreased compared to controls for the 3500 ppm males and females throughout the exposure period. The decreases were more pronounced in the males. At the end of exposure, the body weight was 10 % lower for 3500 ppm males and 6.9 % lower for 3500 ppm females. After the 4 week recovery period, 3500 ppm females had body weights similar to controls, while the males showed a 9.4 % decrease.

Mean body weight and body weight gain were increased for the 250 and 1500 ppm males. Body weight gain was higher than controls throughout, and mean body weight was significantly higher than controls from week 3 (1500 ppm) and week 8 (250 ppm). At the end of the main study, the body weight was increased by 13.4 % for the 1500 ppm males and 6.8 % for the 250 ppm males. A small but significant increase in body weight gain was seen for most of the study for 250 and 1500 ppm females. At the end of the main study, the body weight was increased by 3.1 % for the 1500 ppm females and 0.2 % for the 250 ppm females.

Food consumption was decreased for the 3500 ppm males and females during the first two weeks of the study, followed by a significant increase relative to controls throughout the remainder of the study. No trends in food consumption for the other treated groups were reported.

Clinical observations:
During exposure, most or all of the 3500 ppm animals were prostrate; a few were considered lethargic; laboured breathing was noted in a few animals, particularly in the latter part of the study. Most or all of the 1500 ppm animals were prostrate or lethargic during the first month of the study; a few animals in this group exhibited laboured breathing and lethargy in the latter part of the study. No abnormal signs were seen in the 250 ppm or control groups.

Between exposures, lethargy and prostration were observed following exposure for the 3500 ppm animals, but the animals were considered normal the following morning. Animals in the 3500 ppm group showed moderate anogenital staining, decreased faecal

volume and food consumption and emaciation on occasions. Animals in the 250 and 1500 ppm groups appeared normal between exposures; recovery animals at 3500 ppm also appeared normal. At weekly physical examinations, all animals appeared normal.

Opthalmology:
An apparent dose related increase in corneal scarring was observed in both sexes at 1500 and 3500 ppm (12.5 % of 1500 ppm males and females, 20 % of 3500 ppm males and 28 % of 3500 ppm females). For the controls, the incidence was 3.8 % in males and females; corneal scarring was not seen in the 250 ppm group. In the recovery animals, 30 % of males and 20 % of females showed corneal scarring, compared with no controls. The strain of rats used is susceptible to inherent corneal disturbance.

Neurological observations:
A satellite group of 10/sex/dose was used for acute neurological testing. No test material related changes in motor activity were observed at any doses. FOB tests were performed on the satellite group 1, 6 and 24 hours after acute exposure. CNS depression, indicated by postural changes, drooping or half-closed eyelids, slight stupor or lack of reflex responses, and lack of neuromuscular coordination, indicated by ataxia, impaired locomotion, poor righting reflex, reduced grip strength and increased landing foot splay, were seen in most 3500 ppm animals and a few 1500 ppm males after 1 hour. After 6 hours, one 3500 ppm male was in a low arousal state and a slight decrease in hindlimb grip strength in the 3500 ppm females was observed. After 24 hours, the FOB test results for all groups were comparable to controls.

Following repeated exposures for a second satellite group of 10/sex/dose, an increase in forelimb grip strength was recorded in the 3500 ppm males and 1500 and 3500 ppm females. No other effects on measures of neuromuscular function or CNS depression were observed.

Haematology
A slight test material related increase in platelet count was observed at weeks 5 and 14 in the 1500 and 3500 ppm males and 3500 ppm females, and at week 14 in the 1500 ppm females. The effect was reversed following the 4 week recovery period.

A number of other statistically significant differences between treated animals and controls were observed, where the values were all within the normal physiological ranges. These included a decrease in haemoglobin concentration, haemocrit, and/or red blood cell counts in all treated males at weeks 5 and/or 14, without a corresponding effect being observed in the females. After the recovery period, both sexes at 3500 ppm showed similar results to the controls. A decrease in prothrombin time was observed for males at 1500 and 3500 ppm at week 14; no decrease was seen after the recovery period. The prothrombin time for the females after recovery was slightly higher than for the controls.

No effect of the test material on the total white blood cell count was observed, but an increase in absolute neutrophil count was observed in 3500 ppm males and females at weeks 5 and 14 and a decrease in absolute lymphocyte count in 3500 ppm males and females at week 5 and in 3500 ppm females at week 14 were observed. Values in the 3500 ppm males after the recovery period were similar to controls, but the 3500 ppm females showed slight increases in white blood cell and absolute lymphocyte count.

Clinical chemistry
Test material related increases in total protein were observed in 1500 and 3500 ppm males and females at weeks 5 and 14; these were usually accompanied by increases in albumin and globulin and decreases in the albumin/globulin ratio. Following the 4 week recovery period, total protein, albumin and globulin were all similar to or slightly lower than controls.

Decreases in alkaline phosphatase activity were found in males at 250, 1500 and 3500 ppm at week 5 and at 250 and 3500 ppm at week 14, and in females at 1500 and 3500 ppm at week 14. For recovery animals, this parameter was below the control level for the 3500 ppm males but similar to controls for the 3500 ppm females. Blood urea nitrogen levels were decreased in 250 and 1500 ppm males at week 5 and in 3500 ppm males after the 4 week recovery period.

Other clinical chemistry differences occurred sporadically, without a clear dose relationship, and with all values being within normal control ranges. In males, these included increased creatinine at 1500 ppm at week 14, decreased aspartate aminotransferase activity at 3500 ppm at week 14, decreased glucose at 3500 ppm at week 5 and decreased bilirubin at 3500 ppm after the recovery period. In females, these included decreased aspartate aminotransferase activity at 250, 1500 and 3500 ppm at week 14, increased alanine aminotransferase activity at 3500 ppm at week 5 and decreased alanine aminotransferase activity at 3500 ppm at week 14 and after the 4 week recovery period. Phosphorous was decreased at 1500 ppm at week 14 and increased at 3500 ppm at week 14 and after recovery. Potassium and calcium were increased at 3500 ppm at week 5, and sodium was increased at 1500 ppm at week 5. Glucose was decreased at 3500 ppm after the 4 week recovery period.

Organ Weights:
Decreases of 4-5 % in absolute brain weight relative to controls were recorded for 3500 ppm males and females at the termination of the main study. After the 4 week recovery period, the absolute brain weight for the females was comparable to controls although a decrease of 3 % was observed for the males. Measurements of brain weight and dimensions as part of the neuropathology measurements did not show similar effects. For males at 250 and 1500 ppm, the relative brain weight was reduced relative to controls; this was attributed to the higher terminal body weight for these animals.

Absolute and relative liver weights were increased for all treated males and females at 1500 and 3500 ppm. At the end of the 4 week recovery period, absolute and relative liver weights were similar to controls for the 3500 ppm females, but the relative liver weight for the 3500 ppm males was higher than controls; this was attributed to the lower body weight for this group.

Relative kidney weights were increased for the 3500 ppm males and females at the end of the main study, while both absolute and relative kidney weights were increased for the 1500 ppm females and absolute kidney weights were increased for the 250 and 1500 ppm males. At the end of the 4 week recovery period, the relative kidney weights were increased for the 3500 ppm males and females, although the absolute weights were comparable to controls.

The relative heart weight was increased for 3500 ppm males and females; for males, this

persisted through the recovery period. For the 250 and 1500 ppm males, the absolute heart weight was found to be increased.

Dose-related effects on the absolute and/or relative adrenal weights were observed for both sexes. In the 3500 ppm animals the increase in absolute weight was 55 % in males and 45 % in females. In the 1500 ppm animals the increase was 20 % in males and 16 % in females. In the 250 ppm animals, an increase of 14 % was observed for males only. After the 4 week recovery period, the increase for 3500 ppm females was 11 %. For the 3500 ppm males, the absolute adrenal weights were comparable to controls, although there was an increase in the relative adrenal weight and adrenal to brain weight ratio.

An increase in relative lung weight for the 3500 ppm males at the end of the main study was observed, but after recovery, the relative lung weight was similar to controls. The absolute lung weight was increased in the 250 and 1500 ppm males. The changes in lung, heart and kidney weight in these groups were thought to parallel the increased body weights for these groups. Changes in the testes and epididymis weights were considered to be due to normal variability.

Gross Pathology:
No treatment related macroscopic observations were reported, including for the animals which died during the study. Overall, macroscopic findings occurred with similar incidence in test and control groups, or were sporadic.

Histopathology:
Kidney cell proliferation and nephropathy measurements (in life) were performed on groups of 5 rats/sex/dose. The labelling incidence of the proximal tubules of the kidney was increased in male rats after 4 and 13 weeks of treatment at 1500 and 3500 ppm. Nephropathy, as measured by an increase in the number of regenerative foci, was also increased. Increased protein droplet accumulation in the renal proximal tubule was observed for all treated males at weeks 1 and 4. The protein droplets for all male exposure groups showed α2u-globulin immunoreactivity. No equivalent kidney changes were observed in the treated female rats.

Microscopic necropsy findings concerned the kidneys, nasoturbinal tissues and pharynx. In the kidneys of all control and 3500 ppm male rats, intracytoplasmic eosinophilic/hyaline droplets in the proximal convoluted tubule epithelium were observed; the severity was slightly higher in the treated animals. In stained sections, the intracytoplasmic granules were more numerous, more irregular in size and larger for the 3500 ppm males than for the controls. The reaction to the stain indicated that the hyaline droplets were α2u-globulin. Tubular dilation, with or without intraluminal eosinophilic material, was seen at the cortico-medullary junction and/or in the medulla for several 3500 ppm males and one control male. No similar renal findings were reported for the females.

Hypertrophy or hyperplasia of goblet cells (minimal to moderate) in the respiratory mucosa was seen in almost all animals from the control and 3500 ppm groups; the severity was increased in the 3500 ppm group. Intracytoplasmic eosinophilic material (minimal to moderate) in epithelial cells of the respiratory and olfactory mucosa was seen only in the 3500 ppm animals. Hypertrophy or hyperplasia of goblet cells (minimal to moderate) in the epithelium lining the nasopharynx was seen in all 3500 ppm animals and in two control animals.

No microscopic findings were reported from neuropathological examination of brain, spinal cord and major nerve tissues.

Summary:
A number of effects were observed at the highest dose used, 3500 ppm. These included two deaths, post-exposure clinical signs, acute neurological effects, decreased body weight and body weight gain, increased platelet counts, increases in total protein, albumin and globulin, and a number of effects on organ weights. Many of these resolved after the 4 week recovery period. There were effects on the body weight and brain weight of males after this time. The effects on the kidneys of the male rats were consistent with the male rat specific α2u-globulin syndrome and were not considered to be relevant to risk assessment in humans.

Exposure of rats at 1500 ppm resulted in effects including post exposure clinical signs, acute neurological effects (males only), increased platelet count in males, increases in total protein, albumin and globulin and effects on liver and kidney (only in females) weight. An increase in liver weights of male rats exposed to 250 ppm was also observed.

Result:
No NOEL was established for both sexes combined. A NOEL of 250 ppm was established in this study for female rats only, as effects on liver weight were observed in males at 250 ppm. For acute neurological effects, the NOEL was 250 ppm in males and 1500 ppm in females. For subchronic neurological effects, the NOEL was 3500 ppm for both sexes.

Mice:

Mortality:
At 3500 ppm, 13 of 46 males and 10 of 46 females died after the first exposure and 26 of 46 males and 14 of 46 females died within three exposures to the notified chemical. A trial was conducted with groups of 15 mice/sex exposed at 3000 ppm; 8 males and 4 females died within eight exposures. Accordingly the high dose was set at 2500 ppm and new high dose and control groups established. At this level, 14 of 46 males and 13 of 46 females died over the course of the study. In the concurrent control group, 3 of 46 males and 2 of 46 females died over the course of the study; four of these deaths and the deaths of 4 males and 1 female in the 2500 ppm group were associated with blood collection. Deaths in the 250 ppm and 1500 ppm groups were at similar incidence to the controls, and were attributed to accidental trauma or blood collection.

For animals in the 2500 ppm groups which died, clinical signs included decreased activity in all animals, prostration, laboured breathing, decreased faeces and decreased food consumption in some animals. The causes of death were not determined during postmortem examinations.
Body Weights:
No trends in the body weights of the treated mice were observed. The final body weights of all groups were comparable to the controls. No trends in food consumption for the treated mice were reported.

Clinical observations:
During exposure, most of the 2500 ppm animals were prostrate; a few were considered lethargic and exhibited laboured breathing. In the latter part of the study, the incidence of prostration was lower and some showed increased activity. Uncoordinated gait was seen several times during the last week. Most or all of the 1500 ppm animals were lethargic during the first three weeks of the study while a few were prostrate; a few animals in this group exhibited laboured breathing and lethargy in the latter part of the study. No abnormal signs were seen in the 250 ppm or control groups.

Between exposures, decreased activity was observed in the 2500 ppm animals, with signs including lethargy, prostration, laboured breathing, decreased faeces, decreased food consuption and anogenital stains being seen in a few animals. Animals in the 250 and 1500 ppm groups appeared normal between exposures; recovery animals at 3500 ppm also appeared normal. At weekly physical examinations, all animals appeared normal, except one 3500 ppm male which on one occasion showed distressed symptoms; this was immediately prior to the death of this animal, and one 1500 ppm female which showed lethargy and tremors prior to death which was attributed to blood collection.

Haematology
All changes were considered minimal and were within normal physiological limits. In males at 2500 ppm, there was an increase in the absolute neutrophil count at week 5 and a decrease in the absolute lymphocyte count at week 14. After the 4 week recovery period, there was a slight increase in the mean corpuscular haemoglobin concentration. In males at 1500 ppm, there was an increase in the platelet count at week 5. In females at 2500 ppm, there was a decrease in the absolute lymphocyte count at week 14. Following the 4 week recovery period, females at 2500 ppm showed slight increases in total white blood cells and absolute lymphocyte count.

Clinical chemistry
Total protein, albumin and globulin were increased in 2500 ppm males at week 5. Globulin was increased while albumin was decreased in the 2500 ppm females at week 5. In males at 1500 ppm, globulin was increased at week 6. These parameters were similar to controls at week 14 and after the 4 week recovery period.

In females at 2500 ppm at week 14, increases in alanine aminotransferase and blood urea nitrogen were observed. In two animals for which these parameters were highest, elevated levels of aspartate aminotransferase and glucose were also found. These parameters were similar to controls after the 4 week recovery period.

Other slight clinical chemistry differences occurred with all values being within normal control ranges. These included increased calcium in 2500 ppm males at week 5 and an increase in sodium and decrease in glucose in 2500 ppm males after recovery. In 2500 ppm females, chloride was decreased at week 14. A slight decrease in alkaline phosphatase was observed in all treated females at week 5 or 6.
Organ Weights:
Increases in absolute liver weight, relative liver weight and liver to brain weight ratios were observed in 2500 ppm males and females and in 1500 ppm males. After the recovery period, absolute and relative liver weights for 2500 ppm males and absolute liver weights for 2500 ppm females were comparable to controls, although liver to body weight and liver to brain weight ratios for the females were slightly higher than controls.

An increase in the absolute and relative kidney weight was observed in the 1500 ppm males at the end of the main study.

Gross Pathology:
No treatment related macroscopic observations were reported, including for the animals which died during the study. Overall, macroscopic findings occurred with similar incidence in test and control groups, or were sporadic. Fluid in the trachea and lungs was seen in a number of animals including controls; this was attributed to the use of a carbon dioxide/air mixture for euthanasia.

Histopathology:
Liver cell proliferation measurements (in life) were performed on groups of 5-8 mice/sex/dose. The results indicated time and concentration dependent increases in the labelling incidence of hepatocytes, with statistically significant increases occurring in females at 250 and 1500 ppm and males at 1500 and 2500 ppm. The greatest increases occurred during week 1. The increases were correlated with centrilobular and mid-zone swelling of hepatocytes.

The only microscopic necropsy finding which was considered treatment related was central lobular hepatocellular hypertrophy in the liver at 2500 ppm. This occurred at an incidence of 8 out of 13 control males, 16 out of 21 2500 ppm males, 0 out of 12 control females and 7 out of 21 2500 ppm females. The average severity also appeared higher in the treated animals.

Other microscopic findings occurred with similar incidence in test and control groups, or were sporadic.

Summary:
High mortality was observed in mice exposed to 3500 and 3000 ppm. A number of effects were observed at the highest dose used in the main study, 2500 ppm. These included 27 deaths among 92 mice, post-exposure clinical signs, effects on a number of clinical chemistry parameters, and increased liver weights. Many of these resolved after the 4 week recovery period. Liver cell proliferation studies showed increases in the labelling index of hepatocytes and centrilobular hepatocellular hypertrophy was observed in both sexes.

Exposure of mice at 1500 ppm resulted in effects including post exposure clinical signs, increased globulin in males at week 6 and effects on liver weights in males. Similar findings were made in the liver cell proliferation studies and microscopic examination to those for the 2500 ppm animals. These liver effects were also observed for female rats exposed to 250 ppm.

Result:
No NOEL was established for both sexes combined. A NOEL of 250 ppm was established in this study for male mice only, as effects on liver cell proliferation were observed in females at 250 ppm.

9.3 Genotoxicity

9.3.1 Chromosomal Aberration Assay in Chinese Hamster Ovary (CHO) Cells (American Petroleum Institute, 1997b)

Cells: Chinese hamster ovary (CHO-K_1)

Metabolic activation system: rat liver S9 fraction from animals pretreated with Arochlor 1254, 2 % in standard cofactors

Dosing schedule:

Metabolic Activation	Experiment Number	Test concentration (μg/mL)	Controls
-S9	1	treatment time = 12 hr 313, 625, 1250, 2500, 5000	Positive: Mitomycin C 0.08 μg/mL
	2	treatment time = 12 hr 313, 625*, 1250*, 2500*, 5000*	Negative: ethanol
+S9	1	treatment time = 4 hr with 16 hr recovery 313*, 625*, 1250*, 2500*, 5000	Positive: CP 10, 20 μg/mL Negative: ethanol

CP - cyclophosphamide
* - cultures selected for metaphase analysis

Test method: OECD TG 473

Comment: colcemid (0.1 μg/mL) was added 2 hr before harvest to arrest cells in metaphase

the test material was soluble in the treatment medium at all doses tested

In the first experiment in the absence of S9, there was a discrepancy between the mitotic indexes for the duplicate flasks and the cells were not scored

in the absence of metabolic activation, cell growth inhibition to 70 % compared with solvent control was observed at 5000 μg/mL; the mitotic index was approximately 90 % of control; in the presence of metabolic activation, cell growth inhibition to 48 % compared with solvent control was observed at 2500 μg/mL; no reduction in mitotic index was observed at this concentration; no metaphase cells were observed at 5000 μg/mL

	in the absence of S9, a statistically significant increase in aberrant cells was observed at 2500 and 5000 μg/mL, and a dose response was observed; in the presence of S9, a statistically significant increase in aberrant cells was observed at all concentrations, and a dose response was observed
	the positive controls caused large, statistically significant increases in the proportion of aberrant cells in all cases, indicating that the test system responded appropriately
Result:	the notified chemical was clastogenic under the conditions of the test

9.3.2 *Salmonella typhimurium* Reverse Mutation Assay (Daughtrey & Bird, 1995)

Strains:	TA98, TA100, TA1535, TA1537, TA1538
Metabolic activation:	rat liver S9 fraction from animals pretreated with Arochlor
Concentration range:	100, 316, 1000, 3162 and 10000 μg/plate
Positive controls:	with S9: 2-aminoanthracene 5 μg/plate
	without S9 TA98, TA1538: 2-nitrofluorene 5 μg/plate TA100, TA1535: *N*-methyl-*N*-nitro-*N*-nitrosoguanidine 10 μg/plate TA1537: 9-aminoacridine 100 μg/plate
Test method:	not stated
Comment:	the test was performed in triplicate, using the plate incorporation method; preliminary toxicity screening showed no toxicity up to 10000 μg/plate
	no positive responses were observed with any tester strain in the presence or absence of metabolic activation
	large increases in the number of revertant colonies were seen for the positive controls in all cases, indicating that the test system responded appropriately
Result:	the notified chemical was non mutagenic under the conditions of the test

9.3.3 Micronucleus Assay in the Bone Marrow Cells of the Mouse (Daughtrey & Bird, 1995)

Species/strain:	mouse/CD-1
Number and sex of animals:	15/sex/dose
Doses:	0.15, 0.375, 0.75 g/kg
Method of administration:	intraperitoneal injection, vehicle corn oil; dose volume ≤ 10 mL/kg
Positive control:	cyclophosphamide 40 mg/kg
Test method:	not stated
Comment:	in a range finding study, all mice treated at 5 g/kg, one at 2.5 g/kg and two at 1.0 g/kg died; all mice at lower doses survived for 3 days; 0.75 g/kg was chosen as the highest non-toxic dose bone marrow was collected 24, 48 and 72 hours after administration no increase in micronucleus frequency in polychromatic erythrocytes at any dose level or collection time was observed; no signs of overt marrow toxicity measured by a significant decrease in the percentage of polychromatic erythrocytes was observed the positive control caused large, statistically significant increases in micronucleus frequency, indicating that the test system responded appropriately
Result:	the notified chemical was non clastogenic under the conditions of the test

9.4 Developmental and Reproductive Toxicity

9.4.1 Two Generation Reproductive Toxicity Study (American Petroleum Institute, 1998)

Species/Strain:	rat/CD (Sprague-Dawley)
Number/sex of animals	30/sex/dose (F0 generation) 30/sex/dose (F1 generation)

Method of administration:	whole body exposure to notified chemical vapour exposure 6 hours per day, 5 days per week for 10 weeks (commencing at weaning) prior to a two week mating period with exposure 6 hours per day, 7 days per week continuing through mating, gestation and lactation; males which had mated were returned to 5 days per week exposure; sperm/plug positive dams were not exposed from gestation day (gd) 20; dams with litters were not exposed until post natal day (pnd) 5 litters were culled to 10 pups on pnd 4 and weaned on day 28; no exposure occurred prior to this time; at weaning 30/sex were chosen as F1 parents and exposed to the same concentration as the parents under the same regime; at weaning of F2 pups, 30/sex were chosen for a post-wean retention period without exposure until acquisition of vaginal patency in females or preputial separation in males
Dose:	target concentrations 0, 250, 1500 and 3000 ppm; actual TWA concentrations 0, 245, 1493, 2992 ppm (measured by infrared absorption)
Method:	In-house method: CIIT Protocol 96020 RTI Protocol RTI-545

Mortality:
F0 Parental
Two control males (on study days 24 and 51) and one 1500 ppm female (on pnd 5, study day 99) were found dead.

F1 Parental
One male each at 0 (exposure day 21) and 3000 ppm (exposure day 0) and one female each at 0 (exposure day 67) and 1500 ppm (pnd 2, exposure day 98) was found dead or euthanised moribund; two females were found dead at 3000 ppm (exposure day -4 and gd 12, exposure day 83).

Clinical Observations:
F0 Prebreed Exposure
Body weights of 3000 ppm males and females were significantly reduced; body weight gains were lower than controls during the prebreed period (to week 10); males at 3000 ppm showed an increase in bodyweight gain during week 12; no body weight differences were observed for other exposure levels.

Treatment related clinical observations included ataxia in all animals at 1500 and 3000 ppm, excitability in all animals at 1500 ppm, lethargy in 14 males and 2 females at 1500 ppm and 9 males and 4 females at 3000 ppm. Piloerection was observed in 6 males at 1500 ppm. Alopecia was noted across the groups, including controls, with higher incidence than in

controls at 3000 ppm.

Vaginal cytology measurements indicated that 2 females (one each in the control and 250 ppm groups) had abnormal oestrous cycles. There was a dose related decrease in cycle length.

F0 Gestation
Maternal gestational body weights were reduced at 3000 ppm on gd 0 and 7; on gd 14 and 20, no change was observed; gestational body weight change was unaffected; no changes were observed for other exposure levels.

Treatment related clinical observations were limited to alopecia at higher incidence at 3000 ppm than in controls.

F0 Lactation
Maternal lactational body weights were reduced at 3000 ppm on pnd 0 and 4; weight changes were significantly increased for this group for the lactational period; no changes were observed for other exposure levels.

Treatment related clinical observations included alopecia at higher incidence at 3000 ppm than in controls and mild seizures in 2 females at 1500 ppm and 1 at 3000 ppm.

F0 Parental Holding Period
F0 males and non-pregnant F0 females were exposed after mating until study day 112. Body weights for the males were significantly reduced at 3000 ppm; body weight change at 1500 ppm was significantly increased between days 98 and 105. For the females, no statistically significant trends were seen due to the low numbers. For both sexes, alopecia at higher incidence in the high dose groups was the most common clinical observation. One male at 1500 ppm exhibited seizure.

F1 Prebreed Exposure
Vaginal patency in females was significantly delayed at 250 ppm (32.7 days), 1500 ppm (33.3 days) and 3000 ppm (36.0 days) compared with controls (32.5 days). It was observed that females of lower body weight showed later acquisition of vaginal patency. Covariance of the weekly body weight measurement closest to the time of acquisition with the time of acquisition showing a significant difference only at 3000 ppm. Mean body weight at acquisition of patency was unchanged across the groups.

Preputial separation in males was significantly delayed at 1500 ppm (45.3 days) and 3000 ppm (47.8 days) compared with controls (43.6 days). Covariance analysis showed significant differences when body weight measurements were included for both groups compared with controls. Mean body weight at acquisition of separation was significantly reduced for the 3000 ppm males.

Male and female body weights at 3000 ppm were significantly reduced throughout the prebreed exposure and mating period; for males, the body weight change over the prebreed period was lower than controls. No body weight differences were observed for other exposure levels.

Treatment related clinical observations were only reported for the 3000 ppm animals. These included ataxia in all males and most females, tail abnormalities in most males and 2 females

and lethargy in 3 males. Piloerection was observed in 5 males and 5 females. Audible or laboured respiration, rough coat, sneezing and chromodacryorrhoea were observed in a few animals.

Vaginal cytology measurements indicated that 4 females (two each in the 250 and 3000 ppm groups) had abnormal oestrous cycles. The cycle length for the 1500 ppm females was significantly shorter than controls, but for the 3000 ppm females it was comparable to controls.

F1 Gestation
Maternal gestational body weights were reduced at 3000 ppm at all measurement times; gestational body weight change was unaffected; no changes were observed for other exposure levels.

Treatment related clinical observations were limited to piloerection at 3000 ppm in 3 females.

F1 Lactation
Maternal lactational body weights were reduced at 3000 ppm on pnd 0 to 14; weight changes were significantly increased for this group for the lactational period; reductions in weight gain were observed for 250 and 1500 ppm for pnd 7-14.

Treatment related clinical observations were limited to pale appearance for 3 females at 3000 ppm and 1 each at 250 and 1500 ppm, and piloerection in 6 at 3000 ppm and 1 each at 0, 250 and 1500 ppm. In addition, one dam at 3000 ppm had seizures on pnd 3; one at 1500 ppm was found dead on pnd 2, and one at 1500 exhibited repetitive motion on two days.

F1 Parental Holding Period
A significant reduction in male bodyweight at 3000 ppm was observed; the females also appeared to have lower body weights although statistical analysis could not be performed due to the small group size. Male food consumption was significantly increased at 3000 ppm, and also for the majority of the time for the 1500 ppm group. Clinical observations were restricted to the males, and included chromodacryorrhoea in two at 3000 ppm, piloerection in one each at 250 and 1500 ppm and two at 3000 ppm, and seizures and sneezing, each in one 3000 ppm male.

F2 Weanlings
Vaginal patency in females was significantly delayed at 250 ppm (32.6 days) and 3000 ppm (33.8 days) compared with controls (31.7 days). Covariance of body weight with the time of acquisition showing a significant difference at 250 and 3000 ppm. Mean body weight at acquisition of patency was significantly reduced at 1500 and 3000 ppm.

Preputial separation in males was significantly delayed at 3000 ppm (44.9 days) compared with controls (41.2 days). Covariance of body weight with the time of acquisition showing a significant difference at 3000 ppm. Mean body weight at acquisition of separation was equivalent across all groups.

Male body weights were reduced at 1500 and 3000 ppm on pnd 35, and also on pnd 28 for the 3000 ppm group. Female body weights were reduced at 1500 and 3000 ppm on pnd 28.

No clinical findings were observed for male or female weanlings.

Reproductive and Lactational Indexes:
F0 Parental and F1 Litters
Male and female mating, fertility and pregnancy indexes, gestational index and gestational length in days, implantation sites per dam and numbers of total, dead and alive pups were unaffected by treatment. Prenatal mortality index, stillbirth index, postnatal survival indices and lactation index were also not affected by treatment.

Sex ratios and litter size were statistically equivalent across all groups; birth weights overall did not show a significant difference, although comparison within sex groups showed a significant downward trend with increased dose. Pup body weights were significantly decreased at 3000 ppm on pnd 4, 7, 14 and 21; a decrease was observed for all treated groups on pnd 14 and 21, and for female pups of 1500 ppm parents on pnd 4 and 7. At pnd 28, all pup weights were reduced for 1500 and 3000 ppm and male pup weights were reduced at 250 ppm.

The total numbers of pups found dead during the lactation period were 14, 11, 18 and 22 at 0, 250, 1500 and 3000 ppm, respectively. Most observations included failure to nurse or hypothermic pups. Most deaths occurred during the first 4 days; gross necropsy indicated that common causes of death were no milk in the stomach or primary atelectasis (defective expansion of the pulmonary alveoli at birth).

F1Parental and F2 Litters
Male and female mating, fertility and pregnancy indexes, implantation sites per dam and numbers of total, dead and alive pups were unaffected by treatment. Prenatal mortality index, stillbirth index and lactation index were also not affected by treatment. The mean gestational length in days was slightly increased at 1500 ppm but not at 3000 ppm. Survival indexes at pnd 4 and 21 but not at other times were significantly reduced in the 3000 ppm group.

Sex ratios were statistically equivalent across all groups. Number of pups per litter was significantly reduced at 3000 ppm on pnd 4. Birth weights for the 3000 ppm group were significantly reduced. Pup body weights were significantly decreased at 3000 ppm on pnd 4 (all pups and females, but not males), 7, 14 and 21; a decrease was observed for 1500 ppm pups on pnd 14 and 21. A reduction in anogenital distance at birth was observed for males and females at 3000 ppm separately; the reduction was not statistically significant for the combined sexes. This was attributed to the lower birth weight for this group.

A concentration related increase in the number of pups which died during lactation was observed, with 43, 42, 101 and 186 deaths respectively at 0, 250, 1500 and 3000 ppm. Clinical signs associated with mortality included lethargy, hypothermia, little or no milk, tail chewed off, thin fur, and various haematomas. The majority of the deaths occurred during the first 4 days. Findings at gross necropsy indicated primary atelectasis as a major cause of mortality.

Gross Pathology:
F0 Parental
Increases in absolute and relative liver weight were observed for males at 1500 and 3000 ppm and females at 3000 ppm; absolute kidney weights were increased for males at 250, 1500 and 3000 ppm and females at 3000 ppm; relative kidney weights were increased for males at 1500 and 3000 ppm and females at 3000 ppm. At 3000 ppm, increases were observed in absolute and relative adrenal weights (both sexes), absolute spleen weight (females) and relative spleen

weights (both sexes) and relative testes weight (males).

Andrological parameters showed no differences across the groups, except for an increase in abnormal sperm at 1500 and 3000 ppm; the increase at 3000 ppm was predominantly due to one animal which exhibited minimal testicular atrophy; this animal sired a live litter.

No treatment related gross abnormalities were observed in males; the female (1500 ppm) which died during the study showed effects on adrenals, kidneys, liver, lungs and spleen. No gross abnormalities were observed in females at scheduled necropsy.

F1 Weanlings
Male and female body weights were significantly reduced for all treated groups. Absolute brain weight was reduced for males at 250 and 3000 ppm and all treated females. Absolute thymus weight was reduced for all treated males and females at 3000 ppm; absolute spleen weight was also reduced for all treated males, and for females at 1500 and 3000 ppm. Gross findings were restricted to bilateral hydronephrosis in one 3000 ppm female.

F1 Parental
Increases in absolute liver, kidney and spleen weights were observed for males at 1500 ppm. Males at 3000 ppm showed increased absolute adrenal weights and decreased absolute brain and prostate weights. No significant changes in absolute organ weights were seen for the females. The relative liver, kidney and spleen weights were increased for males at 1500 and 3000 ppm, and relative brain and testes weights were increased for males at 3000 ppm. Females showed a concentration related upwards trend in relative kidney, spleen, adrenal and ovary weights.

Epididymal sperm concentration was reduced in males at 3000 ppm, and the percentage abnormal sperm exhibited an upward trend with exposure level.

For the 3000 ppm animal which died during the study, diffuse dark red lungs were observed. No other treatment related gross abnormalities were observed in males. The control female which died during the study showed enlarged metatarsal joints. The female at 1500 ppm which died showed multiple tan foci in the renal cortex. The females at 3000 ppm which died showed effects on heart, kidneys, liver, lungs, spleen and urinary bladder, and dark red fore toenails. Only scattered gross abnormalities were observed in females at scheduled necropsy. One 3000 ppm female had a lump in the left mammary area; ears were swollen and hard in one 250 ppm female, and renal and bladder effects were seen in one control female.

F2 Weanlings
Male and female body weights were reduced at 1500 and 3000 ppm. Reductions in absolute thymus and spleen weights were observed. For males, the thymus weight was reduced at all treatment levels; for females, the thymus weight was reduced at 3000 ppm. For both sexes, reductions in spleen weight were seen at 1500 and 3000 ppm.

One 250 ppm female exhibited hydrocephaly, and 3 males (one each at 0, 250 and 1500 ppm) exhibited hydronephrosis of the right kidney. No other gross abnormalities were reported.

Histopathology:
F0 Parental
Giant cells (1 male at 3000 ppm) and atrophy (1 male at 3000 ppm and 1 at 1500 ppm) in the

seminiferous tubules of the testes were observed. No significant treatment related effects were observed in females at necropsy.

F1 Parental
No significant treatment related effects were observed at necropsy.

Summary:
F0 and F1 parental generations showed signs of toxicity at 1500 and 3000 ppm. At 3000 ppm, body weight reductions, ataxia and changes in organ weights (including liver, kidney, adrenals, spleen and brain) were observed in both sexes and both generations. At 1500 ppm, ataxia was exhibited in both sexes for the F0 generation, and changes in some organ weights were observed. Major changes at 250 ppm were restricted to increased absolute but not relative kidney weight in F0 males.

The majority of reproductive parameters were not affected by exposure. A reduction in oestrous cycle length was observed for F1 1500 ppm females. A reduction in sperm count for the F1 3000 ppm males and an increase in the percentage of abnormal sperm for the F0 1500 and 3000 ppm males were observed.

Offspring toxicity parameters generally showed no treatment related effects. A significant reduction in birth weight was observed for 3000 ppm F2 pups, and increased mortality of F2 pups with concentration was observed at 1500 and 3000 ppm.

Delays (not correlated with body weight differences) in the age of preputial separation in males (at 1500 and 3000 ppm, F1, and 3000 ppm, F2) and vaginal patency in females (at 3000 ppm, F1, and 250 and 3000 ppm, F2) were observed in both generations. Overall the effects seemed more severe on the F1 generation. Shorter anogenital distances at birth were observed in both sexes of the F2 generation; these appeared to be related to lower birth weights. The pattern exhibited by these results was considered more likely to be due to overall toxicity, rather than endocrine disruption, which would be expected to have more severe effects on one sex than the other.

Result:
For parental toxicity, no NOEL was determined. Only minor changes occurred at 250 ppm, and this was established as the No Observed Adverse Effect Level (NOAEL). For reproductive toxicity, a NOEL of 1500 ppm was observed. For offspring toxicity, no NOEL was determined due to the change in time of vaginal patency in the F2 generation at 250 ppm; a NOAEL of 250 ppm was established.

9.4.2 Developmental Toxicity Study in Rats (American Petroleum Institute, 1997c)

Species/Strain:	rat/CD (SD)BR
Number/sex of animals	25 sperm-positive females/dose
Method of administration:	whole body exposure to notified chemical vapour; exposure 6 hours per day, for 14 consecutive days on gd 6 to 19; dams were sacrificed prior to delivery

Dose:	target concentrations 0, 250, 1500 and 3500 ppm; actual TWA concentrations 0, 246, 1532, 3500 ppm (measured by infrared absorption)
Method:	In-house method: CIIT Protocol 95060/95046 RTI Protocol RTI-542/RTI-541

Mortality:
No dams died, aborted or delivered early.

Clinical Observations:
Seven females (2 controls, 1 each at 250 and 1500 ppm, and 3 at 3500 ppm) were found to be non-pregnant.

Maternal body weight was reduced at 3500 ppm from gd 12. Maternal weight change through the gestational period was reduced for both the 1500 and 3500 ppm groups. Treatment related clinical observations at 3500 ppm included ataxia, dazed appearance, lethargy, eyes squinted or closed, pica, slow respiration, piloerection, rough coat, facial tremors and gasping. At 1500 ppm, lethargy (2 animals) and piloerection (1 animal) were seen. At 250 ppm, pica (1 animal) and piloerection (2 animals) were observed.

Uterine Examination:
No effects of treatment on gestational parameters, including number of ovarian corpora lutea, total number of uterine implantation sites, pre- or post-implantation loss, number of live foetuses per litter and sex ratio were observed. Foetal body weight was significantly reduced at 3500 ppm. Scattered occurrences of foetal malformations were observed; no treatment related changes in the incidence of malformations was identified.

Gross Pathology:
Gravid uterine weight exhibited a downward trend with increased concentration. Maternal liver weight was significantly increased at 3500 ppm.

Comment:
Maternal toxicity was observed at 1500 and 3500 ppm, as indicated by clinical signs of toxicity and changes in body weights. Developmental toxicity at 3500 ppm was indicated by lower foetal weights. No indications of treatment related foetal defects was observed.

Result:
A NOEL of 250 ppm for maternal toxicity and a NOEL of 1500 ppm for developmental toxicity were established in this study.

9.4.3 Developmental Toxicity Study in Mice (American Petroleum Institute, 1997d)

Species/Strain:	mouse/Crl: CD-1(ICR)
Number/sex of animals	25 sperm or plug positive females/dose

Method of administration:	whole body exposure to notified chemical vapour; exposure 6 hours per day, for 11 consecutive days on gd 6 to 16; dams were sacrificed prior to delivery
Dose:	target concentrations 0, 250, 1500 and 3500 ppm; actual TWA concentrations 0, 245, 1551, 3509 ppm (measured by infrared absorption)
Method:	In-house method: CIIT Protocol 95061/95051 RTI Protocol RTI-544/RTI-543

Mortality:
Four dams in the 3500 ppm group died during the study, on gd 6, 7, 8 and 9, and one control dam died on the day before the study commenced. No dams aborted. One dam at 250 ppm delivered early on day 16.

Clinical Observations:
Nine females (1 control, 3 each at 250 and 1500 ppm, and 2 at 3500 ppm) were found to be non-pregnant.

Maternal body weight was reduced at 3500 ppm only on gd 15 and at sacrifice. Maternal weight change through the gestational period was reduced for the 3500 ppm group. Treatment related clinical observations at 3500 ppm included ataxia, hyperactivity, prone position, lethargy, eyes squinted, slow respiration, rough coat, head tremors and gasping. At 1500 ppm, eyes half closed (1 animal) and head tremors (1 animal) were seen. At 250 ppm, one dam delivered early.

Uterine Examination:
No effects of treatment on gestational parameters, including number of ovarian corpura lutea, total number of uterine implantation sites, pre- or post-implantation loss, number of live foetuses per litter and sex ratio were observed. Foetal body weight was significantly reduced at 3500 ppm. Significant increases in percent foetal deaths per litter and percent litters with late foetal deaths were seen at 3500 ppm. Concentration related upward trends in percent non-live implants and percent adversely affected (non-live plus deformed) implants were observed. A significant reduction in foetal body weights was observed at 3500 ppm.

A significant increase in percent litters with foetal external malformations was seen at 3500 ppm (31.58 %) relative to controls (0 %); an increase was also observed at 1500 ppm (18.18 %) although this was not statistically significant. A significant increase in the percent litters with visceral variations was also observed at 3500 ppm. Upwards trends in percent foetuses with variations per litter and percent male (but not female) foetuses with variations per litter were seen.

External malformations observed included cleft palate in 3 foetuses (3 litters) at 1500 ppm and 11 foetuses (6 litters) at 3500 ppm, extra digits on the fore and hind paws in 3 foetuses (1 litter) at 1500 ppm; this litter also included one foetus with exencephaly, open left eye and small lower jaw. Visceral malformations were limited to hydronephrosis, in all groups. Skeletal malformations were also scattered across the groups, and included sternum and rib malformations.

External variations were limited to a few haematomas at various locations for 250 and 1500 ppm. Visceral variations included enlarged lateral ventricles of the cerebrum, with a dose related incidence. This appeared in 8 foetuses (7 litters) at 0 ppm, 6 foetuses (4 litters) at 250 ppm, 7 foetuses (7 litters) at 1500 ppm and 38 foetuses (16 litters) at 3500 ppm. Red foci on the urinary bladder were observed in one control and three 1500 ppm foetuses. Skeletal variations were generally scattered across the groups. Incomplete ossification of the pubis was only observed in 6 foetuses (2 litters) at 3500 ppm.

Gross Pathology:
For the 3500 ppm animals that died during the study, red or dark red nail beds, red foci or red areas on the lungs and solvent smell on the fur were observed. Gravid uterine weight was significantly reduced at 3500 ppm. Maternal absolute liver weight was significantly increased at 1500 ppm, and relative liver weight was increased at 1500 and 3500 ppm.

Comment:
Maternal toxicity was observed at 1500 and 3500 ppm, as indicated by mortalities, clinical signs of toxicity and changes in liver and body weights. Developmental toxicity at 3500 ppm was indicated by lower foetal weights, and the incidence of cleft palate and enlarged lateral ventricles of the cerebrum. While the observation of 3 incidences of cleft palate at 1500 ppm is not statistically significant, it appears to be treatment related in light of the high incidence of this defect at 3500 ppm. Cleft palate was also reported to occur for mice exposed to the analogue MTBE. The increased incidence of enlarged lateral ventricles of the cerebrum was considered to be consistent with developmental delay.

Result:
A NOEL of 250 ppm for maternal toxicity and a NOEL of 250 ppm for developmental toxicity were established in this study.

9.5 Biotransformation Studies

The biotransformation of the notified chemical was studied in rats (Fischer 344, 2/sex) and in one human volunteer by administration of vapour of both normal abundance TAME and TAME which had been ^{13}C labelled at the 2 position, and examination of the biotransformation products in urine (Amberg et al., 1999). Studies were performed by ^{13}C nmr, with confirmation of the structures of the metabolites by gas chromatography/mass spectrometry (GC/MS and secondary ion mass spectrometry (MS/MS). The nmr spectra were compared to those from control urine to determine the peaks due to treatment with the notified chemical. Quantification of the degree of elimination was not carried out.

Rats were also exposed to ^{13}C labelled *t*-amyl alcohol by gavage (250 mg/kg in corn oil) to compare the metabolites from the notified chemical with those from the expected initial metabolism product.

Rats were exposed to the vapour by whole body exposure at an initial concentration of 2000 ppm, which decreased to < 300 ppm over the 6 hour exposure period. Urine samples were collected in 24 hr increments for 48 hr after exposure. No volatile exhaled metabolites were detected in the chamber air by gas chromatography. The human volunteer was exposed to the notified chemical by inhalation of oxygen containing an initial concentration of 27000 ppm

from a gas sampling bag for 4 minutes. Urine samples were collected in 6 hr increments for 48 hr.

In rats, the major metabolites detected were identified as being 2-methyl-2,3-butanediol and a glucuronide of this compound, and a glucuronide of *t*-amyl alcohol. Minor metabolites identified included 3-hydroxy-3-methylbutyric acid, *t*-amyl alcohol and 2-hydroxy-2-methylbutyric acid. No major differences between male and female rats were observed. Differences in relative intensities of the peaks for the different metabolites were observed between the 24 hr and 48 hr urine samples. The results for rats treated with *t*-amyl alcohol were similar to those for rats treated with TAME.

For the human volunteer, the same metabolites were identified. There were differences compared with rats in the relative concentrations of the metabolites; *t*-amyl alcohol was a major metabolite in human urine, while the glucuronide of 2-methyl-2,3-butanediol was present in minor quantities. Metabolites of the notified chemical were observed in human urine up to 48 hr after exposure.

The results indicate that the initial step in the metabolism of TAME is cleavage of the ether linkage following oxidation of the methyl group by cytochrome P450, producing *t*-amyl alcohol. Further oxidation of this product by P450, giving several diols, occurs to a great extent. The metabolites are then conjugated by reaction with glucuronyltransferase, or further oxidised to butyric acid derivatives, prior to elimination in the urine. The study authors conclude that the results indicate that the formation of reactive intermediates in metabolism of the notified chemical is unlikely based on the postulated metabolism pathways.

An additional study concerning biotransformation and excretion kinetics in rats and human volunteers was located by NICNAS (Amberg et al., 2000). Similar metabolites to those described above were identified, and the kinetics of elimination were studied by GC/MS. In rats, all metabolites had excretion half-lives of less than 6 hours, while in humans, the half-lives for excretion of metabolites were in the range of 6 to 40 hours.

In the Finnish study of exposure of tanker drivers involved in unloading gasoline containing the notified chemical (Vainiotalo et al., 1998), a geometric mean concentration of 0.98 mg/m^3 TAME (range 0.22 – 6.9 mg/m^3) over a 30 min exposure time was measured in the breathing zone. Most blood and urine samples taken as part of the study showed levels of TAME and its metabolite, *t*-amyl alcohol, below the quantification limits of 7 nmol/L and 100 nmol/L respectively. For the TAME analogue, methyl *t*-butyl ether (MTBE), which was present at higher concentrations in the gasoline used, the levels in blood and urine and in the breathing zone were correlated. Urinary excretion of the metabolism product of MTBE, *t*-butyl alcohol, did not correlate to the concentration in the breathing zone, and it was considered that this was due to slow excretion of the metabolite. The authors state that their unpublished data indicate that a lower proportion of TAME is excreted in urine compared with MTBE.

9.6 Overall Assessment of Toxicological Data

The notified chemical was of low acute oral toxicity in rats. The acute oral toxicity study submitted by the notifier used a dose of 5000 mg/kg, at which dose 8/10 animals died. The published paper on the 28-day oral toxicity study indicated that preliminary testing had been

performed to obtain an acute LD_{50} of approximately 2100 mg/kg. The MSDS from Sigma Aldrich Pty Ltd supplied by the notifier reports that the Registry of Toxic Effects of Chemical Substances (RTECS) indicates an oral LD_{50} of 1602 mg/kg in rats (National Institute of Occupational Safety and Health, 2000). The notified chemical was of low acute dermal toxicity in rabbits, with no deaths during the study (LD_{50} > 3160 mg/kg). It was of very low acute inhalation toxicity in rats (LC_{50} > 5400 mg/m^3). In a 28 day inhalation study in rats, mortalities of 3 of 14 males and 4 of 14 females were seen over the course of the study at 4000 ppm (16800 mg/m^3). In a 13 week inhalation study in rats and mice, mice were found to be more sensitive to the notified chemical than rats, with 13 of 46 male mice and 10 of 46 female mice dying after one 6 hr exposure at 3500ppm (14700 mg/m^3). A summary of results from a dermal irritation study was submitted; this indicated primary irritation scores of 4.3/8.0 at one hour and 5.0/8.0 at 24 hours.

The skin irritation results from the acute dermal toxicity study (24 hr exposure) indicated high scores for erythema, persisting beyond 14 days after treatment. The notified chemical was found to be a slight eye irritant in rabbits. Conjunctival redness, chemosis and discharge were seen in all animals at 1 and 4 hours after treatment. One animal showed high scores for conjunctival effects to 3 days after treatment, with conjunctival effects persisting through day 7 and corneal ulceration and stippling which resolved by day 3. For the other five animals, conjunctival scores of 1 for redness and in one case chemosis were seen at 24 hours; all effects resolved by day 4 for four of these. The notified chemical was not sensitising to the skin of guinea pigs in a Buehler test.

In an in vitro chromosome aberration study, the notified chemical was found to be clastogenic. However, in an in vitro point mutation test in *S. typhimurium* and in an in vivo mouse micronucleus test, it was not found to be genotoxic.

Three repeat dose toxicity studies were submitted. In a 28 day inhalation study, a NOAEL of 500 ppm (2100 mg/m^3) was established. Minor clinical signs of CNS depression were seen at this level. At levels of 2000 and 4000 ppm, CNS depression and effects on liver weights were observed. The signs of CNS depression cleared by 24 hours after exposure. In a 28 day gavage study, organ weight changes were observed at 500 mg/kg/day, and mortalities at 1000 mg/kg/day. A NOEL of 125 mg/kg/day was established in this study, with 500 mg/kg/day considered to be the NOAEL. In a 90 day repeat dose inhalation study in rats and mice, a NOEL of 250 ppm (1050 mg/m^3) was established for female rats and male mice; no NOEL was established for male rats and female mice. Male rats showed liver weight effects at 250 ppm, while female mice showed effects on liver cell proliferation at this dose. At higher doses (3500 and 1500 ppm for rats, 2500 and 1500 ppm for mice) a number of effects were observed, including high mortality for high dose mice, male rat specific nephropathy, liver cell proliferation in mice, clinical signs of CNS depression during and following exposure, changes in organ weights, particularly liver, and changes in clinical chemistry parameters. The causes of the high observed mortality in mice could not be determined at necropsy.

Several reproductive and developmental studies in rats and mice were also submitted. A two generation inhalation study in rats showed only minor treatment related signs in parental rats at 250 ppm (1050 mg/m^3); this was established as the NOAEL for parental toxicity. For reproductive toxicity, a NOEL of 1500 ppm was established due to decreases in birth weights at 3000 ppm. For offspring toxicity, no NOEL could be determined because delayed vaginal patency was observed in the F2 generation at 250 ppm; this was considered to be the NOAEL. In a developmental study in rats, maternal toxicity (indicated by clinical signs and

changes in body weight) was observed at 1500 and 3500 ppm, and a NOEL of 250 ppm (1050 mg/m^3) was established. Developmental toxicity was indicated by reduced foetal weights at 3500 ppm and a NOEL of 1500 ppm (6300 mg/m^3) was established for developmental toxicity. In a developmental study in mice, maternal mortalities and changes in liver weight were observed at 1500 and 3500 ppm, in addition to the changes observed in rats. Lower foetal weights and higher incidence of cleft palate were seen in mouse foetuses at 3500 ppm; a slight increase in cleft palate was also seen at 1500 ppm. Accordingly, the NOEL was established to be 250 ppm (1050 mg/m^3) for both maternal and developmental toxicity.

The observed mortality in the inhalation studies, particularly in mice, is likely to be indicative of severe CNS depression. The notified chemical should be classified with the risk phrase R67: Vapours may cause drowsiness and dizziness, once this has been adopted in the NOHSC Approved Criteria.

The overall results of the repeat dose, reproductive and developmental toxicity studies are tabulated below.

Exposure route	***Duration***	***Species***	***End Point***	***Sex***	***Quantity Determined***	***Value***
Oral	28-day	rat	Repeat Dose Toxicity	both	NOEL	125 mg/kg
Inhalation	28-day	rat	Repeat Dose Toxicity	both	NOAEL	500 ppm
Inhalation	13-week	rat	Subchronic Toxicity	male female	LOEL NOEL	250 ppm 250 ppm
		mouse	Subchronic Toxicity	male female	NOEL LOEL	250 ppm 250 ppm
Inhalation	2-generation	rat	Parental toxicity	both	NOAEL	250 ppm
			Reproductive toxicity	both	NOEL	1500 ppm
			Offspring toxicity	both	NOAEL	250 ppm
Inhalation	14-day	rat	Parental toxicity	female	NOEL	250 ppm
			Developmental toxicity	both	NOEL	1500 ppm
Inhalation	11-day	mouse	Parental toxicity	female	NOEL	250 ppm
			Developmental toxicity	both	NOEL	250 ppm

NOEL = No Observed Effect Level
NOAEL = No Observed Adverse Effect Level
LOEL = Lowest Observed Effect Level

A study of the biotransformation of the notified chemical in rats and humans showed that it is metabolised to products similar to those found after treatment with *t*-amyl alcohol. These are then largely excreted in urine. Reactive intermediates are not formed. Excretion of metabolites appears to be significantly slower in humans than in rats.

The notified chemical is classified as a skin irritant in accordance with the NOHSC Approved Criteria for Classifying Hazardous Substances (Approved Criteria) (NOHSC, 1999) on the basis of the primary irritation index scores reported in the skin irritation summary and due to the persistence of skin irritation in rabbits during the dermal toxicity study.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

Test	*Species*	*Results*
acute toxicity [OECD 203]	Rainbow trout *(Oncorhynchus mykiss)*	LC50 (96 h) = 580 mg/L NOEC = 310 mg/L
acute toxicity [OECD 202]	*Daphnia magna*	EC50 (48 h) = 100 mg/L NOEC = 83 mg/L
acute toxicity [OECD 201]	Algae *(Selenastrum capricornutum)*	Cell Growth: EC50(96 h) = 0.11 mg/L NOEC = 0.017 mg/L

* NOEC - no observable effect concentration

Fish (American Petroleum Institute, 1995b)
Rainbow trout were exposed to five solutions of the notified chemical at nominal concentrations of 120, 210, 340, 570 and 950 mg/L for a period of 96 hours under flow-through test conditions. Two replicates at each concentration plus a dilution water control were set up in aquaria with ten fish per tank. Each replicate solution was sampled and analysed for TAME concentration at the start of the test and at 96 h. The mean measured exposure concentration for the above nominal solutions were 78, 150, 310, 560 and 640 mg/L. Throughout the test solutions were observed to be clear and colourless and contain no visible undissolved test material.

After 72 h exposure 100 % mortality occurred in the highest concentration. At 96 h 30 % mortality was observed in the second highest concentration with all surviving fish exhibiting sublethal effects. No mortality or sublethal effects were observed in the other concentrations. The LC50 (96 h) was determined to be 580 mg/L with virtually no changes from 24 h.

Aquatic Invertebrates (American Petroleum Institute, 1994a)
Daphnia were exposed to five solutions of the notified chemical at nominal concentrations of 89, 150, 250, 410, and 690 mg/L for a period of 48 hours under flow-through test conditions. Two replicates at each concentration plus a dilution water control were set up in vessels with ten organisms per vessel. Each replicate solution was sampled and analysed for TAME concentration at the start of the test and at 48 h. The mean measured exposure concentration for the above nominal solutions were 15, 28, 55, 83 and 120 mg/L. Throughout the test solutions were observed to be clear and colourless and contain no visible undissolved test material.

After 48 h exposure 90% immobilisation occurred in the highest concentration with all

surviving daphnia exhibiting sublethal effects. No immobilisation or sublethal effects were observed in the other concentrations. The EC50 (48 h) was determined to be 100 mg/L.

Algae (American Petroleum Institute, 1994b)
1 x 10^4 cells/mL of the green algae *Selenastrum capricornutum* were exposed to five solutions of the notified chemical at nominal concentrations of 0.040, 0.080, 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 mg/L for a period of 96 hours under closed system test conditions. A reference control and test medium control were also set up and kept under the same conditions as the test solutions. Each concentration solution was sampled and analyzed for TAME concentration at the start of the test and at 96 h. The mean measured exposure concentration for the above nominal solutions were 0.017, 0.037, 0.067, 0.23, 0.48, 0.52, 1.4 and 3.7 mg/L.

After 96 hours exposure of the notified chemical to green algae *Selenastrum capricornutum* the EC50 was determined to be 0.11 mg/L. The no observed effect concentration at 96 hours was determined to be 0.017 mg/L.

Results from supplemental testing demonstrated that the notified chemical has an algistatic rather than algicidal effect on the growth of green algae once it is diluted to a non-inhibitory concentration (0.040 mg/L).

Conclusion
The ecotoxicity test results submitted for the notified chemical suggest that it is practically non-toxic to fish and aquatic invertebrates but highly toxic to algae. However, the literature (Huttenen et al., 1997) reports that studies on fish, daphnia and algae all have LC50 or EC50 values of >100 mg/L for TAME, indicating a very low aquatic toxicity to all of these organisms.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The intended use pattern of the notified chemical as an unleaded petrol additive in a one-off importation situation is not expected to result in a significant release to the environment. The notified chemical should be completely destroyed by combustion within the petrol engine, resulting in the formation of oxides of carbon and hydrogen. In the event of spills and minor releases during transfer operations and storage, the MSDS contains information on procedures to reduce release to the environment.

If the notified chemical is released to the aquatic environment as result of either a spill or leak from a storage tank, it is expected to be dispersed and diluted rapidly and also to evaporate. If released into soil, the chemical is expected to evaporate or penetrate the soil and eventually reach ground water, due to the low log P_{ow} and low calculated log K_{oc}. Groundwater contamination is an issue which will need to be addressed prior to any ongoing importation of the notified chemical.

The chemical is not readily biodegradable in is therefore likely to be persistent in the environment but is not expected to bioaccumulate. It is practically non-toxic to fish and daphnia but may be highly toxic to algae.

Given the above, environmental exposure and the overall environmental hazard is expected to

be low from the one-off importation of the notified chemical.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Hazard Assessment
The notified chemical showed low to very low acute oral toxicity in rats (LD_{50} = 1602 - 2100 mg/kg) and low acute dermal toxicity in rabbits (LD_{50} > 3160 mg/kg). It showed very low acute inhalation toxicity in rats, with a 4 hour LC_{50} of > 5400 mg/m^3. It was a slight to moderate eye irritant and a moderate skin irritant in rabbits, but was not sensitising to the skin of guinea pigs. It was found to be clastogenic in an in vitro chromosome aberration study, but no evidence of genotoxicity was seen in an in vitro point mutation test or an in vivo mouse micronucleus test. No information on the potential for skin absorption of the notified chemical was supplied, and no information on the skin absorption potential of the more widely studied analogue methyl *tert*-butyl ether (MTBE) is available (International Programme on Chemical Safety, 1998).

A number of repeat dose, reproductive and developmental toxicity studies in both rats and mice, and by oral and inhalation routes, were provided by the notifier. The major findings were effects on the liver and kidney, with clinical symptoms of central nervous system depression and mortalities observed at higher doses. The results of these studies indicated that the notified chemical is not a selective developmental toxicant, with developmental effects seen only at levels corresponding to significant maternal toxicity. For oral administration to rats over 28 days, a NOEL of 125 mg/kg/day was established. By inhalation over 13 weeks, in rats and mice, a NOEL of 250 ppm (1050 mg/m^3) was established for female rats and male mice; no NOEL was established for male rats and female mice, as liver effects were seen in both cases at 250 ppm, the lowest dose used. Long term effects in humans may differ from those observed in rats and mice due to the observed difference in excretion kinetics. The notified chemical is not classified as a hazardous substance in accordance with the Approved Criteria for repeat dose toxicity or for developmental effects.

The observed CNS depression, which was linked by the study authors to the mortalities observed in the 28 day inhalation study, indicates that the notified chemical should be classified with the risk phrase R67 when this is approved for use in Australia.

Based on the results of the skin irritation study and the local dermal effects observed during the dermal toxicity study, the notified chemical is classified as a hazardous substance in accordance with the Approved Criteria, and assigned the risk phrase R38: Irritating to skin. No classification for acute inhalation toxicity could be made because testing was not conducted up to the level of 20 mg/L, above which the chemical would not be considered harmful by inhalation, and because high levels of mortality, particularly in mice, were seen below this level. Due to the observed CNS depression, any MSDS or label for products containing the notified chemical should indicate that vapours may cause drowsiness and dizziness.

The notifier provided Material Safety Data Sheets (MSDS) for TAME prepared by Valero Energy Corporation (a manufacturer of the notified chemical) and by Sigma Aldrich Pty Ltd. These indicate the possibility of severe eye irritation, as well as skin and respiratory irritation. They also indicate the possibility of absorption through the skin, and of CNS depression. The

Sigma Aldrich MSDS gives the classification R36/37/38: Irritating to eyes, respiratory system and skin.

Occupational Health and Safety
The notified chemical is a component of gasoline at 5.45 %. The most probable route of occupational exposure is inhalation, for oil terminal and depot workers, truck drivers and service station attendants. These workers may also have dermal exposure to spilt gasoline containing the notified chemical.

A study of inhalation exposure to the notified chemical for truck drivers in Finland indicated that the amount of notified chemical absorbed was correlated with the breathing zone concentration. This indicated that, for these workers, under the conditions of the study, dermal absorption was not a major contributor to uptake of the notified chemical. A geometric mean concentration of notified chemical in the breathing zone of 0.98 mg/m^3 was measured for these workers, with a maximum breathing zone concentration of 6.9 mg/m^3. For 30 minute exposure, a TWA concentration of 1.1 mg/m^3 was found. The ambient air in two service stations was found to have an average concentration of 0.031 mg/m^3 notified chemical. In the repeat dose studies in rats and mice described above, no effects or minor effects were observed on inhalation of 250 ppm (1050 mg/m^3) notified chemical for up to 13 weeks. This may be compared with the monitoring results from the Finnish studies (Vainiotalo et al., 1998, Vainiotalo et al., 1999, Vainiotalo & Ruonakangas, 1999).

The monitoring studies covered short term exposure only, and therefore the maximum breathing zone concentration would not be expected to be indicative of the time averaged exposure in the longer term. Therefore, taking an indicative breathing zone concentration of 1 mg/m^3, and comparing this with the level at which minor adverse effects or no adverse effects was observed in rats and mice, 1050 mg/m^3, an approximate Margin of Safety (MOS) of 1000 is obtained. Even in view of the facts that the exposure study was not measuring the time weighted average exposure, and that adverse effects were seen in rats and mice in some cases at 1050 mg/m^3, a MOS of at least two orders of magnitude would be expected for the activity described in the monitoring study. A higher MOS, approximately four orders of magnitude, would be expected for service station workers exposed to the ambient air containing 0.031 mg/m^3 notified chemical for 8 hours or more per day. The MOS would be reduced under conditions where dermal absorption may contribute significantly to uptake of the notified chemical, for example if the gasoline is handled without use of gloves. Tanker drivers may be expected to have the highest exposure of any workers handling the notified chemical, during the loading and unloading activities monitored in the study. Detailed monitoring results for the related chemical MTBE, which has a similar use pattern, confirmed that these workers have the highest exposure of any occupational groups handling the notified chemical in gasoline (International Programme on Chemical Safety, 1998).

The USA has set an exposure standard of 40 ppm (144 mg/m^3) for the more volatile and widely used analogue chemical MTBE (American Conference of Government Industrial Hygienists, 1998). An IPCS monograph on MTBE (International Programme on Chemical Safety, 1998) describes toxicity testing with results similar to those seen for the notified chemical. The lowest reported levels for liver and kidney effects from MTBE were 440 mg/kg/day (gavage, 28-day, rats) for ingestion studies and 400 ppm for inhalation studies in rats (104 weeks) and mice (18 months). For TAME, adverse effects were observed at 500 mg/kg/day (gavage, 28-day, rats) and 250 ppm (inhalation, 13-week, rats and mice).

The effects produced by exposure to the notified chemical will be combined with those seen due to exposure to gasoline. Petrol (gasoline) has a NOHSC exposure standard of 900 mg/m^3 TWA, which applies only if individual toxic components such as benzene and n-hexane are not present (NOHSC, 1995). The notified chemical is one of the more volatile hazardous components of gasoline. For example, toluene, xylene and ethylbenzene, with exposure standards of 377 mg/m^3, 350 mg/m^3 and 434 mg/m^3, respectively (NOHSC, 1995), are less volatile than the notified chemical. Therefore TAME is expected to be a significant contributor to the health effects resulting from inhalation of gasoline at the NOHSC exposure standard of 900 mg/m^3 TWA. The reported health effects of gasoline inhalation include intoxication, headaches, blurred vision, dizziness, nausea, eye, nose, and throat irritation, and dizziness and mild anesthesia (American Conference of Government Industrial Hygienists, 1998).

Breathing zone concentrations in the Finnish survey were well below the levels where adverse effects were seen in experimental animals, suggesting that inhalation exposure may not be of concern in the good ventilation accompanying outdoor work. However, if exposure to gasoline containing the notified chemical should occur under less ventilated conditions, respiratory protection should be used to minimise TAME exposure. Dermal uptake of the notified chemical has not been measured, and dermal absorption may also contribute to overall exposure to the notified chemical. The notified chemical is also irritating to skin. Impervious gloves should therefore be used during occupational handling of gasoline containing the notified chemical.

Public Health
Public expose to the chemical will occur at the bowser and in transfer of fuel from personal storage containers. The concentration in the product would be 5.45 %. Exposure will be limited to dermal, through spills, and inhalation of fumes. Since the dermal acute toxicity is low there is unlikely to be any public health hazard resulting from spills. Although acute effects, including development effects, were seen following inhalation exposure a clear NOEL was established at 250 ppm for 6 hours. This is a level likely to be significantly higher than the level to which the public will be exposed, and as exposure is likely to be only of a short duration, the notified chemical is unlikely to pose a significant hazard.

13. RECOMMENDATIONS

Regulatory controls
Use of the notified chemical in gasoline is subject to the Federal Fuel Quality Standards and State and Territory environmental laws;

Hazard classification
The NOHSC Chemicals Standards Sub-committee should consider the following health, environmental and physico-chemical hazard classification for the notified chemical:

R38: Irritating to skin

The following risk phrase should be applied to the notified chemical when the use of the risk phrase has been adopted by NOHSC:

R67: Vapours may cause drowsiness and dizziness

Classify the notified chemical as follows under the ADG Code:

Class 3: Flammable Liquid

Labelling

Suppliers should label the notified chemical as a Class 3 dangerous good with the signal word 'Flammable' and the risk and safety phrases listed above.

Exposure standard

The NOHSC Chemicals Standards Sub-committee should consider setting an exposure standard for the notified chemical.

Control measures (for terminal staff, tanker drivers and service station operators)

OHS

Employees should wear the following personal protective equipment to minimise occupational exposure to the notified chemical:

> Safety goggles, chemical resistant industrial clothing and footwear and impermeable gloves; where engineering controls and work practices do not reduce vapour and particulate exposure to safe levels, an air fed respirator should also be used.

Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

A copy of the MSDS should be easily accessible to employees.

If products and mixtures containing *tert*-amyl methyl ether are classified as hazardous to health in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances*, then workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Secondary notification

Secondary notification of the notified chemical will be required prior to any further importation of the notified chemical. Under Section 64(1) of the Act, the following information will be required:

- Information on the effect of the notified chemical on exhaust emissions;
- An estimate of the likely quantities to be released from fuel storage facilities including fuel station underground storage tanks and discussion of the likely toxic effects of these releases on waterways;
- A worst case localised Predicted Environmental Concentration (PEC) for leakage of the notified chemical from a transport spill or a typical fuel station to ground water;
- Any health effects data in humans, including exposure monitoring and biological monitoring data;

- An acute inhalation toxicity study in mice with determination of the LC_{50} up to a limit concentration of 20 mg/L or greater.

In addition, if any circumstances listed in subsection 64(2) of the Act apply, the notifier must notify the Director in writing within 28 days. The Director will then decide whether secondary notification is required.

14. MATERIAL SAFETY DATA SHEET

Two MSDS for the notified chemical was provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994).

These MSDS was provided by the applicant as part of the notification statement. They are reproduced here as a matter of public record. The accuracy of this information remains the responsibility of the applicant.

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Attachment 1

The Draize Scale (Draize, 1959) for evaluation of skin reactions is as follows:

Erythema Formation	*Rating*	*Oedema Formation*	*Rating*
No erythema	0	No oedema	0
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)	1
Well-defined erythema	2	Slight oedema (edges of area well-defined by definite raising	2
Moderate to severe erythema	3	Moderate oedema (raised approx. 1 mm)	3
Severe erythema (beet redness)	4	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

The Draize scale (Draize *et al*., 1944) for evaluation of eye reactions is as follows:

CORNEA

Opacity	*Rating*	*Area of Cornea involved*	*Rating*
No opacity	0 none	25% or less (not zero)	1
Diffuse area, details of iris clearly visible	1 slight	25% to 50%	2
Easily visible translucent areas, details of iris slightly obscure	2 mild	50% to 75%	3
Opalescent areas, no details of iris visible, size of pupil barely discernible	3 moderate	Greater than 75%	4
Opaque, iris invisible	4 severe		

CONJUNCTIVAE

Redness	*Rating*	*Chemosis*	*Rating*	*Discharge*	*Rating*
Vessels normal	0 none	No swelling	0 none	No discharge	0 none
Vessels definitely injected above normal	1 slight	Any swelling above normal	1 slight	Any amount different from normal	1 slight
More diffuse, deeper crimson red with individual vessels not easily discernible	2 mod.	Obvious swelling with partial eversion of lids	2 mild	Discharge with moistening of lids and adjacent hairs	2 mod.
Diffuse beefy red	3 severe	Swelling with lids half-closed	3 mod.	Discharge with moistening of lids and hairs and considerable area around eye	3 severe
		Swelling with lids half-closed to completely closed	4 severe		

IRIS

Values	*Rating*
Normal	0 none
Folds above normal, congestion, swelling, circumcorneal injection, iris reacts to light	1 slight
No reaction to light, haemorrhage, gross destruction	2 severe

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