I U C L I D

D a t a S e t

Existing Chemical	ID: 637-92-3
CAS No.	637-92-3
EINECS Name	2-ethoxy-2-methylpropane
EC No.	211-309-7
Molecular Formula	C6H14O

Producer Related Part

Company:	A.K. Mallett
Creation date:	02-NOV-2003

Substance Related Part

Company:	A.K. Mallett
Creation date:	02-NOV-2003

Memo:	IUCLID update prepared in response to Commission Regulation (EC) No 1217/2002

Printing date:	26-JAN-2004
Revision date:	
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Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 Applicant and Company Information

Type: lead organisation
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Country: France

Remark: This dataset was prepared under Commission Regulation (EC) No 1217/2002 (5 July 2002) on behalf of the following companies:

Compañia Española de Petroleos S.A, Spain
Ecofuel S.p.A, also on behalf of ENI S.p.A., Italy
Fortum Oil and Gas Oy, Finland
Lyondell Chemical Europe Inc., UK
OXENO Olefinchemie GmbH, Germany
Repsol Petróleo S.A., Spain
Total France, France

26-JAN-2004

1.0.2 Location of Production Site, Importer or Formulator

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1.0.3 Identity of Recipients

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1.0.4 Details on Category/Template

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1.1.0 Substance Identification

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1.1.1 General Substance Information

Substance type: organic
Physical status: liquid

Source: EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
11-FEB-2000

1.1.2 Spectra

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1.2 Synonyms and Tradenames

Synomyms and tradenames

Remark: 1,1-dimethylethyl-ethyl-ether
tert-butyl-ethyl-ether
ethyl-tert-butyl-oxide
2-ethoxy-2-methylpropane
ethyl-1,1-dimethylethyl-ether
30-NOV-2003

1.3 Impurities

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1.4 Additives

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1.5 Total Quantity

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1.6.1 Labelling

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1.6.2 Classification

Remark: F, R11
30-NOV-2003

1.6.3 Packaging

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1.7 Use Pattern

Type: industrial
Category: Fuel industry

Remark: Information on the physico-chemical properties of ETBE and relative performance characteristics of ETBE-gasoline blends is presented.

Phys-chem properties are reported in IUCLID sections 2.2, 2.3, 2.4 and 2.6.

Performance characteristics for ETBE include:
RON (ASTM2699) = 111.9
MON (ASTM D2700) = 97.2

Oxygen content = 15.66 w/w%

Key conclusions include:
* The physico-chemical properties (low RVP, low solubility in water, high boiling point) of ETBE make it suitable for blending into gasoline.
* Its Blending Octane Numbers equal or exceed those of MTBE.
* It is stable and does not oxidise in gasoline blends, however longer-term storage of neat ETBE requires the use of antioxidants to avoid possible peroxide formation.
* Gasoline containing 15% ETBE does not adversely influence lubricant performance or piston and engine cleanliness.

23-JAN-2002 (22)

Type: industrial
Category: Fuel industry

Remark: This paper from the European Commission (Excise, Duties and Transport, Environment and Energy Taxes) reviews the potential socio-economic and environmental (automotive emissions) impact of the use of ETBE in gasoline in Europe. It notes that Directive 85/536/EEC identifies a role for biofuels in reducing Member States' dependence upon oil imports, and authorises the use of up to 15% v/v ETBE in gasoline.

02-JAN-2004 (11)

1.7.1 Detailed Use Pattern

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1.7.2 Methods of Manufacture

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1.8 Regulatory Measures

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1.8.1 Occupational Exposure Limit Values

Remark: ACGIH TLV-TWA: 5 ppm

30-NOV-2003 (1)

1.8.2 Acceptable Residues Levels

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1.8.3 Water Pollution

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1.8.4 Major Accident Hazards

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1.8.5 Air Pollution

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1.8.6 Listings e.g. Chemical Inventories

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1.9.1 Degradation/Transformation Products

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1.9.2 Components

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1.10 Source of Exposure

Remark: This product is used in closed systems.
Exposure may occur during sampling.
Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
From ECB IUCLID dataset for 637-92-3
23-DEC-2003

Source of exposure: Human: exposure of the operator by intended use

Remark: This study examined occupational exposure of service station refuelling attendents and automotive mechanics to gasoline oxygenates in four regions of the USA (New York City area, Minnesota, Arizona, Oregon) during the winter of 1994.

The report introduction states that ETBE was included in these analyses, however no ETBE was reported after analysis of 'bulk' gasoline samples nor were any data or results presented for inhalational exposure to this substance.
15-JAN-2004 (4)

1.11 Additional Remarks

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1.12 Last Literature Search

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1.13 Reviews

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2.1 Melting Point

Value: = -94 degree C

Reliability: (2) valid with restrictions
Secondary literature (data compendium, review article or meeting proceedings).
28-OCT-2003 (72)

2.2 Boiling Point

Value: = 73.1 degree C

Year: 1994

Reliability: (2) valid with restrictions
Secondary literature (data compendium, review article or meeting proceedings).
02-NOV-2003 (22)

Value: = 72.8 degree C

Year: 1990

Reliability: (2) valid with restrictions
Secondary literature (data compendium, review article or meeting proceedings).
02-NOV-2003 (28)

Value: = 70 degree C at 1013 hPa
Decomposition: no

Method: other: ASTM D 86
GLP: no data

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
02-NOV-2003 (2)

2.3 Density

Type: relative density
Value: = .7455

Year: 1994

Reliability: (2) valid with restrictions
Secondary literature (data compendium, review article or meeting proceedings).
02-NOV-2003 (22)

Type: density
Value: = .74 g/cm³

Year: 1990

Reliability: (2) valid with restrictions
Secondary literature (data compendium, review article or meeting proceedings).
02-NOV-2003 (28)

Type: density
Value: = .75 g/cm³ at 20 degree C

Method: other: ASTM D 1298
GLP: no data

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
02-NOV-2003 (2)

2.3.1 Granulometry

-

2.4 Vapour Pressure

Method: other (measured): ASTM D323
Year: 1994

Result: Reid Vapour Pressure for ETBE = 0.298 bar
Reliability: (2) valid with restrictions
Secondary literature (data compendium, review article or meeting proceedings).
02-NOV-2003 (22)

Method: other (measured): comparable to ASTM D323
Year: 1990

Remark: Evaporative emissions for 3 ETBE-gasoline blends were determined by heating the samples from 40.0 to 75.6 degrees C in a model carburettor under laboratory conditions.

Vapour production decreased as the concentration of ETBE in the fuel increased ie

Baseline gasoline = 9.16 psi
10% ETBE blend = 8.57 psi
20% ETBE blend = 8.32 psi

Result: Reid Vapour Pressure = 4.3 - 4.5 psi
Reliability: (2) valid with restrictions
Secondary literature (data compendium, review article or meeting proceedings).
02-NOV-2003 (28)

Value: = 128 hPa at 20 degree C

Method: other (measured)
GLP: no data

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
02-NOV-2003

2.5 Partition Coefficient

Partition Coeff.: octanol-water
log Pow: = 1.48 at 25 degree C

Method: other (measured): FDA Technical assistance Handbook document 3.02
Year: 1994
GLP: yes

Remark: ETBE partitioned between n-octanol saturated with MilliQ purified water, and MilliQ purified water saturated with n-octanol

Phase ratios in preliminary test 50:50 and 25:75 n-octanol/water showed no difference in percent partitioning. Shaking times in preliminary test (0, 0.5,1.0, 2.0 hr) showed equilibration complete in 0.5 hr.

ETBE concentrations: 1 x 10-2 M and 1 x 10-3 M in

water-saturated n-octanol.
Volume of octanol: 50ml
Phase ratio in definitive test 50:50
Equilibration time 0.5hr (3 samples) plus two samples taken at 59 min (high concentration) and 51 min (low concentration) to show equilibrium reached.
Temperature 25 +/-1 degree C

ANALYSIS: Concentrations in each phase were determined by GC-FID (4 replicates)

Comment: Two different sets of test conditions (in this case low and high concentrations of test substance) were used in this study whereas OECD guideline would require three.

Result: Test concentration 1 x 10-2 M, Kow = 28.3

Test concentration 1 x 10-3 M, Kow = 31.4

Test substance: ETBE, 95.31% pure, supplied by ARCO Chemical Co.

Reliability: (2) valid with restrictions
Study available for review. GLP compliant near-guideline investigation, clearly reported, acceptable for assessment.

28-OCT-2003 (59)

Partition Coeff.: octanol-water

log Pow: = 1.56

Year: 1972

GLP: no

Reliability: (2) valid with restrictions
Secondary literature (data compendium, review article or meeting proceedings).

02-NOV-2003 (32)

log Pow: = 1.28

Method: other (calculated)

Year: 1985

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.

02-NOV-2003

Partition Coeff.: water - air

Year: 1995
GLP: no data

Remark: Liquid/air partition coefficients were determined using the closed vial technique (Falk et al., 1990, Br J Ind Med, 47, 62-64). In brief, 0.5 ml olive oil or 2 ml pooled human blood or physiological saline was added to a 22 ml gas tight vial. After addition of 2 ul of aqueous ETBE (concentration not stated) the vial was allowed to equilibrate at 37 degrees C for 40 min. The gas phase was analysed for ETBE content by head-space gas chromatography. Partition coefficients were obtained by comparing peak areas between the sample vial and a reference vial without liquid phase.

The following partition coefficients were obtained:
blood/air: 11.7
water/air: 8.3
oil/water: 190
oil/blood: 16.2 (calculated from [oil/air] / [blood/air])

Test substance: No details.
Conclusion: The blood air partition coefficient indicates efficient uptake of ETBE from, and elimination via, inhaled air. The greater oil/air partition coefficient is consistent with preferential movement of ETBE from blood to fatty tissue.
Reliability: (2) valid with restrictions
Study available for review. Briefly reported but acceptable for assessment.
01-NOV-2003 (45) (64)

Year: 2000

Remark: Vial-equilibration method of Sato et al (1979, Br J Ind Med 36, 231) as used.

Water-air partition coeffcient 10.0 +/- 0.8
Rat blood-air partition coefficient 11.6 +/- 1.5
Olive oil-air partition coefficient 189 +/- 29
Olive oil-water partition coefficient 17.2

Test substance: No details available.
Reliability: (2) valid with restrictions
Study available for review. Briefly reported experimental investigation, acceptable for assessment.
28-OCT-2003 (46)

2.6.1 Solubility in different media

Solubility in: Water
Value: = 2.37 other: w/w% at 20 degree C

Year: 1994

Reliability: (2) valid with restrictions
Secondary literature (data compendium, review article or meeting proceedings).
02-NOV-2003 (22)

Value: = .5 other: % Weight

Remark: Determination of pH according to ASTM D 1087 is not applicable
Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
02-NOV-2003

2.6.2 Surface Tension

-

2.7 Flash Point

Value: < 21 degree C
Type: closed cup

Method: other: ASTM D 56
GLP: no data

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
02-NOV-2003 (2)

2.8 Auto Flammability

Value: = 375 degree C

Method: other: DIN 51794
GLP: no data

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
02-NOV-2003

2.9 Flammability

-

2.10 Explosive Properties

Remark: Lower Explosive Limit : 1.23 % Vol
Upper Explosive Limit : 7.7 % Vol
Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
02-NOV-2003

2.11 Oxidizing Properties

-

2.12 Dissociation Constant

-

2.13 Viscosity

Value: at 40 degree C
Result: viscosity = 0.528 mm2/s (static) at 40 degrees C

Reliability: (2) valid with restrictions
Secondary literature (data compendium, review article or meeting proceedings).
28-OCT-2003 (28)

2.14 Additional Remarks

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3.1.1 Photodegradation

Type: air
Light source: Xenon lamp
Light spect.: = 330 - 370 nm
INDIRECT PHOTOLYSIS
Sensitizer: OH

Year: 1990
GLP: no data

Remark: Light source = High intensity xenon arc lamp

Flow system with hydroxyl radicals from photolysis of methyl nitrate in air containing nitric oxide.

Concentrations of test substances were measured by gas chromatograph.

Rate constants determined relative to diethyl ether.

A values of 4.8 x 10^12 cm3/(molecules*sec) was obtained.
Test substance: No details available.
Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.
08-JAN-2004 (6)

Type: air
Light source: other: fluorescent backlights

Year: 1991
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Experiments were performed using a 140 l pyrex reactor surrounded by 22 fluorescent backlights (GE F15T8-BL). Fourier transform infrared spectroscopy was used to monitor loss of reactants and formation of products in presence of NO, Cl and NO and Cl.
Remark: This research was designed to investigate the mechanism of the oxidation of ETBE, and did not include the determination of rate constants.
Result: Tert-butyl formate (76 +/- 6% yield), formaldehyde and 2-ethoxy-2-methyl propanal were detected as degradation products.
Test substance: Ethyl tert-butyl ether, >99.9% purity (confirmed by GC-MS), source not stated.
Conclusion: The authors conclude that attack by OH radicals on ethyl group yields the corresponding formate; interaction with the tert-butyl group yields 2-ethoxy-2-methyl propanal, or a variety of compounds formed by intramolecular hydrogen abstraction or fragmentation. Formaldehyde can be produced as

a by-product.

Reliability: (2) valid with restrictions
Study available for review. Experimental investigation, acceptable for assessment.

01-NOV-2003 (79)

Type: air

Year: 1991
GLP: no data

Remark: The reactivity of ETBE with different NMOC/NOx ratios was estimated using a previously-derived OH rate constant (Wallington et al. (1989) Int J Chem Kinet 21, 993).

A rate constant of 1.4x10^12 cm3 /(molecule*sec) was obtained.

The following was proposed as a mechanism for OH-initiated oxidation of ETBE:

ETBE + OH + 1.8NO -> 0.8HCOOC(CH3)3 + 0.2C2H5OC(CH3)2CHO + HO2 + 0.8HCHO + 1.8NO2

(From:
Chang and Rudy (1990) Proceedings of the international conference on tropospheric ozone and the environment, Air and Waste Management Association, Los Angeles, CA, March 1990; Wallington et al. Int.J. Chem Kinet 20, 541).

Authors conclude that use of ETBE in fuel may have a beneficial impact on urban ozone levels.

01-NOV-2003 (44)

Type: air

Reliability: (4) not assignable
German language report, not reviewed.

02-NOV-2003 (5)

Year: 1990
GLP: no data

Remark: Results summarised from other publications:

Wallington et al. (1988) Environ Sci Technol 22, 842:
8.12 cm3/(molecules*sec) at 298 degrees K

Bennett and Kerr (1989) J Atmos Chem 7, 87:
5.63 cm3/(molecules*sec) at 294 degrees K

Wallington et al (1989) Int J Chem Kinet 21, 993:
8.83 cm3/(molecules*sec) at 295 degrees K

Reliability: (2) valid with restrictions
Secondary literature (data compendium, review article or meeting proceedings).

08-JAN-2004 (6)

3.1.2 Stability in Water

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3.1.3 Stability in Soil

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3.2.1 Monitoring Data (Environment)

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3.2.2 Field Studies

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3.3.1 Transport between Environmental Compartments

Type: fugacity model level I
Year: 2003
Air: 96.2 % **(Fugacity Model Level I)**
Water: 3.658 % **(Fugacity Model Level I)**
Soil: .098 % **(Fugacity Model Level I)**

Remark: INPUT DATA SET
The following physico-chemical properties of ETBE were used:
Molecular weight = 102
Data temperature = 20 degrees C
Log Kow = 1.48 (at 25 deg C; Montgomery, 1994)
Water solubility = 23700 mg/l (2.37% w/w; 20 degrees C; Chatin et al., 1994)
Vapour pressure = 29800 Pa (temperature not known; IUCLID database)
Melting point = -94 degrees C (EPIWIN database)

The reaction half-life was set at negligible reaction in all compartments.

LEVEL I RESULTS
The environmental distribution, as calculated from the above parameters using the Mackay Level I model (EQC program) is as follows:
To Air 96.2%
To Soil 0.098%
To Water 3.658%
To Fish 5.52E-06%
To Sediment 2.17E-03%

Reliability: (2) valid with restrictions
Recognised modelling method.

08-JAN-2004 (51) (52) (82)

Type: fugacity model level III
Year: 2003

Remark: INPUT DATA SET
The following physico-chemical properties of ETBE were used:
Molecular weight = 102
Data temperature = 20 degrees C
Log Kow = 1.48 (at 25 deg C; Montgomery, 1994)
Water solubility = 23700 mg/l (2.37% w/w; 20 degrees C; Chatin et al., 1994)
Vapour pressure = 29800 Pa (temperature not known; IUCLID database)
Melting point = -94 degrees C (EPIWIN database)

The reaction half-life was set at negligible reaction in all compartments.

LEVEL III RESULTS
The Level III program was used, with the 'default' model and used to calculate the ultimate fate of the substance, taking into account the levels of release into specific compartments.

The distribution between environmental compartments obtained by this method is as follows:

	To Air	To Water	To Soil	To all three
% in air	99.31%	0.42%	2.76%	1.34%
% in soil	0.10%	4.29E-04%	76.82%	13.80%
% in water	0.58%	99.53%	20.40%	84.82%
% in sediment	3.11E-04%	0.05%	0.01%	0.05%

Reliability: (2) valid with restrictions
Recognised modelling method.

08-JAN-2004 (19) (51) (82)

3.3.2 Distribution

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3.4 Mode of Degradation in Actual Use

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3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge, domestic
Concentration: 150 mg/l related to Test substance
Result: other: inherently biodegradable by adapted STP organisms

Method: ISO DIS 9408 "Ultimate aerobic biodegradability - Method by determining the oxygen demand in a closed Respirometer"
Year: 1998
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: Studies were performed in a degradation respirometer (ISO 9408 standard) containing either 30 or 380 mg (dry wt)/l activated sludge. The inoculum was obtained from an urban STP in the Paris area. Degradation was followed by electrolytic respirometry using a D-12 Sapromat apparatus (Voith, Germany). Respiration was measured in the presence of 150 mg/l ETBE was corrected for endogenous respiration (oxygen uptake in the absence of ETBE).
Remark: Gordona terrae and Rhodococcus equi, isolated from cultures used in these studies, were able to grow on ETBA as sole carbon source.
Result: No degradation of ETBE was observed at an inoculum concentration of 30 mg/l.

At 380 mg/l, oxygen consumption measurements showed that degradation of ETBE commenced after a lag of approx. 22 hr. The amount of oxygen consumed at 360 hr was consistent with total removal of ETBE.

Addition of a second amount of ETBE resulted in degradation with no lag, and a degradation rate of 2.8 mg ETBE/l/hr.

Similar results were obtained in triplicate samples of activated sludge over a period of several weeks (no further details).
Test substance: ETBE, Aldrich Chemical Co. (no further details).
Conclusion: Results from these studies demonstrate degradation of ETBE (and TBA) by a concentrated, adapted inocculum of microorganisms from an urban wastewater treatment plant.
Reliability: (2) valid with restrictions
Study available for review. Experimental investigation, acceptable for assessment.
01-NOV-2003 (27)

Type: aerobic
Inoculum: activated sludge, adapted
Concentration: 89 mg/l related to Test substance
Degradation: = 100 % after 80 hour(s)
Result: other: inherently biodegradable by adapted WWTP organisms

Year: 1996
GLP: no data

Method: Acclimated culture from a petroleum refinery WWTP (adapted to degrading MTBE) was used as inoculum. The test substance concentration was89 mg/l, and incubations were performed in replicate at 30 degrees C in a 16 place Comput-Ox respirometer (N-CON Systems Inc, GA). Oxygen uptake, substrate removal and intermediate accumulation measured. ETBE concentration was determined by GC. Based on the results, degradation kinetics were modeled using standard procedures and models.

Result: Graphical data indicate that oxygen uptake by the cultures increased from 0 to 140 mg/l in 80 hr while the concentration of ETBE decreased from 80 to 0 mg/l over the same period. Small amounts of TBA (approx. <1 mg/l) were detected between 50-100 hr.

Degradation rate for ETBE = 0.027/h

Comment: additional studies on TBA included in this experimental series showed complete degradation of 82 mg/l TBA over 80 hr, with a calculated degradation rate of 0.029/hr.

Test substance: No details available.

Conclusion: High levels of ETBE degradation were achieved with sludge from a WWTP acclimated to MTBE.

Reliability: (2) valid with restrictions
Study available for review. Experimental investigation, acceptable for assessment.

01-NOV-2003 (24)

Type: aerobic
Inoculum: other: mixed bacterial population isolated from gasoline-polluted soil

Year: 1998
GLP: no data

Method: A mixed bacterial population capable of degrading ETBE were isolated from gasoline-polluted soil (Kharoune et al. (1997) Int Symp Environ Technol Oostende, Belgium, 381-384).

Degradation studies were conducted at 28 degrees C in a BSB digi V 1.01 respirometer (Buhler) at an agitation speed of 125 rpm. Ten respirometer flasks containing ETBE (200 mg/l) were inoculated with defined amounts of biomass (see results), with two endogenous respiration control flasks (no ETBE). Oxygen consumption, total cell protein, pH, oxygenate- and COD concentrations measured.

ETBE and TBA were analysed by GD-FID.

Mineralisation of ETBE was assessed by measuring COD.

Result: At a concentration of 243 mg/l total cell protein, mineralisation of 200 mg/l ETBE was complete within 21 hr (as demonstrated by disappearance of substrate, zero carbon mass balance (COD) and cessation of oxygen uptake).

Rate of ETBE degradation increased with inoculum size (sub-linear relationship):

Inoculum	Oxygen uptake (mg/l/hr)	ETBE (mg/l/hr)	Removal rate (mg ETBE/hr/g cell protein)
12.9 mg/l	5	1.1	1666
19.5	5	1.86	1706
120	15	5.60	1750
243	30	9.75	1666

Accumulation of TBA was reported (but not quantified) in systems containing 120 mg total cell protein/l and above.

The rate of ETBE degradation (9.3-9.9 mg/l/hr) was approximately constant over a substrate concentration range of 200-1200 mg/l, however this decreased to 3.2 or 2.4 mg/l/hr at ETBE concentrations of 2000 or 3000 mg/l, respectively. (Equivalent removal rates were 1800-1830 mg/hr/g total protein at 200-1200 mg ETBE/l, and 1570 or 1400 mg/hr/g at higher substrate concentrations.)

Test substance: ETBE, purity not stated, Elf Aquitaine, France or Fluka France (no further details).

Conclusion: A decrease in ETBE concentration, removal of COD along with substrate-dependent oxygen uptake were observed when ETBE by was incubated with a microbial consortium obtained from a gasoline-polluted site. Degradation rates of 1800-1900 mg

ETBE/g cell/hr were achieved at substrate concentrations of 200-1200 mg/l, however higher concentrations of ETBE were toxic.

Reliability: (2) valid with restrictions
Study available for review. Briefly reported experimental investigation, acceptable for assessment.

02-JAN-2004 (48)

Type: aerobic
Inoculum: other: mixed bacterial population isolated from gasoline-polluted soil

Year: 1998
GLP: no data

Method: Degradation of ETBE was investigated at 28 degrees C in a 2 l continuous fixed-bed recycling biofilm reactor (60x8 cm internal diameter) containing a sintered SIRAN glass ring as biomass carrier (55-60% pore volume, 0.15 m2/g surface area). A recycle rate of 650 l/d and airflow of 4.5 l/d were employed, along with a feed rate was 2 l/d (salts solution and ETBE at 50-345 mg/l in distilled water).

The reactor was inoculated with ETBE-mineralising bacteria isolated from gasoline polluted soil (Kharoune et al. (1997) Int Symp Environ Technol Oostende, Belgium, 381-384). Cell growth was monitored at 600 nm, while the amount of biomass (protein dry wt) present on the sintered glass carrier was quantified after sonication.

Degradation of ETBE, together with identification of its major breakdown products, was quantified by GC-FID while mineralisation was followed using COD and oxygen uptake.

Result: The concentration of ETBE in the reactor was increased from 50 mg/l to 345 mg/l between days 15 and 86.

The operation of the reactor was defined by three states:
- State 1: between days 15-40, a removal rate of 299 mg/l/d was achieved at an ETBE loading rate of 327 mg/l/d. Efficiency of conversion was approx. 92% with a COD removal rate of 801 mg/l/d.
- State 2: between days 40-67, the reactor destabilised, the concentration of residual ETBE in effluent increased and a removal rate of 280 mg/l/d was achieved at a loading rate of 345 mg/l/d (80.9% yield).
- State 3: the ETBE loading rate was reduced and reactor performance returned to high performance by day 69, with 99% removal efficiency at a loading rate of 280 mg/l/d.

Graphical data demonstrate good correlation (R2=0.8984) between removal of ETBE and removal of COD.

Test substance: ETBE, purity not stated, Elf Aquitaine, France or Fluka France (no further details).

Reliability: (2) valid with restrictions

Study available for review. Briefly reported experimental investigation, acceptable for assessment.

02-JAN-2004 (49)

Type: aerobic
Inoculum: other: soil microcosms
Result: other: biodegradable by soil microcosms under specialised conditions

Year: 1995
GLP: no data

Method: Degradation was evaluated in soil microcosms containing 5 g dry weight soil and water in 13x100 mm septum-capped tubes containing 100 mg/l ETBE (saturating concentration). The tubes were filled to the top (to prevent loss by evaporation) and incubated at 20 degrees C in the dark.

Hydrogen peroxide added to the incubations every 7-10 d to a concentration of 0.01% as oxygen source.

ETBE concentrations were measured by gas chromatography. Direct chemical oxidation of ETBE to TBA by hydrogen peroxide was also assessed.

Result: Soil with no additional hydrogen peroxide, or soil pretreated with hydrogen peroxide (to oxidise and precipitate soil iron and prevent chemical oxidation), was also used to assess biodegradation in some studies. Control incubations contained soil that had been subject to repeated cycles of autoclaving.

After 90 d incubation, no biodegradation of ETBE was seen in aerobic systems containing soil, nutrient solution and ETBE.

Hydrogen peroxide catalysed the very rapid chemical oxidation of ETBE by soil samples (and also in simple solution), with the appearance of TBA in minutes.

Soils pretreated with hydrogen peroxide prior to addition of ETBE showed steady degradation over 30 d, with a calculated degradation rate of 2.5 mg/l.d per g soil. The authors suggest that hydrogen peroxide provided a source of oxygen for the microorganisms and also removed ferrous iron form the system (ie preventing competitive chemical oxidation of ETBE).

Test substance: No details available.
Conclusion: Biological oxidation of ETBE to TBA was demonstrated in soil microcosms supplemented with hydrogen peroxide.
Reliability: (2) valid with restrictions
Study available for review. Experimental investigation, acceptable for assessment.

01-NOV-2003 (84)

Type: aerobic
Inoculum: other: two bacterial strains isolated from gasoline-polluted soil

Year: 2001
GLP: no data

Method: Aerobic degradation of ETBE by two bacterial strains (E1 and E2), isolated from gasoline-contaminate soil, was investigated.

Incubations contained 200 mg/l ETBE (sole carbon source) and mineral salts and were performed in triplicate at 28 degrees C. Residual amounts of ETBE and presence of TBA was quantified by GC. Oxidation of ETBE was also followed using COD. Abiotic control incubations contained heated-killed cells, while others included metyrapone (inhibitor of cytochrome P-450) or methimazole (inhibitor of flavin-containing monooxygenase).

Bacterial cytochrome P-450 content was quantified from the CO-difference spectrum of post-30,000 g cell-free hommogenates.

Result: Strain E1 (tentatively identified as Comamonas testosteroni):
Complete degradation in 10 d, no lag
Degradation rate 80 mg/g cell protein/day
Protein concentration increased x2.5

Stain E2 (unidentified):
Complete degradation in 15 d.
Degradation rate 58 mg/g cell protein/day
Protein concentration increased x 3
COD analyses indicate complete degradation of ETBE

Abiotic controls indicated 3-4% of ETBE lost by volatilisation.

There was no accumulation of TBA with either strain.

Degradation of ETBE was strongly inhibited by 5 mM metyrapone (both strains, 13-18% decrease) but not methimazole. Reduced CO-difference spectra contained a major absorbance peak at 450 nm (consistent with presence of cytochrome P-450; specific content not reported).

Test substance: ETBE, purity not stated, Elf Aquitaine, France or Fluka France (no further details).

Conclusion: The results indicate that ETBE is utilised as sole carbon source (complete degradation) by the two bacterial strains isolated from a gasoline contaminated site, possibly by a cytochrome P-450-dependent pathway.

Reliability: (2) valid with restrictions
Study available for review. Experimental investigation, acceptable for assessment.

02-JAN-2004 (47)

Type: aerobic

Year: 1997
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: Several propane-oxidizing bacteria were examined for their ability to degrade ETBE.

A pure culture of Mycobacterium vaccae JOB5 (ATCC 292678) was capable of degrading 80-100% ETBE (30 mg/l; analysis by GC) in 24 hr after aerobic incubation at 28 degrees C; degradation by other microorganisms was highly variable (0-30%).
Test substance: ETBE, 99%, Aldrich Chemical Co., Milwaukee, WI.
Reliability: (2) valid with restrictions
Study available for review. Experimental investigation, acceptable for assessment.
01-NOV-2003 (73)

Type: anaerobic
Inoculum: other: from methanogenic anoxic aquifer
Concentration: 70 mg/l related to Test substance
Result: under test conditions no biodegradation observed

Year: 1993
GLP: no data

Remark: ETBE was incubated with sediment and groundwater collected from a methanogenic portion of a shallow anoxic aquifer polluted by municipal landfill leachate.

Slurries of 50 g sediment and 75 ml groundwater were placed in sterile 160ml bottles together 70mg/l ETBE. Bottles (triplicate incubations) were sealed, incubated at room temperature, in the dark.

EBTE concentration was measured by GC.

No degradation was detected following 168 d.
Test substance: No details available.
Reliability: (2) valid with restrictions
Study available for review. Experimental investigation, acceptable for assessment.
01-NOV-2003 (74)

Type: anaerobic
Inoculum: other: sediment and water from landfill leachate impacted aquifer
Result: under test conditions no biodegradation observed

Year: 1994
GLP: no data

Remark: Sediment and groundwater were collected from a landfill leachate impacted aquifer and from a gasoline polluted aquifer.

Anaerobic slurries containing 50g sediment in 75 ml water were incubated under sulphate-reducing conditions for 244 d, nitrate-reducing conditions for 85 d or methanogenic conditions for 90 d at degrees C, dark. The initial concentration of ETBE was 70 mg/l.

GC was used to following depletion of ETBE, HPLC for sulphate and nitrate depletion.

Under these condition, no anaerobic biodegradation was observed.

Test substance: No details available.
Reliability: (2) valid with restrictions
Study available for review. Experimental investigation, acceptable for assessment.

01-NOV-2003 (60)

Type: anaerobic
Inoculum: other: soil
Result: other: biodegradable by soil microcosms

Year: 1994
GLP: no data

Remark: Soil from various depths from 3 sites used in static soil/water microcosms in sealed test tubes (in triplicate, autoclaved control) at 20 degrees C, in the dark. The soils were characterised as to their anaerobic microbial populations, soil anions, moisture content, organic content, nitrogen availability, ammonium fixation rate and soil pH.

ETBE concentration approx. 80 mg/L, and degradation followed under denitrifying and methanogenic conditions by GC-FID for >250 days.

Graphical data indicated that ETBE degradation occurred with a lag of about 120 d under anoxic conditions in soil of low organic content and low pH (5.5):

	0 d	100 d	250 d	
Site 1	80	60	10	mg/l ETBE
Site 2	70	60	40	

The amount of TBA formed once degradation commenced was quite

variable, possibly indicating degradation of TBA.

No degradation was seen in organic rich soils.

Addition of nitrate and nutrients did not significantly increase degradation under denitrifying conditions.

Graphical data show that under methanogenic conditions, degradation of ETBE occurred only in oligotrophic soils with low organic matter and was maximal at approx. pH 5.5:

	0 d	100 d	250 d
Site 1	80	70	50 mg/l ETBE

Test substance: No details available.
Conclusion: ETBE was degraded slowly under anaerobic conditions by some soil microcosms included in this study.
Reliability: (2) valid with restrictions
Study available for review. Experimental investigation, acceptable for assessment.
01-NOV-2003 (83)

Remark: The product is highly volatile and has a very low biodegradability. Ecological data are not available, owing to the high volatility of the product which does not allow to carry out the test procedures.
Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
02-NOV-2003

3.6 BOD5, COD or BOD5/COD Ratio

-

3.7 Bioaccumulation

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3.8 Additional Remarks

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AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: semistatic
Species: Cyprinodon variegatus (Fish, estuary, marine)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
NOEC: = 2500 - measured/nominal
LC50: > 2500 - calculated

Method: EPA OTS 797.1400
Year: 1994
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: TEST ORGANISM
Juvenile sheepshead minnows were obtained from Aquatic Biosystems Inc., Co, and maintained under flow-through conditions in a 40 l glass tank prior to testing. At the conclusion of the test, the control fish had a mean wet weight of 0.34 g (loading rate 0.23 g/l) and a mean length of 28 mm.

DILUTION WATER
Natural seawater (pH 8.0) was adjusted with dechlorinated tap water to a salinity of 15-17 ppt during a 14 d acclimation period, and to 20 ppt during the test.
Other characteristics (measured):
Organochlorine pesticides <2 ug/l (limit of detection)
Organophosphorus pesticides <0.5 ug/l (limit of detection)
PCBs <1.0 ug/l (limit of detection)

ACCLIMATION PERIOD
The following conditions were recorded during a 14 d acclimation period:
Temperature = 21.0-22.3 degrees C
Salinity = 15-17 ppt
pH = 7.2-7.9
Dissolved oxygen = at least 7.1 mg/l
Fish were fed live brine shrimp and/or a commercial dry diet during acclimation.

SCREENING TEST
The screening test used exposure concentrations of 0, 1.0, 10 or 100 mg/l. It was terminated after 24 hr due to 100% survival at all tested concentrations.

DEFINITIVE TEST
Thirty sheepshead minnows were distributed between three replicate test vessels (20 l glass aquaria containing 15 l of test solution, depth approx. 18 cm) and exposed to a nominal concentration of 2500 mg/l ETBE. Twenty additional fish were assigned to two control tanks. The test medium was replaced with fresh solution after 24, 48 or 72 hr. A 16 hr light (8 hr

dark) cycle was maintained (15 minute transition between light and dark).

Dissolved oxygen, pH, salinity and temperature were recorded in each vessel before and after renewal of the medium, and also at 0 hr and 96 hr. Aeration was not required.

The mean number of survivors and occurrence of sub-lethal effects were assessed in the control and test vessels every 24 hr.

Reviewer's comment: Unlike the acute mysid study performed by this laboratory (see Section 4.2), the report does not state whether or not the vessels were covered to prevent evaporative loss of ETBE.

STATISTICAL METHODS
Since no mortality was recorded, no EC50 could be derived form the data. The NOEC was defined as the concentration of ETBE that did not cause any sublethal effects and allowed at least 90% survival of the test organisms.

Result: ENVIRONMENTAL CONDITIONS (range for all vessels)
Salinity = 20 ppt
pH = 7.5-8.0
Dissolved oxygen = 5.6-8.4 mg/l
Temperature = 21.3-22.5 degrees C

SURVIVAL
There was no mortality in the control or test vessels.

SUBLETHAL EFFECTS
There were no sublethal effects in the control or test vessels.

MEDIAN LETHAL CONCENTRATIONS
The EC50 was >2500 mg/l at all time points (ie 24, 48, 72 and 96 hr).

NOEC CONCENTRATIONS
The NOEC was 2500 mg/l at all time points.

Test substance: ETBE, 95.3% pure, lot no. 12/6/93, supplied by ARCO Chemical Company, PA, USA.

Conclusion: Under the conditions of this test, the 96 hr EC50 of ETBE in the sheepshead minnow (Cyprinodon variegatus) was greater than 2500 mg/l. The NOEC was 2500 mg/l, the highest concentration tested.

Reliability: (2) valid with restrictions
Study available for review. GLP compliant guideline investigation. Test solution renewed every 24 hr, however uncertainty over potential evaporative loss limits overall reliability of result.

02-NOV-2003 (10)

Type: other: No data available
Species: other: No data available
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
LC50: > 100 -

GLP: no data

Remark: No toxicity was observed at 100 mg/l.
Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test substance: ETBE
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
15-JAN-2004 (23)

4.2 Acute Toxicity to Aquatic Invertebrates

Type: static
Species: Daphnia magna (Crustacea)
Exposure period: 48 hour(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: = 56 -
EC50: = 110 -

Method: OECD Guide-line 202
Year: 2003
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: TEST ORGANISM
First instar daphnia from a single in-house source were maintained in 2-3 litre polypropylene beakers at a density of 30-80 daphnids/vessel prior to testing.

DILUTION WATER
Reconstituted water (containing analytical grade CaCl2, MgSo4, NaHCO3 and KCL), hardness 250 mg/l, pH 7.8, conductivity <5 uS/cm, aerated to a dissolved oxygen concentration of approx. half saturation value.

DEFINITIVE TEST
Based on results from a screening test (using concentrations of 0.1-1000 mg/l), 20 daphnia were divided between two 250 ml vessels containing ETBE at nominal concentrations of 0 (water), 10, 18, 32, 56, 100, 180, 320, 560 or 1000 mg/l. The vessels (300 ml conical flasks) were completely filled and stoppered to minimise evaporative losses of ETBE. In order to reduce losses due to volatilisation, the test vessels remained sealed throught the 48 hr of the study. The test was performed at 21 degrees C, with a 16 hr light / 8 hr dark photoperiod,

with 20 min dusk/dawn transition periods.

The mean number of survivors and occurrence of sub-lethal effects were assessed in all vessels every 24 hr.

Temperature was recorded daily in the control vessels. Dissolved oxygen and pH were measured for each vessel at the start and the end of the study.

VERIFICATION OF CONCENTRATION
Chemical analysis (GC) was performed over the entire concentration range (ie all vessels) at 0 hr and 48 hr. Duplicate samples were stored frozen (-20 degrees C) for reanalysis, if required.

VALIDATION CRITERIA
The test was considered invalid if 10% or more of the control organisms were immobilised or stressed after 48 hr, or if the oxygen concentrtaion of the control vessels fell below 60% of air saturation values at the end of the test.

STATISTICAL METHODS
The 24 and 48 hr EC50 and 95% confidence limits were calculated using standard statistical methods (Probit analysis using ToxCalc computer package). The NOEC was defined as the concentration of ETBE that did not cause any significant immobilisation or sublethal effects.

Result:

ANALYSED CONCENTRATIONS
Analysis at 0 hr and 48 hr showed that measured test concentrations were generally 81-96% of nominal, with the exception of the 18 mg/l test concentration which showed measured concentrations of 71% and 73% at 0 hr and 48 hr, respectively. As this concentration was below the NOEC (see below), these findings were not considered by the Study Directors to have affected the outcome of the study. (A low measured concentration of 63% nominal was also reported for the 32 mg/l solution at 0 hr, however reanalysis of a frozen sample gave a value of 84% nominal.)

ENVIRONMENTAL CONDITIONS
pH = 7.9-8.0
Dissolved oxygen = 8.3 to 8.2-8.4 mg/l (0 hr and 48 hr, respectively)
Temperature = 21.2-21.6 degrees C

SURVIVAL
There was no mortality or immobilisation in the controls or test cultures exposed to 10-180 mg/l for 24 hr, or in the controls or test cultures exposed to 10-56 mg/l for 48 hr.

The percentage immobilisation at study termination (48 hr) was as follows:
Control: 0/20 (0%)
10 mg/l: 0/20

18 mg/l: 0/20
32 mg/l: 0/20
56 mg/l: 0/20
100 mg/l: 13/20 (65%)
180 mg/l: 16/20 (80%)
320 mg/l: 19/20 (95%)
560 mg/l: 20/20 (100%)
1000 mg/l: 20/20 (100%)

MEDIAN LETHAL CONCENTRATIONS (nominal)
24 hr: 440 mg/l (95% CI = 370-520)
48 hr: 110 mg/l (95% CI = 91-130)

NOEC CONCENTRATION (nominal)
48 hr: 56 mg/l

Test substance: ETBE, CAS No. 637-92-3, 99.97%% pure, Total France.
Conclusion: Under the conditions of the test, the 48 hr EC50 for ETBE in Daphnia magna was 110 mg/l and the NOEC 56 mg/l.
Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, well documented methods and results, raw data available, acceptable for assessment.
23-DEC-2003 (80)

Type: semistatic
Species: Mysidopsis bahia (Crustacea)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:**
NOEC: = 25 - measured/nominal
EC50: = 37 - calculated

Method: EPA OTS 797.1930
Year: 1994
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: TEST ORGANISM
Juvenile mysids from a single in-house source (original culture from Aquatic Biosystems Inc., Co) were maintained under flow-through conditions in a 38 l glass tank prior to testing. At the conclusion of the test, the control mysids had a mean wet weight of 0.3 mg (loading rate 0.003 g/l).

DILUTION WATER
Natural seawater (pH 8.0-8.1) was adjusted with dechlorinated tapwater to a salinity of 15-17 ppt during a 14 d acclimation period, and to 20 ppt during the test.
Other characteristics (measured):
Organochlorine pesticides <2 ug/l (limit of detection)
Organophosphorus pesticides <0.5 ug/l (limit of detection)
PCBs <1.0 ug/l (limit of detection)

ACCLIMATION PERIOD
The following conditions were recorded during a 14 d

acclimation period:
Temperature = 24.4-25.8 degrees C
Salinity = 15-17 ppt
pH = 7.7-8.0
Dissolved oxygen = at least 7.4 mg/l

SCREENING TESTS
Three screening tests were performed:
1. Mysids <24 hr old, 0 or 2500 mg/l ETBE, 24 hr exposure;
2. Mysids <24 hr old, 0, 25, 250 or 2500 mg/l ETBE, 24 hr exposure;
3. Mysids <24 hr old or 5 d old, 0, 1, 10, 100 or 1000 mg/l, 96 hr exposure period

DEFINITIVE TEST
Twenty mysids (<24 hr old) were distributed between two replicate vessels (3.8 l glass jars containing 1 l of test solution, depth approx. 6 cm) and exposed to nominal concentrations of 0, 15, 25, 40, 60 or 100 mg/l ETBE. The test vessels were tightly covered (method not specified) to prevent evaporative losses. The test medium was replaced with fresh solution after 24, 48 or 72 hr. A 16 hr light (8 hr dark) cycle was maintained.

Dissolved oxygen, pH, salinity and temperature were recorded in each vessel before and after renewal of the medium, and also at 0 hr and 96 hr. Aeration was not required.

The mean number of survivors and occurrence of sub-lethal effects were assessed in all vessels every 24 hr.

STATISTICAL METHODS
The EC50 and slope of the dose-response curve was determined using standard statistical methods (Probit analysis). The NOEC was defined as the concentration of ETBE that did not cause any sublethal effects and allowed at least 90% survival of the test organisms.

Result: ENVIRONMENTAL CONDITIONS (range for all vessels)
Salinity = 20 ppt
pH = 7.9-8.1
Dissolved oxygen = 6.6-8.3 mg/l (mean 7.6 mg/l)
Temperature = 23.1-25.8 degrees C (mean 25.2)

SURVIVAL
There was no mortality in the control cultures. Immobilisation of a single organism was seen in one replicate from the 15 or 25 mg/l exposures at 72 or 96 hr. Immobilisation was noted at 48 hr in the 40 mg/l vessels, and at 24 hr in the 60 or 100 mg/l vessels. Survival at study termination (96 hr) was as follows:
Control : 20/20
15 mg/l: 19/20
25 mg/l: 18/20
40 mg/l: 9/20

60 mg/l: 2/20
100 mg/l: 0/20

SUBLETHAL EFFECTS
No sublethal effects were noted in cultures exposed to 0-40 mg/l ETBE.

Sublethal effects were observed in the 60 mg/l culture (erratic swimming at 48 and 72 hr; erratic swimming and lethargy in survivors at 96 hr).

No sublethal effects were noted at 0 or 24 hr in cultures exposed to 100 mg/l ETBE; full immobilisation thereafter.

MEDIAN LETHAL CONCENTRATIONS
24 hr: 96 mg/l (95% CI = 80-140)
48 hr: 60 mg/l (95% CI = 54-68)
72 hr: 41 mg/l (95% CI = 36-47)
96 hr: 37 mg/l (95% CI = 32-43)

NOEC CONCENTRATIONS
24 hr: 40 mg/l
48 hr: 25 mg/l
72 hr: 25 mg/l
96 hr: 25 mg/l

Test substance: ETBE, 95.3% pure, lot no. 12/6/93, supplied by ARCO Chemical Company, PA, USA.

Conclusion: Under the conditions of this test, the 96 hr EC50 of ETBE in the mysid (Mysidopsis bahia) was 37 mg/l and the NOEC 25 mg/l.

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.

05-OCT-2003 (9)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species: other algae: Pseudokirchneriella subcapitata
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: mg/l **Analytical monitoring:** yes
NOEC: = 7.5 -
EC50: = 1100 -

Method: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year: 2003
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: TEST ORGANISM
Pseudokirchneriella subcapitata (CCAP 276/20; formerly Scenedesmus subspicatus) was cultured for 4-5 d at 21 degrees C (cell density approx. 2x10^4 to 2x10^5 cell/ml) prior to use. (Pre-culture conditions gave an algal suspension in log phase growth characterised by a cell density of 4.03 x 10^6 cells per ml, which was diluted to a cell density of 8.66 x 10^5 cells per ml prior to use.)

TEST MEDIUM
The culture medium (mineral salts) was prepared using reverse osmosis purified deionised water (Elga Optima 15+), the pH adjusted to 7.0 ± 0.1 with 0.1N NaOH or HCl and sterilised using a 0.2 µm membrane filter. Sodium bicarbonate (500 mg/l) was added after preparation to provide a source of carbon for algal growth in the sealed test vessels.

DEFINITIVE TEST
Based on results from two screening tests (using ETBE concentrations of 0, 0.1, 1.0, 10, 100, 100 mg/l and 0, 9.1, 91, 910, 9100 mg/l) the definitive test was conducted using ETBE concentrations of 0, 9.2, 29, 92, 290, 920, 2900 or 9100 mg/l (3 replicates per concentration). This concentration range was selected based on recommendations contained in the draft revised OECD TG201 (July 2002), and was designed to result in 0-90% inhibition of growth.

Following the recommendations of Mayer et al (2000; Environ. Toxicol Chem 19, 2551), a nominal cell density of 3 x 10^3 cells per ml was used at initiation of the test. The flasks (250 ml, completely filled with test medium) were sealed (ground glass stoppers) and incubated at 24 degrees C under continuous illumination (approx. 7000 lux) for 72 hours with shaking (150 rpm). Samples were removed at 0, 24, 48 and 72 hours for the determination of cell densitity (haemocytometer).

The pH of each control and test flask was determined at initiation of the test and after 72 hours exposure. The pH was measured using a WTW pH 320 pH meter. The temperature

within the incubator was recorded daily.

Since some loss of test substance may have occurred when the vessels were openeded for cell sampling, additional flasks were incubated unopened alongside the definitive test vessels then analysed for ETBE concentration at 72 hours.

VERIFICATION OF CONCENTRATION
Chemical analysis (GC) was performed over the entire concentration range (ie all test vessels) at 0 hr and 72 hr, and also on the unopened duplicate vessels at 72 hr only. Duplicate samples were stored frozen (-20 degrees C) for reanalysis, if required.

ANALYSIS OF DATA
The area under the curve was used as a measure of cell growth, and the percentage inhibition obtained by comparison of the AUC for the control and test vessels. The average maximum growth rate for each culture was also calculated from the linear section of the growth curve.

STATISTICAL METHODS
One way analysis of variance incorporating Bartlett's test for homogeneity and Dunnett's multiple comparison procedure was used applied to the AUC data to determine any statistically significant differences between the test and control groups.

Result:

RANGE FINDING TEST
Results from the initial range finding test showed no significant effect on growth at 1 or 10 mg/l, however growth was reduced at 100 and 1000 mg/l. In the second range-finder, growth reduction was observed at all concentrations tested.

DEFINITIVE TEST
The percentage inhibition values for biomass and growth rate were plotted against test concetration, and the following results obtained:
EbC50 (biomass, 72 hr) = 37 mg/l; 95% CI 28-49 mg/l;
ErC50 (growth, 0-72 hr) = 1200 mg/l, 95% CI 890-1600 mg/l
NOEC = 9.2 mg/l

Since analytical measurements showed a slight decline in concentration of ETBE in some of the test vessels over the 72 hr of the study (see below), hence the EC50 values were recalcalculated based upon the geometric mean of the analysed concentration. The following results were obtained:
EbC50 (biomass, 72 hr) = 32 mg/l; 95% CI 22-46 mg/l;
ErC50 (growth, 0-72 hr) = 1100 mg/l, 95% CI 810-1500 mg/l
NOEC = 7.5 mg/l

OBSERVATIONS and PHYSICO-CHEMICAL MEASUREMENTS
Cell density in the control cultures increased by a factor of 386 during the definitive test (exceeds Guideline requirement for a 16-fold increase over 72 hr):
Density at 0 hr: 3.33 X 10^3 cells/ml

Density at 72 hr: 1.29 x 10^6 cells/ml

The pH values of the control cultures increased from pH 7.7 at 0 hr to pH 7.9-8.1 at 72 hr. This was less than 1.5 units and therefore within the limits given in OECD Guideline 201. The pH of the test vessels was 7.7-7.8 at 0 hr and 7.5-7.9 at 72 hr. In all cases, the pH shift was less than 1.5 units.

VERIFICATION OF TEST CONCENTRATIONS
Analysis of test preparations at 0 hr demonstrated concentrations were 91-116% of nominal. After 72 hr, there was a slight decline in measured concentration to 72-94% of nominal. Analysis of samples from unopened replicate vessels returned measured concentrations that were 77-103% of nominal at 72 hr. This slight decline was considered by the Study Director to reflect possible adsoption of ETBE to algal cells and/or losses due to volatility when sampling for chemical analysis.

Given this slight decline in measured test concentration, the STudy Director considered it was justificable to base the EC50 and NOEC values on geometric mean measured concentrations.

Nominal (mg/l)	Analysed 0 hr (mg/l)	Analysed 72 hr (mg/l)	Geometric mean (mg/l)
9.2	8.4	6.7	7.5
29	27	23	25
92	91	66	78
290	336	274	303
920	900	748	820
2900	3300	2470	2850
9200	10300	7900	9020

INTERPRETATION
A difference between the EbC50 and ErC50 values is not uncommon in algal inhibition tests since small changes in growth rate result in large changes in biomass. Hence the EbC50 is often lower than the ErC50. Current regulatory advice (European Chemicals Bureau (2003) Technical Guidance Document Part II, Chapter 3, Environmental Risk Assessment; OECD (2002) draft revised Guideline 201, July 2002) notes that for both risk assessment and classification purposes the ErC50 value should be used.

Test substance: ETBE, CAS No. 637-92-3, 99.97%% pure, Total France.

Conclusion: Under the conditions of the test, the 72 hr ErC50 for ETBE in Pseudokirchneriella subcapitata was 1200 mg/l (NOEC 9.2 mg/l) based on nominal concentrations, while calculations based on geometric mean measured concentrations gave an ErC50 of 1100 mg/l (NOEC 7.5 mg/l).

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, well documented methods and results, raw data available, acceptable for assessment.

23-DEC-2003 (57)

4.4 Toxicity to Microorganisms e.g. Bacteria

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4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

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4.5.2 Chronic Toxicity to Aquatic Invertebrates

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TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

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4.6.2 Toxicity to Terrestrial Plants

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4.6.3 Toxicity to Soil Dwelling Organisms

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4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

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4.7 Biological Effects Monitoring

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4.8 Biotransformation and Kinetics

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4.9 Additional Remarks

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5.0 Toxicokinetics, Metabolism and Distribution

In Vitro/in vivo: In vitro
Type: Metabolism
Species: mouse

Year: 1999
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method:

ANIMALS AND MAINTENANCE
Male and female homozygous 2E-/- mice (deficient in cytochrome P-450 2E1) were obtained from the National Cancer Institute (Bethesda, MD) and used to establish an in-house breeding colony.

Female C57BL/6N and female 129/Sv mice were purchased from Taconic Farms (Germantown, NY). [Comment: these are the parental lineage strains of the 2E- knock-out mice, and contain intact functional 2E1 alleles.]

Diet: Purina Laboratory Chow, ad libitum
Water: unspecified, ad libitum

PREPARATION OF MICROSOMAL FRACTION
Animals were killed by decapitation, livers removed and stored frozen at -80 degrees C. After thawing, livers were homogenised (Ultra-Turrax polytron) in Tris-HCl buffer (50 mM, 1.15% KCl, pH 7.4). The microsomal fraction was isolated by differential centrifugation. Protein content was determined by the method of Lowry et al.

NDMA DEMETHYLATION ASSAY
N-demethylation of 14C-N-nitrosodimethylamine (an assay that is highly specific for cytochrome P-450 2E1) was determined by following release of 14C-formaldehyde (Hong et al., 1989, Cancer Res., 49, 2973-2979).

METABOLISM OF ETBE
The incubation mixture (sealed vial, 37 degrees C) contained a NADPH regenerating system, microsomal protein (1.5 mg/ml) and ETBE (0.1 mM) in a total volume of 0.4 ml Tris-HCl (50 mM, pH 7.4). The reaction was halted after 30 min by addition of 25% zinc sulphate (50ul) and saturated barium hydroxide (50 ul). Formation of tert-butanol (TBA) was determined by headspace GC analysis.

STATISTICAL METHODS
Values are presented as mean and SD. Data analysed by ANOVA followed by Newman-Keuls test.

Result:

NDMA DEMETHYLASE ACTIVITY
No NDMA demethylase activity was detectable in hepatic

microsomes from 2E-/- knock-out mice. In contrast microsomal NDMA demethylase activity was present in C57BL/6N (99.8 pmol/min/mg protein) and 129/Sv (100.2 pmol/min/mg protein) mice.

HEPATIC MICROSOMAL METABOLISM OF ETBE
No metabolism of ETBE was found in hepatic cytosol (data not presented by Authors).

The following rates of metabolism (conversion of ETBE to TBA) were recorded for the three strains of mouse:
C57B/6N (female): 0.70 +/- 0.12 nmol/min/mg protein
129/Sv (female): 0.66 +/- 0.14
2E1 -/- (female): 0.51 +/- 0.24
(Mean and SD for 4 or 5 replicate incubations)

Comparable rates of hepatic microsomal conversion of ETBE to TBA were present in males and females of different ages:
4 month old
- males: 0.45 +/- 0.11 nmol/min/mg protein
- females: 0.11 +/- 0.04

8 month old
- males: 0.68 +/- 0.04
-females: 0.40 +/- 0.19

(Mean and SD for triplicate incubations)

Test substance: ETBE, c.99%, Aldrich Chemical Co., Milwaukee, WI.

Conclusion: Results from this in vitro investigation indicate that cytochrome P-450 2E1 plays a minor role in conversion of ETBE to TBA by mouse hepatic microsomal enzymes.

Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.

27-OCT-2003 (34) (36)

In Vitro/in vivo: In vitro
Type: Metabolism
Species: rat

Year: 1997
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS AND MAINTENANCE
Male SD rats (9 wk old) were obtained from Taconic Farms (Germantown, NY) and acclimatised for 1 wk.

Diet: Lab Diet 5012 (PMI Feeds Inc., St Louis, MO), ad libitum
Water: tap water, ad libitum

PREPARATION OF MICROSOMAL FRACTIONS
Animals were killed by decapitation, and the nasal mucosa (including olfactory and respiratory epithelium) collected and stored frozen at -80 degrees C (Hong et al., 1991, Cancer Res, 51, 1509-1514). The liver, lungs and olfactory bulb were also immediately removed and stored at -80 degrees. After thawing, tissues were homogenised (Ultra-Turra polytron) in Tris-HCl buffer (50 mM, 1.15% KCl, pH 7.4). The microsomal fraction was isolated by differential centrifugation. Protein content was determined by the method of Lowry et al.

METABOLISM OF ETBE
The incubation mixture (sealed vial, 37 degrees C) contained a NADPH regenerating system, microsomal protein (1.5 mg/ml except for nasal mucosa = 0.25 mg/ml) and ETBE (0.25 mM) in a total volume of 0.4 ml Tris-HCl (50 mM, pH 7.4). The reaction was halted after 30 min by addition of 25% zinc sulphate (50ul) and saturated barium hydroxide (50 ul).

Some incubations were conducted in the absence of cofactor (NADPH) or in presence of carbon monoxide. Enzyme kinetics were investigated using 0.01-2.5 mM ETBE. The influence of TAME (tert-amyl methyl ether, a structurally-related substance) on metabolism of ETBE by olfactory mucosal microsomes was also investigated.

Formation of tert-butanol (TBA) was determined by headspace GC analysis.

STATISTICAL METHODS
Values are presented as mean and SD.

Result: METABOLISM OF ETBE (0.25 mM) BY MICROSOMAL FRACTIONS FROM DIFFERENT TISSUES
The following specific activities were obtained:

Nasal mucosa
- olfactory: 8.78 +/- 0.55 nmol TBA/min/mg protein
- respiratory: 0.95 +/- 0.18
Liver: 0.24 +/- 0.01
Lung: not detected
Kidney: not detected
Olfactory bulb: not detected
(limit of detection not reported)

Carbon monoxide inhibited conversion of ETBE to TBA by rat olfactory mucosal microsomes; omission of NAPDH eliminated metabolism:
Control: 9.5 nmol TBA/min/mg protein
plus CO: 1.2
minus NADPH: no activity detected

KINETIC STUDIES
Metabolism of ETBE by rat olfatory microsomes exhibited Michaelis-Menton kinetics over the concentration range 0.01 to

2.5 mM ETBE:
Km = 125 uM
Vmax = 11.7 nmol TBA/min/mg

INFLUENCE OF TAME (0.1-0.5 mM) ON METABOLISM OF ETBE (0.25 mM) BY RAT OLFACTORY MICROSOMES
TAME inhibited microsomal metabolism of ETBE:
Control: 9.2 nmol TBA/min/mg protein
plus 0.1 mM TAME: 4.8 (48% inhibition)
plus 0.25 mM TAME: 3.7 (60% inhibition)
plus 0.5 mM: 2.2 (76% inhibition)

Test substance: ETBE, c.99%, Aldrich Chemical Co., Milwaukee, WI.

Conclusion: Results from these studies demonstrate that cytochrome P-450 dependent metabolism of ETBE was approx. 36 fold greater in rat nasal olfactory epithelium than in rat liver.

Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.

30-NOV-2003 (35) (36)

In Vitro/in vivo: In vitro
Type: Metabolism
Species: rat

Year: 1998
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: MICROSOMES
Liver microsomal fractions were prepared from male Sprague-Dawley rats (6-8 wk old) pretreated as follows:
- ETBE in corn oil administered i.p. (dose volume not specified) at dose levels of 200 and 400 mg/kg for 4 days;
- ETBE dosed orally at 2 mL/kg in 50% corn oil solution for 2 days;
- phenobarbitone i.p. at 80 mg/kg in saline for 3 days;
- B-naphthoflavone i.p. at 40 mg/kg in corn oil for 3 days;
- dexamethasone at 50 mg/kg in corn oil for 4 days;
- pyrazole at 200 mg/kg in saline for 4 days;
- t-butyl alcohol (TBA) 200 or 400 mg/kg for 4 days.

The washed microsomal pellets were resuspended in 100 mM phosphate buffer, 1 mM EDTA (pH 7.4) and stored at -80°C.

PURIFIED ENZYME STUDIES
CYP P450B1 and NADPH-P450-reductase (following phenobarbitone pretreatment), P4502E1 (after pyrazole pretreatment) and P4502C11 from control animals were isolated and purified from rat liver according to established published methods. The purified enzymes were reconstituted in a system comprising 0.1 nmol purified P450, 0.3 nmol of P450 reductase, 30 mg of

dilauroylphosphatidylcholine together with substrate in 1 mL of 0.1 mM phosphate buffer, pH 7.4. The reaction was initiated by the addition of NADPH (1 mM).

ANTIBODY STUDIES
Polyclonal antibodies (rabbit anti-rat P450B1 and anti-P4502E1) were obtained from Oxygene (Dallas, TX, USA). Goat anti-rabbit IgG were purchased from Dako (Copenhagen, Denmark). Anti-P4502E1 IgG (shown by Western blotting to recognize the same antigen as the commercially prepared P4502E1 polyclonal antibodies purchased from Oxygene) was prepared from female New Zealand white rabbits.

ENZYME ASSAYS
- aniline hydroxylase was determined by quantifying formation of p-aminophenol
- benzphetamine demethylase (BzD) and erythromycin demethylase (ErD) activities from formation of formaldehyde
- ethoxyresorufin-O-deethylase (EROD) and PROD activities were determined by measuring formation of resorufin
- para-nitrophenol hydroxylase (pNPH) activity followed formation of 4-aminocatechol
- testosterone hydroxylase activity was measured using an HPLC method
All activities were determined according to published methods).

METABOLISM AND KINETIC STUDIES
The extent of ETBE deethylation was followed from production of acetaldehyde using the semicarbazide/semicarbazone method, and quantified by HPLC (undertaken according to published methods). Concentrations in the range 0.05 to 20 mM were used to assess microsomal kinetic constants (Km and Vmax).

INHIBITION STUDIES
Specific chemical P450 inhibitors were added to microsomal incubations to assess the significance of specific P450 isozymes in ETBE metabolism:
- triacetyloleandromycin (TAO) for P4503A4
- 4-methyl pyrazole (4-MP) for P4502E1
- metapyrone (MET) for P4502B

WESTERN BLOT ANALYSIS
Hepatic microsomes from control and ETBE-treated rats were analysed by Western blotting using polyclonal antibodies against P450B1/2 and 2E1.

STATISTICAL METHODS
Students t test was used to compare activity present in various microsomal fractions etc (no further details).

Remark: Reviewers comment: The authors concluded that since ETBE induced P4502B1 only when dosed orally at high levels (2 ml/kg bw), it was unlikely that low environmental exposures would

impact ETBE metabolism in vivo. The reliability of this conclusion in the absence of appropriate studies (prolonged low level inhalation exposure) is not certain to this reviewer.

Result: EFFECTS OF ETBE AND TBA ADMINISTRATION ON P450 ACTIVITIES
ETBE at doses of 200 or 400 mg/kg (i.p.) for 4 days had no effect on total P450, BzD, ErD, pNPH, PROD or EROD. PROD and pNPH were increased (approx. 7-fold and approx. 2-fold, respectively) after pretreatment with 2 mL/kg/day for 3 days.

PROD, EROD, ErD, and pNPH activities were used as selective markers for P4502B1/2, 1A1/2, 3A1/2 and 2E1 respectively.

WESTERN BLOT ANALYSIS
ETBE (dosed orally but no i.p.) at 2mL/kg appeared to induce both P4502B1 and 2E1.

KINETIC PARAMETERS
ETBE was metabolized in control and induced microsomal enzymes to actealdehyde. This reaction had an absolute requirement for molecular oxygen and NADPH, while acetaldehyde production was inhibited by approx. 80% by carbon monoxide.

Production of acetaldehyde followed simple Michaelis-Menten kinetics and was linear up to 30 min in the presence of 1 mg microsomal protein. Only microsomes from PB induced rats showed enhanced rate of acetaldehyde metabolism.

Purified CYP2B1 catalysed acetaldehyde production in a reconstituted system, with a high turnover number and a Km similar for control or induced microsomes. 2E1, 2C11 and 1A1 had modest turnover and the authors suggest that P4502B1 may be the major contributor (in rat) to ETBE metabolism in vivo.

INHIBITION STUDIES
TAO (P4503A4 inhibitor) did not diminish metabolism of ETBE in microsomes from PB and PYR induced rats. Met (P4502B inhibitor) caused ~60-75% inhibition but only in PB induced microsomes. 4-MP (P4502E1 inhibitor) had little or no effect on ETBE metabolism.

Immuno-inhibition studies using PYR-induced microsomes and anti-P4502E1 IgG did not inhibit ETBE metabolism.

Testosterone (0.1 mM) inhibited metabolism of ETBE by 75% in (untreated) female rat microsomes indicating that P4502A1 may be important in female rats.

Test substance: ETBE, purity not stated, Fluka, Buchs, Switzerland.

Conclusion: CYP2B1 and 2E1 were induced in rat hepatic microsomes following oral (but not i.p.) treatment with ETBE at 2 ml/kg bw, with an increase in immunodetectable 2B1 and 2E1 apoproteins in these fractions. Following treatment with other inducing agents, only PB increased metabolic capacity compared to controls. Reconstituted purified 2B1 showed

higher turnover than 2E1, 2C11 and 1A1. The authors conclude that 2B1 has a prominent role in ETBE metabolism and the other isoforms a secondary role. Purified P4502A1 was not available for study but investigations in female rat with testosterone indicated that P450 isoforms belonging to the 2A subfamily might be important in rat.

Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.

30-NOV-2003 (77)

In Vitro/in vivo: In vitro
Type: Metabolism
Species: human

Year: 1997
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: EXPRESSION OF HUMAN CYTOCHROME P-450 ENZYMES
Human CYP2A6 and CYP2E1 were obtained by expressing the corresponding cDNAs in a baculovirus expression system (Pattern and Koch, 1995, Arch Biochem Biophys, 317, 504-513). Sf9 insect cells were infected simultaneously either with the CYP2A6 and P-450 reductase recombinant viruses or with the CYP2E1 and reductase recombinant viruses (no further details provided). A microsomal fraction was prepared by sonication of the infected cells followed by centrifugations at 40,000 rpm. P450 content and P450 reductase activity were determined as described by Patten and Koch (see earlier record).

METABOLISM OF ETBE
The incubation mixture (sealed vial, 37 degrees C) contained a NADPH regenerating system, microsomal protein (1.5 mg/ml) and ETBE (0.1 mM) in a total volume of 0.4 ml Tris-HCl (50 mM, pH 7.4). The reaction was halted after 30 min by addition of 25% zinc sulphate (50ul) and saturated barium hydroxide (50 ul). Formation of tert-butanol (TBA) was determined by headspace GC analysis.

STATISTICAL METHODS
Values are presented as mean and SD.

Result: Metabolism of ETBE to TBA by human CYP2A6 was approx. 17-fold greater than metabolism of ETBE by human CYP2E1:

CYP2A6: 13.6 nmol TBA formed/min/nmol P450
CYP2E1: 0.8 nmol TBA formed/min/nmol P450

Test substance: No details available.
Conclusion: The results demonstrated that cloned human CYP2A6 is more active than cloned human CYP2E1 in metabolising ETBE to tert-butanol in vitro.

Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.
27-OCT-2003 (37)

In Vitro/in vivo: In vitro
Type: Metabolism
Species: human

Year: 2001
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: LIVER SAMPLES AND PREPARATION OF MICROSOMAL FRACTION
Liver samples were obtained from 11 adult donors (9 male, 2 female, age 41+/- 11 yr) at onset of brain death, immediately frozen on dry ice then stored in liquid nitrogen. Microsomal fractions were prepared (Berthou et al., 1989, Xenobiotica, 19, 401-417) and stored at -80 degrees C until use.

METABOLISM OF ETBE
The incubation mixture (sealed vial, 37 degrees C) contained NADPH, microsomal protein (1.2 mg/ml) and ETBE (0.5 or 10 mM) in a total volume of 0.5 ml Tris-HCl (50 mM, pH 7.4). The reaction was halted after 30 min by addition of 0.3M perchloric acid (125 ul) for HPLC analysis or zinc sulphate (200 ul; concentration unspecified) and saturated barium hydroxide (200 ul) for GC analysis. Blanks were run without NADPH.

Michaelis-Menten kinetic parameters for ETBE metabolism were determined by following formaldehyde formation in incubations containing 0.01-20 mM ETBE and microsomal fractions from 3 human donors.

METABOLISM OF ETBE BY HETEROLOGOUSLY EXPRESSED CYTOCHROME P450
Heterologously-expressed human CYP proteins were purchased from Gentest Corp. (Woburn, MA, USA). The incubation (0.5 ml) contained CYP2A6, CYP2B6, CYP2E1 or CYP3A4 (40, 57, 72 or 17 pmol, respectively), NADPH-P450 reductase (concentration unspecified), NADPH (concentration unspecified) and ETBE (0.5 or 10 mM).

ANALYSIS OF METABOLITES
Formation of tert-butanol (TBA) was quantified by GC, and deethylation of ETBE to formaldehyde followed by HPLC (after reaction of formaldehyde with 2,4-dinitrophenylhydrazone (DNP), crotonaldehyde-DNP internal standard).

OTHER CYTOCHROME P450 DEPENDENT ACTIVITIES
Methoxyresorufin O-demethylase (MROD; marker for CYP1A2

activity), ethoxyresorufin O-deethylase (EROD; CYP1A1), 4-nitrophenol hydroxylation (4-NP; CYP2E1),nifedipine dehydrogenation (NIF; CYP3A4) and coumarin 7-hydroxylation (7-OH; CYP2A6) were determined using standard methods.

INHIBITION STUDIES
Hepatic microsomal metabolism of ETBE (0.5 or 3 mM) was investigated in the presence of 8-methoxypsoralen (8-MOP; 0.02 mM; selective inhibitor of CYP2A6), diethyldithiocarbamate (DEDTC; 0.3 mM; CYP2E1) or ketoconazole (5 uM; CYP3A4).

STATISTICAL METHODS
Least squares regression was used to determine correlation coefficients. t values for the null hypothesis of no linear association were calculated, and found to be equivalent to r = 0.7 for P<0.02.

Result:

METABOLISM OF ETBE
Metabolism of ETBE by human microsomal fractions exhibited complex kinetics. An Eadie-Hostee plot (v versus v/[ETBE]) revealed biphasic kinetics, consistent with a 2-site model, with a Km1 of 0.11 mM and a Vmax1 of 0.14 nmol/min/mg protein, and a Km2 of 5.0 mM and a Vmax2 of 1.7 nmol/min/mg protein.

Results obtained for the 11 subjects showed an approx. 4-fold range in activity when metabolism was assessed using 0.5 or 10 mM ETBE (no quantitative data reported).

ENZYME CORRELATION STUDIES
Enzyme correlation studies showed that demethylation of ETBE correlated well with NIF and 7-OH activity:

Correlation at 0.5 mM ETBE:
EROD 0.14
MROD 0.25
4-NP 0.58
NIF 0.72*
7-OH 0.85**

Correlation at 10 mM ETBE:
EROD 0.41
MROD 0.65
4-NP 0.52
NIF 0.68
7-OH 0.88*

(* P<0.02; ** P<0.01)

INHIBITOR STUDIES
Inhibition studies demonstrated that ETBE metabolism was strongly inhibited by 8-MPO (inhibitor of CYP2A6) and DEDTC (CYP2E1), and less affected by Ketoconazole (CYP3A4):

Inhibitor = 8-MPO
0.5 mM ETBE: 55.4% inhibition

3 mM ETBE: 79.1% inhibition

Inhibitor = DEDTC
0.5 mM ETBE: 64.3% inhibition
3 mM ETBE: 83.4%

Inhibitor = Ketoconazole
0.5 mM ETBE: 20.9% inhibition
3 mM ETBE: 7.4% inhibition

METABOLISM OF ETBE BY HETEROLOGOUSLY EXPRESSED CYTOCHROME P450
CYP2A6 exhibited the greatest turnover number, and CYP2B6 the lowest, for ETBE:

CYP2A6
0.5 mM ETBE: 2.85 min-1
10 mM ETBE: 9.8 min-1

CYP2B6
0.5 mM ETBE: 0.28 min-1
10 mM ETBE: 3.2 min-1

CYP3A4
0.5 mM ETBE: 0.45 min-1
10 mM ETBE: 7.6 min-1

CYP2E1
0.5 mM ETBE: 1.7 min-1
10 mM ETBE: 5.6 min-1

Test substance: ETBE, 97%, Aldrich Chemical, Saint-Quentin-Fallavier, France.
Conclusion: Kinetic data, correlation studies, chemical inhibition and use of purified heterologously expressed cytochrome P-450 haemoproteins showed that CYP2A6 was the primary enzyme involved in the metabolism of ETBE. CYP3A4 and CYP2B6 were also found to be involved, whereas CYP2E1 was found to be less important.
Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.
30-NOV-2003 (50)

In Vitro/in vivo: In vitro
Type: Metabolism
Species: human

Year: 2001
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: METABOLISM OF ETBE BY HUMAN HEPATIC MICROSOMAL FRACTIONS

Liver samples were obtained from consenting liver cancer patients (age 37-80 yr). The samples were from tissue adjacent to the tumour, and were snap frozen in liquid nitrogen within 30 minutes of removal. The microsomal fraction was isolated by differential centrifugation.

EXPRESSION OF HUMAN CYTOCHROME P-450 ENZYMES
Metabolism of ETBE by human CYP2A6, CYP2E1, CYP2B6 and CYP1A2 expressed in the human AHH-1 TK+/- B-lymphoblastoid line (Gentest, Woburn, MA) was assessed in vitro. The report states that the cells contained very low levels of endogenous cytochrome P450 enzyme activity, and each cell line was engineered to consistently express a specific human CYP cDNA. The cells were stored frozen at -80 degrees C until use.

ENZYME CORRELATION STUDIES
The following substrate-specific cytochrome P-450 dependent enzyme were determined in 15 human hepatic microsomal samples (HepatoScreen kit, Human Biologics, Phoenix, AZ; no assay details provided):

7-ethoxy resorufin O-delakylation: marker for CYP1A2
coumarin hydroxylation: CYP2A6
S-mephenytoin N-demethylation: CYP2B6
tolbutamide hydroxylation: CYP2C9
S-mephenytoin hydroxylation: CYP2C19
dextromethorphan O-demethylation: CYP2D6
chlorzoxazone hydroxylation: CYP2E1
dextromethorphan N-demethylation: CYP3A4
testosterone oxidation: CYP3A4/5

Demethylation of ETBE by these 15 samples was also quantified.

METABOLISM OF ETBE
The incubation mixture (sealed vial, 37 degrees C) contained a NADPH regenerating system, expressed CYP proteins (1.5 mg/ml) or microsomal protein (250-375 ug/ml) and ETBE (0.1 mM) in a total volume of 0.4 ml Tris-HCl (50 mM, pH 7.4). The reaction was halted after 30 min by addition of 25% zinc sulphate (50ul) and saturated barium hydroxide (50 ul). Formation of tert-butanol (TBA) was determined by headspace GC analysis (detection limit 15 ng TBA).

STATISTICAL METHODS
Values are presented as mean and SD. Correlation analysis was performed using CA-Criketgraph III version 1.01 software (Computer Associates International, Islandia, NY).

Result: METABOLISM OF ETBE BY HEPATIC MICROSOMAL FRACTIONS
Metabolism of ETBE by human hepatic microsomal enzymes was variable: range 179 to 3,134 pmol formaldehyde formed/min/mg microsomal protein.
Comment: number of samples not specified; graphical data suggest that at least 10 were used.

METABOLISM OF ETBE BY HETEROLOGOUSLY EXPRESSED HUMAN CYTOCHROME P450 ENZYMES
CYP 2A6 expressed the greatest activity toward ETBE:

CYP2A6: 1.61 +/- 0.29 pmol TBA/min/pmol CYP
CYP2E1: 0.34 +/- 0.0
CYP2B6: 0.18 +/- 0.05
CYP1A2: 0.13 +/- 0.0
Note: the report states that activities for CYP1B1, CYP2C8, CYP2C9, CYP2C19 and CYP2D6 were not detectable in these samples. No results are presented.

ENZYME CORRELATION STUDIES
ETBE metabolism correlated most closely with expression of CYP2A6 and, to a lesser degree, with CYP2B6, CYP3A4 and CYP3A4/5 present in human hepatic microsomes:
CYP2A6: 0.95 (correlation coefficient)
CYP2E1: 0.19
CYP2B6: 0.69
CYP1A2: 0.01
CYP3A4: 0.71
CYP3A4/5: 0.64
CYP2C9: 0.28
CYP2C19: 0.18
CYP2D6: 0.01
There was no correlation with CYP1A2 or CYP2D6 activities.

Test substance: ETBE, >99%, Aldrich Chemical Co., Milwaukee, WI.

Conclusion: Metabolism of ETBE (O-demethylation) by human hepatic microsomal fractions exhibited a high degree of interindividual variation. This activity was associated with the expression of cytochrome P450 2A6 in human liver microsomes and engineered cell lines expressing human recombinant cDNA. Other isozymes (including CYP2E1, CYP2B6, CYP3A4 and CYP3A4/5) appeared of minor importance.

Reliability: (2) valid with restrictions
Study available for review. Briefly reported experimental investigation, acceptable for assessment.

30-NOV-2003 (33) (36)

In Vitro/in vivo: In vitro
Type: Toxicokinetics
Species: other: human PBTK model

Year: 1999
Test substance: as prescribed by 1.1 - 1.4

Method: PHYSIOLOGICALLY BASED TOXICOKINETIC MODEL
Organs were grouped together into compartments according to blood flow (lung and arterial blood; readily perfused tissues; working muscles; resting muscles; liver) and fat content; the model for tert butanol (TBA) also included urine. Two muscle compartments were included since human TK used to validate the

model (from Nihlen et al (1998) Tox Sci 46, 1; see entry in this section) were collected under conditions of light exercise. First order metabolism was assumed since linear kinetics had been observed experimentally in humans exposed to 5, 25 or 50 ppm ETBE vapour (Nihlen et al., 1998).

Physiological characteristics of the model were based upon previously published equations (Droz (1992) Ann Occup Hyg, 36, 295). Partition coefficients for ETBE and TBA were based on published results obtained by the authors and others. Information on body composition and physiological parameters were obtained from Nihlen et al (1998).

A sensitivity analysis was performed (Pierce et al (1996) Toxicol Appl Pharmacol, 139, 49) to identify which physical/physiological parameters most strongly influenced the model.

PREDICTIONS IN HUMANS
The PBTK model was used to predict biomarker levels in blood, urine and exhaled air at the end of a hypothetical work shift (8 hr duration) and the next morning (24 hr after the start of the first exposure, immediately prior to the next work shift). In other simulations, it was assumed that subjects were exposed to 50 ppm ETBE from 8am to noon, and from 1 pm to 5 pm 5 d/wk for 2 wk. The impact of fluctuations in ETBE exposure concentration (mean modelled time weighted average = 50 ppm, with a SD of 25 ppm in scenario 1 and a SD of 100 ppm in scenario 2) were investigated, as was the influence of work load (0, 50, 100 or 150 W) and changes in body composition.

Result: Reviewer's comment: Results in this report are generally presented either as descriptive information or graphical plots. Quantitative data are given, where available.

The model adequately described real-life human exposure to 5, 25 or 50 ppm ETBE vapour for 2 hr.

Simulated exposure over 2 weeks was associated with a minor predicted accumulation of ETBE and TBA at the end of each work week, with elimination over the weekend.

Physical exercise markedly increased the update of ETBE, with an approx. 2-fold increase at 100 W (heavy exercise) compared to that predicted at rest (0 W).

The predicted concentration of ETBE in blood and exhaled air at the end of a work shift was sensitive to modelled fluctuations in external exposure:
- 4.4 or 44 uM ETBE predicted in blood, and 17 or 85 umol ETBE/min in exhaled air, after simulated exposure to 50+/-25 ppm or 50+/-100 ppm ETBE respectively for 8 hr;
- 0.8 or 7.3 uM TBA predicted in blood, 0.7 or 7.3 umol TBA/min in exhaled air, and 1.0 or 8.0 umol TBA/min in urine after simulated exposure to 50+/-25 ppm or 50+/-100 ppm ETBE

respectively for 8 hr.

The predicted concentration of TBA in urine at the end of a work shift or the following morning was relatively unaffected by fluctuations in external (inhaled) ETBE concentration.
50+/-25 ppm ETBE:
- endshift urine concentration = 0.8 uM
- next morning urine concentration = 1.1 uM
50+/-100 ppm ETBE:
- endshift urine concentration = 7.9 uM
- next morning urine concentration = 12 uM

The coefficient of variation (CV) for the predicted end-shift or next morning mean concentration of ETBE in blood (uM) or elimination of ETBE in exhaled air (umol/min) for 8 subjects exposed to 50 ppm ETBE for 5 consecutive days was around 10% or less. The inter-individual CV for predicted end-shift exhalation of TBA (umol/min) was 9% while that for urinary excretion (umol/min) was 19%; a larger inter-individual CV of 29-30% was obtained for the equivalent next morning predictions.

The sensitivity analysis showed the concentration of ETBE in blood and exhaled air was most strongly influenced by the blood/air partition coefficient of ETBE and body fat content, while body weight, partition coefficients and hepatic metabolism influenced the fate of TBA.

Conclusion: The authors describe a PBTK model for inhalation exposure to ETBE in humans. The results indicate that the concentration of ETBE in blood and exhaled air at the end of a work-shift is sensitive to fluctuations in the external environment, whereas TBA levels the next morning were less sensitive. TBA in urine is proposed as a suitable biomarker for the estimation ETBE exposure.

Reliability: (2) valid with restrictions
Study available for review. Accepted modelling method, suitable for assessment.

30-NOV-2003 (61)

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: mouse
No. of animals, males: 3
No. of animals, females: 3
Doses, males: 0 (control), 500, 750, 1000, 1750, 2500, 5000 ppm
Doses, females: 0 (control), 500, 750, 1000, 1750, 2500, 5000 ppm
Route of administration: inhalation
Exposure time: 6 hour(s)

Year: 1995
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method:

ANIMALS AND TREATMENTS
Animals: male and female CD-1 mice (6-8 weeks)
Supplier: Charles River, Portage, MI, USA
Group size: n=3
Diet: Agway Prolab Animal Diet Rat, Mouse, Hamster 3000 (Agway Inc.), ad libitum
Water: unspecified, ad libitum
Treatment: 0, 500, 750, 1000, 1750, 2500, 5000 ppm [14C]-ETBE, 6 hr, nose-only
Exposure chamber: stainless steel (Cannon design) nose-only unit. (Uniformity of exposure concentration was continuously monitored by gas chromatography (GC) and LSC during the experiments.)

Immediately after exposure 1 mouse/sex/group was transferred to an individual glass metabolism cage while another was sacrificed by exsanguination and the liver removed. The remaining animal remained in a metabolism cage (for up to 96 hr).

SAMPLE COLLECTION
The following were collected (the longer collection periods applied only to the 5000 ppm groups):
- expired volatiles (charcoal traps) at 1,3, 6, 12, 24, 48, 72, 96 and 118 hr;
- 14CO2 (KOH trap) at 1, 3, 6, 12, 24, 48, 72, 96, and 118 hr;
- urine (over dry-ice) at 12, 18 24, 48, 72, 96 and 118 hr;
- faeces (room temperature) at 12, 18 24, 48, 72, 96 and 118 hr;
- cage wash at 118 hr.

After the last collection point animals were euthanized by CO2 asphyxiation.

SAMPLE PROCESSING
Radioactivity present in 14CO2 and cage wash was quantified by direct liquid scintillation counting (LSC), while faeces and liver homogenates were combusted prior to counting. Exhaled volatiles were extracted from charcoal traps with methanol and an aliquot assayed by LSC.
Comment: The total dose of radioactivity was not calculated.

STATISTICAL METHODS
No specific methods or software given.

Remark: The study was initially designed to incorporate a repeat dosing phase but this was cancelled.

Result:

EXPOSURE
Achieved exposure levels throughout the 6 h period were +/- 10% of target (478, 763, 991, 1695, 2507, 5125 ppm)

ELIMINATION OF [14C]-ETBE
Comment: The total absorbed dose was not calculated and elimination values are expressed as % of total radioactivity recovered in excreta.

The majority of absorbed radioactivity was eliminated over 48 hr, and 83-93% of the total excreted in urine or as exhaled volatile organics at all exposure levels. The majority of exhaled volatile organics were eliminated by 12 hr with highest proportions in the 1 to 3 h collection. Minor amounts were eliminated as 14CO2 or in faeces.

The proportion of radioactivity in urine decreased from 74% to 46% as exposure concentrations increased from 500 ppm to 1750 ppm, with a concomitant increase in volatile organics from 10% to 42%. The relative proportions remained quite similar as exposure concentrations increased from 1750 ppm to 5000 ppm.

Although not noted by the authors it appeared that there was no consistent gender difference in elimination characteristics.

Blood and liver concentrations (mg equiv. ETBE/g) increased from 154 to 481 (blood) and 208 to 724 (liver) as exposure concentration increased from 500 ppm to 1750 ppm. Values then remained similar from 1750 ppm to 5000 ppm.

Test substance: Non-labeled ETBE (98%) was obtained from Pittsburgh Applied Research Corp. (PA, USA). 14C-ETBE was obtained from Wizard Laboratories (CA, USA), chemical purity 99.8%.

Conclusion: The majority (83-93%) of an inhaled dose of 14C-ETBE was eliminated by the CD-1 mice within 48 hr. The proportion of radioactivity in urine decreased from 74% to 46% as exposure concentrations increased from 500 ppm to 1750 ppm, with a concomitant increase in volatile organics from 10% to 42%. The relative proportions remained quite similar (plateau) as exposure concentrations increased from 1750 ppm to 5000 ppm. The authors conclude that processes responsible for the absorption and elimination of ETBE in the mouse appear to become saturated at an exposure concentration of 1750 ppm.

Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.

30-NOV-2003 (75)

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: mouse
No. of animals, males: 6
Doses, males: 5000 ppm
Route of administration: inhalation
Exposure time: 6 hour(s)

Year: 1966
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method:

ANIMALS AND TREATMENTS
Six male CD-1 mice (Charles River, NC, USA; bwt 29-33 g) were exposed to [14C]- ETBE (5000 ppm, 6 hr) using a nose-only exposure tower. Environmental conditions were monitored and recorded automatically. The 12 h light/dark cycle was interrupted briefly on study days for collection of samples during a dark phase. All animals received pelleted Standard NIH-07 rodent chow (Zeigler Bros., PA, USA) and drinking water ad libitum prior to and following nose-only exposures.

Each mouse received approx. 0.8 uCi of [14C]-ETBE.

Immediately post-exposure, 3 animals were sacrified, carcasses digested and the total content of radioactivity determined by liquid scintillation counting (LSC). The remaining animals were transferred to individual glass metabolism cages for 96 hr, then sacrificed (CO_2) and carcasses digested for quantitation of total radioactivity.

SAMPLE COLLECTION
The following were collected:
- expired volatiles (charcoal traps) at 1,3, 6, 12, 24, 48, 72 and 96 hr;
- $^{14}CO_2$ (KOH trap) at 1, 3, 6, 12, 24, 48, 72 and 96 hr;
- urine (over dry-ice) at 24, 48, 72 and 96 hr;
- faeces (room temperature) at 24, 48, 72, and 96 h
cage wash at 96 hr

SAMPLE PROCESSING
Urine, $^{14}CO_2$, cage wash and nose-only tube wash were assayed for radioactivity by LSC. Faeces and carcasses were digested prior to LSC and volatiles removed from charcoal traps with dimethylformamide (DMF) before direct radioassay by LSC. Thus, total recovery of radioactivity was determined as a percentage of the calculated dose.

EXPOSURE APPARATUS
Exposure was conducted using a stainless steel Cannon nose-only unit (Lab products, Maywood, NJ, USA). Uniformity of concentration throughout the apparatus was established before the experiments and was continuously monitored during the experiments. Radioactivity in the apparatus atmosphere was measured by LSC and the total ETBE concentration by gas chromatography (GC).

METABOLISM
Urine was analysed for ETBE, TBA, a-hydroxybutyrate (HBA) and 2-methyl-1,2-propandiol (MPD) by GC-MS. Control urine and feces were collected from an additional animal for establishing background concentrations.

DATA ANALYSIS
A Microsoft Excel spreadsheet was used to calculate recoveries of radioactivity expressed as percentage of total

radioactivity recovered from the animal or by using the total radioactivity determined in an animal concurrently exposed and sacrificed immediately following the 6 hr exposure.

Result: EXPOSURE
The mean achieved exposure level was 4927 +/- 209 ppm.

ELIMINATION OF [14C]-ETBE
The major pathways of elimination of radioactivity (ETBE equivalents) were urine (57.32%) and expired air (37.31%, as volatile organics). Approximately 87% of the absorbed dose was eliminated within 24 h following the end of exposure and approximately 98% by 96 h. Only minor proportions were eliminated as 14CO2 (1.59%) or in feces (1.62%).

BIOTRANSFORMATION OF ETBE
In urine levels of TBA were greater than those of ETBE, the latter decreasing quickly after exposure. In exhaled air ETBE was present at higher concentration (44.7 mmoles) than TBA (16.9 mmoles) at 1 h but ETBE levels decreased quickly and were lower thereafter. In addition the presence of HBA and MPD were was detected in urine.

Test substance: Non-labeled ETBE (97.5%) was obtained from Pittsburgh Applied Research Corp. (PA, USA). 14C-ETBE was obtained from Wizard Laboratories (CA, USA), chemical purity 99.2%.

Conclusion: Following single nose-only exposure to 5000 ppm [14C]-ETBE, mice eliminated radioactivity (mainly as TBA) in urine and exhaled air. Amounts of unchanged ETBE were also detected (greatest at 1 h) but decreased quickly with time. HBA and MPD, being further products of metabolism of TBA, were also detected in urine.

Reliability: (2) valid with restrictions
Study available for review. GLP compliant method developmnent study, clearly reported, acceptable for assessment.

02-JAN-2004 (14)

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: mouse
No. of animals, males: 8
No. of animals, females: 8
Doses, males: 0, 500, 1750 and 5000 ppm
Doses, females: 0, 500, 1750 and 5000 ppm
Route of administration: inhalation
Exposure time: 6 hour(s)

Year: 1996
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS AND TREATMENTS

Animals: male (20-44 g) and female (15-35 g) CD-1 mice

Supplier: Charles River, NC, USA;
Group size: n=8/sex
Diet: Standard NIH-07 rodent chow (Zeigler Bros., PA, USA), ad libitum;;
Water: unspecified, ad libitum;
Treatment: 0, 500, 1750 and 5000 ppm [14C]- ETBE, 6 hr, nose-only;
Environmental: conditions were monitored and recorded automatically; the 12 hr light/dark cycle was interrupted briefly on study days for collection of samples (during dark phase);
Exposure chamber: stainless steel Cannon nose-only unit (Lab products, Maywood, NJ, USA). Uniformity of concentration throughout the apparatus had been established before the experiments and was continuously monitored during the experiments (14C by LSC, total ETBE by GC)

Immediately post-exposure, 4 animals/sex/exposure level were transferred to individual glass metabolism cages and 4 animals/sex/exposure level were euthanized. The carcasses of the latter were digested and the total carcass content of radioactivity determined by liquid scintillation counting (LSC). The surviving animals remained in the metabolism cages (air flow rate 200 mL/min) for 48 hr, after which they were sacrificed by CO2 asphyxiation and carcasses digested for radioassay.

It was estimated (from exposure levels, breathing rate and specific activity measurements) that the mice were exposed to 15-18 mCi of [14C]-ETBE.

SAMPLE COLLECTION
The following were collected:
- expired volatiles (charcoal traps) at 1,3, 5, 8, 16, 24 and 48 hr;
- 14CO2 (KOH trap) at 3, 8, 16, 24 and 48 hr;
- urine (over dry-ice) at 8, 24 and 48 hr;
- faeces (room temperature) at 24 and 48 hr;
- cage wash at 48 hr

At 48 h after the end of the nose-only exposure the animals were euthanized

SAMPLE PROCESSING
Urine, 14CO2, cage wash and nose-only tube wash were assayed for radioactivity by LSC. Feces and carcasses were digested prior to LSC and volatiles removed from charcoal traps with dimethylformamide (DMF) before direct radioassay by LSC. Thus, total recovery of radioactivity was determined as a percentage of the calculated dose.

METABOLISM
Urine was analysed for ETBE, TBA, a-hydroxybutyrate (HBA) and

2-methyl-1,2-propandiol (MPD) by GC-MS. Concentrations of ETBE and TBA were quantitated in urine and in DMF extracts from charcoal traps. Control urine and feces were collected from an additional animal for establishing background concentrations.

DATA ANALYSIS
A Microsoft Excel spreadsheet was used to calculate recoveries of radioactivity expressed as percentage of total radioactivity recovered from the animal or by using the total radioactivity determined in an animal concurrently exposed and sacrificed immediately following the 6 hr exposure.

Result:

EXPOSURE
The mean achieved exposure levels (activity inhaled) were:
555 ppm (17.98 uCi)
1717 ppm (15.39 uCi)
4957 ppm (17.15 uCi)

ELIMINATION OF [14C]-ETBE
The major pathways of elimination of radioactivity (ETBE equivalents) were urine and expired air. The totals eliminated increased with increasing exposure but in a less than dose proportional fashion. At all three exposure levels >95% of the absorbed dose was eliminated within 48 h following the end of exposure.

Males, nose only:

	% absorbed eliminated (48 hr)		
	500 ppm	1750 ppm	5000 ppm
Exhaled volatiles	12.7	23.3	39.9
Exhaled CO2	1.1	2.2	2.9
Urine	81.3	70.7	52.6
Faeces	3.2	1.1	0.6
Total	98.3	97.4	95.9

Females, nose only:

	% absorbed eliminated (48 hr)		
	500 ppm	1750 ppm	5000 ppm
Exhaled volatiles	26.7	35.8	47.2
Exhaled CO2	1.2	1.9	3.3
Urine	67.2	60.7	47.4
Faeces	2.2	0.6	0.7
Total	97.4	99.0	98.7

(Values = mean for 4 mice/group)

At the 500 ppm exposure urine accounted for majority of dose whilst at 5000 ppm exhaled air and urine accounted for approximately similar proportions. Only minor proportions were eliminated as 14CO2 or in feces.

RETENTION
Carcass accounted for approx. 2-2.5%, 1-1.5% and 0.6-0.8% of the absorbed radioactivity in the low, intermediate and high dose groups, respectively.

BIOTRANSFORMATION OF ETBE
In urine and exhaled air levels of [14C]-TBA were greater than those of [14C]-ETBE, the latter decreasing quickly after exposure. The period of elimination of TBA in exhaled air was more extended in the intermediate and high dose groups (detectable amounts present in both sexes at 16 hr post-exposure) than in the 500 ppm group (undetectable after 1 hr post-exposure). In contrast detectable amounts of TBA were present in urine from all three treatment groups at 24 hr post-dose (both sexes).

In addition the presence of HBA and MPD were confirmed qualitatively in urine.

Test substance: Non-labeled ETBE (97.5%) was obtained from Pittsburgh Applied Research Corp. (PA, USA). 14C-ETBE was obtained from Wizard Laboratories (CA, USA), chemical purity 99.2%.

Conclusion: Following single nose-only exposure to [14C]-ETBE at 500, 1750 or 5000 ppm, mice eliminated radioactivity mainly in urine and exhaled air. At 500 ppm the majority of the absorbed dose was eliminated in urine whilst at 5000ppm urine and exhaled air accounted for approximately similar proportions. [14C]-TBA was the main elimination product in both urine and exhaled air, and HBA and MPD were identified qualitatively in urine. There were no gender differences.

Reliability: (2) valid with restrictions
Study available for review. GLP compliant near-guideline investigation, clearly reported, acceptable for assessment.

30-NOV-2003 (15)

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: rat
No. of animals, males: 3
No. of animals, females: 3
Doses, males: 0 (control), 500, 750, 1000, 1750, 2500, 5000 ppm
Doses, females: 0 (control), 500, 750, 1000, 1750, 2500, 5000 ppm
Route of administration: inhalation
Exposure time: 6 hour(s)

Year: 1995
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS AND TREATMENTS
Animals: male and female Fischer 344 rats (10-12 weeks)
Supplier: Harlan-Sprague Dawley, Indianapolis, IN, USA
Group size: n=3
Diet: Agway Prolab Animal Diet Rat, Mouse, Hamster 3000 (Agway Inc.), ad libitum
Water: unspecified, ad libitum
Treatment: 0, 500, 750, 1000, 1750, 2500, 5000 ppm [14C]-ETBE, 6 hr, nose-only

Exposure chamber: stainless steel (Cannon design) nose-only unit. (Uniformity of exposure concentration was continuously monitored by gas chromatography (GC) and LSC during the experiments.)

Immediately after exposure 1 rat/sex/group was transferred to an individual glass metabolism cage while another was sacrificed by exsanguination and the kidneys removed. The remaining animal remained in a metabolism cage (for up to 96 hr).

SAMPLE COLLECTION
The following were collected (the longer collection periods applied only to the 5000 ppm groups):
- expired volatiles (charcoal traps) at 1,3, 6, 12, 24, 48, 72, 96 and 118 hr;
- 14CO2 (KOH trap) at 1, 3, 6, 12, 24, 48, 72, 96, and 118 hr;
- urine (over dry-ice) at 12, 18 24, 48, 72, 96 and 118 hr;
- faeces (room temperature) at 12, 18 24, 48, 72, 96 and 118 hr;
- cage wash at 118 hr.

After the last collection point animals were euthanazed by CO2 asphyxiation.

SAMPLE PROCESSING
Radioactivity present in 14CO2 and cage wash was quantified by direct liquid scintillation counting (LSC), while faeces and liver homogenates were combusted prior to counting. Exhaled volatiles were extracted from charcoal traps with methanol and an aliquot assayed by LSC.
Comment: The total dose of radioactivity was not calculated.

STATISTICAL METHODS
No specific methods or software given.

Remark: The study was initially designed to incorporate a repeat dosing phase but this was cancelled.

Result: EXPOSURE
Achieved exposure levels throughout the 6 h period were +/- 10% of target (478, 763, 991, 1695, 2507, 5125 ppm).

ELIMINATION OF [14C]-ETBE
Comment: The total absorbed dose was not calculated and elimination values are expressed as % of total radioactivity recovered in excreta.

The majority of absorbed radioactivity was eliminated by 48 hr with 96-98% of the total excreted in urine or as exhaled volatile organics at all exposure levels. The majority of exhaled volatile organics were eliminated by 12 hr with the greatest proportions in the 1 to 3 h collection. Minor amounts were eliminated as 14CO2 or in faeces.

Although not noted by the authors it appeared that there was

no consistent gender difference in elimination characteristics.

As exposure concentrations increased from 500 ppm to 1750 ppm the proportion of radioactivity in urine decreased from 60% to 38% with a concomitant increase in volatile organics from 37% to 58%. However as exposure concentrations increased from 1750 ppm to 5000 ppm these relative proportions remained quite similar.

Blood and kidney concentrations (mg equiv. ETBE/g) increased from 37 to 155 (blood) and 74 to 185 (kidney) as exposure concentration increased from 500 ppm to 1750 ppm. Values then remained similar from 1750 ppm to 5000 ppm.

Test substance: Non-labeled ETBE (98%) was obtained from Pittsburgh Applied Research Corp. (PA, USA). 14C-ETBE was obtained from Wizard Laboratories (CA, USA), chemical purity 99.8%.

Conclusion: The majority (96-98%) of an inhaled dose of 14C-ETBE was eliminated by the Fischer rat within 48 hr. The proportion of radioactivity in urine decreased from 60% to 38% as exposure concentrations increased from 500 ppm to 1750 ppm, with a concomitant increase in volatile organics from 37% to 58%. The relative proportions remained quite similar (plateau) as exposure concentrations increased from 1750 ppm to 5000 ppm. The authors conclude that processes responsible for the absorption and elimination of ETBE in the rat appear to become saturated at an exposure concentration of 1750 ppm.

Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.

27-OCT-2003 (76)

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: rat
No. of animals, males: 6
Doses, males: 5000 ppm
Route of administration: inhalation
Exposure time: 6 hour(s)

Year: 1996
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS AND TREATMENTS
Six male F-344 rats (Charles River, NC, USA; 234-249 g) were exposed nose only to 5000 ppm [14C]-ETBE. Enviromental conditions were monitored and recorded automatically. The 12 h light/dark cycle was interrupted briefly on study days for collection of samples during a dark phase. All animals received pelleted Standard NIH-07 rodent chow (Zeigler Bros.,

PA, USA) and drinking water ad libitum prior to and following nose-only exposures.

Each rat received approx. 3 uCi of [14C]-ETBE.

Immediately post-exposure, 3 animals were sacrified, carcasses digested and the total content of radioactivity determined by liquid scintillation counting (LSC). The remaining animals were transferred to individual glass metabolism cages for 96 hr, then sacrificed (CO2) and carcasses digested for quantitation of total radioactivity.

SAMPLE COLLECTION
See details for mouse study (identical reference number).

SAMPLE PROCESSING
See details for mouse study (identical reference number).

EXPOSURE APPARATUS
See details for mouse study (identical reference number).

METABOLISM
See details for mouse study (identical reference number).

DATA ANALYSIS
See details for mouse study (identical reference number).

Result: EXPOSURE
The mean achieved exposure level was 4927 +/- 209 ppm.

ELIMINATION OF [14C]-ETBE
The major pathways of elimination of radioactivity (ETBE equivalents) were urine (43.55%) and expired volatiles (50.54%). Approximately 90% of the absorbed dose was eliminated within 24 hr following the end of exposure and approximately 98% by 96 h. Only minor proportions were eliminated as 14CO2 (0.87%) or in faeces (3.30%).

BIOTRANSFORMATION OF ETBE
In urine levels of TBA were greater than those of ETBE, the latter decreasing quickly after exposure. In exhaled air ETBE was present at higher concentration than TBA up to 3 hr (144.1 mmoles and 34.4 mmoles respectively at 3 hr). ETBE levels decreased quickly and were lower thereafter. In addition the presence of HBA and MPD were was detected in urine.

Test substance: Non-labeled ETBE (97.5%) was obtained from Pittsburgh Applied Research Corp. (PA, USA). 14C-ETBE was obtained from Wizard Laboratories (CA, USA), chemical purity 99.2%.

Conclusion: Following single nose-only exposure to 5000 ppm [14C]-ETBE, rats eliminated radioactivity (mainly as TBA) in urine and exhaled air. Amounts of unchanged ETBE were also detected (greatest at 1 h) but decreased quickly with time whereas levels of TBA increased to 6 hr. HBA and MPD, being further products of metabolism of TBA, were also detected in urine.

Reliability: (2) valid with restrictions

Study available for review. GLP compliant method developmnent study, clearly reported, acceptable for assessment.

27-OCT-2003 (14)

In Vitro/in vivo:	In vivo
Type:	Toxicokinetics
Species:	rat
No. of animals, males:	8
No. of animals, females:	8
Doses, males:	0, 500, 1750 and 5000 ppm (single or multiple exposures)
Doses, females:	0, 500, 1750 and 5000 ppm (single or multiple exposures)
Route of administration:	inhalation
Exposure time:	6 hour(s)

Year:	1996
GLP:	yes
Test substance:	as prescribed by 1.1 - 1.4

Method: ANIMALS AND TREATMENTS

Animals: male (200-250 g) and female (130 - 180 g) F-344 rats
Supplier: Charles River, NC, USA;
Group size: n=8/sex
Diet: Standard NIH-07 rodent chow (Zeigler Bros., PA, USA), ad libitum;
Water: unspecified, ad libitum;
Acute treatment regime: 0, 500, 1750 and 5000 ppm [14C]-ETBE, 6 hr, nose-only;
Repeat treatment regime: whole body exposure (6 hr/d) to 0, 500 and 5000 ppm unlabeled ETBE prior to a single 6 hr nose-only to 500 or 5000 ppm 14C-ETBE;
Environmental: conditions were monitored and recorded automatically; the 12 hr light/dark cycle was interrupted briefly on study days for collection of samples (during dark phase);
Exposure chamber: stainless steel Cannon nose-only unit (Lab products, Maywood, NJ, USA). Uniformity of concentration throughout the apparatus had been established before the experiments and was continuously monitored during the experiments (14C by LSC, total ETBE by GC)

Immediately post-exposure, 4 animals/sex/exposure level were transferred to individual glass metabolism cages and 4 animals/sex/exposure level were euthanazed. The carcasses of the latter were digested and the total carcass content of radioactivity determined by liquid scintillation counting (LSC). The surviving animals remained in the metabolism cages (air flow rate 200 mL/min) for 48 hr, after which they were sacrificed by CO2 asphyxiation and carcasses digested for radioassay.

It was estimated (from exposure levels, breathing rate and specific activity measurements) that the rats were exposed to 45-51 mCi of [14C]-ETBE in the nose-only experiments.

SAMPLE COLLECTION
See details for mouse study (identical reference number).

SAMPLE PROCESSING
See details for mouse study (identical reference number).

METABOLISM
See details for mouse study (identical reference number).

DATA ANALYSIS
See details for mouse study (identical reference number).

Result:

EXPOSURE
The mean achieved exposure levels (activity inhaled) were:
555 ppm (17.98 uCi)
1717 ppm (15.39 uCi)
4957 ppm (17.15 uCi)

ELIMINATION OF [14C]-ETBE
In the nose-only experiment and in the pre-exposed animals the major pathways of elimination of radioactivity (ETBE equivalents) were urine and expired air. The totals eliminated increased with increasing exposure but in a less than dose proportional fashion. At all exposure levels >90% of the absorbed dose was eliminated within 48 h following the end of exposure.

Males, 6 hr nose only:

	---% absorbed eliminated (48 hr)---		
	500 ppm	1750 ppm	5000 ppm
Exhaled volatiles	27.9	40.8	50.8
Exhaled CO2	1.1	1.5	1.6
Urine	58.6	53.1	44.7
Faeces	2.8	0.1	0.2
Total	90.4	95.6	97.3

(Values = mean for 8 rats/group)

Although a minor pathway the relative proportion eliminated as 14CO2 increased with increasing exposure concentration.

At the 500 ppm exposure urine accounted for majority of dose whilst at 5000 ppm exhaled air was the major pathway for elimination. This shift was more pronounced in females (6 hr, nose only):

	---% absorbed eliminated (48 hr)---		
	500 ppm	1750 ppm	5000 ppm
Exhaled volatiles	31.7	52.2	64.1
Exhaled CO2	1.3	1.7	2.0
Urine	59.3	41.3	30.4
Faeces	1.0	0.5	0.2
Total	93.5	95.8	96.7

(Values = mean for 8 rats/group)

Pre-exposure to ETBE for 13 days prior to exposure to 14C-ETBE decreased the percentage of absorbed material eliminated in exhaled air and increased the proportion in urine. This effect was most pronounced in animals exposed to 5000 ppm (results for males given below, response in females comparable):

	--% absorbed eliminated (48 hr)--	
Treatment regime =	0/5000	5000/5000
Exhaled volatiles	48.6	24.1
Exhaled CO2	2.1	1.4
Urine	45.1	71.5
Faeces	0.5	0.3
Total	96.3	97.4

(Values = mean for 8 rats/group)

RETENTION
Carcass accounted for approx. 1.5-3% of the absorbed radioactivity irrespective of exposure level or sex.

BIOTRANSFORMATION OF ETBE
In urine and exhaled air levels of [14C]-TBA were greater than those of [14C]-ETBE, the latter decreasing quickly in exhaled air after exposure.

Pre-exposure to 5000 ppm ETBE for 13 d increased the proportion of absorbed material eliminated as [14C]-TBA (less pronounced effect at 500 ppm):

	---------uM TBA in urine ---------			
	------males------		-----females-----	
	0/5000	5000/5000	0/5000	5000/5000
8 hr	2344	4666	806	1439
24 hr	1058	2982	911	2429

In addition the presence of HBA and MPD were confirmed qualitatively in urine.

Test substance: Non-labeled ETBE (97.5%) was obtained from Pittsburgh Applied Research Corp. (PA, USA). 14C-ETBE was obtained from Wizard Laboratories (CA, USA), chemical purity 99.2%.

Conclusion: Following single nose-only exposure to [14C]-ETBE at 500, 1750 or 5000 ppm rats eliminated radioactivity mainly in urine and exhaled air with feces and 14CO2 being minor pathways. At 500 ppm most was eliminated in urine whilst at 5000 ppm exhaled air accounted for the greatest proportion. Total radioactivity eliminated increased with increasing exposure, but in a less than exposure proportional manner. Pre-exposure to ETBE increased the proportion eliminated as [14C]-TBA and the overall weight of evidence indicated that pre-exposure to ETBE induced its metabolism. [14C]-TBA was always the main elimination product in urine and exhaled air. HBA and MPD, being further products of metabolism were also detected in urine.

Reliability: (2) valid with restrictions
Study available for review. GLP compliant near-guideline

investigation, clearly reported, acceptable for assessment.
30-NOV-2003 (15)

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: rat

Year: 2001
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS AND TREATMENTS
Animals: F344 rats (12 wk old; males 210-240 g; females 190-220 g)
Supplier: Harlan Winkelmann (Borchen, Germany)
Group size: 5 per sex per exposure level
Diet: no details
Water: no details
Treatment: 4 hr inhalation exposure to target concentrations of 4 ppm or 40 ppm ETBE vapour
Exposure chamber: exposure of the rats coincided with exposure of a group of human volunteers, within the same chamber (see following record for further details)
Blood collection: from tail vein (100 ul)
Urine collection: animals housed in metabolism cages (unspecified); urine (4 degrees C) collected every 6 hr for 72 hr; animals accustomed to metabolism cages for 3 d prior to exposure; control urine collected for 12 hr prior to exposure; report states no urine excreted during the exposure period

ANALYTICAL PROCEDURES
Chamber air was sampled every 15 min and the concentration of ETBE vapour present determined by GC-MS. Actual concentrations were 4.5 +/- 0.6 ppm and 40.6 +/- 3.0 ppm (mean and SD for 16 determination taken over 4 hr).

The concentration of ETBE in blood was quantified using headspace GC-MS, with MTBE as internal standard; limit of detection 0.1 nmol ETBE per ml blood (signal to noise ratio 5:1; variation not stated). TBA in blood was determined using GC-MS, with deuterated TAB as standard; limit of detection 0.2 nmol per ml blood (signal to noise ratio 5:1; variation 10%).

The concentration of 2-methyl-1,2-propanediol in urine was determined using GC-MS, with 1,2-propanediol as standard; limit of detection 1 nmol/ml urine (signal to noise ratio 5:1; variation <15%). 2-Hydroxyisobutyrate in urine was quantified after conversion to the methyl ester using GC-MS; limit of detection 3 nmol per ml urine (signal to noise ratio 5:1; variation <18%).

STATISTICAL METHODS

Data were analysed using Student's t-test (P values <0.05 considered significant). Half-lives were calculated using exponential regression.

Remark: Some of this information has also been published in:

Amberg, A, Rosner, E and Dekant, W (2000) Biotransformation and kinetics of excretion of ethyl-tert-butyl ether in rats and humans. Toxicol Sci. 53, 194-201.

Dekant, W, Bernbauer, U, Rosner, E, Amberg, A. (2001) Toxicokinetics of ethers used a fuel oxygenates. Toxicol Lett 124, 37-45.

Result: RECEIVED DOSE IN THE RAT
Received doses of 2.3 and 21 umol were calculated for rats exposed to 4 ppm or 40 ppm ETBE for 4 hr, respectively.

TOXICOKINETICS AND BIOTRANSFORMATION OF ETBE BY RATS
-BLOOD
ETBE was not detected in blood samples taken before exposure commenced. A maximal concentration of 1.0 uM was achieved following 4 hr exposure to 4 ppm ETBE, with a maximum of 5.3 uM present after 4 hr exposure to 40 ppm. Elimination half-lives of 0.4 hr and 0.8 hr were obtained in the 4 ppm and 40 ppm groups, respectively.

Following exposure to ETBE, the concentration of TBA in rat blood increased significantly (P<0.01; control level not reported) reaching a maximum of 5.7 or 21.7 uM for the 4 ppm or 40 ppm exposures, respectively (elimination half-lives not determined). The authors note that the measured concentration of TBA in blood was greater in the rats compared to humans exposed under identical conditions.

- URINE
TBA, 2MP and 2HIB were present in control urine prior to exposure to ETBE. The concentration of urinary metabolites increased post-exposure, with 2HIB the main metabolite. The following recovery data were obtained over 72 hr post-exposure:

ETBE (low exposure, high exposure)
- background: none detected
- corrected total excreted: none detected
- half-life: not relevant

TBA (low exposure, high exposure)
- background: 0.2, 0.2 umol over 6 hr
- corrected total excreted: 0.2, 0.7 umol
- half-life: not determined, 4.6 hr

2MP (low exposure, high exposure)
- background: 0.02, 0.02 umol
- corrected total excreted: 0.3, 1.6 umol
- half-life: 4.0, 2.6 hr

2HIB (low exposure, high exposure)
- background: 1.4, 1.9 umol
- corrected total excreted: 2.3, 10.6 umol
- half-life: 4.7, 3.0 hr

Reviewer's comments:
- urinary excretion of metabolites was less the rats than in humans exposed under identical conditions;
- urinary half-lives in rats were also shorter than in humans.
- unchanged ETBE not detected in rat urine (present in humans)

Test substance: ETBE, >99%, Aldrich Chemical Co., Deisenhofen, Germany.

Conclusion: ETBE vapour (4 ppm or 40 ppm) is readily absorbed by the rat lung and distributed into the blood from which it was eliminated with a half-life of 0.4-0.6 hr. It is metabolised to tert-butanol (primary metabolite; half-life not determined) which reaches a higher blood concentration in the rat than is seen humans exposed under identical conditions. Urinary metabolites were consistent with cytochrome P450-mediated metabolism of tert-butanol, and comprised 2-hydroxyisobutyrate (predominant metabolite) and 2-methyl-1,2-propanediol along with minor amounts of TBA.

Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.

02-JAN-2004 (25)

In Vitro/in vivo: In vivo
Type: Metabolism
Species: other: rats, human

Year: 1998
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS AND TREATMENTS
Male and female Fischer F344 rats (220-260g) were obtained from Harlan Winkelmann (Borchen, Germany). The animals were maintained on a 12h light/dark cycle, Altromin food and tap water were available ad libitum. Animals were accustomed to metabolism cages for 3 days before the experiments and control urine was collected at this time.

Male (n=2) and female rats (n=2) were individually exposed to [2-13C]-ETBE by inhalation for 6 h in a static exposure chamber with an initial concentration of 2000 ppm. An comparable study was also conducted with non-isotopic ETBE. The concentration of ETBE in the chamber was monitored every 10 min GC-FID. After 6 h the animals were transferred to individual glass metabolism cages with free access to food and water where they were maintained for 72 h.

In a separate experiment male animals (n=3) were dosed with [2-13C]-TBA (dissolved in corn oil) at a dose level of 250g/kg. These animals were also maintained in metabolism cages for 72 h with urine collection.

A single male human subject (aged 44 years, weight 80 kg) was given 5 mg/kg bw [2-13C]-TBA (study design approved by the institutional review board of the University of Wurzburg).

SAMPLE COLLECTION
In the animal studies urine was collected over 0-24 h and 24-48 h. In the human experiment, urine was collected up to 48 hr (12 hr intervals).
Comment: It is not clear if samples were collected at ambient temperature or chilled, or how they were stored.

SAMPLE ANALYSIS
Urine was analysed directly by NMR in the presence of D2O. Spectra were recorded on a Bruker AC 250 or a Bruker Avance DMX 600 spectrometer under standard conditions. Some samples were pre-treated with b-glucuronidase or sulfatase enzymes (Sigma) or treated with HCl (pH 2) for 1 h at 37°C to release putative conjugates.

Headapace GC/MS was performed on a Fisons MD 800 mass spectrometer coupled with a Carlo Erba GC 8000 GC (split injection ratio 1:5).

Result:

BIOTRANSFORMATION OF ETBE
TBA glucuronide, 2-methyl-1,2-propanediol, 2-hydroxyisobutyrate and TBA sulfate were identified in 0-24 hr urine from rats exposed to [2-13C]-ETBE, together with small amounts of 13C-acetone.
Comment: The authors note that these were identical to the metabolites in urine from rats dosed with MTBE in a parallel experiment.

After 24-48 hr, only 2-hydroxyisobutyrate was observed in urine. There was no apparent gender difference (qualitative or quantitative) in the metabolite pattern.

No metabolites deriving from oxidation of the b-carbon of the ethyl group in ETBE (e.g. tert-butyl glycol or tert-butoxyacetic acid) were observed.

BIOTRANSFORMATION OF TBA
Comment: These studies were included to confirm the structure of metabolites "downstream" from TBA.

NMR and GC/MS showed the presence of [2-13C]-TBA and the same range of components present in urine from rats exposed to [2-13C]-ETBE. TBA-sulphate predominated.

The urine samples from the human subject given [2-13C]-TBA exhibited a pattern of metabolites that was qualitatively the

same as for rat. The major metabolite was however 2-hydroxyisobutyrate rather than TBA sulfate.

Test substance: [2-13C]-ETBE (>95% pure) and [2-13C]-TBA (>97% pure) were prepared by custom synthesis.

Conclusion: The metabolism of [2-13C]-ETBE in the rat was shown to proceed by oxidation of the ethyl group to TBA, with TBA-glucuronide, TBA-sulfate and 2-hydroxyisobutyrate and trace amounts of [13C]-acetone present in urine. Later time-points (24-48 h) contained only 2-hydroxyisobutyrate suggesting that this component had a longer half-life of elimination. Metabolism of [2-13C]-TBA in rat and human gave rise to the same range of metabolites as was noted in rats exposed to [2-13C]-ETBE. The relative proportions of metabolites differed, however, with only small amounts of TBA-sulfate and a greater preponderance of 2-hydroxyisobutyrate in humans.

Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.

30-NOV-2003 (8)

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: human
No. of animals, males: 8
Doses, males: 0 (control), 5, 25, 50 ppm
Route of administration: inhalation
Exposure time: 2 hour(s)

Deg. product: yes

Year: 1988
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: SUBJECTS AND TREATMENTS
8 healthy male volunteers (Caucasian; age 21 to 41 years, mean 29 years; bodyweight 70 to 97 kg, mean 82 kg). All were non-smokers, had no occupational exposure to ETBE and refrained from alcohol and drugs 2 days before and during the study. Exposures were conducted during 2 h of light physical exercise in an exposure chamber. Each subject exposed on 4 occasions to nominal levels of 0, 5, 25, and 50 ppm ETBE with at least 2 weeks between successive exposures. All sampling of blood and expired air was performed during exercise.

The concentration of ETBE in the chamber air was analysed every 5 min during exposure (mean measured concentrations = 4.8, 25 and 50 ppm). Other variables (temperature, humidity, air exchanges, average rate of exercise) were closely monitored and found to be consistent during each exposure and between different exposures.

SAMPLE COLLECTION
Complete urine collections were made prior to exposure then at 0, 2, 4, and approx. 7, 11, 20 and 22 h post-exposure with a spot sample at 46 h. Those at 7, 11 and 20 h were collected at home and stored at room temperature. Collection condition of the other samples is not clear.

Blood (capillary from fingerprick) taken at multiple time-points (>20 samples over 24 h) commencing before exposure, then during and up to 48 h post-exposure. Samples (200 ul) were transferred immediately to sealed headspace vials. Timepoints were not specified but were adequate to provide concentration versus time profiles and comprehensive toxicokinetic estimates.

Exhaled air was collected once before exposure, four times during (30 min intervals) and 6 times after exposure. Samples were carefully collected according to a set procedure and ETBE measurement undertaken as air was being exhaled. TBA in exhaled air was trapped in sorbent tubes for later measurement.

SAMPLE PROCESSING
ETBE in exhaled air was assayed by direct capillary column GC-FID. TBA in exhaled air was measured (after desorption) by GC using FID.

ETBE, TBA and acetone in blood and urine were determined by capillary GC-FID. All three analytes were shown to be stable on storage in the matrices up to 24 h.

STATISTICAL METHODS
Toxicokinetic calculations for exhaled air used a previously published method (linear four compartment model with zero order uptake) applied previously to MTBE. The absorbed dose was calculated as Respiratory Intake (new approach) and Net Respiratory Intake (old approach), the former regarded by the researchers (with good reason) as more accurate as it allowed for material exhaled during exposure.

Elimination curves for ETBE in urine were fitted to biexponential functions using non-linear regression analysis. The elimination phase for TBA in blood and urine was fitted to a monoexponential function by non-linear regression analysis. Area under the concentration-time curve (AUC) for ETBE in blood was calculated using the four compartment model whilst AUCs for TBA and acetone were calculated by the trapezoidal rule in Microsoft Excel.

Analysis of variance (SuperANOVA) and Student's paired t test were used to compare results from different exposures with a level of significance set to 0.05. Data are presented as mean

values with 95% confidence intervals.

Remark: Half lives:

	---5 ppm---		---25 ppm---		---50 ppm---	
	ETBE	TBA	ETBE	TBA	ETBE	TBA
Blood						
1st:	1.8 min	n.d	1.2 min	n.a	2.0 min	n.a.
2nd:	20 min	n.d	15 min	n.a	1.9 min	n.a.
3rd:	2.1 h	n.d	1.5 h	n.a	1.5 h	n.a.
4th	33 h	n.d.	24 h		12 h	24 h	12 h
Urine						
1st	6.3 min	n.d	9.1 min	n.a	9.3 min	n.a.
2nd	8.8 h	n.d	8.7 h	8.4 h	8.2 h	7.6 h

Comment: The concentration versus time plot for blood indicates that this long half-life probably accounts for a relatively small proportion of absorbed ETBE and may represent a deep compartment (body fat e.g.).

Result: TOXICOKINETICS IN BLOOD
The concentration of ETBE in blood increased throughout exposure period (tending to level-off but no plateau achieved). Average maximum concentrations of 1.1, 5.4 and 10 mM were achieved at 5, 25 and 50 ppm respectively (i.e. essentially dose proportional). The elimination of ETBE from blood was well described by the four compartment model used. The apparent terminal elimination half-life was 28 h with no statistically significant differences between exposure levels. The AUC for ETBE was linearally related to exposure level, indicating linear kinetics up to 50 ppm. Clearance of ETBE in blood was in the range approximately 0.6 to 0.9 l/h/kg.

In contrast, the concentration of TBA in blood increased steadily throughout exposure and remained high for several hours post exposure. Average maximum blood concentrations at 30 min post exposure were 6.9 mM and 12 mM (25 ppm and 50 ppm respectively), and began to decline slowly from 4 h after exposure. Concentrations at 5 ppm were too low for quantification. AUC for TBA was linear with exposure level. Average blood elimination half-life for TBA was 12 h.

Acetone was detected at elevated levels in blood, being greatest (concentration, AUC) after exposure to 50 ppm ETBE, however concentrations in general were poorly correlated with exposure.

TOXICOKINETICS IN URINE
Urinary excretion of ETBE over 24 h was low (0.120, 0.061 and 0.056 % of respiratory uptake at 5, 25 and 50 ppm respectively). The elimination half-life of ETBE in urine was in the range 6.3 to 9.3 h. Corresponding urinary excretion of TBA over 24 h was 0.71, 0.66 and 0.90% at 5, 25 and 50 ppm respectively). The elimination half-life of TBA in urine was in the range 7.6 to 8.4 h. Acetone excretion (data not

reporetd) was widely variable between subjects and treatments but concentrations appeared exposure related.

ELIMINATION
The authors estimate that a total of approx. 50% of the retained dose of ETBE was subsequently excreted as ETBE and TBA in exhaled air and in urine.

Test substance: ETBE obtained from Ecofuel S.P.A., Italy. It was redistilled to a purity of 98.5% prior to use. Major impurities (identified by GC-MS) were TBA (0.4%) and MTBE (1.1%).

Conclusion: ETBE was absorbed from the respiratory tract with no evidence of metabolic saturation (linear kinetics over the exposure range tested), with rapid distribution and subsequent rapid elimination (exhaled air and urine) of ETBE and its major metabolite TBA. Approx. 50% of the absorbed dose was eliminated as ETBE and TBA in exhaled air and urine, suggesting that products of further further metabolism were present but not detected in this study.

Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.

15-JAN-2004 (62)

In Vitro/in vivo: In vivo
Type: Toxicokinetics
Species: human

Year: 2001
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Method: SUBJECTS AND TREATMENTS
Six healthy volunteers (3 female, age 26-29 yr, bwt 58-61 kg; 3 male, age 26-31 yr, bwt 62-83 kg) were exposed to ETBE vapour at concentrations of 4 or 40 ppm in a dynamic exposure chamber for 4 hr. The subjects refrained from consuming alcohol or medication for 2 d prior to the study, did not abuse alcohol and were non-smokers or occasional smokers. Subjects did not refuel their cars for 2 d prior to the study.

The exposure chamber had an internal volume of 8 m3, an air-flow rate of 28 m3/hr, a temperature of 22 degrees C and relative humidity of 50-60%. Preliminary analyses showed that exposure concentrations were uniform throughout the chamber. Exposures commenced at 0800 hr, with a 4 wk interval between the 4 ppm and 40 ppm exposures.

A group 10 rats was also placed inside the chamber to ensure identical exposure conditions were achieved during the animal phase of these investigations.

Blood collection: 10 ml samples, heparinised syringes)
Urine collection: collected (4 degrees C) every 6 hr for 72 hr

ANALYTICAL PROCEDURES
See record above

STATISTICAL METHODS
See record above

Remark: Some of this information has also been published in:

Amberg, A, Rosner, E and Dekant, W (2000) Biotransformation and kinetics of excretion of ethyl-tert-butyl ether in rats and humans. Toxicol Sci. 53, 194-201.

Dekant, W, Bernbauer, U, Rosner, E, Amberg, A. (2001) Toxicokinetics of ethers used a fuel oxygenates. Toxicol Lett 124, 37-45.

Result: RECEIVED DOSE IN HUMANS
Received doses of 121 and 1092 umol were calculated for subjects exposed to 4 ppm or 40 ppm ETBE for 4 hr, respectively.

TOXICOKINETICS AND BIOTRANSFORMATION OF ETBE BY HUMANS
-BLOOD
ETBE was not detected in blood samples taken before exposure commenced. A maximal concentration of 1.3 uM was achieved following 4 hr exposure to 4 ppm ETBE, with a maximum of 12.1 uM present after 4 hr exposure to 40 ppm. An elimination half-life of 1.1 hr was obtained for individuals exposed to 4 ppm, whereas two half-lives (1.1 hr; 6.2 hr) were present after exposure to 40 ppm. ETBE concentrations had decreased to the limit of detection 4 hr (4 ppm) or 24 hr (40 ppm) after exposure ended.

TBA was present at low levels (approx. 0.5 nmol/ml) in the pre-exposure blood samples, and increased significantly ($P<0.01$) following exposure to ETBE (max. 1.8 uM or 13.9 uM for the 4 ppm or 40 ppm exposures, respectively). Elimination half-lives of 8.2 hr or 9.8 hr were derived for the 4 ppm or 40 ppm exposures, respectively, with detectable amounts of TBA remaining 24 hr after exposure ended (numerical data not presented in report; from graphical results, the Reviewer estimates that the concentration of TBA in blood 24 hr post-exposure was approx. 0.5 uM or 5 uM for the low and high exposures, respectively). Clearance followed first order kinetics.

- URINE
TBA, 2MP and 2HIB were present in control urine prior to exposure to ETBE. The concentration of urinary metabolites increased post-exposure, with 2HIB the main metabolite. The following recovery data were obtained over 72 hr post-exposure:

ETBE (low exposure, high exposure)
- background: none detected
- corrected total excreted: 0.3, 0.9 umol
- half-life: 5.6, 3.5 hr

TBA (low exposure, high exposure)
- background: 2.0, 2.5 umol over 6 hr
- corrected total excreted: 5.1, 22.6 umol
- half-life: 14.6, 11.2 hr

2MP (low exposure, high exposure)
- background: 3.5-5.8 umol
- corrected total excreted: 13.6-96.6 umol
- half-life: 10.2, 12.3 hr

2HIB (low exposure, high exposure)
- background: 93.6, 167.4 umol
- corrected total excreted: 130.4, 522.6 umol
- half-life: 20.3, 28.3 hr

Test substance: ETBE, >99%, Aldrich Chemical Co., Deisenhofen, Germany.

Conclusion: ETBE vapour (4 ppm or 40 ppm) is readily absorbed by the human lung and distributed into the blood from which it was eliminated in a bi-phasic manner with half-lives of approx. 1 hr and 6 hr. It is readily converted to tert-butanol (primary metabolite) which exhibits an elimination half-life of 8-9 hr in blood. (The authors comment that the concentration of TBA in blood was less than that quantified in rats exposed under identical conditions.) Urinary metabolites were consistent with cytochrome P450-mediated metabolism of tert-butanol, and comprised 2-hydroxyisobutyrate (predominant metabolite) and 2-methyl-1,2-propanediol along with minor amounts of TBA.

Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.

02-JAN-2004 (25)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type:	LD50
Species:	rat
Strain:	other: OFA
Sex:	male/female
No. of Animals:	30
Vehicle:	CMC
Doses:	25, 200 and 2000 mg/kg bwt
Value:	> 2000 mg/kg bw
Method:	OECD Guide-line 401 "Acute Oral Toxicity"
Year:	1981
GLP:	yes
Test substance:	as prescribed by 1.1 - 1.4
Method:	Male and female OFA rats (100 - 120 g) were obtained from a commercial supplier and acclimatised for 5 d prior to treatment. ETBE was administered as a solution in 1% carboxymethyl cellulose at doses of 25, 200 and 2000 mg/kg bwt. There were 5 rats/sex/dose, and a dosing volume of 10 ml/kg was employed.
Result:	There were no deaths during the 14 day post-treatment observation period. Body weight and body weight gain in males was similar for the 3 treatment groups, whereas growth in females given 2000 mg/kg bwt appeared lower in comparison to the other treated female groups from day 10 onward. Macroscopic examination at necropsy revealed desquamation of the gastric mucosae in 2 male and 1 female given 2000 mg/kg ETBE, and also in 2 males from the 200 mg/kg bwt dose group.
Test substance:	ETBE, purity not stated, lot no. 408-2404, Total Raffinage Distribution, France.
Conclusion:	Under the conditions of the test, the acute oral LD50 of ETBE in the OFA rat was >2000 mg/kg bwt.
Reliability:	(1) valid without restriction Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.

02-NOV-2003 (42)

Type:	other: limit test
Species:	rat
Strain:	Sprague-Dawley
Sex:	male/female
No. of Animals:	10
Vehicle:	other: undliuted
Doses:	2.67 ml/kg, equivalent to 2003 mg/kg
Value:	> 2003 mg/kg bw
Method:	OECD Guide-line 401 "Acute Oral Toxicity"
Year:	1987
GLP:	yes
Test substance:	as prescribed by 1.1 - 1.4
Method:	Five male and five female SD rats (mean bwt 209 g and 171 g, respectively) were given a single dose of 2.67 ml/kg bwt undiluted ETBE by gavage. Body weights and clinical signs were monitored for 14 days post-dosing, and the animals taken to necropsy on day 15.
Result:	The behaviour of the animals was subdued 1-4 hr post-dosing, but there were no deaths and they gained weight in a normal manner. There were no unusual macroscopic findings at necropsy.
Test substance:	ETBE, 95.8%, batch no. 93050601, supplied by Elf France.
Conclusion:	Under the conditions of the test, the acute oral LD50 of ETBE in the SD rat was >2000 mg/kg bwt.
Reliability:	(1) valid without restriction Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.

04-JAN-2004 (69)

Type: LD50
Species: rat
Strain: Wistar
Sex: male/female
No. of Animals: 16
Vehicle: other: administered undiluted
Doses: 500, 1000, 2500, 5000 mg/kg bwt
Value: > 5000 mg/kg bw

Year: 1988
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Male and female Wistar rats (208-241 males; 212-230 females; age 8 wk) were obtained from a commercial supplier.

ETBE was administered undiluted at doses of 500, 1000, 2500 or 5000 mg/kg bwt (variable volume: 0.15-1.6 ml/rat). There were 2 rats/sex/dose level. Animals were observed for 14 d, then subject to gross necropsy.

Result: There were no deaths or clinical signs reported during the 14 day observation period. All animals appeared normal at necropsy.

Test substance: Supplied by ARCO Chemical Co., clear liquid, SG 0.73 (no further details)

Conclusion: Under the conditions of the test, the acute oral LD50 of ETBE in the OFA rat was >5000 mg/kg bwt.

Reliability: (1) valid without restriction
Study available for review. GLP compliant study following US-EPA guidelines, acceptable for assessment.

30-NOV-2003 (53)

Type: LD50
Species: rat
Value: > 2000 mg/kg bw

Method: other: no data
GLP: no data

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Test substance: ETBE

Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.

04-JAN-2004 (23)

5.1.2 Acute Inhalation Toxicity

Type: other: limit test
Species: rat
Strain: Crj: CD(SD)
Sex: male/female
No. of Animals: 10
Vehicle: other: filtered air
Doses: 5.99 mg/l
Exposure time: 4 hour(s)
Value: > 5.88 mg/l

Method: OECD Guide-line 403 "Acute Inhalation Toxicity"
Year: 1989
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Male and female SD rats (age approx. 4 wk) were obtained from a commercial supplier and held in quarantine for 2 wk. The animals weighed approx. 180 g (females) to 260 g (males) at the time of the study.

The vapour generating system comprised a U-shaped glass tube, heated to approx. 69 degrees C. ETBE was delivered into the tube at a constant rate (not specified) via 1/8 inch OD teflon tubing and a Fluid Metering Inc pump. The ETBE was completely vaporised under these conditions, and delivered to the exposure chamber (160 l, stainless steel; nose only exposure) at 59 l/min.

The concentration of test substance in air was monitored using IR spectrometry every 30 min.

The animals were exposed for 4 hr, followed by a 14 day observation period. Body weight was recorded weekly. A gross necropsy was performed at study termination.

Result: IR analysis demonstrated an actual mean+/-SD concentration of 5.88 +/- 0.077 mg/l; TWA = 5.88 mg/l.

There were no deaths during the exposure period or during the 14 d observation period.

Clinical signs comprised redness around the nose/eyes and discoloured facial fur immediately after exposure (appearance of most animals was normal the following day).

Weight gain was normal in all animals during the 2 wk observation period.

There were no treatment-related findings at necropsy. (Lung foci and/or red areas in the lung were noted in 6/10 rats. The report states these were of a type and severity common in control rats and therefore were considered unrelated to treatment.)

Test substance: ETBE, 99%, product no. LF-9072 from Aldrich Chemical Co., Milwaukee.
Conclusion: Under the conditions of the test, the acute inhalation LC50 of ETBE in male and female SD rats was >5.88 mg/l.
Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.
21-NOV-2003 (39)

Type: other: ocular and nasal effects after acute inhalation exposure
Species: human

Year: 1998
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: SUBJECTS AND TREATMENTS
8 healthy male volunteers (Caucasian; age 21 to 41 years, mean 29 years; bodyweight 70 to 97 kg, mean 82 kg). All were non-smokers, had no occupational exposure to ETBE and refrained from alcohol and drugs 2 days before and during the study. Exposures were conducted during 2 h of light physical exercise in an exposure chamber. Each subject exposed on 4 occasions at nominal levels of 0, 5, 25, and 50 ppm ETBE with at least 2 weeks between successive exposures. All sampling of blood and expired air was performed during exercise.
Reviewer's comment: ETBE is an odorous material, hence subjects would have been aware of when they were exposed to the control and test atmospheres.

The concentration of ETBE in the chamber air was analysed every 5 min during exposure and mean measured concentrations were 4.8, 25 and 50 ppm. Other variables such as temperature, humidity, air exchanges and average rate of exercise were closely monitored and found to be very consistent during each exposure and between different exposures.

SUBJECTIVE SYMPTOMS
A questionnaire was used to assess symptoms of subjective irritation (discomfort in the eyes, nose, throat and airways; difficulty breathing; smell) and effects on the central nervous system (headache, fatigue, nausea, dizziness, intoxication). Responses were self-recorded on a visual analogue scale graded from "not at all" to "almost unbearable". Responses were recorded on 8 occasions (pre-exposure, during exposure while exercising, 4 hr post-exposure).

OCULAR MEASUREMENTS
Blinking frequency (from video tape record), eye redness (photographic comparison; 50 ppm only), tear film break-up time (slit-lamp with fluorescein; 50 ppm only) and conjunctival epithelial damage (Lissamine green) were assessed

using published methods (Nihlen et al (1998) Toxicol Appl Pharmacol, 148, 281). Assessments were recorded by a single observer to reduce variability.

NASAL MEASUREMENTS
Nasal mucosal swelling (acoustic rhinometry) and analysis of nasal lavage (leukocytes, epithelial cells, inflammatory markers) were assessed using published methods (Nihlen et al (1998) Toxicol Appl Pharmacol, 148, 281).

PULMONARY FUNCTION
Peak expiratory flow (PEF), forced vital capacity (FVC), one-second forced expiratory volume (FEV1) and vital capacity (VC) were assessed following guidelines of the American Thoracic Society Medical Section of the American Lung Association (1994, Am j Respir Crit Care med, 152, 1107). Lung transfer factor (TLco; a measure of the gas exchange characteristics of lung parenchyma) was determined using carbon monoxide (0.3%) as marker) (Cotes et al. (1993) Eur Respir 6, 41-52).

STATISTICAL METHODS
The data were analysed using repeated ANOVA.

Result:

SUBJECTIVE SYMPTOMS
Graphical data indicated that subjective discomfort of the throat and airways was increased significantly after 40 exposure to 50 ppm ETBE, and remained elevated for the remainder of the 2 hr exposure period (P=0.02; control score = approx. 2.5 units; exposed score = approx. 6-8 units). Symptoms had resolved 80 min after exposure ceased. The taste and odour of the test atmosphere was unpleasant and unacceptable to the subjects.
Reviewer's comment: The authors state that subjective symptoms of ocular and nasal discomfort, fatigue, nausea dizziness and intoxication were greater in the 50 ppm group, but no dose-response relationship or statistically significant difference was present. The intensity of the response at 50 ppm appeared to be very mild (stated to be between "not at all" and "hardly at all").

OCULAR MEASUREMENTS
There was no statistically significant effect on eye redness, tear-film break-up, conjunctival damage or blinking frequency.

NASAL MEASUREMENTS
Nasal volume was decreased by 6-15% (P = 0.001) in all groups, including controls, compared to pre-exposure values. Exposure to ETBE vapour was without effect on the composition of nasal lavage fluid (i.e. leukocytes, epithelial cells, inflammatory markers etc).

PULMONARY FUNCTION
A small (3-4%) but significant (P<0.05) decrease in FVC and VC was reported in subjects exposed to 25 or 50 ppm ETBE vapour.

Reviewer's comment: The author's state that FEV1 and TLco were also decreased significantly (approx. 2% and 2-4%, respectively) after exposure, however data included in the publication are assigned P values of 0.06-0.5 ie non-significant. The reason for this discrepancy is not known.

Test substance: ETBE, technical grade, supplied by Ecofuel S.P.A, Italy.

Conclusion: The authors conclude that exposure to 50 ppm ETBE vapour for 2 hr was associated with subjective irritation of the throat and airways and slightly impaired lung function. This reviewer notes that these changes were mild in nature and minor in extent.

Reliability: (4) not assignable
Study available for review. Highly odourous nature of the test substance sample suggests that subjective findings may be unreliable. Inconsistencies present in reporting of statistical significance of changes in key lung parameters.

15-JAN-2004 (63)

5.1.3 Acute Dermal Toxicity

Type: other: limit test
Species: rabbit
Strain: New Zealand white
Sex: male/female
No. of Animals: 10
Vehicle: other: applied undiluted
Doses: 2000 mg/kg bwt, under occlusion
Value: > 2000 mg/kg bw

Method: other: comparable to OECD 402
Year: 1988
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Male and female New Zealand rabbits (5 per sex; age approx. 8 wk) were obtained from a commerical supplier. The animals weighed 2.3-2.9 kg (males) and 2.5-2.8 kg (females) at the start of the study.

ETBE (6.9-7.9 ml, equivalent to 2 g/kg bwt) was applied undiluted to at least 10% of the body surface (clipped free of fur), covered by gauze and then secured with tape (occlusion) for 24 hr. The treatment site was cleaned (water) after removal of the patch and observed for signs of dermal irritation on 1, 7 and 14 d after post-dosing.

Animals were also observed 1, 2, 4 hours post-dosing and twice daily thereafter for 14 days for mortality, toxicity and pharmacological effects. Dermal responses were recorded using the Draize scoring system.

Body weights recorded pre-test, weekly and at termination.

All animals were subject to a gross examination at necropsy on day 14. Abnormal tissues preserved for future microscopic examination.

Result: One male rabbit died on day 3.

Clinical signs included one male with diarrhoea from day 8; one female with yellow nasal discharge from day 2; one female with lachrymation on days 6-9.

Dermal reactions included:
- very slight to slight erythema (grade 1-2) on day 1, very slight to severe erythema (grade 1-4) on day 7, and absent to severe erythema (grade 0-4) on day 14;
- slight to moderate-to-severe oedema (grade 2-3) present day 1, very slight to moderate-to-severe oedema (grade 1-3) on 7, and absent or slight oedema (grade 0-2) on day 14.
- flaking skin was present at days 7 and 14.

All animals exhibited skin irritation and poor hair regrowth (considered indicative of injuries in-depth by the report) at necropsy. Males which died had congested lungs with brown areas, excess fluid in pleural and peritoneal cavities, pale margins in liver.

Test substance: ETBE, purity not stated, SG 0.73, clear liquid, from ARCO Chemical Co., PA (no further details).

Conclusion: Under the conditions of this test, the acute dermal LD50 of ETBE in male and female New Zealand white rabbits was >2000 mg/kg bwt.

Reliability: (1) valid without restriction
Study available for review. GLP compliant near-guideline investigation, clearly reported, acceptable for assessment.

02-JAN-2004 (56)

Type: other: limit test
Species: rabbit
Strain: New Zealand white
Sex: male/female
No. of Animals: 10
Vehicle: other: applied undiluted
Doses: 2000 mg/kg bwt, under occlusion
Value: > 2000 mg/kg bw

Method: other: comparable to OECD 402
Year: 1989
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Male and female New Zealand rabbits (5 per sex; age approx. 8 wk) were obtained from a comerical supplier. The animals weighed approx. 3.7 kg (males) to 4.0 kg (females) at the start of the study.

ETBE (undiluted, equivalent to 2 g/kg bwt) was applied uniformly to the shaved dorsum of each rabbit, covered with a

surgical dressing under plastic film (occlusion) and held in place by elastic adhesive bandage for 24 hr. The application site was cleaned with light mineral oil and towel dried on removal of the patch. The treatment site was observed for signs of dermal irritation on 1, 7 and 14 d after post-dosing.

Animals were observed regularly for up to 5 hr post-dosing, then daily for 14 days.

Body weights recorded pre-test, weekly and at termination.

All animals were subject to a gross examination at necropsy on day 14.

Result: There were no deaths.

Clinical signs included a transient increase in respiration in 3 male and 4 female rabbits approx. 30 minutes after treatment.

Dermal irritation (erythema and oedema) was present at the application site of all rabbits on removal of the patch. Eschar formation was present at the test site of most animals within 5-7 days, and eventually in all the animals at day 14. (Comment: no quantitative information on the severity of the response is given in the report)

No other overt signs of toxicity were present.

Mean bwt increased slightly during the study (no further details).

Three females had multiple red foci on the lungs at necropsy, but this was not considered to be treatment related.

Test substance: ETBE, 99%, product no. LF-9072 from Aldrich Chemical Co., Milwaukee.

Conclusion: Under the conditions of this test, the acute dermal LD50 of ETBE in male and female New Zealand white rabbits was >2000 mg/kg bwt.

Reliability: (1) valid without restriction
Study available for review. GLP compliant near-guideline investigation, clearly reported, acceptable for assessment.

02-NOV-2003 (38)

Type: LD50
Species: rat
Value: > 20 mg/kg bw

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
02-NOV-2003

5.1.4 Acute Toxicity, other Routes

-

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

Species: rabbit
Concentration: 100 %
Exposure: Semiocclusive
Exposure Time: 4 hour(s)
No. of Animals: 6
Result: not irritating
EC classificat.: not irritating

Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 1981
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: The skin irritation potential of ETBE was investigated in 6 male New Zealand White rabbits (2.7 +/- 0.2 kg bwt).

0.5 ml ETBE was applied to clipped skin (6 cm2) on the right flank for 4 hr under a semi-occlusive dressing.

Result:

Mean scores at:	1 hr	24 hr	48 hr	72 hr
Erythema	1.0	1.5	0.5	0.0
Oedema	1.3	0.0	0.0	0.0

Test substance: ETBE, purity not stated, supplied by Total Raffinage Distribution.
Conclusion: Under the conditions of the test, ETBE was not irritating to rabbit skin.
Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.
02-NOV-2003 (20)

Species: rabbit
Concentration: undiluted
Exposure: Occlusive
Exposure Time: 4 hour(s)
No. of Animals: 6
Vehicle: other: applied undiluted
PDII: 3.08
Result: moderately irritating

Method: other: comparable to Guideline 404
Year: 1988
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: New Zealand white rabbits (2 male, 4 females; age approx. 8 wk; bwt 2-3 kg) were obtained from a commercial supplier.

The dorsum and flanks were clipped free of fur, and the left side lightly abraded with a needle.

0.5 ml of test substance was applied to two regions areas of intact skin and two of abraded skin (are not specified), covered with gauze under plastic (occlusion), and held in place with adhesive tape.

The patch was removed after 4 hr, and dermal responses recorded at 4, 24, 48 and 72 hr using the Draize scoring system. The primary irritation index was calculated, based upon the mean score for erythema/eschar and oedema formation at both intact and abraded sites at the 4 time points.

The post-treatment observation period was extended to 7 d and 14 d to examination reversibility of any skin effects present.

Result: Erythema, generally absent at 4 hr, was well-defined at 24 hr and 48 hr, and slight-to-well defined at 72 hr. By day 7, erythema was absent to well-defined. At day 14, no erythema was present.

Oedema was slight to well defined at 4, 24 or 48 hr, and absent to well defined at 72 hr.

Flaking skin was present at majority of sites on day 7 (absent at preceding and later timepoints).

The PII was 3.08.

The following results were recorded at the intact skin sites:

Mean scores at:	4 hr	24 hr	48 hr	72 hr
Erythema	0.00	2.00	2.00	1.83
Oedema	1.75	1.75	1.42	1.25

Test substance: ETBE, purity not stated, clear liquid, from ARCO Chemical Co., PA (no further details).

Conclusion: Under the conditions of the study, ETBE was moderately irritating to intact and abraded rabbit skin following 4 hr occluded contact.
Reliability: (1) valid without restriction
Study available for review. GLP compliant near-guideline investigation, clearly reported, acceptable for assessment.
30-NOV-2003 (54)

Species: rabbit
Concentration: 100 %
Exposure: Semiocclusive
Exposure Time: 4 hour(s)
No. of Animals: 3
Result: not irritating
EC classificat.: not irritating

Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 1992
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: The skin irritation potential of ETBE was investigated in 3 male New Zealand White rabbits (2 - 3 kg bwt).

0.5 ml ETBE was applied to a gauze pad (2.5 x 2.5 cm) which was held in contact with clipped healthy skin under a semi-occlusive dressing for 4 hr.
Result:

Mean scores at:	1 hr	24 hr	48 hr	72 hr
Erythema	1.0	1.0	0.67	0.33
Oedema	0.67	0.33	0.0	0.0

One animal showed slight desquamation at the application site on day 7
Test substance: ETBE, 95.8%, batch no. 93050601, supplied by Elf France.
Conclusion: Under the conditions of the test, ETBE was not irritating to rabbit skin.
Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.
02-NOV-2003 (68)

Species: rabbit
Result: slightly irritating
EC classificat.: not irritating

Method: other: EEC Directive 92/69
GLP: no data
Test substance: other TS

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test substance: ETBE
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
04-JAN-2004 (23)

5.2.2 Eye Irritation

Species: rabbit
Concentration: 100 %
Dose: .1 ml
Exposure Time: 24 hour(s)
No. of Animals: 6
Result: not irritating
EC classificat.: not irritating

Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: 1987
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: The eye irritation potential of ETBE was investigated in 6 male New Zealand White rabbits (2.9 +/- 0.25 kg bwt).

0.1 ml ETBE was instilled into the conjunctival sac of the left eye, the right eye serving as a control. Ocular examinations were carried out 1 hr after administration, then at 24, 48 and 72 hr post treatment.
Result: Chemosis (mean score = 0.8) present at 1 hr post treatment had resolved by the 24 hr observation period. Scores for discharge, iridial effects, corneal opacity and surface opacity were zero at all time points included in the investigation.
Test substance: ETBE, purity not stated, supplied by Total Raffinage Distribution.
Conclusion: Under the conditions of the test, ETBE was not irritating to rabbit eye.
Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.
02-NOV-2003 (21)

Species: rabbit
Concentration: 100 %
Dose: .1 ml
Exposure Time: 24 hour(s)
No. of Animals: 3
Result: not irritating
EC classificat.: not irritating

Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: 1987
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: The eye irritation potential of ETBE was investigated in 3 male New Zealand White rabbits (2 - 3 kg bwt).

0.1 ml ETBE was instilled into the conjunctival sac of the right eye, the left eye serving as a control. Ocular examinations were carried out 1 hr after administration, then at 24, 48 and 72 hr post treatment. Readings were continued on days 7 and 14 to determine the reversibility of any lesions present at 72hr.

Result: Mean individual scores at (24+48+72hr):

	1	2	3
Chemosis	0.00	0.67	0.33
Redness	0.33	1.67	0.33
Congestion	0.33	1.00	0.67
Degree of opacity	0.00	0.67	0.00

Residual redness in one animal (score = 1) and residual congestion in another (score = 1) had resolved completely by day 7.

Test substance: ETBE, 95.8%, batch no. 93050601, supplied by Elf France.
Conclusion: Under the conditions of the test, ETBE was not irritating to rabbit eye.
Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.
02-NOV-2003 (67)

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time: 24 hour(s)
Comment: rinsed after (see exposure time)
No. of Animals: 9
Vehicle: other: used undiluted
Result: moderately irritating

Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: 1988
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Male and female New Zealand white rabbits (age approx. 8 wk; 2-3 kg at study initiation) were purchased from a commercial supplier.

0.1 ml ETBE was instilled into the conjunctival sac of the left eye of 6 rabbits, the right eye serving as a control. Ocular examinations were carried out 1 hr after administration, then at 24, 48 and 72 hr post treatment.

Treated eyes of a further 3 rabbits were flushed with water one minute after dosing.

The eyes were subject to an ophthalmic examination for corneal, iridial and conjunctival effects on days 1, 2, 3, 7 and 14 using a Draize scoring system.

Result: UNWASHED
In the unwashed eyes corneal opacity (grade 2) was noted in 1 out of 6 eyes on days 1-3, fully resolved by day 7. Minor iridial involvement (score 1) was also present (same animal) at day 1 only.

Conjunctival redness was present in all animals, resolved by day 7:
Day 1 1, 1, 2, 2, 2, 1
Day 2 0, 1, 2, 2, 2, 1
Day 3 0, 1, 1, 1, 1, 1
Day 7 0, 0, 0, 0, 0, 0
Day 14 0, 0, 0, 0, 0, 0

Conjunctivae swelling (chemosis) was present in all animals, resolved by day 7:
Day 1 2, 2, 2, 2, 2, 1
Day 2 0, 0, 2, 2, 2, 1
Day 3 0, 0, 2, 0, 0, 0
Day 7 0, 0, 0, 0, 0, 0
Day 14 0, 0, 0, 0, 0, 0

Scores for 3 washed eyes were generally slightly lower.

Overall mean irritation Draize score (maximum possible 110):
1 day 12.2
2 days 8.3
3 days 4.0
7 days 0
14 days 0

Test substance: ETBE, purity not stated, clear liquid, from ARCO Chemical Co., PA (no further details).

Conclusion: Under the conditions of this investigation, ETBE was moderately irritating to rabbit eye.

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.

30-NOV-2003 (55)

Species: rabbit
Result: slightly irritating
EC classificat.: not irritating

Method: other: EEC Directive 92/69
GLP: no data
Test substance: other TS

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test substance: ETBE
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
04-JAN-2004 (23)

5.3 Sensitization

Type: Guinea pig maximization test
Species: guinea pig
Concentration 1st: Induction 10 % intracutaneous
2nd: Challenge 100 % occlusive epicutaneous
No. of Animals: 30
Result: not sensitizing
Classification: not sensitizing

Method: OECD Guide-line 406 "Skin Sensitization"
Year: 1992
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS
Hartley guinea pigs (approx. 6 wk old and weighing 250 - 550 g) were randomly assigned to control (5 male + 5 female) and test (10 male + 10 female) groups.

INDUCTION
- intradermal injection:
- 50% Freund's complete adjuvant (2 x 0.1 ml)
- 10% v/v ETBE in sterile liquid paraffin (2 x 0.1 ml)
- 10% v/v ETBE in 50% Freund's complete adjuvant (2 x 0.1 ml)
The control animals received sterile liquid paraffin (2 x 0.1 ml), also by intradermal injection.

- topical occlusive application:
0.5 ml undiluted ETBE was applied to the injection site (under occlusion) after treating the skin with 10% SLS. The control animals received sterile liquid paraffin in place of ETBE.

CHALLENGE

	0.5 ml undiluted ETBE was applied to a 2 x 2 cm area of clipped and depilated skin on the left flank of each control and test animal. Skin responses were recorded at 24 and 48 hr post-challenge.
Result:	Visual examination of the challenge site revealed no evidence of any redness or oedema in any of the control or test animals (all scores = 0).
Test substance:	ETBE, 95.8%, batch no. 93050601, supplied by Elf France.
Conclusion:	Under the conditions of the test, ETBE did not provoke any sign of cutaneous sensitisation in the guinea pig.
Reliability:	(1) valid without restriction Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.

02-NOV-2003 (66)

Type:	no data
Species:	other: presumably rabbit
Result:	not sensitizing
Classification:	not sensitizing

Method:	other: EEC Directive 92/69
GLP:	no data
Test substance:	other TS

Source:	Agip Petroli SpA ROMA EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test substance:	ETBE
Reliability:	(4) not assignable This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.

04-JAN-2004 (23)

5.4 Repeated Dose Toxicity

Type: Sub-chronic
Species: rat **Sex:** male/female
Strain: Fischer 344
Route of administration: inhalation
Exposure period: 90 d
Frequency of treatment: 6 h/d, 5 d/wk for 13 wk
Doses: 0 (filtered air), 500, 1750 or 5000 ppm
Control Group: yes, concurrent vehicle
NOAEL: = 500 ppm
LOAEL: = 1750 ppm

Method: EPA OTS 798.2450
Year: 1996
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS AND MAINTENANCE
Animals: Fischer F344 rats (Charles River Laboratories, Raleigh, NC, USA), males and females, age approx. 5 wk on receipt
Acclimation period: 10 d
Housing: individually housed, stainless steel cages (wire mesh bottom) within exposure chamber (one per sex per exposure level) for duration of study
Diet: NIH-07 (Zeigler Brothers Inc), ad libitum
Water: deionised water, ad libitum
Light cycle: 12 hr light/dark period (lights on 0700-1900)
Assignment to treatment groups: computer-generated weight randomisation

GENERAL EXPERIMENTAL DESIGN
Animals were exposed to target concentrations of 0 (filtered air), 500, 1750 or 5000 ppm. The highest exposure level was approx. 50% of the lower explosive limit for ETBE. All animals were observed for mortality and overt clinical signs just before and shortly after exposure, and once daily on non-exposure days. An ophthtalmic examination was performed on all rats prior to the start of the study and again at study termination. Body weights from all rats were recorded weekly. The study comprised the following elements:
- main study group (11 rats/sex/exposure level)
- neurotoxicity subgroup (12 rats/sex/exposure level; see separate record)
- interim clinical chemistry and haematology sub-group (10 rats/sex/exposure level)
- cell replication subgroup (15 rats/sex/exposure level)

MAIN STUDY
Main study animals were exposed to target concentrations of 0, 500, 1750 or 5000 ppm ETBE vapour 6 hr/d, 5 d/wk for at least 13 wk (equivalent to at least 65 exposures). Animals were anaesthetised (sodium pentobarbital) and sacrificed by

exsanguination. All major organs were subject to macroscopic examination, and samples retained in 10% neutral buffered formalin (NBF). Organ weights were recorded for selected tissues (adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes). All retained tissues were processed (H&E staining) and those from the control and high dose groups subject to microscopic examination. Lungs, liver, kidneys, testes(* see below) and femoral bone marrow (females only) from low and intermediate level animals were also examined.

*Note: one hundred round cross sections of seminiferous tubules were examined per testis and assigned to a specific group of developmental stages according to Hess (1990) (Biology of Reproduction, 43, 525-542). The number of tubules that contained debris and degenerating or necrotic cells was also recorded.

CLINICAL CHEMISTRY AND HAEMATOLOGY DETERMINATIONS
Animals (10/sex/group) scheduled for interim clinical chemistry and haematology investigations were exposed to 0, 500, 1750 or 5000 ppm for 6 wk (32-33 exposures for males or females, respectively). Analyses were also performed on main study animals at termination (wk 13). Blood was collected by cardiac puncture (sodium pentabarbital anaesthesia).

Haematological evaluations were performed on blood containing EDTA as anticoagulant using a Serono Hematology System 910 analyser, and included haematocrit, total leukocyte count, haemoglobin concentration, erythrocyte count, platelet count, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Differential leukocyte counts were performed on a blood smears.

Clinical chemistry determinations (serum) were performed using a Roche Cobass Fara II analyser and included glucose (non-fasting), urea, nitrogen, bilirubin, creatinine, calcium, total protein, albumin, sodium, chloride, potassium, phosphorus, aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase and gamma-glutamyl transferase.

CELL REPLICATION SUBGROUP
Five animals/sex/group were exposed to 0, 500, 1750 or 5000 ppm ETBE for 1, 4 or 13 wk (equivalent to 4, 19 or 63 exposures). Alzet 2ML1 pumps were implanted (s.c.) after the first exposure in each investigation week and delivered BrdU (16 ug/hr) for 3.5 d prior to sacrifice. Animals were removed from the exposure chamber on the morning of day 5 of each investigation week (prior to exposure), anaesthetised (sodium pentobarbital) and perfused in situ with 10% NBF. The kidneys (potential target tissue) and a section of duodenum (control tissue) were removed, fixed (10% NBF) and embedded in paraffin wax. BrdU-labelled cells (5 um sections) were identified by immunohistochemical staining. Labelling index (LI; the percentage of total nuclei counted that were positive for

BrdU) was determined by counting a minimum of 1000 renal cortical epithelial cells from 2 kidneys per animal using the Cytology/Histology Recognition System (Sverdrup Technology Inc, Walton Beach, FL).

OTHER RENAL HISTOPATHOLOGY
Additional studies were performed on individual kidneys to determined the presence and extent of protein droplet accumulation (one section haematoxylin and eosin; another Mallory Heidenhain stain) and the presence of a2u-globulin (immunohistochemical staining) in renal cortical cells.

EXPOSURE AND GENERATION OF TEST ATMOSPHERE
Animals were exposed in 1000 litre multi-tier, stainless steel, whole body exposure chambers (one chamber per sex per exposure level). Vapour concentration was determined (GC-FID) at 5 positions within each chamber prior to introduction of animals to check uniformity of exposure (variation <8%), with cages rotated weekly to compensate for any minor variations that might be present. Airflow was 200-250 l/min (12-15 changes per hr). Environmental conditions were maintained in the range 71.0-72.1 degrees C, relative humidity 48.0-53.1%.

The test atmosphere was generated by passing liquid ETBE through a metering pump and into a heated stainless steel J-tube; nitrogen gas carried the vapour into the exposure chamber. The concentration of ETBE within the chamber (animals' breathing zone) was analysed by GC-FID at least 6 times per day.

STATISTICAL METHODS
The Shapiro-Wilks' test (normality) and the O'Brien and Brown-Forsythe (homogeneity) test were followed by ANOVA and Dunnetts's test (parametric data) or the Kruskal-Wallis test (non-parametric data) on group means. A P value <0.05 was considered significant. Histopathology incidence data were evaluated using Fisher's exact test with Bonferroni correction. Labelling index and testicular data were transformed (arcsine) prior to analysis using William's test.

Result:

GENERAL
Gas chromatographic analysis showed that the mean concentration of ETBE within the test chambers over the course of the study was 505(13), 1748(59) and 4971(155) ppm (SD given in brackets). No ETBE was detected in the control chamber.

All treated animals survived to scheduled study termination. Transient ataxia the only clinical sign noted (high dose males only, post-exposure only). Absolute body weight was decreased significantly in males (3-6%) and females (2-5%) during study weeks 1-3, while body weight gain was decreased by approx. 25% in intermediate and high dose animals of both sexes during the first week of treatment.

HAEMATOLOGY

Platelet counts were increased 14-16% (P<0.05) and MCHC decreased 2-4% (P<0.05) in high dose males at wk 6 and wk 13. MCHC was also slightly decreased (2.5%; P<0.05) in intermediate dose males at study termination only. MCV was increased 1% (P<0.05) in high dose females at the same time points (but RBC count unaffected, hence not indicative of regenerative anaemia). There were no other treatment related alterations in haematological parameters in either sex.

CLINICAL CHEMISTRY
Serum chloride was decreased 3-6% (P<0.05) and total protein increased 9-11% (P<0.05) in male rats exposed to 1750 ppm or 5000 ppm ETBE for 6 wk or 13 wk. Serum phosphorus (decreased 17%) and bilirubin (decreased 39%) were altered in high dose females at wk 6 (both P<0.05), however these parameters were unremarkable at study termination. There were no other treatment related alterations in clinical chemistry endpoints in either sex.

NECROPSY OBSERVATIONS AND ORGAN WEIGHTS
No treatment related gross abnormalities were present in either sex at scheduled necropsy. Absolute mean kidney (10-19%) and liver (22-32%) weights were increased significantly (P<0.05) in intermediate and high dose males following 13 wk exposure to ETBE, while adrenal weights were increased 34% (P<0.05) in high dose males only. For females, absolute heart (12%) and liver (26%) weights were increased significantly in high dose animals, while kidney weights were elevated 12-21% (P<0.05) in the 1750 ppm and 5000 ppm groups, respectively.

HISTOPATHOLOGY
Kidneys and testes from males and femoral bone marrow from females were the only tissues to exhibit treatment related microscopic lesions.
Reviewer's comment: The source references contain only incidence data, and generally no information on severity.

- males, kidney (LOAEL 500 ppm)
The incidence of nephropathy (occurrence of regenerative foci) was increased significantly (P<0.05) in all treatment male groups relative to the controls. Results summarised by exposure level:
4/11 (36%), 10/11 (91%) 11/11 (100%), 11/11 (100%)

- testes (NOAEL 500 ppm)
Exposure to 1750 ppm or 5000 ppm ETBE resulted in a significant (P<0.05) increase in the incidence of degeneration of the seminiferous tubules of the testes of male rats. Results (mean incidence per 100 examined with SD in brackets) summarised by exposure level:
2(1), 2(2), 8(4), 13(11)

Reviewer's comment: The large standard deviation indicates

that this finding was inconsistently expressed in high dose males. Overall range by exposure level:
1-4, 1-7, 3-16, 0-39.
Review of the individual animal data (pathology report) reveals that some animals were unaffected by treatment (incidence 0 or 2) while others exhibited a pronounced 10-20 fold increase the incidence of degeneration.

ETBE treatment was without effect on the percentage of tubules with debris was present in the lumen. Results (mean percent with SD in brackets) summarised by exposure level:
2(2), 1(2), 3(8), 1(1)

There was no change in the percentage of seminiferous tubules within the various functional stages of development when treated animals were compared with controls, consistent with no treatment related effect on spermatocyte maturation. Results (mean percent with SD in bracket) summarised by exposure level:
Stage I-V: 36(5), 32(5), 34(6), 36(6)
Stage VI-VIII: 30(6), 27(4), 31(3), 29(6)
Stage IX-XIII: 29(7), 37(3), 30(5), 31(6)
Stage XIV: 5(2), 5(2), 5(2), 5(2))

The report notes in those testes with tubular degeneration, aberrant spermatids appeared most common in seminiferous tubules in stages IX-XIII (no further details).

- females, femoral bone marrow (NOAEL 500 ppm)
An increased incidence of congestion was observed in femoral bone marrow from female rats exposed to 1750 ppm or 5000 ppm ETBE for 13 wk. Incidence by exposure level:
0/10, 0/11, 5/11 (45%), 11/11 (100%)

TIMECOURSE AND CELL PROLIFERATION STUDIES IN KIDNEY
- MALES
H&E staining revealed a concentration dependent increase in nephropathy, as determined from the increase in mean number of regenerative foci. Results (mean number of regenerative foci per 2 kidney sections/rat) by week and exposure level:
- 1 wk: 0, 0, 0, 0
- 6 wk: 0, 0, 2, 4
- 13 wk: 2, 11, 17, 34

Proximal tubule labelling indices was increased in high dose male rats after 1 wk or 4 wk exposure to ETBE, and in all treated males at wk 13. Results by week and exposure level:
- 1 wk: 3.5, 4.9, 4.3, 7.1 ($P<0.01$)
- 4 wk: 3.3, 4.0, 2.8, 9.0 ($P<0.001$)
- 13 wk: 0.9, 2.2 ($P<0.01$), 3.4 ($P<0.001$), 2.5 ($P<0.001$)
(The report notes that tissue growth and maturation accounts for the variation in the control data from the different timepoints.)

Mallory-Heidenhain stain demonstrated increased severity of protein droplet accumulation in the renal tubule from male rats exposed to ETBE. Results by week and exposure level:
- 1 wk: 1.2, 3.4, 4.0, 4.6
- 4 wk: 1.8, 2.6, 3.4, 3.8
- 13 wk: 1.8, 3.0, 3.2, 3.8
Grading: 1 = minimal; 2 = <10% of cortex involved; 3 = 10-25% of cortex; 4 = 25-50% of cortex; 5 = >50% of cortex.

Immunoreactivity toward a2u-globulin was present in the protein droplets present in kidneys from all exposed males.

- FEMALES
There was no microscopic evidence of any treatment related increase in nephropathy in female rats.

Proximal renal tubule labelling indices were elevated in all females rats for all treatment groups at wk 1 and wk 4 but were indistinguishable from controls at wk 13. Results by week and exposure level:
- 1 wk: 2.6, 4.2 (P<0.05), 5.0 (P<0.05), 4.0 (P<0.05)
- 4 wk: 1.4, 1.4, 1.6, 1.8 (P<0.05)
- 13 wk: 0.6, 1.0, 1.0, 0.9

The Mallory-Heidenhain stain was negative for protein droplet accumulation in proximal tubules.

There was no a2u-globulin immunoreactivity in kidneys from any of the females exposed to ETBE.

Test substance: ETBE, 97.5% pure (by GC-FID) was supplied by the Sponsor (ARCO Chemical Company, PA, USA) and manufactured by Phillips 66 (no further details). The chemical composition of the test substance was verified using NMR.

Conclusion: Survival, body weight and the majority of clinical chemistry and haematological parameters were unremarkable in male and female F344 rats exposed to 5000 ppm ETBE by inhalation for up to 13 weeks. Degenerative changes in testicular seminiferous tubules were present in males exposed to 1750 ppm or 5000 ppm, while concurrently exposed females responded with increased congestion of femoral bone marrow. An increased incidence of renal nephropathy, cell proliferation and accumulation of protein droplets in proximal tubules from all exposed males was associated with positive immunoreactivity toward a2u-globulin, suggesting the findings were specific to the male rat. Overall these results are consistent with a sub-chronic NOAEL for ETBE in the rat of 500 ppm, based upon histopathological changes in testes and bone marrow at 1750 ppm.

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, well documented methods and results, raw data available, acceptable for assessment.

30-NOV-2003 (13) (58)

Type: Sub-chronic
Species: mouse **Sex:** male/female
Strain: CD-1
Route of administration: inhalation
Exposure period: 90 d
Frequency of treatment: 6 h/d, 5 d/wk for 13 wk
Doses: 0 (filtered air), 500, 1750 or 5000 ppm
Control Group: yes, concurrent vehicle
NOAEL: = 1750 ppm

Method: EPA OTS 798.2450
Year: 1996
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS AND MAINTENANCE
Animals: CD-1 mice (Charles River Laboratories, Raleigh, NC, USA), males and females, age approx. 5 wk on receipt
All other details identical to the preceding record (rat 90 d inhalation study)

GENERAL EXPERIMENTAL DESIGN
Animals were exposed to target exposure concentrations of 0 (filtered air), 500, 1750 or 5000 ppm. The study comprised the following elements:
- main study group (15 mice/sex/exposure level)
- clinical chemistry and haematology sub-group (10 mice/sex/exposure level)
- cell replication subgroup (15 mice/sex/exposure level)
See preceding record (rat 90 d inhalation study) for other details.

MAIN STUDY
Main study animals were exposed to target concentrations of 0, 500, 1750 or 5000 ppm ETBE vapour 6 hr/d, 5 d/wk for at least 13 wk (equivalent to at least 65 exposures). Animals were anaesthetised (sodium pentobarbital) and sacrificed by exsanguination. All major organs were subject to macroscopic examination, and samples retained in 10% neutral buffered formalin (NBF). Organ weights were recorded for selected tissues (adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes). All retained tissues were processed (H&E staining) and those from the control and high dose groups subject to microscopic examination. Lungs, liver (with gall bladder), kidneys and all gross lesions from low and intermediate level animals were also examined.

CLINICAL CHEMISTRY AND HAEMATOLOGY DETERMINATIONS
Ten mice per sex per exposure level were subject to clinical chemistry and haematological evaluation at study termination (see preceding record, rat 90 d inhalation study). Animals were subsequently subject to a full necropsy.

CELL REPLICATION SUBGROUP

Fifteen animals/sex/group were exposed to 0, 500, 1750 or 5000 ppm ETBE for 1, 4 or 13 wk (equivalent to 4, 19 or 63 exposures). Alzet 2001 pumps were implanted (s.c.) after the first exposure in each investigation week and delivered BrdU (16 ug/hr) for 3.5 d prior to sacrifice. Animals were removed from the exposure chamber on the morning of day 5 of each investigation week (prior to exposure), anaesthetised (sodium pentobarbital). The livers (potential target tissue) and a section of duodenum (control tissue) were removed, fixed (10% NBF) and embedded in paraffin wax. BrdU-labelled cells (5 um sections) were identified by immunohistochemical staining. Labelling index (LI; the percentage of total nuclei counted that were positive for BrdU) was determined by counting a minimum of 1000 hepatocytes per animal.

EXPOSURE AND GENERATION OF TEST ATMOSPHERE
See preceding record (rat 90 d inhalation study)

STATISTICAL METHODS
See preceding record (rat 90 d inhalation study)

Result:

GENERAL
Gas chromatographic analysis showed that the mean concentration of ETBE within the test chambers over the course of the study was 505(13), 1748(59) and 4971(155) ppm (SD given in brackets). No ETBE was detected in the control chamber. Six male and six female mice died during the study; five were found dead (controls + exposed animals) and 7 died from accidental trauma. Transient ataxia was occasionally observed post-exposure in high dose animals of both sexes. Absolute body weight and body weight gain were unaffected by treatment.

HAEMATOLOGY
Haemoglobin and haematocrit values were increased significantly in male mice exposed to 1750 ppm ETBE relative to the controls, however values for the high dose group and intermediate or high dose females were unaffected suggesting these findings were unrelated to treatment. There were no other treatment related alterations in haematological parameters in either sex.

CLINICAL CHEMISTRY
All changes in ETBE exposed mice were minimal in extent and considered not indicative of any adverse toxicological process by the authors of the report.

NECROPSY OBSERVATIONS AND ORGAN WEIGHTS
No treatment related gross abnormalities were present in either sex at scheduled necropsy. Absolute liver weights were increased significantly in male and female mice exposed to 1750 ppm or 5000 ppm ETBE vapour. Mean values (g) by exposure level were as follows:
- males: 2.1, 2.3, 2.4 ($P<0.05$), 2.5 ($P<0.05$)

- females: 1.6, 1.6, 1.9 (P<0.05), 2.1 (P<0.05)

HISTOPATHOLOGY
- liver (NOAEL 1750 ppm)
An increased incidence of centrilobular hypertrophy was observed in livers of mice exposed to ETBE. This was characterised by enlargement of hepatocytes in the centrilobular to midzonal areas, with homogeneously eosinophilic cytoplasm. Results summarised by exposure level:
- males: 0/15, 0/15, 2/15, 8/15 (P<0.05)
- females: 0/13, 2/15, 1/15, 9/14 (P<0.05)

- kidney (NOAEL, females, 1750 ppm)
The incidence of minimal renal nephropathy (characterised by thickened tubular basement membranes, lymphocytic interstitial infiltrates, fibrosis and/or regenerative, basophilic tubules) was slightly increased in exposed males and in 5000 ppm females. Results summarised by exposure level:
- males: 4/15 (27%), 7/15 (47%), 8/15 (53%), 4/10 (40%)
- females: 4/13 (31%), 6/15 (40%), 6/15 (40%), 9/14 (64%)
Reviewer's comment: The study report notes these findings were not considered indicative of a definitive effect of ETBE on mouse kidney. No statistical analysis was performed on these findings. In the opinion of this Reviewer, no obvious dose-response relationship is present in males, while in females the response appears limited to the high exposure group only.

TIMECOURSE AND CELL PROLIFERATION STUDIES IN LIVER
The liver of male and female mice exhibited a treatment and concentration dependent increase in cell proliferation. LI remained elevated in males following 4 wk treatment but returned to control levels by wk 13. In females, LI was comparable to control values at wk 4, but elevated at wk 13 (males unaffected). Results (LI) are summarised below by sex and by exposure level:

- males:
- 1 wk: 0.41, 1.64, 6.16 (P<0.001), 18.08 (P<0.001)
- 4 wk: 0.52, 0.99, 1.37 (P<0.05), 2.49 (P<0.001)
- 13 wk: 0.98, 0.89, 1.13, 0.67

- females:
- 1 wk: 2.75, 4.10, 20.24 (P<0.001), 38.31 (P<0.001)
- 4 wk: 2.17, 3.80, 1.65, 2.56
- 13 wk: 1.49, 1.63, 3.40 (P<0.05), 4.91 (P<0.05)

Reviewer's comment: The study report notes that a transient increase in hepatocyte LI is a common response observed in mice following exposure to mitogenic substances, with a more exaggerated response typically seen in females.

Test substance: ETBE, 97.5% pure (by GC-FID) was supplied by the Sponsor (ARCO Chemical Company, PA, USA) and manufactured by Phillips 66 (no further details). The chemical composition of the test

substance was verified using NMR.

Conclusion: Survival, body weight, clinical chemistry determinations and haematological parameters were unremarkable in male and female CD-1 mice exposed to 500, 1750 or 5000 ppm ETBE vapour for up to 13 weeks. Absolute liver weights were increased in animals exposed to 1750 ppm and above, while an increased incidence of centrilobular hypertrophy was present in high dose animals of both sexes. Hepatic cell proliferation (a common non-adverse change seen in mouse liver after exposure to mitogenic substances) was increased following 1-13 weeks exposure to 1750 ppm and above. These findings are consistent with a sub-chronic NOAEL for ETBE in the mouse of 1750 ppm, based on the occurrence of increased liver weight and mild histopathological changes present in animals exposed to 5000 ppm.

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, well documented methods and results, raw data available, acceptable for assessment.

30-NOV-2003 (12) (58)

Type: Sub-chronic
Species: rat **Sex:**
Strain: Fischer 344

Method: EPA OTS 798.2450
Year: 1966
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: GENERAL
See record by Bond et al (1996) for further experimental details.

NEUROLOGICAL EVALUATION
A functional observation battery (FOB) evaluation was performed 4 days prior to the first exposure, and after 1, 6, 10, 20, 42, 65 days. Data were collected at least 18 hr after ETBE exposure.

Animals evaluated for posture, tremors, spasms, convulsions, palpebral closure, handling reactivity and muscle tone together with an assessment of general condition (piloerection, fur appearance, skin temperature and colour, breathing pattern, salivation and lachrymation).

During then open field test, rats were observed for 2 min and spontaneous activity and behaviour recorded; arousal, ataxia, gait, body position, excessive vocalization, stereotypy and any unusual behaviour were also noted at this time.

An assessment of visual approach response, auditory startle response, tail pinch response, surface righting reflex, visual placing response, forelimb and hindlimb grip strength, hind

leg splay and papillary reflex were also performed.

Motor activity was measured during ten 6 min intervals using a photobeam activity system.

ORGANS EXAMINED AT NECROPSY
Brains were perfused in situ by intra-aortic perfusion (0.7% sodium nitrite) and fixed in situ with glutaraldehyde and formaldehyde. Brains were then weighed and size measured. Gasserian ganglia, dorsal root ganglia, spinal root fibres, brain, spinal cord, eye, optic nerve, sciatic nerve were subject to histopathological examination.

STATISTICAL METHODS
Levene's test for homogeneity followed by ANOVA and Dunnett's t-test were applied on homogeneous data. Non-homogeneous data were log-transformed. If transformed data were non-homogeneous, Kruskall-Wallis H-test and Wilcoxon two-sample rank-sum test were used. Categorical FOB data were analysed using a log-linear model.

Result: There were no gross macroscopic changes in brain or nervous tissue.

The FOB evaluation found no evidence of sensorimotor dysfunction, neuromuscular dysfunction, ataxia, piloerection, excessive vocalization, muscle tremors or spasms, clonic or tonic seizures, increased salivation, abnormal respiration or abnormal pupil reflex.

Decreased mean hindlimb grip strength and increase in mean hindlimb splay were observed in low exposure group males at first exposure, with a statistically significant increase in mean forelimb grip strength in high exposure males on day 10. A statistically significant decrease in mean forelimb grip strength was observed in low dose females following 65 exposures. The absence of any consistent dose-related trend suggest these observations are of doubtful toxicological significance.

There was no treatment-related effect on overall motor activity during any observation period.

Test substance: ETBE, 97.5% pure (by GC-FID) was supplied by the Sponsor (ARCO Chemical Company, PA, USA) and manufactured by Phillips 66 (no further details). The chemical composition of the test substance was verified using NMR.

Conclusion: No gross, functional or microscopic abnormalities were observed in males and female rats exposed to 5000 ppm ETBE vapour for up to 13 wk.

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, well documented methods and results, raw data available, acceptable for assessment.

02-NOV-2003 (13) (26)

Type: Sub-acute
Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 28 d
Frequency of treatment: 6 hr/d, 5 d/wk
Doses: 0 (air), 500, 2000 or 4000 ppm
Control Group: yes, concurrent vehicle
NOAEL: = 500 ppm

Method: OECD Guide-line 412 "Repeated Dose Inhalation Toxicity: 28-day or 14-day Study"
Year: 1990
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS AND MAINTENANCE:
Animals: SD rats (Charles River Breeding Laboratories Inc., Portage, MI), males and females, age approx. 9 wk on receipt
Acclimation: 3 wk
Weight at study initiation: mean 309g (males), 206g (females)
Housing: individually housed, stainless steel cages (wire mesh bottom)
Diet: Purina Rodent Chow 5001 (Ralston Purina Co.) ad libitum (except during inhalation exposure)
Water: Reverse osmosis tap water, automatic watering system, ad libitum (except during inhalation exposure)
Light cycle: 12 hr light, 12 hr dark
Assignment to treatment groups: random allocation of rats with bwt deviation <3 SD from population mean

GENERAL EXPERIMENTAL DESIGN
Animals (n=10/sex/treatment level) were exposed whole body in 1 m3 glass and stainless steel chamber to target concentrations of 0, 500, 2000 or 4000 ppm ETBE 6 hr/d, 5 d/wk for 28 d. The concentration of ETBE vapour in each exposure chamber was determined by IR analysis (Wilkes Miran 1A-1R analyser). Animals were observed for morbidity/mortality (7 d/wk) and clinical signs (5 d/wk) once daily. Body weight was recorded at study initiation, weekly thereafter and at termination. Comment: no food/water intake data collected.

CLINCIAL CHEMISTRY
Blood (abdominal aorta) was collected following an 18 hr fast under sodium pentobarbital anaesthesia, prior to necropsy. Analyses were performed for glucose, creatine kinase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea nitrogen, sodium, potassium, calcium, chloride, total protein, triglyceride, cholesterol, albumin, creatinine (methods not given).

HAEMATOLOGY DETERMINATIONS
Blood (as above) examined for total erythrocyte count, haemoglobin, mean corpuscular volume, total leucocytes,

differential leucocytes and platelets (methods not given).

POST-MORTEM OBSERVATIONS
Animals were sacrificed (pentobarbital) 18 hours after the final exposure, following an overnight fast, and subject to a macroscopic examination. Organ weights were recorded for adrenal glands, brain, gonads, heart, kidneys, liver, lungs and spleen. Lung volume was assessed from liquid displacement. Samples of adrenals, brain, epididymides, eyes, oesophagus, femur and bone marrow, gonads, heart, duodenum, jejunum, ileum, caecum, colon, kidneys, larynx, liver, lungs, lymph nodes, mammary gland, nasal turbinates, pancreas, parathyroids, pituitary, prostate and seminal vesicles, salivary glands, sciatic nerve, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, thymus, thyroids, tongue, trachea, urinary bladder, uterus, ear and any gross lesions were preserved in 10% neutral buffered formalin. Tissues from the control and high exposure groups were subject to microscopic evaluation (no details of tissue processing or staining).

NEUROLOGICAL EVALUATION
A functional observation battery evaluation (FOB) was performed one week before first exposure and following 1, 5, 20 exposures. It included home-cage observation (posture/appearance), hand-held observation, open field (mobility/gait), eye blink, pupil response, extensor thrust, visual placing, tactile placing, negative geotaxis, grip strength, foot splay, hind limb extension, startle response, catalepsy, righting reflex, body temperature, rotorod performance and tail pinch.

STATISTICAL METHODS:
Data were log-transformed and analysed by ANOVA. Body weights, body temperatures, hind limb splay, grip strength and rotorod performance evaluated using a multivariate repeated measures analysis of variance to determine dose-response relationship over time. For analysis of interactions Dunnett's test used.

Remark: No NOAEL is identified in the report from IIT Research Institute (1991).

White et al (1995) nominate a NOAEL of 500ppm but do not identify the critical effect(s). It is assumed that this was derived from an increase in relative liver weight in females exposed to 2000 ppm or 4000 ppm, however an absence of accompanying changes in clinical chemistry or histopathology suggests these alterations are of questionable toxicological significance.

A more appropriate NOAEL appears to be 2000ppm, based on the CNS effects (sedation and ataxia) seen in animals exposed to 4000 ppm ETBE vapour.

Result: GENERAL

Exposure concentrations in a range 350-555ppm were recorded in low exposure chamber (mean 501+/-17.8), with 1720-2370 in intermediate chamber (2090+/-111) and 3200-4540 ppm in the high exposure chamber (3920+/-206).

All animals survived to scheduled study termination. Signs of general sedation and reduced motor activity were noted in animals exposed to 4000 ppm ETBE vapour, with some animals exhibiting mild to moderate ataxia. After 3 hours exposure no startle response was evident in the majority of high exposure animals. All treated animals appeared to be in 'sleeping position' (muscle relaxation not evident) during exposure but were normal 15 minutes post-exposure. Other treatment-related clinical signs included salivation, redness around nose/mouth/face and discoloured paws/forelimbs.

There were no statistically significant effects of treatment on bw or bw gain.

CLINICAL CHEMISTRY
No treatment related effects or trends were apparent in the data.

HAEMATOLOGY
White blood cell counts were significantly increased in intermediate (elevated 61%; P<0.05) and high exposure (elevated 81%; P<0.05) females. All other parameters were comparable to control for both sexes. Histopathological examination found no changes in bone marrow or evidence of inflammation which could account for these observation.

NECROPSY OBSERVATIONS
Lung foci, present in all groups (hence not considered treatment related) were the only macroscopic observation of note. Absolute and relative liver weights were significantly increased in high exposure male (16% inc; P<0.05) and female rats (abs. 10% inc; rel. 12% inc; P<0.05 for both). Relative liver weight was also increased in females from the intermediate exposure group (10% inc; P<0.05). Absolute kidney (inc 12%; P<0.05) and adrenal weights (inc 14%; P<0.05)were also increased in high exposure males.

HISTOPATHOLOGY
Lymphoid and plasma cell hyperplasia in mandibular lymph nodes, and lymphoid hyperplasia and haemorrhage in the respiratory lymph nodes was commonly seen but not treatment related. There were no histopathological changes in liver, kidney or adrenal gland (ie those tissues showing weight changes). Comment: pyelonephritis, unilateral hydropelvis and tubular basophilia, present sporadically in both sexes (including controls), were not considered related to treatment. Unilateral tubular atrophy of the testes was present in one control male, while males from the 4000 ppm exposure group were unremarkable. Bone marrow had a normal

microscopic appearance. Overall there were no treatment related histopathological changes.

FUNCTIONAL OBSERVATION BATTERY
There were no differences in clinical signs, mobility/gait, sensory perception and reflex responses. Body temperature was decreased by approx. 0.5 degrees C (P<0.05) in 4000 ppm males on day 5 only. Hind limb splay at day 20 was significantly increased (+27%; P<0.05) when results for high exposure males and females were combined and compared with the controls (individual sex-specific values were comparable to control). Comment: Combined values at day 20 (7.51+/-1.98) are essentially identical to combined day 0 control values (8.36+/-1.91) and combined day 0 high exposure animal values (7.45+/-1.94). These observations therefore appear of marginal biological significance.

Test substance: ETBE, supplied by Amoco Corporation, purity not stated.

Conclusion: Survival, body weight and the majority of clinical chemistry and haematological parameters were unremarkable in male and female F344 rats exposed to 4000 ppm ETBE by inhalation for up to 28 weeks. An increase in relative liver weight in females exposed to 2000 ppm with no accompanying clinical chemistry or histopathological changes appears of doubtful toxicological relevance. Overall the results are consistent with a sub-acute NOAEL for ETBE in the rat of 2000 ppm, based upon CNS depression (sedation and ataxia) seen in animals exposed to higher exposure levels.

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.

05-OCT-2003 (40) (81)

Type: Sub-acute
Species: rat **Sex:** male/female
Strain: Sprague-Dawley
Route of administration: inhalation
Exposure period: 4 wk
Frequency of treatment: 6 h/d, 5 d/wk for 4 wk
Doses: 0, 500, 2000 or 4000 ppm
Control Group: yes, concurrent vehicle
NOAEL: = 2000 ppm

Year: 1991
GLP: no data
Test substance: as prescribed by 1.1 - 1.4

Remark: Published abstract:

Crl:CD rats (n=10/sex/treatment level) exposed to 0, 500, 2000 or 4000 ppm ETBE vapour 6 hr/d, 5 d/wk for 4 wk.

Parameters monitored included bwt, organ wt, lung volume, haematology, clinical chemistry, gross and microscopic histopathology as well as function observation battery for

neuromuscular function, reflex response and sensory perception (before and after 1, 5 and 20 exposures).

CNS depression (ataxia, sedation) observed immediately post-exposure in 4000 ppm group, with rapid and full recovery post-exposure.

Changes in body temperature and hindlimb splay pattern present in high dose group. (The authors note that the latter finding is 'possibly' indicative of an effect on hindsplay.)

Liver, kidney, and adrenal wt (absolute and/or relative) increased in high dose males, and liver wt increased in intermediate and high dose females. No histopathological changes were associated with these findings.

White cell counts increased in females exposed to 2000 or 4000 ppm ETBE vapour for 4 wk.

Test substance: No details available.
Conclusion: The author's conclude that repeated exposure to 4000 pm ETBE produced transient signs of CNS depression and associated changes in body temperature and possibly hindlimb splay.
Reliability: (4) not assignable
Study available for review. Abstract, highly limited information on methods and findings.
30-NOV-2003 (71)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA98, TA 100 (with independent repeat)
Concentration: First test: 0, 8, 40, 200, 1000 and 5000 ug/plate; Second test: 0, 313, 625, 1250, 2500 and 5000 ug/plate
Cytotoxic Concentration: > 5000 ug/plate
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 471
Year: 1983
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Commercially-available freeze-dried S9 fraction (MOLTOX Inc) from Arochlor 1254-induced rats was used in these studies. Absolute ethanol was used as carrier solvent, and added at a final volume of 0.05 ml/plate.

Each dose was tested in triplicate, with independent repeat.

2-Nitrofluorene (TA 98 and TA 1538), 9-aminoacridine (TA 1537) and sodium azide (TA 1535 and TA 100) were used as positive control substances in the absence of S9 mix, and 2-amino

anthracene (all strains) as positive control in the presence of S9.

Results were considered positive if a suitable response had been obtained with the negative and positive control substances, and the number of revertants in the presence of ETBE was at least twice that of the negative control, with a dose-response correlation.

Result: Results from a preliminary test using TA 100 (without S9) revealed no toxicity at doses up to 5000 ug/plate.

All data met the acceptability criteria established for the test. No increase in the number of revertants was seen with any of the tester stains, both in the absence and presence of S9 fraction, in either the first or second study. A satisfactory response was obtained with the negative and positive controls.

Test substance: ETBE, 95.8%, batch no. 93050601, supplied by Elf-Antar, France.

Conclusion: Under the conditions of these tests, ETBE was not mutagenic to Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 or TA 100 at doses up to 5000 ug/plate, both in the absence and presence of Arochlor 1254-induced rat S9 fraction.

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.

02-NOV-2003 (65)

Type: Ames test
System of testing: TA 1535, TA 1537, TA 1538, TA98, TA 100 (with independent repeat)
Concentration: 0, 5, 15, 50, 150 and 500 ug/plate
Cytotoxic Concentration: >1500 ug/plate for TA 1537, TA 1538 and TA 98; > 500 ug/plate for TA 1535 and TA 100
Metabolic activation: with and without
Result: negative

Method: other: Maron and Ames, Mut Res 113, 173 - 215, 1983
Year: 1983
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: S9 fraction was prepared from the livers of Arochlor 1254-induced male Sprague Dawley rats. 95% ethanol was used as carrier solvent, and added at a final volume of 0.1 ml/plate.

Each dose was tested in triplicate, with independent repeat.

B-propiolactone (TA1535), hycanthone (TA 1537), 2-nitrofluorene (TA 1535 and TA 98) and sodium azide (TA 100) were used as positive control substances in the absence of S9 mix, and 2-amino anthramine (all strains) was used as positive control in the presence of S9.

Statistically significant increases in revertant numbers were identified using Dunnett's test.

Result: Results from a preliminary test demonstrated that doses > 1500 ug/plate were cytotoxic to TA 1537, TA 1538 and TA 98, while doses > 500 ug/plate were cytotooxic to TA 1535 and TA 100.

No increase in the number of revertants was seen with any of the tester stains, both in the absence and presence of S9 fraction, in either the first or second study. A satisfactory response was obtained with the negative and positive controls.

Test substance: ETBE, purity not stated, lot no. 408-2404, Total Raffinage Distribution, France.

Conclusion: Under the conditions of these tests, ETBE was not mutagenic to Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98 or TA 100 at doses up to 500 ug/plate, both in the absence and presence of Arochlor 1254-induced rat S9 fraction.

Reliability: (2) valid with restrictions
Study available for review. Non-GLP near-guideline investigation, clearly reported, acceptable for assessment.

02-NOV-2003 (43)

Type: Ames test
System of testing: Salmonella typhimurium TA 97, TA 98, TA 100, TA 1535 (no independent repeat)
Concentration: 0, 100, 333, 1000, 3333, 10000 ug/plate
Cytotoxic Concentration: >10000 ug/plate
Metabolic activation: with and without
Result: negative

Year: 1992
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: S9 fraction was prepared from the livers of Arochlor 1254-induced rat or hamster. Assays performed using 10% or 30% S9 from each source.

Initial toxicity was determined using TA100 only (test performed on single occasion.

Each dose was tested in triplicate (no independent repeat).

Sodium azide (TA100 and TA1535), 9-aminoacridine (TA97 and TA1537) and 4-nitro-o-phenyldimaine (TA98) were used as positive control substances in the absence of S9, while 2-aminoanthracene was used in the presence of S9.

A result was judged to be indicative of mutagenicity if there was a reproducible, dose-related response over the solvent control, under a single metabolic condition, in replicate trials.

Result: Only limited results are reported in this publication.

Results are presented for TA 100 and 1535 only in absence of S9 in a single test using 2 concentrations of S9 from rat and hamster. No increases in numbers of revertants were seen in either strain.

Note: the report states that ETBE was evaluated using all strains, but results are only presented for TA 100 and TA 1535.

Test substance: ETBE, 99%, Aldrich Chemical Co., Milwaukee, WI.
Conclusion: Under the conditions of this test, ETBE was not mutagenic in a bacterial reverse mutation assay at concentrations up to 10000 ug/plate, in the absence or presence of rat or hamster S9.
Reliability: (2) valid with restrictions
Study available for review. Briefly reported experimental investigation from US-NTP, acceptable for assessment.
02-NOV-2003 (85)

Type: Ames test
System of testing: no data
Concentration: no data
Metabolic activation: no data
Result: negative

Method: other: no data
GLP: no data
Test substance: other TS

Remark: Chatin, L. (Elf Antar France).

As reported in the Acts of 6th EFOA Conference "ETBE in Europe", Brussels, October 27-28 1994, p. 9
Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Test substance: ETBE
Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.
02-NOV-2003

Type: Chromosomal aberration test
System of testing: Metaphase analysis in CHO cells
Concentration: 0, 300, 1000, 3000, 5000 ug/ml
Cytotoxic Concentration: >5000 ug/ml
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 473
Year: 1995
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Logarithmically growing cultures were treated (in duplicate) for 4 hours with ETBE and harvested 10 hr after onset of

treatment. Optimal harvest time determined in a preliminary study by cell cycle kinetics using bromodeoxyuridine. Incubations were performed in the absence or presence of Aroclor induced rat liver S9 fraction. CAs were scored in 50 cells per culture.

Toxicity determined by colony-forming potential and mitotic inhibition in the preliminary toxicity test and by mitotic index analysis in the main study.

All treatments and culturing performed in glass bottles to avoid solvent effects of ETBE.

Mitomycin C (minus S9) and cyclophosphamide (plus S9) were used a positive control substances.

Results were analysed using Fisher's exact test, and considered positive if there was:
- a statistically significant, dose-related increase in chromosome aberrations (excluding gaps);
- a statistically significant increase at one dose level which was reproducible between experiments and of large magnitude.

Result: No cytotoxicity was present as judged by the lack of mitotic inhibition and cell cycle delay.

No statistically significant increases in chromosome aberrations were seen at any treatment level.

Positive and negative control results were acceptable.

Test substance: ETBE, 98% pure (containing 13 ppm antioxidant) supplied by the Sponsor (ARCO Chemical Company, PA, USA) and manufactured by Phillips 66 (no further details).

Conclusion: Under the conditions if this investigation, ETBE did not induce chromosomal aberrations in CHO cell in vitro at concentrations up to 5000 ug/ml, in the presence and absence of Arochlor-induced S9.

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, some minor deviations, well documented methods and results, raw data available, acceptable for assessment.

02-NOV-2003 (17)

Type: HGPRT assay
System of testing: mammalian gene mutation assay in CHO cells
Concentration: 100, 300, 1000, 3000, 5000 ug/ml
Cytotoxic Concentration: >5000 ug/ml
Metabolic activation: with and without
Result: negative

Method: OECD Guide-line 476
Year: 1995
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Duplicate cultures (8 x 10e5 cells) were grown for 20-24 hours before treatment with ETBE (up to 5000 ug/plate; 4 hr) in presence and absence of S9 (rat, Arochlor 1254-induced). Subcultures were made at 2-3 day intervals (5-10 x 10e5 cells per culture). At 8 days after treatment 2 x 10e5 cells plated into each of 5 plates containing selective medium for each culture. Counted after 6-8 days.

There were three independent repeat tests in the absence of S9 and two independent repetitions in the presence of S9.

Cytotoxicity was determined by comparing the numbers of cells in treated cultures with those in controls 24 hours after treatment (cloning efficiency).

Ethylmethanesulphonate and dimethylnitrosamine were used as positive control substances, DMSO as negative control.

Data were log transformed and analysed by the method of Irr and Snee (1979, Banbury Report II, 263).

A result was considered valid if the spontaneous mutation frequency less than 20 x 10e-6, the positive control frequency was at least 3 times control and control cloning efficiency of at least 80%.

Result: No cytotoxicity was present at concentrations up to the maximum recommended for the assay (5000ug/ml). A satisfactory response was obtained with the positive control cultures.

No statistically significant or dose-related increases in mutation frequency were seen in any test.

Comment: the second test without S9 was repeated due to high values in a vehicle control culture and in the cultures treated with 100, 3000 and 5000ug/ml. A negative result was obtained. Results from all three tests performed in the absence of S9 are summarised below:

Mutation frequency (x 10e-6):
Test 1, -S9:
Control - 0.45
100ug/ml - 0.5

300ug/ml - 1.3
1000ug/ml - 2.9
3000ug/ml - 1.2
5000ug/ml - 2.8.

Test 2, -S9:
Control - 17.7
100ug/ml - 26.7
300ug/ml - 12.2
1000ug/ml - 14.9
3000ug/ml - 25.9
5000ug/ml - 31.7

Test 3, -S9:
Control - 6.2
100ug/ml - 0.6
300ug/ml - 0.5
1000ug/ml - 2.3
3000ug/ml - 1.1
5000ug - 3.0

Test substance: ETBE, 98% pure (containing 13 ppm antioxidant) supplied by the Sponsor (ARCO Chemical Company, PA, USA) and manufactured by Phillips 66 (no further details).

Conclusion: Under the conditions of the assay, ETBE (up to 5000 ug/ml) did not induce mutations at the HGPRT locus in CHO cells in the absence or presence of Arochlor-induced rat S9.

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, well documented methods and results, raw data available, acceptable for assessment.

02-NOV-2003 (18)

Remark: The structures of methyl- and ethyl-ter-buthyl-ethers were analyzed by CASE, an expert system, and compared to the structural determinants previously recognized as being associated with carcinogenicity in rodents, mutagenicity in Salmonella or the induction of sister chromatid exchanges and chromosomal aberrations in cultured mammalian cells.

On the basis of this analysis the two chemicals are predicted to be neither genotoxicants nor carcinogens.

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.

23-DEC-2003 (70)

5.6 Genetic Toxicity 'in Vivo'

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: other: OF1
Route of admin.: gavage
Exposure period: 24 and 48 hr
Doses: 5000 mg/kg bwt
Result: negative

Method: Directive 84/449/EEC, B.12 "Other effects - Mutagenicity (micronucleus test)"
Year: 1984
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS
Groups of 5 male and 5 female OF1 mice (22 - 29g, age 4 - 6 wk) were given 0 or 5000 mg/kg ETBE by gavage in 1% carboxymethyl cellulose (dose volume 25 ml/kg bwt) and sacrificed at 24 hr and 48 hr post-treatment.

PROCEDURE
Femoral bone marrow smears were prepared from each mouse, and 2000 PCEs scored for the presence of micronuclei. The ratio of PCE:NCE was quantified in 1000 erythrocytes.

POSITIVE CONTROL
Cyclophosphamide (50 mg/kg bwt in distilled water by ip injection, dose volume 25 ml/kg bwt) was used as a positive control substance, with animals sacrificed at 24 hr only.

STATISTICS
Results were analysed using the Mann Whitney U test and Student's T test.

Result: CLINICAL SIGNS AND TOXICITY
All animals given ETBE survived 48 hr post-dosing with no apparent signs of toxicity.

MICRONUCLEUS TEST
There was no increase in the number of micronuclei per 1000 PCEs in mice treated with 5000 mg ETBE/kg bwt in either sex at either time point, whereas the incidence of micronuclei was increased significantly ($P < 0.01$) following cyclophosphamide treatment.

Test substance: ETBE, purity not stated, lot no. 408-2404, Total Raffinage Distribution, France.

Conclusion: Under the conditions of the test, no increase in micronuclei was found in male and female mice 24 hr and 48 hr after oral administration of 5000 mg ETBE/kg bwt (oral limit dose).

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, clearly reported, acceptable for assessment.

05-OCT-2003 (41)

Type: Micronucleus assay
Species: mouse **Sex:** male/female
Strain: CD-1
Route of admin.: inhalation
Exposure period: 6 hr/d for 5 d
Doses: 0, 400, 2000 or 5000 ppm
Result: negative

Method: OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year: 1995
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS AND TREATMENTS
Groups of 5 male (approx. 28g) and 5 female (approx. 25 g) CD-1 mice were exposed whole body to 0, 400, 2000 or 5000 ppm ETBE vapour (6 hr/d for 5d). The highest exposure represented one half of the lower explosive limit for ETBE. Body weights were recorded before first exposure and at scheduled termination. Individual clinical observations were recorded before and after exposure, and on a group basis during exposures.

GENERATION OF TEST ATMOSPHERE
Liquid ETBE was delivered by a metered piston pump into a heated glass evaporator and the resultant vapour delivered into a 900l stainless steel and glass exposure chamber. The nominal vapour concentration in the chambers was calculated from the volume of ETBE vapourised and the total airflow. The actual vapour concentration was determined using GC-FID (23-24 measurements during each 6 hr exposure period).

MICRONUCLEUS EVALUATIONS
Femoral bone marrow smears were prepared from each mouse 24 hr after the final exposure, and 2000 PCEs scored for the presence of micronuclei. The ratio of PCE:NCE was quantified in 1000 erythrocytes. Toxicity was determined from the number of PCEs per 1000 erythrocytes.

POSITIVE CONTROL
Cyclophosphamide (15 mg/kg bwt in distilled water; ip injection) was administered 24 hr prior to sacrifice.

STATISTICS
Results were analysed using the Mann Whitney U test.

Result: ACHIEVED CONCENTRATION
Daily exposure concentrations (mean+/-SD) were 400+/-8.5, 1996+/-37 and 5053+/-80 ppm (limit of detection 10 ppm).

CLINICAL SIGNS AND TOXICITY
All animals survived until study termination. Clinical signs included abnormal gait, hypoactivity, incoordination, abdominal breathing and lack of startle reflex in the 5000ppm group and to a lesser extent in the 2000ppm group.

Hypoactivity was also present following 4.5 hr exposure to 400ppm ETBE vapour. There were no differences in body weight between the groups.

MICRONUCLEUS TEST
No statistically significant increase in micronucleus frequency were seen in animals exposed to ETBE. A statistically significant increase (P<0.01) was observed in the positive control group.

There was no significant change in proportion of PCEs to NCEs.

Test substance: ETBE, 98% pure (containing 13 ppm antioxidant) supplied by the Sponsor (ARCO Chemical Company, PA, USA) and manufactured by Phillips 66 (no further details).

Conclusion: Under the conditions of the test, no increase in micronuclei was found in male and female mice following 5 consecutive daily exposures to 400-5000 ppm ETBE vapour.

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, well documented methods and results, raw data available, acceptable for assessment.

05-OCT-2003 (16)

5.7 Carcinogenicity

Remark: The source reference (Maltoni, C (1995) The contribution of experimental (animal) studies to the control of industrial carcinogenesis. Appl. Occup. Environ. Hyg. 10, 749-760.) includes reference to a carcinogenicity study on ETBE.

At the time of preparation of this IUCLID dataset (November 2003), the study was not located on ToxLine following a literature search using the first named author.

Source: Maltoni, C (1995) The contribution of experimental (animal) studies to the control of industrial carcinogenesis. Appl. Occup. Environ. Hyg. 10, 749-760.

Reliability: (3) invalid
Study available for review. Contains no relevant information.

30-NOV-2003

Remark: The source reference (Belpoggi, G, Soffriti, M, Minardi,F, Bua, L, Cattin, E and Maltoni, C (2002). Ann. NY Acad. Sci., 982, 7-86.) includes reference to a carcinogenicity study on ETBE. This is cited as:

Maltoni, C et al. (1999) Comprehensive long-term experimental project of carcinogenicity bioassay on gasoline oxygenated additives: plan and first report of results from the study on ethyl-tertiary butyl ether (ETBE). Eur J Oncol, 4, 493-508.

At the time of preparation of this IUCLID dataset (November 2003), the article does not appear to have been published in this journal, nor was the study located on ToxLine following a literature search using the first named author.

Source: Belpoggi, G, Soffriti, M, Minardi,F, Bua, L, Cattin, E and Maltoni, C (2002). Ann. NY Acad. Sci., 982, 7-86.

Reliability: (3) invalid
Study unavailable for review.

02-JAN-2004

Remark: The structures of methyl- and ethyl-ter-buthyl-ethers were analyzed by CASE, an expert system, and compared to the structural determinants previously recognized as being associated with carcinogenicity in rodents, mutagenicity in Salmonella or the induction of sister chromatid exchanges and chromosomal aberrations in cultured mammalian cells.

On the basis of this analysis the two chemicals are predicted to be neither genotoxicants nor carcinogens.

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.

23-DEC-2003 (70)

5.8.1 Toxicity to Fertility

Type: Two generation study
Species: rat
Sex: male/female
Strain: Sprague-Dawley
Route of administration: gavage

Method: OECD Guide-line 416 "Two-generation Reproduction Toxicity Study"
Year: 2004
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: Study in progress
Test substance: ETBE, CAS No. 637-92-3, >98% pure, Totalfinaelf France.
01-NOV-2003 (31)

Type: other: fertilisation in vitro
Route of administration: drinking water
Exposure Period: 2 wk

Year: 2003
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS AND MAINTENANCE
Animals: Simonson albino rats (Sprague-Dawley derived; supplier not specified); males at least 100 days old, females 28-45 days old.
No further details available.

TREATMENTS
Females received either 0.3% ETBE in drinking water or drinking water alone for 2 wk preceeding oocyte recovery (n=6 per group). Female bwt, number of females ovulating and the number of ooctyes collected was recorded.

RECOVERY OF OOCYTES
Ovulation was induced using 15 IU pregnant mare's serum gonadotropin i.p. followed 2 d later by 15 IU human chorionic gonadotrophin i.p. Animals were sacrificed by cervical dislocation 16-18 hr later, oviducts and attached ovaries removed and the oviducal cumulus dispersed with hyaluronidase in saline-BSA. Isolated oocytes were rinsed with 3 drops of saline-BSA, the zona pellucida removed by incubation with acid Tyrone's medium (pH 2.5) for 10 seconds followed by further rinsing.

RECOVERY OF SPERM
Males were sacrificed (carbon dioxide), and cauda epididymides and attached vas deferens dissected, rinsed in modified Tyrode's medium and placed in warmed medium in a culture dish. Sperm were removed by flushing with medium from a blunt needle

inserted into the vas deferens. Sperm motility was evaluated subjectively using phase contrast microscopy (no further details) and sperm concentration using a haemocytometer. The suspension was diluted to 7 or 0.5 x 10^6 sperm/ml and incubated for 3 hr (37 degrees C, 5% CO2) before use.

OOCYTE PENETRATION ASSAY
Following removal of the zona pellucida, oocytes were mixed with diluted sperm in modified Tyrone's medium (final volume of 120 ul). Oocytes in all treatments within a replicate were inseminated with aliquots from the same suspension. After 20 hr incubation (37 degrees C, 5% CO2) oocytes were rinsed to remove loosely adherent spermatozoa, incubated (10 min) with 0.08 mg/ml Hoechst 33342 then placed on slides and stored (in the dark) until microscopic examination (UV illumination, x400 magnification) for decondensed sperm heads and attached sperm.

STATISTICAL METHODS
Data were analysed by ANOVA.

Remark: The author's note that in vitro fertilising potential of a fixed concentration of sperm was found to vary from male to male, and was affected by other assay conditions such as composition of the medium. Higher sperm concentrations (7 x 10^6/ml) typically had more than enough high quality sperm to produce 100% oocyte penetration, and fertilisation was not affected by minor reductions in oocyte quality. A reduced sperm concentration (0.5 x 10^6/ml) was necessary in order to detect variations in oocyte quality.

Result: ETBE treatment was without significant effect on any of the parameters evaluated in this study:

	Control	ETBE
Final bwt (g)	155	148
Percentage females ovulating	72	84
No. oocytes recovered per ovulating female	30	29
Percentage oocytes remaining after removal of zona pellucida	57	66
Percentage oocytes fertilised	84	82
Penetrated sperm/oocyte	1.84	1.72

Test substance: ETBE, purity not specified, Aldrich Chemical Co., Milwaukee, WI.

Conclusion: Under the conditions of this study, administration of ETBE (0.3% in drinking water) to female rats was without effect on oocyte fertilisation in vitro.

Reliability: (2) valid with restrictions
Study available for review. Experimental research study, acceptable for assessment however reliability/reproducibility of methodology not known.

02-JAN-2004 (7)

5.8.2 Developmental Toxicity/Teratogenicity

Species: rat **Sex:** female
Strain: Sprague-Dawley
Route of administration: gavage
Exposure period: GD5-19
Frequency of treatment: daily
Doses: 0 (corn oil), 250, 500 or 1000 mg/kg bw/d
Control Group: yes, concurrent vehicle
NOAEL Maternal Toxity: = 500 mg/kg bw
NOAEL Teratogenicity: = 1000 mg/kg bw
NOAEL Embryotoxicity : = 1000 mg/kg bw
NOAEL Fetotoxicity : = 1000 mg/kg bw
Result: No effect on fetal development

Method: OECD Guide-line 414 "Teratogenicity"
Year: 2003
GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: ANIMALS AND MAINTENANCE
Animals: time-mated female Sprague Dawley rats (Charles River Laboratories, L'Arbresle, France);
Age at start of treatment: approx. 11 wk (bw 215-297 g)
Acclimation period: 5 d
Housing: individually housed, stainless steel cages (wire mesh bottom) for duration of study
Diet: A04 C pelleted maintenance diet (UAR, Villemoisson, France), ad libitum
Water: deionised water, ad libitum
Light cycle: 12 hr light/dark period (lights on 0700-1900)
Assignment to treatment groups: computer-generated weight randomisation

DESIGN
ETBE was administered by gavage (dosing needle) at doses of 0 (corn oil; 4 ml/kg bw) 250, 500 or 1000 mg/kg/day on GD 5-19, inclusive.

DOSING SOLUTIONS
The dosing solutions were prepared weekly (based upon stability data demonstrating no losses of ETBE over 9 d) and samples taken for analysis (GC) to confirm received dose.

GENERAL OBSERVATIONS
The dams were examined twice daily mortality, and for the occurrence of clinical signs 3 times per day on GD5-14, and twice daily thereafter. Body weight and food consumption were recorded on 10 occasions between GD2-20.

TERMINAL OBSERVATIONS
Dams were sacrificed by carbon dioxide asphyxiation on GD20 and subject to a macroscopic evaluation of internal organs and placenta. The gravid uterine weight recorded, and the ovaries

and uterue examined for:
- no. corpora lutea
- no./distribution of dead/live fetuses
- no./distribution of early/later resorptions
- no./distribution of implantations sites/uterine scars
(ammonium sulphide)

FETAL EXAMINATION
All litters with at least 1 live fetus were weighed and subject to a detailed external examination (including oral cavity). Any findings were recorded according to the glossary of the International Federation of Teratology Societies, and classified as malformations or variations. Dead fetuses were discarded, live fetuses were sacrificed with thiopental sodium (s.c.) and the sex recorded. Approx. half the fetues were fixed in Harrison's fluid and examined using a free-hand serial sectioning technique (Wilson, 1965, Teratology Principles and Techniques, Univ. Chicago Press, pp 262-277). The remainder were fixed (ethanol), eviscerated and bone and cartilage examined after staining with alizarin red S and alcian blue (Peters, 1977, Methods in Prenatal Toxicology, Georg Thieme Publ., pp 153-154).

STATISTICAL METHODS
Data were expressed as group means (+/- SD) or as percentages. The unit of comparison was the litter, with fetal body weight analysed by sex as well as for both sexes combined. Means (normally distributed) were compared by ANOVA and Dunnett's test, percentage values by Fisher's exact probability test.

Result:

ANALYSIS OF DOSING SOLUTIONS
Solutions displayed good homogeneity, and deviations from nominal concentration were +/- 10%.

PREGNANCY STATUS
There were no significant or biologically relevant differences in pregnancy status between the groups:

Dose-level (mg/kg/day)	0	250	500	1000
Mated females	24	24	24	24
Non-pregnant females	3	5	4	2
Pregnant, alive at GD20	21	19	20	22
Pregnancy rate	87%	79%	83%	92%
Litters	21	19	20	22

MATERNAL OBSERVATIONS
There were no deaths in any group. Excess salivation was present immediately post-dosing (resolved by one hour) in 5, 13 or 17 females from the 250, 500 and 1000 mg/kg/day dose groups at various times during the study.
Comment: the report considers this a response to the unpalatable 'taste' of ETBE rather than an adverse clinical effect.

There was a transient reduction in maternal body weight gain

of high dose dams on GD 5-9 (-20%), with mean body weight gain decreased significantly over the whole dosing period (-11%, p<0.05). Net body weight gain in the 1000 mg/kg/day group (corresponding to the maternal carcass weight at day 20 minus maternal bw on GD5) was also significantly lower (-17%, p<0.01) when compared with the controls.

Dose-level (mg/kg/day)	0	250	500	1000
Bw change GD 5-9	24	23	23	20
% diff. from control		-4	-4	-20
Bw change GD 5-20	135	132	134	120*
% diff. from control		-2	-1	-11
Net bw change GD 5-20	61.8	59.4	60.0	51.5**
% diff. from control		-4	-3	-17

* = p<0.05; ** = p<0.01

The food consumption was unaffected by the treatment.

No treatment-related macroscopic changes were present in the dams at necropsy.

LITTER DATA
There were no differences in the number of corpora lutea and implantation sites between the groups (hence no effects on pre-implantation losses).

There was no total litter resorption in any group.

The number (21) and rate (1 per female) of early resorptions was slightly greater in the 1000 mg/kg/day when compared to the control group (number = 14, rate = 0.7 per female), hence the extent of post-implantation loss was minimally increased in the high-dose females (7.5%) versus controls (5.2%; non-significant). The report notes that this incidence was in the range of historical control data for the laboratory (mean post-implantation loss=3.4%, minimum=1.0%, maximum= 8.5%) and was therefore considered unrelated to treatment.

The number of live fetuses were in a range 11.7 to 12.3 per female, with no treatment-related or statistically significant differences present between the groups. No dead fetuses were found in any group.

The sex ratio in the low dose group (53.5% male, 46.5% female) was significantly different from the control value. This difference appeared principally related to an unusually low proportion of males (40.8%) and a correspondingly higher number of females (59.2%) in the control group. The sex ratio in the other treated groups (47.3-48.8% male, 51.2-52.7% female) was within the normal range (no dose-response relationship present). The report considered this observation in the low dose litters a chance event, unrelated to treatment with ETBE.

Fetal weight was unaffected by the treatment.

FETAL EXAMINATION
A single occurrence of umbilical hernia (malformation) and a bent tail (variation) were present in the 250 mg/kg/day group (no other findings in any group). These were considered unrelated to treatment due by the study report due to isolated nature of the findings and absence of any dose-response.

SOFT TISSUE EXAMINATION
- MALFORMATIONS
There were no malformations in the control or the low or intermediate treatment groups. At 1000 mg/kg/day an absence of kidneys, ureters and adrenals was observed in single fetus. These were considered unrelated to treatment by the study report due to isolated nature of the findings and absence of any dose-response.

- VARIATIONS
The following findings were noted among the groups:
- dilated ureters in one fetus from the control, 250 m/kg/day and 1000 mg/kg/day groups;
- short uterine horns in one fetus from the 500 mg/kg/day;
- dilated renal pelvis in one fetus from the 250 and 1000 mg/kg/day.
These few findings, which were not dose-related and randomly distributed across the groups, were considered by the report to be unrelated to the treatment.

SKELETAL EXAMINATION
- MALFORMATIONS
The following malformations were recorded:
- misaligned sternebra in one control fetus;
- at 250 mg/kg/day, fused rib and misshapen sacral vertebra in one fetus; split sternebra and fused ribs in one other fetus;
- at 500 mg/kg/day, bilateral misshapen ilium noted in one fetus;
- at 1000 mg/kg/day, absence of one pair of ribs observed in two fetuses, one of which also had an absence of thoracic vertebra.
Because of their low incidence and the absence of any dose-relationship, these malformations were not considered by the report to be related to the treatment with ETBE.

- VARIATIONS
There was a significant increase in the incidence of unossified 4th metacarpal at the highest dose: 43/136 fetuses (31.6%, $p<0.05$) were affected versus 27/135 (20%) in the control group. This was not statistically significant when expressed on a fetus/litter basis. Cartilage was generally present suggesting that this was due to slightly delayed ossification rather than to a persistent alteration. As the findings were isolated, not statistically significant or

dose-related at the level of the litter and not associated with any significant delay in ossification of other bones, and since the total incidence of skeletal variations in treated animals did not increase, the report concludes these are of no toxicological significance. There were no other findings.

Test substance: ETBE, CAS No. 637-92-3, >98% pure, Totalfinaelf France.

Conclusion: No evidence of maternal or embryo-fetal toxicity was recored when ETBE was administered to pregnant SD rats at 250 or 500 mg/kg bw/d (gavage) on GD 5-19. Maternal toxicity (statistically significant decrease in bw gain and net bw gain) was observed at 1000 mg/kg/day. There were no adverse effects on embryofetal development at this dose-level. Consequently, under the conditions of the study, the NOAEL for maternal toxicity was 500 mg/kg/day and the NOAEL for embryofetal development was 1000 mg/kg/day.

Reliability: (1) valid without restriction
Study available for review. GLP compliant guideline investigation, well documented methods and results, raw data available, acceptable for assessment.

30-NOV-2003 (30)

5.8.3 Toxicity to Reproduction, Other Studies

Type: other: dose range-finding study
In Vitro/in vivo: In vivo

Method:

ANIMALS AND MAINTENANCE
Animals: Male Fischer F344 rats; male and female Sprague Dawley rats (Charles River Laboratories, L'Arbresle, France);
Age at start of treatment: Males approx. 7 wk, females approx. 11 wk
Acclimation period: 1 wk
Housing: individually housed, stainless steel cages (wire mesh bottom) for duration of study
Diet: A04 C pelleted maintenance diet (UAR, Villemoisson, France), ad libitum
Water: deionised water, ad libitum
Light cycle: 12 hr light/dark period (lights on 0700-1900)
Assignment to treatment groups: computer-generated weight randomisation

DESIGN
ETBE was administered by gavage (dosing needle) at doses of 0 (corn oil; 3 ml/kg bw) 50, 250, 500 or 1000 mg/kg/day.

Male F344 rats: n=12/dose level; daily administration for 12 weeks
Male SD rats: n=12/dose level; daily administration for 12 weeks
Female SD rats: n=24/dose level; daily administration through:
- pre-mating (2 weeks)
- mating (2 weeks)

- pregnancy (until day 19 post-coitum for 12 females/dose level)
- until the end of lactation (day 21 post-coitum, remainder of females per dose level).

Dosing solutions were analysed (GC) to confirm received dose.

GENERAL OBSERVATIONS
The parental animals were examined for mortality and clinical signs daily, and body weight and food consumption were recorded weekly.

MATING
The mating index, pre-coital time and fertility index were calculated. After mating, the males were sacrificed and the females divided into two sub-groups.

PREGNANCY AND REPRODUCTION PARAMETERS
One half of the females were subjected to hysterectomy on day 20 of pregnancy. The gravid uterus was weighed to allow calculation of the net body weight gain of the pregnant females. The fetuses were removed by hysterectomy and the litter parameters recorded: namely number of corpora lutea, implantation sites, early and late resorptions, dead and live fetuses. The fetuses were weighed, sexed and examined externally.

The remainder of the females were allowed to deliver normally. Duration of gestation and fetal parameters were recorded, and the litters observed daily during the lactation period (to post-natal day 21) for survival and clinical signs.

TERMINAL OBSERVATIONS IN PARENENTAL ANIMALS
At study termination, parental animals were subjected to a macroscopic post-mortem examination, with particular attention paid to the reproductive organs. In addition, in males, the testes and epididymides were weighed and sperm sampled and examined for total count and viability.

STATISTICAL METHODS
Data were analysed by ANOVA and Dunnett's test. Percentage values were compared using the Fisher exact probability test.

Remark: The objective of this study was to provide preliminary information on the potential effects of ETBE on reproductive and developmental indices in the rat. Particular attention was paid to the male reproductive system since published results (see record for Medinsky et al., 1999; Section 5.4) suggested this was a possible target in Fischer rats.
The report notes, however, that the investigation of Medinsky et al. (1999) used F344 rats; this is an inbred strain of low fecundity and therefore unacceptable for use in reproduction studies designed to meet international regulatory requirements (e.g. OECD Guideline 416; EU Annex V method B35; US-EPA 40CFR-798.4700).

For this reason, testis and epididymal evaluations included in the current investigation were conducted in two strains of male rat: Fisher 344 (included for consistency with Medinsky et al.) and Sprague Dawley (for compliance with regulatory testing requirements).

Result: GENERAL
There were no deaths in any group. Excessive secretion of saliva post-dosing with ETBE was the only clinical sign present, affecting primarily high-dose animals of both sexes. There were no toxicologically relevant changes in body weight or food consumption for any of the treated males, however female SD rats treated with 1000 mg ETBE/kg bw/d exhibited a slight (-11%) but statistically significant (P<0.05) decrease in body weight gain although food intake was unaffected.

MATING DATA - SD rats
The pre-mating period was of similar duration in the control and treated groups. The male and female mating and fertility indexes were similar in the control and the treated groups:

Dose level (mg/kg/d)	0	50	250	500	1000
Mating index (%)	95.8	95.8	100	83.3	100
Fertility index (%)	95.7	95.7	95.8	95.0	95.8

SPERM PARAMETERS - SD and F344 rats
The count, viability and morphology of spermatozoa were not affected by treatment in either strain at any dose-level:

Dose level (mg/kg/d)	0	50	250	500	1000
F344 rats					
Epididymal sperm					
Spermatozoa (10^3/mm^3)	337	338	367	330	364
Motility (% motile)	75	91	86	82	89
Normal morphology (%)	97	96	96	97	96
Testicular sperm					
Sperm heads (10^6/g)	125	126	127	129	123
Production (10^6/g/d)	20.5	20.7	20.8	21.1	20.0
SD rats					
Epididymal sperm					
Spermatozoa (10^3/mm^3)	378	336	315	345	324
Motility (% motile)	90	87	91	96	86
Normal morphology (%)	97	97	97	97	96
Testicular sperm					
Sperm heads (10^6/g)	129	134	123	104	123
Production (10^6/g/d)	21.1	21.9	20.2	17.0	20.1

HYSTEROCTOMY, DELIVERY AND LITTER DATA - SD rats
Treatment with the test item had no influence on ovulation, implantation, fecundity or embryo-fetal development. Treatment with the test item had no adverse effect on post-implantation or neonatal losses, delivery litter size, fetal weight or sexual differentiation.

OBSERVATION OF PROGENY DURING LACTATION
The survival, growth and development of the pups were similar in the control and the treated groups. There were no relevant clinical signs or any weight change in any pup.

PATHOLOGY
No relevant macroscopic post-mortem findings were noted in males of either strain or females at any dose-level.

Test substance: ETBE, CAS No. 637-92-3, >98% pure, Totalfinaelf France.

Conclusion: The test item, ETHYL TERTIARY BUTYL ETHER (ETBE), was administered daily by oral gavage at 50, 250, 500 or 1000 mg/kg/day to male Fischer rats for 12 weeks or to male and female Sprague-Dawley rats through the pre-mating and mating periods and until sacrifice (males, 12 weeks treatment) or until day 19 post-coitum for half of the females or day 21 post-partum for the other half.

Minor signs of maternotoxicity (lower body weight gain) were noted at 1000 mg/kg/day during pregnancy whereas no effect was observed at lower dose-levels. There were no adverse changes in male gonadal function, mating behavior, fertility, embryo-fetal development or parturition at any dose-level. The survival and development of the pups was also unaffected. Consequently, under the conditions, the author concluded the results supported a parental NOAEL of 1000 mg/kg bw/d (for parental and reproductive effects), a fetal NOAEL of 1000 mg/kf bw/d and maternal NOAEL of 500 mg/kg bw/day (based on significantly decreased bw gain at 1000 mg/kg bw/d).

Reliability: (2) valid with restrictions
Study available for review. GLP compliant dose-range finding study, clearly reported methods and results, suitable for assessment.

07-NOV-2003 (29)

5.9 Specific Investigations

Endpoint: other: Odour and taste threshold studies with ETBE

Method:

SUBJECTS
Seven individuals (1 male, six female; no further details) were selected from a panel of experienced subjects maintained by the test laboratory. Based on preliminary screening, the individuals were said to be representative of the "normal distribution of olfactory sensitivity" found in the general population. Prior to the odour threshold study, the olfactory response of each subject was calibrated using 1-butanol.

ODOUR THRESHOLD IN AIR
A known volume of ETBE (0.6 ul) was mixed with a known volume of carbon-filtered, hydrocarbon-free air (0.380 cubic feet) in a Tedlar bag (final concentration = 9.25 ppm; confirmed by GC). This vapour sample was mixed with additional hydrocarbon-free air using a dynamic dilution triangle olfactometer (IITRI System, 1979 Model) to give a further 6 diluted samples, spaced at log3 intervals (range: 0.0068-1.156 ppm). Each ETBE/air sample was accompanied by two samples of filtered air. The subjects were asked to 'sniff' all three samples and identify the one that contained ETBE. The results were used to derive:
- detection threshold: the dilution at subjects could differentiate between the ETBE/air sample and filtered air;
- recognition threshold: the dilution at which subjects could rate the intensity of the odour relative to that of 1-butanol.

ODOUR THRESHOLD IN WATER
A series of dilutions of ETBE in distilled water (5 samples, range = 0.066-0.866 ppm, confirmed by GC) was presented to 7 panellists in ascending concentration order. Each sample was accompanied by two flasks that contained plain distilled water. The panellist sniffed the headspace of each flask, and indicated which one differed from the other two. The odour threshold in water was calculated from a linear regression plot.

TASTE THRESHOLD IN WATER
An aliquot of the samples used for the aqueous odour threshold tes was presented to the subjects in order of increasing concentration. Each sample was paired with a water reference. The panellists were required to taste the sample and decide if it tasted different to the water blank. The taste threshold in water was calculated from a linear regression plot.

Result: The mean odour detection and recognition threshold values for ETBE in air were 0.013 ppm and 0.024 ppm, respectively. (MTBE was also included in the study, and returned values of 0.053 ppm and 0.08 ppm for detection and recognition

thresholds, respectively).

The mean odour detection threshold for ETBE in water was 0.049 ppm, with a recognition threshold of 0.106 ppm. (The equivalent values for MTBE in water were 0.095 ppm and 0.193 ppm, respectively.)

The mean taste detection threshold for ETBE was 0.047 ppm, and was described as highly objectionable. (The taste detection threshold for MTBE was 0.134 ppm).

Test substance: ETBE, 99.0-99.5% pure supplied by ARCO Chemical Company, PA, USA.

Reliability: (2) valid with restrictions
Study available for review. Well documented research investigation, meets generally accepted scientific principles, acceptable for assessment.

05-OCT-2003 (78)

5.10 Exposure Experience

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5.11 Additional Remarks

Type: other: SAR modelling

Remark: This paper predicts toxicological properties of ETBE using a SAR model (CASE/MULTICASE). It considers the potential biological reactivity of ETBE, along with that of putative predicted metabolites (derived using META).

The authors conclude that several metabolites are potential sensory irritants, contact sensitisers, mutagens, developmental toxicants or carcinogens.

Reviewer's comment: The reliability of some of the metabolic predictions included in publication (i.e. putative formation of ethylene oxide from ETBE) is unknown, but appears inconsistent with results from metabolism and toxicokinetic studies summarised elsewhere in this IUCLID dataset. As a result, the toxicological profile proposed by these authors cannot be considered reliable or suitable for assessment.

Reliability: (3) invalid
Study available for review. Modelled data of unknown reliability, not suitable for assessment.

02-NOV-2003 (86)

Type: other: Narcotic effects

Remark: General anesthetic effects (Inhalation, Mouse):

LC50 = 123 g/m3/15'

Source: Agip Petroli SpA ROMA
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Test substance: ETBE

Reliability: (4) not assignable
This information originates from the Eurpean Chemicals Bureau IUCLID dataset for CAS No. 637-92-3. Its reliability has not been assessed.

02-NOV-2003 (3)

6.1 Analytical Methods

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6.2 Detection and Identification

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7.1 Function

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7.2 Effects on Organisms to be Controlled

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7.3 Organisms to be Protected

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7.4 User

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7.5 Resistance

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8.1 Methods Handling and Storing

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8.2 Fire Guidance

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8.3 Emergency Measures

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8.4 Possib. of Rendering Subst. Harmless

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8.5 Waste Management

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8.6 Side-effects Detection

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8.7 Substance Registered as Dangerous for Ground Water

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8.8 Reactivity Towards Container Material

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10.1 End Point Summary

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10.2 Hazard Summary

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10.3 Risk Assessment

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