European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

Existing Substances

European Union Risk Assessment Report

CAS No: 75-05-8

EINECS No: 200-835-2

acetonitrile

1st Priority List

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ACETONITRILE

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RISK ASSESSMENT

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RISK ASSESSMENT

Final Report, 2002

Spain

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Review of report by MS Technical Experts finalised:	**1999**
Final report:	**2002**

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

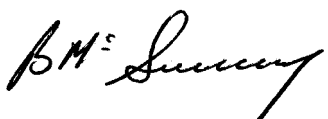
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a Meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) which gives its opinion to the European Commission on the quality of the risk assessment.

If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.

The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.



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[1] O.J. No L 084, 05/04/199 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS Number: 75-05-8
EINECS Number: 200-835-2
IUPAC Name: acetonitrile

Environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to atmosphere.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (iii) applies to the aquatic, terrestrial ecosystems and microorganisms in the sewage treatment plant as a consequence of exposure rising from the use in the pharmaceutical industry.

Human health

Human health (toxicity)

Workers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to acute toxicity, irritation/corrosivity, sensitisation, mutagenicity, carcinogenicity and reproductive toxicity for all occupational scenarios.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (iii) applies to general systemic toxicity as a consequence of dermal exposure arising from use as a solvent and as an intermediate.

Consumers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Human health (risks from physico-chemical properties)

Conclusion (**ii)** There is at present no need for further information and/or testing and no need for risk reduction measures beyond those that are being applied already.

Unintentional sources

The risk assessment has identified other sources of exposure of the substance to humans and to the environment, in particular, the substance is produced during biomass burning and is present in automobile exhaust, which do not result from the life-cycle of the substance produced in or imported into the European Community. The assessment of the risks arising from these exposures are not part of the this risk assessment. The comprehensive Risk Assessment Reports as forwarded to the Commission by the Member State Rapporteur however provides information about these risks.

CONTENTS

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 75-05-8
EINECS Number: 200-835-2
IUPAC Name: acetonitrile
Synonyms: cyanomethane, ethanenitrile, ethyl nitrile, methanecarbonitrile
methyl cyanide
Structural formula:

$H_3C - C \equiv N$

Molecular formula: C_2H_3N
Molecular weight: 41.05

1.2 PURITY/IMPURITIES, ADDITIVES

Degree of purity: ≥ 99.5 %

Identity and percentage of impurities:

- Propionitrile (0.02 %)
- Water, distilled, conductivity or of similar purity (0.01%)

1.3 PHYSICO-CHEMICAL PROPERTIES

Acetonitrile is a volatile, colourless liquid with sweet, ether-like odour. It is infinitely soluble in water and readily miscible with ethanol, ether, acetone, chloroform, carbon tetrachloride and ethylene chloride. It is immiscible with many saturated hydrocarbons (petroleum fractions). Acetonitrile has high dielectric constant and high polarity and it is strongly reactive (Hawley's Dictionary).

Acetonitrile produces hydrogen cyanide when heated to decomposition or when reacting with acids or oxidising agents (Reynolds, 1982).

1.3.1 Physical state at normal temperature and pressure (ntp)

Acetonitrile is a volatile, colourless liquid with a sweet ether-like odour.

1.3.2 Melting point

Grayson (1985) gives a value of -45.7°C. Verschuerenk (1983), the Merck Index, 11th Edition, (1989) and Aldrich, catalogue handbook, (1994-1995) give values between -41°C and -48°C.

1.3.3 Boiling point

The Merck Index, (11th Edition) and Howard (1993) give the figure of 81.6°C at 1,013.25 hPa.

1.3.4 Relative density

IUCLID presents a density of 0.786 at 20°C (Aldrich, 1994-1995).

1.3.5 Vapour pressure

The ca. value provided in IUCLID is between 94.51-98.64 hPa at 20°C (Verschueren, 1983; Envirofate, 1994). The 98.64 hPa value was selected at 25ºC because it gives a Henry's law constant of 2.91 Pa m^3/mol, which is in the range of experimental values given in the literature.

1.3.6 Water solubility

Acetonitrile is infinitely soluble in water (Clayton & Clayton, 1982). Sax (1989) gives a value for water solubility 999999.999 mg/l at 25°C. Montgomery (1991) gives a value of 139,000 mg/l in saturated solution.

1.3.7 Partition coefficient (log n-octanol/water)

IUCLID provides a value of -0.34 (Leo et al., 1971).

1.3.8 Flash point

Two values (5°C and 5.6°C) are given as flash point (open cup) (Aldrich, 1994-1995; Reynolds, 1982). In closed cup the value is 12.8°C (Reynolds, 1982). These values conclude in the R-phrase: R11.

1.3.9 Autoflammability

Sax (1989) provides a value of 524°C.

1.3.10 Flammability

Acetonitrile is highly flammable, with a lower flammability limit of 4.4% in volume and an upper flammability limit of 16% in volume. These data are obtained from Grayson (1985) and Sax (1989).

1.3.11 Explosive properties

Sax (1989) presents that acetonitrile forms explosive mixtures with air. The lower explosive limit is 3.05% in volume and the upper explosive limit 17% in volume.

1.3.12 Surface tension

29.04 dynes/cm at 20°C (Merck Index, 11th Edition, 1989).

1.3.13 Dissociation constant

pKa = 29.1 (Howard, 1993).

Table 1.1 Physico-chemical properties of acetonitrile

Property	Value	Remarks
Physical state (at ntp)	Liquid	Colourless with a sweet, etherlike odour. Produces hydrogen cyanide when heated or when reacte with acids or oxidising agents.
Melting point °C	- 45.7	
Boiling point °C	81.6	At 1,013.25 hPa
Relative density (d_4^{20})	0.786	
Vapour pressure hPa	98.64	At 25°C
Water solubility (at 25°C) mg/l	139,000	
Partition coefficient Log n-octanol/water	- 0.34	
Flash point °C	5-5.6 12.8	(open cup) (closed cup)
Autoflammability °C	524	
Flammability % in volume	4.4 16	Lower flammability limit (LFL) Upper flammability limit (UFL)
Explosive properties % in volume	3.05 17	Lower explosive limit (LEL) Upper explosive limit (UEL)
Conversion factor mg/m^3	1.68	At 25°C
Henry's Law Constant Pa.m^3/mol	2.91	At 25°C
Relative vapour density (air=1)	1.42	
Refractive index	1.34604 1.33934	At 15°C At 30°C
Surface tension dynes/cm	29.04	At 20°C
Dissociation constant pKa	29.1	

WHO (1993), Howard (1993), Verschueren (1983), Montgomery (1991), Snider and Dawson (1985)

1.4 CLASSIFICATION

Classification and labelling according to the 28th ATP of Directive 67/548/EEC[4]:

Classification:	F; R11	Highly flammable.
	Xn; R20/21/22	Harmful by inhalation, in contact with skin and if swallowed.
	Xi; R36	Irritating to eyes.
Labelling:	F; Xn R: 11-20/21/22-36 S: (1/2-)16-36/37	

According to the data mentioned below and to the criteria of Classification and Labelling, acetonitrile has not been classified as dangerous to the environment.

4 The classification of the substance is established by Commission Directive 2001/59/EC of 6 August 2001 adapting to the technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (OJ L 225, 21.8.2001, p.1).

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

Acetonitrile is obtained as a by-product of acrylonitrile synthesis, by a method known as the SOHIO (Standard Oil Company of Ohio) process, which involves a high temperature catalytic reaction between propylene and ammonia and produces crude acrylonitrile containing acetonitrile, hydrogen cyanide and carbon oxides as the main impurities. The reported ratio of acetonitrile/acrylonitrile in this product varies between 3% (Lowenheim and Moran, 1975; Kirk-Othmer, 1978) and 10% (Riegel, 1974; Reinders, 1983). Acetonitrile is obtained from the reaction product, after cooling, by fractional distillation. The main organic impurity in commercial acetonitrile is propionitrile together with small amounts of allyl alcohol (Kirk-Othmer, 1978; USEPA, 1992).

Other potential routes of acetonitrile synthesis are the dehydration of acetamide, the reaction of ammonia and acetylene, the ammonolysis of glacial acetic acid and the catalytic reaction of ethanol and ammonia (Merck, 1989; USEPA, 1980).

Acetonitrile is produced in the EU by two companies in two European countries and one company imports this product from outside, **Table 2.1**.

2.2 USE

Acetonitrile is used in different processes in the chemical industry and in research laboratories. The use pattern of this compound is shown in **Table 2.2**.

This compound is used as a starting material for the synthesis of many chemicals, pharmaceutical and pesticides and in the manufacturing of photographic film.

Acetonitrile is used as a solvent in various extraction processes like butadiene extraction from C_4 streams and isoprene from C_5 streams, dissolution of cationic textile dyes, recrystallization of steroids, extraction of fatty acids from animal and vegetable oils and removal of tars, phenols and colouring matter from petroleum hydrocarbons.

Acetonitrile is widely used in research and analytical laboratories. It is used as a solvent for genetic engineering research and in the analytical determination of a great number of chemicals by high performance liquid chromatography (HPLC).

In addition, it is used as an inert medium in physico-chemical investigations and as a solvent in non-aqueous titrations.

Table 2.1 Acetonitrile production and import in the EU in 1993

Country	Quantity (tonnes/year) produced or imported
Germany [a]	1,000 – 5,000
Italy	1,000 – 5,000
United Kingdom [b]	1,000 – 5,000

[a] 1990 data
[b] Imported from outside, 1992 data

Table 2.2 Use pattern of acetonitrile

Use	Approximate percentage
Pharmaceutical industry	50
Extraction solvent (butadiene production)	15
Analytical laboratories (HPLC)	7
Photographic film	7
Pesticide manufacture	3
Other uses	18

Merck (1989), USEPA (1980), BP (1992), Hawley (1971), Borman (1990)

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

Sources of acetonitrile release to the environment can be anthropogenic or natural. Acetonitrile occurs in coal tar in small amounts, has been detected in volcanic gases and quantified in emissions from the combustion of wood and other biomass. Acetonitrile is also released during its manufacture and use, from some industrial operations like shale oil retorting and coal gasification and from combustion processes in gas turbines, ignition engines and automobile exhaust.

3.1.2 Environmental releases

3.1.2.1 Releases from production

Emission factors for acetonitrile during production have been reported by several authors. Bouscaren et al. (1986) quoted an emission factor of 2kg acetonitrile/tonne acrylonitrile, Reinders (1983) reported a range of factors, for acetonitrile release, between one and 7 kg acetonitrile/tonne acrylonitrile and USEPA (1980) reported a factor of 7.02 kg/tonne acrylonitrile produced.

The emission factor to air, as well as to water and soil, has been estimated by using default values from the Technical Guidance Document (TGD) and assuming that acetonitrile is in category Ic (intermediates stored off-site). **Table 3.1** shows the calculated emissions factors and releases of acetonitrile to air, water and soil compartments during production. The estimated emission factor to air (10kg/tonne acetonitrile) is lower than those previously reported (2-7.02 kg/tonne acrylonitrile) considering, as indicated in Section 2.1, that the reported ratio of acetonitrile/acrylonitrile in crude acrylonitrile varies from 3 to 10%.

Site-specific information on releases of acetonitrile to air during production has been provided for one site and the total emission (fugitive and point emission) reported for two production plants was 77 tonnes/year in 1993, which corresponds to about 256.6 kg/day per site and 128.3 kg/day per production plant. The production volume is stated to be 2,000 – 10,000 tonnes/year (**Table 2.1**). In the EUSES estimation, the regional production volume is 1/10 of the production volume, that is 1,000 tonnes/year. However, it is more appropriate to set the regional production volume to 5,000 tonnes/year, based on information from the main manufacturers production sites. This will result in a local emission to air closer to the estimated value based on emission data provided by industry, 256.6 kg/day, and this value will be considered as a worst-case release value.

Table 3.1 Release of acetonitrile into the environment during production

Environment	Compartment	Emission factor	Production volume (t/a)	Release (kg/day)
Local	Air	0.01	5,000	256.6 [a]
Regional	"	"	5,000	137
Continental	"	"	10,000	137
Local	Water	0.003	5,000	50
Regional	"	"	5,000	41.1
Continental	"	"	10,000	41.1

[a] 256.6 kg/day (1993 emission value provided by a company)

3.1.2.2 Releases from use

Use in the pharmaceutical industry

Approximately 50% of acetonitrile produced is used by the pharmaceutical industry to manufacture different products like insulin, antibiotics and vitamins, **Table 2.2**. In the manufacture of these products, the solvent is handled in close reaction and distillation sections, purified and recycled a number of times and the waste solvent is disposed of by licensed waste contractors. Site-specific information has been provided by industry regarding breakdown of the site usage of acetonitrile by pharmaceuticals companies and the highest usage of acetonitrile per site in 1996 was 700 tonnes. In this year about 1,340 tonnes were exported. Acetonitrile may be considered to be used as solvent in the pharmaceutical industry. Releases for this usage have been calculated using TGD default values for industrial category 2 (chemical industry: basic chemicals). Local emissions have been calculated according to EUSES by means of the equation:

$$E_{local,\ ij} = Fmainsource_i \cdot RELEASE\ reg_{ij} \cdot 365 / Temission_i$$

These values have been included in **Table 3.2**.

Data on the emissions of acetonitrile from the pharmaceutical industry have been provided for various sites and summarised in **Table 3.3**. In some sites, there is not a recycling of solvent or an onsite treatment of wastewater and in these cases emission factors to water can be very high as it occurs in sites 3 and 19, which confirms the TGD default value on emissions. On the other hand, the total annual usage of acetonitrile for the different sites accounts for approximately 20% of the total annual volume of acetonitrile used by the European pharmaceutical industry. Therefore, the TGD default value of 65% was used as a worst-case assumption.

Table 3.2 Release of acetonitrile from use in the pharmaceutical industry using TGD default values

Environment	Compartment	Emission factor	Release (kg/day)
Local	Air	0.25	1,000
Regional	"	"	514
Continental	"	"	4,620
Local	Water	0.65	2,600
Regional	"	"	1,340
Continental	"	"	12,000

Table 3.3 Release of acetonitrile from use in the pharmaceutical industry based on data provided by industry

Site	Processing volume (t/a)	Release to water (t/a)	Emission factor
1	272.3	18.5	0.068
3	30	20	0.667
5	393	3.93	0.01
6	119	6.8	0.057
7	80	0.1	0.001
19	50	20	0.4
20	190	<0.93	<0.005
Total	1,134.3		

Use in butadiene production

Approximately 15% acetonitrile is used as extraction solvent in the obtention of 1-3-butadiene from the mixed C_4-hydrocarbon fraction in petrochemical cracking processes for ethylene manufacture. The emission factors reported by Reinders (1983) ranged from 0-0.1 kg acetonitrile/tonne butadiene produced to air and the same range of emission factors to water. If it is assumed that acetonitrile is used exclusively in the butadiene extraction process and considering a butadiene production volume of 195,128 tonnes for the UK in 1990 (Chem-Fats 1991), the maximum release possible, using these emission factors, is about 65 kg acetonitrile/day for each compartment, air and water, for the production volumes of 1990.

Information on emissions data from a company have been provided during 1996 and aqueous discharge was 8 tonnes/year, corresponding to 26.7 kg/day. Air point emissions of 35 tonnes acetonitrile/year (116.6 kg/day) were reported together with accidental discharges of 27 tonnes/year (90 kg/day) producing a release of 206.6 kg/day.

Table 3.4 Release of acetonitrile from use in butadiene production

Environment	Compartment	Emission factor	Release (kg/day)
Local	Air	0.03 [1]	206.6 [1]
Regional	"	"	18.5
Continental	"	"	166
Local	Water	0.036 [1]	26.7 [1]
Regional	"	"	2.22
Continental	"	"	20

[1] Emision data provided by industry

Other uses

Acetonitrile is also used in the manufacture of photographic film, in the synthesis of some pesticides and in other uses, **Table 2.2**. In these processes, acetonitrile is used as a solvent in closed reaction or processing systems. Information on acetonitrile emissions during 1998 from the photographic industry has been provided for two sites. Reported daily releases to water were 0.2 kg for one site and 88 kg for the other, having this site a WWTP capacity of 6,000 m^3 per day. These releases are covered by values shown in **Table 3.5**, assuming that 28% of acetonitrile produced is used in these processes and using default values from the TGD for main category Ic, taking into account that the default value for the WWTP capacity is 2,000 m^3 per day.

There is no actual information on acetonitrile emissions from the other uses, including agrochemical industries, but considering the low percentages used and the breakdown of the site usage, emission values given in **Table 3.5** can cover the possible utilisation of acetonitrile as solvent in those uses.

Table 3.5 Release of acetonitrile from other uses

Environment	Compartment	Emission factor	Release (kg/day)
Local	Air	0.001	2.5
Regional	"	"	1.15
Continental	"	"	10.4
Local	Water	0.02	50
Regional	"	"	23
Continental	"	"	207

Use as laboratory chemical

Acetonitrile is used in research and analytical laboratories as HPLC solvent, as a solvent for genetic engineering research, as an inert medium and as a solvent in non-aqueous titration. When used in HPLC the solvent should be collected after use and disposed of by licensed waste contractors, although this may not always be accomplished. Emission factors can be estimated assuming that a 7% of acetonitrile is used in these processes and using default values from the TGD for chemicals used in synthesis main category 3, **Table 3.6**.

Table 3.6 Release of acetonitrile from use in laboratory chemicals

Environment	Compartment	Emission factor	Release (kg/day)
Local	Air	0.025	62.5
Regional	"	"	7.19
Continental	"	"	64.7
Local	Water	0.02	50
Regional	"	"	5.75
Continental	"	"	51.8

3.1.2.3 Releases from other sources

Biomass burning

Biomass burning is widespread, particularly in the tropics and in developing countries, and serves various purposes such as clearing of forest, control of pests, nutrient mobilisation, production of charcoal and energy production for cooking and heating (Crutzen and Andreae, 1990). These authors estimated in $3 - 6 \cdot 10^{15}$ g the biomass carbon burned annually, corresponding to $24 - 57 \cdot 10^{12}$ g of biomass nitrogen. Lobert et al (1990), based on these estimates, reported an acetonitrile emission from biomass burning to the atmosphere equivalent to 410,000 - 2,220,000 tonnes/year. Hamm and Warneck (1990) estimated an emission of 850,000 tonnes/year, which is in the same range, while the emission reported by Arijs and Brasseur (1986) of 24,000 - 174,000 tonnes/year is smaller. According with these estimates, a global acetonitrile release from biomass burning of 24,000 - 2,200,000 tonnes/year can be estimated. Assuming an EU population ratio of 6.34%, with respect to world population, a 6.34% of this release can take place in Europe. Then, a continental estimate of 1,522 - 139,480 tonnes of acetonitrile release per year from biomass burning can be considered. This corresponds to about 4.2 - 382 tonnes/day for the EU. The release at regional level can be estimated in one tenth, which corresponds to 0.42 – 38.2 tonnes/day. The lower values of these ranges will be used for the regional and continental calculation, due to the fact that the highest proportion of biomass burning takes place in the tropics.

Automobile exhaust

Various nitrogen compounds, including acetonitrile and hydrocyanic acid, have been identified as combustion products (Schuchmann and Laidler, 1972). The global release of acetonitrile from automobile exhaust has been estimated in 80,000 tonnes/year by Arijs and Brasseur (1986). Dulson (1978) reported that exhaust gases from gasoline-powered engines contained 1.32% by mass acetonitrile. Assuming an emission of 617,400 VOC tonnes/year at one area (UK) and a content of 1.32% by mass acetonitrile, the release of acetonitrile from motor exhaust would be 8,150 tonnes/year, equivalent to 22.3 tonnes/day. The overall amount for the EU, considering a population ratio of 6, would be 134 tonnes/day.

These releases from motor exhaust are likely to be overestimates, due to the use of diesel in a significant number of cars and the use of new technologies, such as catalytic converters, used to reduce emissions from automobiles. In addition, the origin of acetonitrile detection in automobile exhaust may be associated with contamination from the sampling system (e.g. preparation/clean-up of the sampling cartridges), according to information provided by industry.

Table 3.7 Summary of release estimates

Source	Main compartment	Amount/site kg/day	Amount/regional kg/day	Amount/Continental kg/day
Production	Air	256.6 [1]	137	137
	W.Water	50	41.1	41.1
Use: Pharm. Ind.	Air	1,000	514	4,620
	W.Water	2,600	1,340	12,000
Use: Butad. Prod.	Air	206.6 [1]	18.5	166
	W.Water	26.7 [1]	2.22	20
Other uses	Air	2.5	1.15	10.4
	W.Water	50	23	207
Use: Laboratory	Air	62.5	7.19	64.7
	W.Water	50	5.75	51.8
Disp. Sourc.: Biomass bur. Motor exh.	 Air Air	 - -	 420 - 38,200 13,400	 4,200 - 382,000 134,000
Total	Air	-	14,360-52,140	143,598-521,398
	W.Water/Surf.water	-	985/422	8,640/3,700

[1] Emission data provided by industry

3.1.3 Environmental fate

3.1.3.1 Degradation

3.1.3.1.1 Abiotic degradation

Air

The maximum of absorption for acetonitrile in the UV range is lower than 160 nm, therefore direct photolysis of acetonitrile in the atmosphere is not expected to be an important fate process (Silverstein and Bassler, 1967; Howard, 1991).

Acetonitrile is reactive to oxidising materials and compounds in the atmosphere, being the reaction with hydroxyl radicals quoted as one of the main mechanisms for its removal from the environment (Lobert et al., 1990). The rate constant for the reaction of acetonitrile with hydroxyl radicals has been determined by several authors to range from $1.9 - 4.94 \cdot 10^{-14}$ $cm^3 \cdot molec^{-1} \cdot sec^{-1}$ for the temperature range 20-27ºC. In a moderately polluted atmosphere, with a mean concentration of 10^7 hydroxyl radicals/cm^3, the calculated half-life was about 20 days (Atkinson, 1985; Güsten et al., 1981 and 1984; Harris et al. 1981; Wallington et al., 1988; Lyman et al., 1982). The Arrhenius activation energy, as determined by Harris et al. (1981), was 1,500 cal mol^{-1}.

In conditions of an average tropospheric OH radical concentration, $[OH]= 5 \cdot 10^5$ cm^{-3}, Klöpffer et al. (1988) obtained a half-life about 10 times longer, $t_{1/2}$>60 days. Acetonitrile reaction with

chlorine radicals is not thought to be significant in relation to hydroxyl radical reaction (Arijs et al., 1983).

The reaction of acetonitrile with ozone is slow, with a reported reaction rate constant of $\leq 1.5 \cdot 10^{-19}$ $cm^3 \cdot molec^{-1} \cdot sec^{-1}$ (Harris et al., 1981), which will yield a half-life of ≥54 days, considering an average ozone abundance of $1 \cdot 10^{12}$ molec/cm^3 (Munshi et al., 1989).

A reaction rate constant between acetonitrile and single oxygen of $2.4 \cdot 10^{-16}$ $cm^3 \cdot molec^{-1} \cdot sec^{-1}$ has been reported (Graedel, 1978), which yields an atmospheric half-life of >5,000 years.

An overall half-life for acetonitrile in the troposphere of about 42 days has been estimated, considering both the OH radical and ozone reaction rate constants (USEPA, 1987), based on rate constants quoted by Harris et al. (1981). Nevertheless, as indicated above, that value should be about 10 times higher for average tropospheric conditions, which will give a half-live of about one year (321 days).

Water

The hydrolysis of acetonitrile is unimportant for the aquatic fate of this compound at the normal pH range of natural waters (Ellington et al., 1988). The half-life for acetonitrile in water has been estimated to be >150,000 years based on a hydrolysis rate constant at ph 7 and 25ºC of $5.8 \cdot 10^{-3}$ M^{-1} $hour^{-1}$ (Howard, 1991).

3.1.3.1.2 Biodegradation

The information on the biodegradability of acetronitrile is scarce and no studies using the recommended guidelines have been presented. Therefore, an in-depth consideration of the available information and the expert judgement of the weight of the available evidence are required.

There is enough evidence on the rapid degradation of acetronitrile by adapted microbial populations. Ludzack and Ettinger (1960) reported previous works from Ludzack et al. (original papers not submitted) indicating 100% removal (60% theoretical oxidation) after 4 days in acclimated river water, and more than 98% removal (more than 70% theoretical oxidation) in acclimated activated sludge in 28 days. In addition, Babeu and Vaishnav (1987) reported 57.5% of degradation (BOD/ThOD) in five days using mixed microbial cultures acclimated to several chemicals including acetonitrile.

The evidence for biodegradation of acetonitrile by non-adapted bacterial populations is scarce. However, two pieces of information suggest a rapid biodegradation. In a report by Sasaki (1978), acetonitrile is reported as well biodegradable; no specific data are presented but this classification should indicate over 30% of degradation (BOD/THOD) in 14 days. A reported paper (not submitted) from Plack and Ruchhoft (1947) indicates 17% of removal in one day (based on oxygen demands) in a bench-scale fill and draw study with non-acclimated (less than one day) activated sludge.

This information has been summarised in **Table 3.8.**

The biodegradability of acetonitrile can be confirmed by the comparison of these data with those available for other nitriles and particularly for acrylonitrile. The chemical structure and the data from Ludzack and Ettinger (1960) and Ludzack et al. (1961) suggest that acetonitrile is at least as biodegradable as acrylonitrile.

No original data have been submitted on the anaerobic degradation of acetonitrile, although the information included in IUCLID suggests no digestion of acetonitrile in a 1-month study with digestion bottles and an estimated (no information on the estimation method is reported) half-life of 4-16 weeks under anaerobic conditions.

It is concluded that acetonitrile should be considered as ready biodegradable for local scenarios if WWTP is available, while, due to the higher uncertainty and the application of the Precautionary Principle, the rate constants reported in the TGD for inherent biodegradable chemicals must be applied for regional/continental scenarios and to derive the rate constant for soil.

Table 3.8 Biodegradation test results for acetonitrile

Type	Source of microorganisms	Method	Result	Detection	Ref
Aerobic	activated sludge <1 day acclimation	Bench-scale fill and draw study	17% 1day	O_2 demand	1
Aerobic	river water, acclimated (19 days) population	Other	100% 4day	O_2 demand	1
Aerobic	Other	Other	98% 28day	O_2 demand	1
Aerobic	Acclimated mixed microbial cultures	Other	>58% 5day BOD/ThOD	O_2 demand	2
Aerobic	Active sludge	Japanese method	30% 14day BOD/ThOD	O_2 demand	3

1 Reported by Ludzack and Ettinger (1960)
2 Babeu and Vaishnav (1987)
3 Sasaki (1978)

3.1.3.2 Distribution

Acetonitrile is a volatile liquid with a vapour pressure of 9,864 Pa at 25ºC and a rapid volatilisation of this compound to the atmosphere is expected when released to the environment.

The Henry's Law constant of 2.07-2.9 $Pa \cdot m^3 \cdot mol^{-1}$ (Snider and Dawson, 1985; Hine and Mookerjee, 1975) indicates that volatilisation of acetonitrile from surface waters and moist soils is likely to be significant (Howard, 1993). Chen et al. (1981) reported a decrease to 5% of the original acetonitrile level in river water after 72 hours.

The removal of acetonitrile from the atmosphere by precipitation has been quoted in several reports as one of the main fate processes for this compound, as important as the photooxidation reaction with hydroxyl radicals (Hamm et al., 1989; Hamm and Warneck, 1990). A residence time of about three years has been estimated for acetonitrile in the troposphere due to wet deposition (Hamm et al., 1984; Arijs and Brasseuur, 1986).

An overall residence time for acetonitrile near 1.5 years was calculated considering the two principal removal processes, precipitation and reaction with hydroxyl radicals (Hamm and Warneck, 1990; Arijs and Brasseur, 1986). Nevertheless, a shorter residence time was calculated based on the tropospheric mass balance of this compound (Hamm and Warneck, 1990). They estimated a mass content (G) in the troposphere of $370\text{-}570 \cdot 10^3$ tonnes acetonitrile and a global

source strength (Q) of 600-1,100 $\cdot 10^3$ tonnes acetonitrile/year, which would yield an overall residence time, calculated as G/Q, of 0.23-0.95 years. The dissolution in the ocean was proposed as an effective process for acetonitrile removal, that would take up 65% of the total acetonitrile emission, and the combined residence time for the process (precipitation, hydroxyl radical reaction and ocean dissolution) was estimated to be 0.45 years, which is in agreement with the mass balance estimate.

The adsorption coefficient, K_{oc}, can be estimated for acetonitrile using the octanol-water partition coefficient, log P_{ow}= -0.34 (Leo et al., 1971), and K_{oc} values of 0.3-16 l/kg were obtained using regression equations reported in Lyman et al. (1982). These K_{oc} values indicate a low potential for adsorption and then leaching of acetonitrile to groundwater may be important when spilled on soil (Howard, 1993). K_{oc} calculated according to the TGD for nonhydrophobics (log K_{oc} = 0.52 log K_{ow} + 1.02) gives a K_{oc} value of 7.

3.1.3.3 Accumulation

No experimental data on bioaccumulation are available but a very low bioaccumulation potential is expected. The value calculated according to the TGD is 0.1 but the log P_{ow} of acetonitrile is considered too low to use this equation, which according to the TGD should be used for substances with a log P_{ow} value in the range 2-6. A bioconcentration factor for fish of 0.3 l/kg was calculated using the log P_{ow} = -0.34 value and regression equations defined in Lyman et al. (1982). This bioconcentration factor is in agreement with other calculated values which are in the range of 0.3-0.4 (EPA, 1991).

Regardless the specific QSAR equation applied for these calculations the information indicates a lack of bioaccumulation potential in the environment.

3.1.4 Aquatic compartment

3.1.4.1 Calculation of Predicted Environmental Concentrations

3.1.4.1.1 Calculation of PEC local

The PEC_{local} for water has been calculated using the equations given in the TGD, assuming that the release occurs to a wastewater treatment plant.

$$PEC_{local,water} = Ceff / (1 + K_{p(susp)} \cdot C_{susp}) \cdot D \text{ [g/l]}$$

where:
Ceff = concentration of the chemical in the WWTP effluent [g/l]
$K_{p(susp)} = K_{oc} \cdot f_{oc,susp} = K_{oc} \cdot 0.1 = 7 \cdot 0.1 = 0.7$ (l/kg)
C_{susp} = concentration of suspended matter in the river = 15 mg/l = $15 \cdot 10^{-6}$ kg/l
D = dilution factor = 10

The WWTP effluent concentration is calculated using the following equation:

$$Ceff = W \cdot (100 - P) / 100 \cdot Q$$

where:
W = emission rate (kg/day)
Q = volume of waste water (m^3/day) = 2,000 m^3/day
P = percentage removal in the WWTP (%) = 87.7%

Acetonitrile is readily biodegradable under aerobic conditions and using the tables in Appendix II of the TGD based on the SIMPLETREAT model, log P_{ow} = -0.34 and log H = 0.46, the removal of acetonitrile in the WWTP is 87.7%. Fate of acetonitrile in the WWTP is 86.6% degraded, 12.3% dissolved in water and 1.1% directed to air (EUSES). The release of acetonitrile to wastewater during production was estimated to 50 kg/day, **Table 3.6**.

$$Ceff = 50 \cdot 12.3 / 100 \cdot 2{,}000 = 3.07 \cdot 10^{-3} \text{ g/l}$$

$$PEC_{local,\ water} = 3.07 \cdot 10^{-3} / (1 + 0.7 \cdot 15 \cdot 10^{-6}) \cdot 10 = 3.07 \cdot 10^{-3} / 10 \cdot 00024 = 0.307 \cdot 10^{-3} \text{ g/l} = 307 \text{ µg/l}$$

Acetonitrile is used in various processes in the chemical industry. The highest percentage is used in the pharmaceutical industry (50%). Data on acetonitrile emissions from the pharmaceutical industry have been provided by industry and summarised in **Table 3.9**.

Table 3.9 Data on the emissions of acetonitrile from the pharmaceutical industry

Site	Release to water (t/a)	Total WWTP flow (m3/d)	Receiving water dilution	PEC (µg/l)
1	18.5		260-280	<1 (measured)
3	20	21,000-23,000	Default	390
5	3.93	52,800	9.2	3.3
6	6.8	2,314	Default	162
7	0.1	None	100	111
19	20	Default	Default	209
20	<0.93	535	2,415	0.3

However, as explained in the releases from use, the emission factors to water can be very high when acetonitrile is used as solvent in the pharmaceutical industry. This fact has been confirmed by emission factors near 65%, the TGD default value (66.7% reported from site 3 and 40% from site 19), mainly in sites not using solvent recycling or onsite wastewater treatment. Therefore, the TGD default values are used as a worst-case estimate and the calculated release of acetonitrile to water is 2,600 kg/day, **Table 3.10**.

$$Ceff = 2{,}600.\ 12.3 / 100 \cdot 2{,}000 = 160 \text{ mg/l}$$

$$PEC_{local,\ water} = 160. / 10.00024 = 16 \text{ mg/l}$$

Other important percentage (15%) is used in butadiene production. The release of acetonitrile to water provided by industry is 26.7 kg/day, **Table 3.7**.

$$Ceff = 26.7 \cdot 12.3 / 100 \cdot 2{,}000 = 1.64 \cdot 10^{-3} \text{ g/l}$$

$$PEC_{local,\ water} = 1.64 \cdot 10^{-3} / 10.00024 = 0.164 \cdot 10^{-3} \text{ g/l} = 164 \text{ µg/l}$$

Another percentage (7%) is used as laboratory chemicals and the calculated release to water was 50 kg/day, **Table 3.10**.

$$Ceff = 50 \cdot 12.3 / 100 \cdot 2{,}000 = 3.07 \cdot 10^{-3} \text{ g/l}$$

$PEC_{local, water} = 3.07 \cdot 10^{-3} / (1 + 0.7 \cdot 15 \cdot 10^{-6}) \cdot 10 = 3.07 \cdot 10^{-3} / 10.00024 = 0.307 \cdot 10^{-3}$ g/l = 307 μg/l

The rest of acetonitrile produced volume (28%) was considered to be used as other uses and the calculated release to water was 50 kg/day, **Table 3.10**.

$$Ceff = 50 \cdot 12.3 / 100 \,.\, 2{,}000 = 3.07 \cdot 10^{-3} \text{ g/l}$$

$PEC_{local, water} = 3.07 \cdot 10^{-3} / (1 + 0.7 \cdot 15 \cdot 10^{-6}) \cdot 10 = 3.07 \cdot 10^{-3} / 10.00024 = 0.307 \cdot 10^{-3}$ g/l = 307 μg/l

Table 3.10 shows the $PEC_{local, water}$ calculated for the aquatic environment.

Table 3.10 Local PECs for the aquatic environment

Process	Release to waste water (kg/day)	Ceff (μg/l)	PEC_{local} (μg/l)
Production of acetonitrile	50	3,070	307
Pharmaceutical Industry	2,600	160,000	16,000
Butadiene production	26.7	1,642	164
Other uses	50	3,070	307
Lab. Chem.	50	3,070	307

The $PEC_{local, sed}$ can be calculated using the equations given in the TGD and the values for $PEC_{local, water}$.

$$PEC_{local, sed} = [K_{susp, water} / RHO_{susp}] \cdot PEC_{local, water} \cdot 1{,}000$$

$$K_{susp, water} = 1.07 \text{ m}^3 \cdot \text{m}^{-3}$$

$$RHO_{susp} = 1{,}150 \text{ kg.m}^{-3}$$

Using this equation and the various PECs $_{local, water}$ calculated above, the PECs $_{local, sed}$ have been calculated and are shown in **Table 3.11**.

Table 3.11 Local PECs for sediment

Process	$PEC_{local, water}$ μg/l	$PEC_{local, sed}$ μg/kg
Production of acetonitrile	307	287
Pharmaceutical industry	16,000	14,900
Butadiene	164	153
Other uses	307	287
Lab. Chem.	307	287

3.1.4.1.2 Calculation of $PEC_{regional}$ and $PEC_{continental}$

The calculation of predicted regional and continental environmental concentrations of acetonitrile has been done using EUSES, considering emissions from production and processing, as well as emissions from biomass burning and motor exhaust. A summary of the results is shown in **Table 3.12**.

Table 3.12 Summary of regional and continental modelling

	Regional	Continental
PEC_{air} (mg/m^3)	$4.13 \cdot 10^{-4}$	$2.21 \cdot 10^{-4}$
$PEC_{surface\ water}$ (mg/l)	$2.41 \cdot 10^{-3}$	$4.51 \cdot 10^{-4}$
$PEC_{sediment}$ (mg/kg)	$1.77 \cdot 10^{-3}$	$3.26 \cdot 10^{-4}$
$PEC_{agric.soil}$ (mg/kg)	$1.24 \cdot 10^{-4}$	$6.3 \cdot 10^{-5}$

3.1.4.2 Measured levels

Table 3.13 Levels of acetonitrile in water and sediment

Country	Location	Sample	Concentration
USA	Rock Sprigs, Wyoming	Retort water [a], shale oil processing	42 μg/l
	Hanna, Wyoming	Process water [a], coal gasification plant	620 μg/l
	Gillette, Wyoming	Process water [a], coal gasification plant	1,246 μg/l
	Colorado	Waste water [b], shale oil retorting	23-43 mg/l
		Coal gasification condensate [c]	365 mg/l
	Tucson, Arizona	Rainfall [d]	0.18 μg/l
Japan	47 Prefectures	Water [e]	1.1 – 7.4 mg/l
		Sediment [e,f]	0.02 – 1.9 mg/kg

[a] Pellizzari et al. (1979)
[b] Benton (1992)
[c] Mohr and King (1985)
[d] Snider and Dawson (1985)
[e] Environment Agency, Japan (1996)
[f] Office of Health Studies (1990)

No data on measured levels in water or sediment was found for European countries.

Acetonitrile concentration found in water and sediment samples in the USA and Japan are shown in **Table 3.13**. Acetonitrile levels were determined in aqueous samples collected from different operations related with shale oil processing and coal gasification in the USA. A Japanese environment agency made a study on acetonitrile in fresh and marine surface waters across Japan (Office of Health Studies, 1991; Environment Agency, 1996). In addition, acetonitrile was detected in four rainfall samples collected at a rural site in Tucson, Arizona (Snider and Dawson, 1985).

3.1.4.3 Comparison of PECs with measured levels

The local PECs calculated for surface water ranged from 164 to 307 μg/l at acetonitrile production or use sites and the acetonitrile concentration in WWTP effluents (Ceff) ranged from 1.64 to 3.07 mg/l. In the case of acetonitrile used as solvent in the pharmaceutical industry, the calculated values were 16 mg/l for local PEC and 160 mg/l for Ceff. These values are in the range of those reported in the literature for acetonitrile levels in wastewater of various industrial processes in the USA, ranging from 0.04 to 365 mg/l, **Table 3.10**. In relation with sediment levels, calculated values for local PEC_{sed} varied from 153 to 287 μg/kg and are in the lower range of those found in Japan, which ranged between 20 and 1,900 μg/kg, when the calculated value for the pharmaceutical industry (14.9 mg/kg) was clearly higher.

3.1.5 Atmosphere

3.1.5.1 Calculation of Predicted Environmental Concentrations

According to the TGD, in the calculation of PEClocal for air, both emission from a point source and emission from a STP are taken into account. The concentration on the regional scale (PECregional) is used as background concentration and summed to the local concentration. The maximum from the two concentrations (direct and via STP) is used as the PEClocal.

$$C_{local,\ air} = \max\left(E_{local,\ air}, E_{stp,\ air}\right) \cdot C_{std,\ air}$$

where:

$E_{local,\ air\ i,j} = F_{mainsource\ i} \cdot 1{,}000 / T_{emission\ i} \cdot RELEASE_{\ i,j}$

$E_{stp,\ air} = F_{stp,\ air} \cdot E_{local,\ water}$

$C_{std,\ air}$ = standard concentration in air at source strength of 1 kg/s = $24 \cdot 10^{-6}$ kg/m^3

A PEClocal for air for acetonitrile emissions from production and various uses has been calculated by using these equations and emissions given in **Table 3.7**. The obtained values are summarised in **Table 3.14**.

Table 3.14 Annual average local PECs calculated for the atmospheric environment

Source	Emission to air Kg/day	PEC local μg/m^3
Production	256.6	59
Use in Pharmaceutical Industry	1,000	57.1
Use in Butadiene Production	206.6	7.48
Other uses	2.5	0.56
Laboratory Chem.	62.5	1.4

EUSES has been used to calculate regional and continental concentrations. The $PEC_{regional}$ obtained for air was 0.4 μg/m^3 and the $PEC_{continental}$ was 0.21 μg/m^3, **Table 3.12**.

3.1.5.2 Measured levels

Levels of acetonitrile in air have been determined in the atmosphere over Europe (**Table 3.15**) and other parts of the world (**Table 3.16**). Some of these results show the contribution of biomass burning to the atmospheric levels in rural areas (Becker and Ionescu, 1982) and also over the Atlantic Ocean, where the tendency of levels to be higher around the equator (Hamm and Warneck, 1990) can be explained by the high percentage of the world biomass burning that takes place in the tropics, estimated in more than 80% by Lobert et al. (1990).

3.1.5.3 Comparison of PECs with measured levels

The local PECs calculated for air ranged from 0.56 to 59 μg/m^3 at acetonitrile use or production sites. These values are in agreement with those reported in the literature for acetonitrile levels in urban and rural areas in Europe, which ranged from 0.2 to 58.6 μg/m^3, **Table 3.15**.

The calculated regional PEC is also in the range of values reported for an urban area in Germany (0.2 to 1.45μg/m^3) and the estimated continental PEC is similar, although somewhat higher, than values reported for the atmosphere of the Atlantic Ocean near Europe (0.035 to 0.138 μg/m^3).

Table 3.15 Levels of acetonitrile in the European atmosphere

Location / Sample	Concentration (ng/m^3)	Reference
Rural area, Germany	5,210 – 10,420	Becker and Ionescu (1982)
Rural area, Germany before agric. Burning during agric. burning	 6,720 58,630	
Urban area, Wupertal, Germany	12,430 ± 4,030	
Coastal area, Ireland	10,420	
Mountain area, Switzerland	3,700 - 4,870	
Urban area, Mainz, Germany	197 – 1,453	Hamm and Warneck (1990)
North sea	109 - 328	
Deuselbach	197 - 279	Hamm et al. (1989)
Aircraft flight over Germany	218 - 260	
Aircraft flight over Scandinavia	186 - 341	

Table 3.16 Levels of acetonitrile in the atmosphere of other countries

Location / Sample	Concentration (ng/m^3)	Reference
Rural area, Arizona, USA	94.1 ± 33.6	Snider and Dawson (1984)
Samples collected across Japan	210 - 42,000	Office of Health Studies (1991)
Atlantic Ocean 30-17^{o}S 14^{o}S-Eq Eq-16^{o}N 22-50^{o}N	122 – 192 208 – 302 170 – 257 35 – 138	Hamm and Warneck (1990)

3.1.6 Terrestrial compartment

3.1.6.1 Calculation of Predicted Environmental Concentration

PEC_{local} has been calculated according to the TGD by means of the equation:

$$PEC_{local\ ,\ soil} = C_{local,\ soil} + PEC_{regional,\ natural,\ soil}$$

The obtained results are sumarised in **Table 3.17**.

Table 3.17 Local PECs calculated for the terrestrial compartment

	Averaged over 30 days	Averaged over 180 days
Production	0.0647 mg/kgwwt	0.036 mg/kgwwt
Pharma. Industry	2.56 mg/kgwwt	1.07 mg/kgwwt
Butadiene	0.0281 mg/kgwwt	0.0128 mg/kgwwt
Other uses	0.0491 mg/kgwwt	0.0204 mg/kgwwt
Lab. Chem.	0.0493 mg/kgwwt	0.0207 mg/kgwwt

Regional and continental PECs have been calculated according to the TGD giving the following values: $PEC_{agric,\ soil,\ regional}$ = 0.124 µg/kgwwt and $PEC_{agric,\ soil,\ continental}$ = 0.06 µg / kgwwt.

The PEC for soil porewater has been calculated using the equation in the TGD:

$$PEC_{local,\ soil,\ porewater} = PEC_{local,\ soil} \cdot RHO_{soil} / K_{soil,\ water} \cdot 1{,}000$$

where:
$K_{psoil,\ water}$ = soil/water partition coefficient = Foc comp $\cdot$ K_{oc} = 0.02 $\cdot$ 7 = 0.14 l/kg

This equation and the PECs calculated above have been used to calculate a PEC for the soil porewater.

Table 3.18 Local PECs calculated for agricultural soil porewater

Production	0.149 mg/l
Pharmaceutical Industry	4.43 mg/l
Butadiene	0.0532 mg/l
Other uses	0.0847 mg/l
Laboratory Chemicals	0.0856 mg/l

$PEC_{regional,\ soil,\ porewater}$ = 0.514 µg/l
$PEC_{continental,\ soil,\ porewater}$ = 0.26 µg/l

3.1.6.2 Measured levels

No information on measured levels in soil was found.

3.1.7 Secondary poisoning

A summary of values provided by EUSES, at the regional scale, for exposed biota is presented in **Table 3.19** and values calculated for daily human intake are given in **Table 3.20**.

Table 3.19 Regional concentration for intake media

Wet fish	$7.24 \cdot 10^{-4}$ mg/kg
Root tissue of plant	$4.8 \cdot 10^{-4}$ mg/kg
Leaves of plant	$3.14 \cdot 10^{-4}$ mg/kg
Drinking water	$2.41 \cdot 10^{-3}$ mg/kg
Meat	$1.6 \cdot 10^{-7}$ mg/kg
Milk	$1.6 \cdot 10^{-6}$ mg/kg

Table 3.20 Daily doses in intake media

Drinking water	$6.89 \cdot 10^{-5}$ mg/kg
Fish	$1.19 \cdot 10^{-6}$ mg/kg
Leaf crops	$5.39 \cdot 10^{-6}$ mg/kg
Root crops	$2.64 \cdot 10^{-6}$ mg/kg
Meat	$6.98 \cdot 10^{-10}$ mg/kg
Milk	$1.3 \cdot 10^{-8}$ mg/kg
Air	$8.85 \cdot 10^{-5}$ mg/kg

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT) ASSESSMENT

3.2.1 Aquatic compartment

3.2.1.1 Aquatic organisms

The provided information includes a set of data on the acute toxicity of acetonitrile for aquatic organisms. These data have been summarised in **Tables 3.21, 3.22** and **3.23**. It has not been possible to check all data going back to the original publications (particularly for fish), and some values have been included in the tables when cited in good quality reports. The non validated data have been used as additional information to support the assessment produced from the available reports and all values used to establish the proposed PNEC have been checked against the original publications.

The acute toxicity of acetonitrile on fish has been assessed on different species. The IUCLID data base includes values for *Pimephales promelas, Lepomis macrochirus, Leuciscus idus melanotus, Poecilla reticulata* and *Oryzias latipes*. The lowest value is the 48 h TLm, 730 mg/l, reported in IUCLID as toxicity for *Oryzias latipes* while all 48-96 h LC_{50} values are in the g/l

range. Information on additional fish species can be obtained from other sources. The WHO report (1993) includes 14 acute LC_{50} values on seven fish species. Significant values are 24-48 h LC_{50} values higher than 1g/l for *Oryzias latipes*, and two 48-h LC_{50}s, 730 and 880 mg/l on *Cyprinus carpio* and *Ctenopharyngodon idellus*, respectively. The data set includes flow through and static test as well as different water pH and hardness conditions. Relevant differences on acetonitrile toxicity regarding water quality conditions were not observed. Although not all original publications have been presented to the rapporteur, the coherence of the submitted data and the fact that most data have been previously reported in other reviews suggest that the available data set is suitable for a proper risk assessment.

The lower acute toxicity for fish is 730 mg/l as reported by Nishiuchi, although discrepancies between the fish species showing this toxicity appear when comparing IUCLID and the other reviews. The original paper has been submitted but it is in Japanese; however, the title and the scientific names also appear in English and indicate that the toxicity data are for the carp, Cyprinus capio, and therefore is reported as toxicity for carp in the table. In any case, the fish species is not relevant for this assessment and the value will be used as the lowest acute fish toxicity value.

The acute toxicity of acetonitrile on aquatic invertebrates was investigated by Ewell et al. (1986) on six species from different taxonomic groups including Arthropoda, Platyhelminthes, Mollusca and Annelida; all LC_{50} values were reported as > 100 mg/l. Additional data reported by Bowman et al. (1981) on *Daphnia pulex, Culex restuans, Hyalella azteca* and *Palaemonetes kadiakensis* indicate LC_{50} values in the g/l range.

A reduced number of toxicity results on algae have been found. Toxicity threshold based on the first detectable inhibition of cell multiplication for the green alga *Scenedesmus quadricauda* and for the blue-green algae *Microcystis aeruginosa* has been reported by Bringmann and Künn (1978) as 7,300 and 520 mg/L, respectively. No EC_{50} or NOEC values have been reported.

Table 3.21 Toxicity of acetonitrile to fish

Species	Test type	Comments	Duration (hour)	Toxicity end point (mg/l)	Reference
Pimephales promelas (fresh water)	Static nominal concentration	Used as additional information	96	LC_{50}=1,000-1,150	Henderson et al. (1961) (cited in UK and IPCS reports)
Pimephales promelas (fresh water)	Static nominal concentration	Valid	96	LC_{50} > 100	Ewell et al. (1986)
Lepomis macrochirus (fresh water)	Static nominal concentration	Used as additional information	24-46	LC_{50} = 1,850	Henderson et al. (1961) (cited in UK and IPCS reports)
Ctenopharyngodon idellus	Static nominal concentration	Used as additional information	24-48	LC_{50} = 1,950 LC_{50} = 880	Chen (1981) (cited in IPCS)
Poecilia reticulata (fresh water)	Static nominal concentration	Used as additional information	24-96	LC_{50} = 1,650	Henderson et al., (1961) (cited in UK and IPCS reports)
Oryzias latipes (fresh water)	Static nominal concentration	Used as additional information	24-48	LC_{50} > 1,000	Tonogai et al. (1982) (cited in UK and IPCS reports)
Cyprinus carpio (fresh water)	--	Valid	48	LC_{50} = 730	Nishiuchi (1981)
Pimephales promeles (fresh water)	Flow through measured concentration	Valid	96	LC_{50} =1,640	Centre for Lake Superior Environmental studies
Leciscus idus melanotus (fresh water)	Static	Used as additional information	48	LC_{50} = 5,850-7,050	Juhnkeand Ludemann (1978) (cited in UK and IPCS report)

Table 3.22 Toxicity of acetonitrile to aquatic invertebrates

Species	Test type	Comments	Duration (hour)	Toxicity end point (mg/l)	Reference
Asellus intermedius (fresh water crustacean)	Static nominal concentration	Valid	96	EC_{50} >100	Ewell et al. (1996)
Daphnia magna (fresh water crustacean)	Static nominal concentration	Valid	24	LC_{50} > 10,000	Bringmann and Kuhn (1977)
Dapnia pulex (fresh water crustacean)	Static nominal concentration	Valid	18	LC_{50} = 5,838	Bownan et al. (1981)
Dapnia magna (fresh water crustacean)	Static nominal concentration	Valid	96	LC_{50} > 100	Ewell et al. (1986)
Gammarus fasciatus (fresh water crustacean)	Static nominal concentration	Valid	96	LC_{50} > 100	Ewell et al., (1986)
Culex restuans (insect larvae)	Static nominal concentration	Valid	18	LC_{50} = 6,420	Bownan et al., (1981)
Dugesia tigrina flatworus (Phatyhelminthe)	Static nominal concentration	Valid	96	LC_{50} > 100	Ewell et al., (1986)
Helisoma trivolvis (fresh water snail)	Static nominal concentration	Valid	96	LC_{50} > 100	Ewell et al. (1986)
Hyalella azteca (fresh water crustacean)	Static nominal concentration	Valid	18	LC_{50} = 6,565	Bowman et al. (1981)
Lumbriculus variegatus (segmented worm)	Static nominal concentration	Valid	96	EC_{50} > 100	Ewell et al., (1976)
Palaemonetes kadiakensis fresh water crustacea)	Static nominal concentration	Valid	18	EC_{50} = 5,170	Bowman et al., (1981)

Table 3.23 Toxicity of acetonitrile to algae

Species	Test type	Comments	Toxicity end point (mg/l)	Reference
Microcystis aeruginosa (blue-green algae)	Inhibition of cell multiplication	Valid	TT = 520 [1]	Bringmann and Kühn (1978)
Scenedesmus quadricauda (green algae)	Inhibition of cell multiplication	Valid	TT = 7,300	Bringmann and Kühn (1977)

[1] TT = toxicity threshold for inhibition of cell multiplication

There are no data on the chronic toxicity of acetonitrile for aquatic organisms. In addition, the information provided for the inhibition of alga growth does not include NOEC calculations or enough data for these estimations. Thus, the effect assessment focuses exclusively on acute toxicity data.

The lowest L(E)C_{50} figure is the 48 h LC_{50} on *Cyprinus carpio*, 730 mg/l. The TTs for algae are in the same range. Therefore, it seems appropriate in this assessment to consider 730 mg/l as the lowest end of the acute toxicity range for aquatic organisms. This range covers three taxonomic groups, fish, invertebrates and algae, and taking into account the lack of chronic figures, the recommendations of the TGD suggest the application of the factor 1,000 to the lowest end of the acute toxicity range (730 mg/l), obtaining a PNEC for aquatic organisms of 0.73 mg/l.

$$PNEC_{aquatic\ organisms}=\text{lowest end acute toxicity range}/1{,}000=0.73\ mg/l$$

The data set only includes freshwater species. Therefore, in absence of data on saltwater species the above PNEC can be used for both, freshwater and marine environments, while data on marine species are produced.

3.2.1.2 Effects on microorganisms

The toxicity of acetonitrile on microorganisms (**Table 3.24**) has been previously reviewed by different authors. Blum and Speece (1991) present data on three groups of environmental bacteria that can be relevant when considering the microbial activity of domestic WWTPs. The IC_{50} values (the concentrations of acetonitrile causing 50% bacterial inhibition compared to controls) are considered valid for this assessment and can be summarised following the proposal included in the internal report from Nielsen and Howe (1995) as follows:

- Aerobic heterotrophs. These bacteria predominate in activate sludge systems and in natural aerobic environments where they convert organic material into carbon dioxide and water. The inhibition in oxygen uptake by the bacteria was monitored after 15 hours exposure and used to calculate an IC_{50} of 7500 mg/l.

- *Nitrosomas sp*. These bacteria convert ammonia nitrogen to nitrite as the first most sensitive step in the biological oxidation of inorganic nitrogen. The inhibition in ammonia consumption by the bacteria was monitored after 24 hours exposure and used to calculate an IC_{50} of 73 mg/l.

- Methanogens. Methanogens form part of the consortium of bacteria that convert organic matter to carbon dioxide and methane under anaerobic conditions. The inhibition of gas production by the bacteria was monitored after 48 hours exposure and used to calculate an IC_{50} of 28,000 mg/l.

Additional data on *Pseudomona putida* and several protozoa species have been published by Brigmann and Kühn (1980a, 1980b, 1981). The lowest toxicity threshold is 680 mg/l reported for *P. putida,* while TT values for protozoa species are in the range of g/l.

The recommendations of the TGD suggest the application of the factor 10 to the EC_{50} and one to the NOEC if the test has been performed with nitrifying bacteria and a factor 100 for the EC_{50} obtained using respiration inhibition or similar endpoints. However, the amount of information available for acetonitrile is clearly higher than that usually available, covering most significant bacterial population and several protozoan species. Nitrifying bacteria seems to be particularly

sensitive under laboratory conditions, considering all the available information, the weight of evidence justifies the use of a factor 1 on the IC_{50} reported for nitrifying bacteria. The value obtained in this way for the most sensitive test on nitrifying bacteria, 73 mg/l, agrees with the application of the factor 100 to the inhibition of oxygen uptake by aerobic heterotrophs, which produces a figure of 75 mg/l, and with the derivation expected from the Pseudomona data. Thus a $PNEC_{microorganisms}$ of 73 mg/l is proposed in this assessment.

$$PNEC_{microorganisms} = 73 \text{ mg/l}$$

This value is more than 10 times lower than the lowest toxicity threshold reported for protozoa and therefore is considered appropriate to cover the potential effects on this taxonomic group.

Nevertheless, in the risk characterisation, the consequences of a much more conservative approach, following strictly the TGD recommendations, and applying a factor of 10 to the EC_{50} for nitrifying bacteria, giving a $PNEC_{microorganisms}$ of 7.3 mg/l, will be also considered.

Table 3.24 Toxicity of acetonitrile to aquatic microorganisms

Species	Test type	Comments	Duration (hour)	Toxicity end point (mg/l)	Reference
Chillmonas paramecium (Protozoa)	Inhibition of cell multiplication	Valid as additional information	48	TT[1] = 942	Bringmann and Kühn (1981)
Entosiphon sulcatum (Protozoa)	Inhibition of cell multiplication	Valid as additional information	72	TT = 1,810	Bringmann and Kühn (1980a)
Pseudomona putida (Bacteria)	Inhibition of cell multiplication	Valid as additional information	16	TT = 680	Bringmann and Kühn (1980a)
Uronema parduzci (Protozoa)	Inhibition of cell multiplication	Valid as additional information	20	TT = 5,825	Bringmann and Kühn (1980b)
Nitrosomas (Bacteria)	Inhibition of ammonia consumption	Valid for the assessment	24	IC_{50} = 73	Blum and Speece (1991)
Aerobic microorganisms	Inhibition of oxygen uptake AFNOR and ETAD	Valid for the assessment	15	IC_{50} = 7,500	Blum and Speece (1991)
Metanogenic bacteria	Inhibition of gas production	Valid for the assessment	48	IC_{50} = 28,000	Blum and Speece (1991)

[1] TT = toxicity threshold

3.2.1.3 Effects assessment for the sediment

The available data set includes a single data on the effect of acetonitrile on the sediment organism *Hyalella azteca*, but for waterborne exposure. The EC_{50} obtained for this organism is similar to that reported for other aquatic invertebrates suggesting that the 1st assumption of the partitioning method presented by the TGD can be accepted. In addition, the very low Pow of acetonitrile indicates that the use of water phase as the single via for uptake is also adequate. Thus, the PNEC may provisionally be calculated using the equilibrium partitioning method with the PNEC for aquatic organisms.

$$PNEC_{sed} = (K_{(sed, water)} / RHO_{sed}) \cdot PNEC_{aquatic\ organisms} \cdot 1{,}000$$

The value estimated with the EUSES program is:

$$PNEC_{sed} = 0.55 \text{ mg/kg}.$$

3.2.2 Atmosphere

There are not data available for this assessment.

3.2.3 Terrestrial compartment

The available data set does not include a single data for the terrestrial compartment other than those on mammals, which would be used for the assessment of secondary poisoning.

Taken into account the lack of data, and according to the TGD, the equilibrium partitioning method can be applied as a conservative calculation method to identify a potential risk to the soil compartment. Thus, the PNEC may provisionally be calculated using the equilibrium partitioning method with the PNEC for aquatic organisms.

$$PNEC_{soil} = (K_{(soil, water)} / RHO_{soil}) \cdot PNEC_{aquatic\ organisms} \cdot 1{,}000$$

The value estimated with the EUSES program is:

$$PNEC_{sed} = 0.176 \text{ mg/kg}.$$

3.2.4 Secondary poisoning

According to the TGD, the risk characterisation for secondary poisoning is required if three specific criteria are fulfilled.

These criteria can be summarized as:

- Indirect exposure likely,
- Indication of bioaccumulation potential,
- Mammalian toxicity risk.

Acetonitrile is toxic for mammalian species but its low potential for bioaccumulation indicates that secondary poisonings are of low concern.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment

The potential local risks for aquatic (water column) organisms related to the different phases of the life cycle of acetonitrile included in this assessment has been summarised in **Table 3.25**.

Table 3.25 Local risk assessment for the aquatic environment: water column

Process	PEC_{local} (µg/l)	PNEC(µg/l)	PEC/PNEC
Production of acetonitrile	307	730	0.42
Pharmaceutical Industry	16,000	730	21.9
Butadiene production	164	730	0.22
Other uses	307	730	0.42
Lab. Chem.	307	730	0.42

For the production of acetonitrile and its use in butadiene production, in chemical laboratories and minor uses, the PEC/PNEC ratios are below 1. However, a high potential risk has been identified for the use of acetonitrile as solvent by the pharmaceutical industry. A similar situation is observed for the local risk characterisation for sediment dwelling organisms (**Table 3.26**), using the PNEC value derived from the toxicity for aquatic organisms according to the equilibrium partitioning method.

Table 3.26 Local risk assessment for the aquatic environment

Process	$PEC_{local(sed)}$ (µg/kg)	PNEC (µg/kg)	PEC/PNEC
Production of acetonitrile	287	550	0.52
Pharmaceutical industry	14,900	550	27.1
Butadiene	153	550	0.28
Other uses	287	550	0.52
Lab. Chem.	287	550	0.52

The values for the regional and continental assessment are included in **Table 3.27**.

Table 3.27 Risk assessment for the aquatic environment

Surface Water	PEC_{lswl} (µg/l)	PNEC(µg/l)	PEC/PNEC
Regional	2.41	730	0.003
Continental	0.45	730	0.0006
Sediment	PEC_{sed} (µg/kg)	PNEC (µg/kg)	PEC/PNEC
Regional	1.77	550	0.003
Continental	0.33	550	0.0006

Regional and continental risks are estimated to be very low.

The exposure assessment uses the default dilution value of 10 and the effect assessment only has information on the toxicity of acetonitrile for freshwater species. In absence of specific information for the marine environment, this assessment is considered to cover both, freshwater and saltwater ecosystems.

Therefore, it should be considered that for the aquatic compartment, including sediment, the risk characterisation indicates that the present risk of acetonitrile for the aquatic environment is only of concern for the local emissions from the pharmaceutical industry.

The highest concentration of acetonitrile in the effluent of WWTP has been estimated to be 166 mg/l for the use of acetonitrile as solvent by the pharmaceutical industry. This value is higher that the proposed $PNEC_{microorganisms}$ of 73 mg/l. Therefore a potential risk for the biological activity of WWTP must be expected.

Table 3.28 shows the risk estimations for WWTP for production and all other uses included in this assessment. The PEC/PNEC ratios are lower than 1.

Table 3.28 Risk assessment for WWTP microorganisms excluding the use by the pharmaceutical industry

Assessment	PEC (mg/l)	PNEC (mg/l)	PEC/PNEC
Effluent Concentration	3.07	73	0.04
Inflow Concentration	30	73	0.41

Assuming a much more conservative approach, including an additional factor of 10 in the PNEC derivation, the $PEC_{effluent/conservative}$ / $PNEC_{microorganisms}$ is 0.4 and therefore it is still below 1. Therefore it is concluded that no significant risk for WWTP microorganisms is expected for the production of acetonitrile and its use excluding the use by the pharmaceutical industry.

Results

For the aquatic compartment, including water, sediments and biological processes of WWTP:

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion applies to local risks for production and processing in butadiene production, use as a laboratory chemical, use in the photographic industry and other uses included in the risk assessment except the use in the pharmaceutical industry. It also applies to the regional and continental risks related to the acetonitrile life cycle.

Conclusion (iii) There is a need for limiting the risk; risk reduction measures which are already being applied shall be taken into account.

This conclusion applies to the use of acetonitrile as a solvent in the pharmaceutical industry.

3.3.2 Atmosphere

No information has been provided for a proper risk assessment for the atmosphere.

3.3.3 Terrestrial compartment

No information on the toxicity of acetonitrile for terrestrial organisms has been provided, therefore the effect assessment has been estimated using the equilibrium partitioning method. The risk characterisation for the local assessment is included in **Table 3.29**.

Table 3.29 Local risk assessment for the soil compartment

	PEC (mg/kgwwt) averaged over 30 days	PNEC (mg/kgwwt)	PEC/PNEC
Production	0.0647	0.176	0.36
Pharma. Industry	2.56	0.176	14.5
Butadiene	0.0281	0.176	0.16
Other uses	0.0491	0.176	0.28
Lab. Chem.	0.0493	0.176	0.28

The PEC/PNEC value for the use of acetonitrile by the pharmaceutical industry is higher than one suggesting a potential risk for soil dwelling organisms. All other PEC/PNEC ratios are lower than 1 suggesting a low local risk for soil organisms.

Regional and continental PECs have been calculated according to the TGD giving the following values: $PEC_{agric, soil, regional}$ = 0.124 µg/kgwwt and $PEC_{agric, soil, continental}$ = 0.06 µg / kgwwt. The risk assessment is included in **Table 3.30**.

Table 3.30 Regional and continental risk assessment for the soil compartment

	PEC (mg/kgwwt)	PNEC (mg/kgwwt)	PEC/PNEC
Regional	0.00012	0.176	0.00068
Continental	0.00006	0.176	0.00034

These ratios are low enough to consider that the risk for terrestrial organisms at the regional and continental level is of low concern.

Results

For the terrestrial environment:

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion applies to local risks for production and processing in butadiene production, use as a laboratory chemical, use in the photographic industry and other uses included in the risk assessment except the use in the pharmaceutical industry. It also applies to the regional and continental risks related to the acetonitrile life cycle.

Conclusion (iii) There is a need for limiting the risk; risk reduction measures which are already being applied shall be taken into account.

This conclusion applies to the use of acetonitrile as a solvent in the pharmaceutical industry.

3.3.4 Secondary poisoning

The low potential of acetonitrile for bioaccumulation indicates that secondary poisonings are of low concern.

Result

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

Acetonitrile is a volatile liquid with high water solubility. It is readily absorbed from the gastrointestinal tract and through the skin and lungs. All three routes of exposure have been reported to lead to systemic effects.

4.1.1.2 Occupational exposure

Occupational exposure to acetonitrile could mainly occur through inhalation of vapours. Dermal exposure due to direct skin contact is also possible.

Acetonitrile is produced as a by-product of acrylonitrile manufacture. It is a highly polar solvent with a high dielectric constant. It is used as an industrial and laboratory solvent and as a chemical intermediate.

It is mainly used:

- In the petrochemical industry, to separate olefin-diolefin mixtures and C_4-hydrocarbons.
- To extract fatty acids from animal and vegetable oils.
- For extractive distillation, crystallisation, as a starting material or intermediate of vitamins, steroids, bactericides and pesticide manufacture.
- As a solvent for spinning fibers and casting and molding plastics, due to its superior solvency with polymers.
- In the manufacture of photographic film.
- It is a common laboratory solvent, and is widely used for HPLC and as a solvent for DNA synthesis and peptide sequencing.

Acetonitrile has a sweet ether-like odour. The odour threshold is 170 ppm (284 mg/m^3) (Amoore and Hautala, 1983).

The acetonitrile is synthesised and used in closed systems. Therefore, occupational exposure could occur when the system is breached. Its high volatility and relatively high odour threshold favour the worker exposure potential.

Occupational exposure data were obtained from industry and from several exposure databases provided by some European countries. No relevant monitoring data have been obtained from the

literature. EASE model has been used to estimate inhalation and dermal exposure in all possible scenarios.

The occupational exposure limits in different countries are summarised in **Table 4.1**.

Table 4.1 Occupational exposure limits in different countries

Country	8 h-TWA exposure limit		STEL short-term exposure limit		Skin notation
	ppm	mg/m³	ppm	mg/m³	
USA (ACGIH, OSHA)	40	67	60	101	No
USA (NIOSH)	20 *	34 *	-----	-----	No
Austria	40	70	-----	-----	No
Belgium	40	67	60	101	Yes
Denmark	40	70	60	105	No
Finland	40	70	60	105	No
France	40	70	-----	-----	No
Germany	40	70	80	140	No
Spain	40	68	60	102	No
Sweden	30	50	60	100	No
The Netherlands	40	70	-----	-----	No
United Kingdom	40	70	60	105	No

* TWA limit for up to a 10-hour workshift in a 40-hour workweek

Conversion factor: 1 ppm = 1.68 mg/m^3 (25ºC, 760 mmHg)

4.1.1.2.1 Manufacture

The European production of acetonitrile is limited to Germany and Italy (2 production plants), since the United Kingdom plant was closed for production in 1990. The number of workers potentially exposed to this compound during its manufacture is estimated to be about 300 – 400, (200 in the Italian plants).

The acetonitrile is mainly obtained as a by-product of acrylonitrile manufacture via propylene ammoxidation. This process, named as SOHIO, is carried out in a closed system and involves temperatures of 400 - 500 ºC. The acetonitrile:acrylonitrile ratio is reported to be about 1:35 (Kirk-Othmer, 1978), i.e. acetonitrile produced is about 3% of acrylonitrile production.

After cooling, the reaction product is absorbed in water and the resulting solution is fractionally distilled. The column bottoms, consisting of acetonitrile and water, are azeotropically distilled. The distillate is dried at 70ºC and redistillate to produce technical acetonitrile.

The plants are outdoors, taking advantage of natural ventilation. In this situation, significant exposures would be accidental. The likelihood of such exposures increases during activities involving breaching of the closed system such as maintenance operations, repair of equipment, transferring and quality control sampling of acetonitrile.

According to the two European producers the process is continuous and automated. Filling operations consist in filling railway tank cars with gas exhaust pipe for purifying.

Industry has reported that acetonitrile is produced and processed in plants where there are some R45 substances (acrylonitrile, butadiene) and therefore, all the operations in which there is a potential for exposure are carried out with special care to minimise the risk to the workers. Measures to prevent exposure comprise the use of adequate personal protection equipment, decontamination procedures before maintenance, wide use of automatic systems for sampling, information to workers on risk, etc.

Measured data

Table 4.2 shows the measurements made by the two European producers, and sent by Industry:

Table 4.2 Long-term occupational exposure during production

Site	Year	Task	No. Samples	Type	Range (ppm)	Mean value	Comment	RPE
Site 1 [1]	1987-1993	Different work positions [3]	10	Stationary	< 0.1			
Site 2 [1]	1987	Different work positions [3]	----	Stationary	< 0.1 – 1.1	0.5		
	1990-1993	Different work positions [3]	17	Stationary	< 0.1			
Site 3 [2]	1995	Production	2	Personal/ stationary	< 0.006		Normal production conditions	No
	1995	Filling operations	2	Personal/ stationary	< 0.006		Filling rail way tank cars with gas exhaust pipe	No

[1] Samples analysed by gas chromatography
[2] Samples taken in activated carbon and analysed by gas chromatography (detection limit: 0.003 mg, GLP)
[3] It is assumed that tasks in which exposure is expected to be high are included as "different work positions" (such as filling operations, sampling and maintenance)

Data show that exposures are lower than 0.1 ppm. Along 1987 exposures rise to 1.1 ppm in site 2 (mean value in this period: 0.5 ppm). In the following years (1990-1993) the situation returns to exposures lower than 0.1 ppm.

Table 4.3 Short-term occupational exposure during production

Site	Year	Task	No. of samples	Type of samples	Range (ppm)	Respiratory protection
Site 3	1995	Take sample of the product	2	Stationary/ personal	< 0.006 · 20 min	No

In summary, 0.1 ppm represents a worst-case exposure situation and it will be used for purposes of risk characterisation. This concentration is much lower than the most representative exposure limit value of 40 ppm (8h-TWA).

Modelled data

The EASE model predicts that inhalation exposure to gas or vapour used in a closed system will be in the range of 0 – 0.1 ppm. The model also predicts that, under these conditions, dermal exposure will be negligible.

High exposure may occur during activities that involve breaching the closed system such as sampling, maintenance and filling activities. The estimated inhalation exposure, according to the EASE model (non-dispersive use with LEV), will be 10 – 50 ppm. However, these concentrations will be received as brief and intermittent exposures rather than over a full workshift.

Dermal exposure is also possible during sampling, filling and maintenance activities:

- According to industry information there is a wide use of automatic systems for sampling.
- Filling operations involve filling railway tank cars with gas exhaust pipe for purifying.
- Contact with contaminated surfaces could occur during the maintenance activities. Every company establishes decontamination procedures before maintenance to prevent exposures. Acetonitrile is infinitely soluble in water and therefore, the efficacy of flushing would be very high, making the equipment decontamination procedure very effective. The frequency of these activities is very low. Usually, they are carried out once a year and it is unlikely that they last more than 25 days.

Taking into account these considerations, EASE gives an estimation within the range of 0 – 0.1 mg/cm^2/day for these activities (non-dispersive use, incidental contact, direct handling).

The following surface exposed areas have been estimated for the situations above described:

- Sampling: 420 cm^2 (half of two hands)
- Filling activities: 420 cm^2 (half of two hands)
- Maintenance: 840 cm^2 (both hands)

In the worst case, a worker could be dermally exposed to 84 mg/day of acetonitrile.

Literature data

No data were found in the literature about the levels of acetonitrile in the manufacturing setting.

4.1.1.2.2 End Uses

The actual number of persons exposed or potentially exposed to acetonitrile in this scenario is unknown. It could be a high figure in view of the wide pattern of uses.

Use as a solvent or chemical intermediate

Acetonitrile is used as a solvent in the chemical industry. According to a producer, acetonitrile is handled in closed systems with the "spent" solvent being continuously distilled and recycled.

The main use as a solvent is the extraction of 1,3 - butadiene.

Pure 1,3 - butadiene is separated from the mixed C_4 – hydrocarbon fraction arising from cracking by extractive distillation using solvents that include acetonitrile.

Measured data

Industry has provided measured data from a chemical company that uses acetonitrile to extract 1,3 – butadiene and they are presented in **Table 4.4**.

Table 4.4 Long-term exposure data from a chemical company

Company	Task	Year	Range (ppm)	Type of sampling	No of samples	No of workers monitored
Chemical company	Sampling, maintenance, cleaning	1990	< 0.1 – 1.1	Personal	10	4
		1993	< 0.0001	Personal	4	2
		1994	< 0.1	Personal	5	4
	-----	1997	1.3 - 2.7	Stationary (15 points in the plant)	At least 15	---

Table 4.5 presents data sent by Industry, from a company purifying acetonitrile by redistillation. The companies are laboratory suppliers which purify acetonitrile by chemical treatment and distillation to the ultra high purity grades required for use in HPLC. This process is also carried out in closed systems.

Table 4.5 Long-term occupational exposure data in a company purifying acetonitrile

Year	Location/Occupation	Type of sampling	Range (ppm)	No. of samples (ppm)	
				< 5	5-20
1988	Reaction vessel (charging additions)/ Receiving vessel/ Plant operator	Personal/Area samples	<1.78-12.5	4	1
1990	Reaction vessel/ Receiving vessel	Area samples	17.3 –21	---	2

Data have been collected in line with the UK Control of Substances Hazardous to Health (COSHH) Regulations. All samples have been taken by activated charcoal and analysed by gas chromatography. The company has reported that area samples have been obtained in the atmosphere close to the equipment and represent worst-case exposures, since workers are generally distant from the points of emission. These exposures were reported to be the result of old control systems and bad practices and the company was advised to modify them.

Industry has also sent data from a company that distributes acetonitrile. In 1998, two samples were collected in the close vicinity of the drum filling installation during filling operations. The reported results were 774 $\mu g/m^3$ and 136.6 $\mu g/m^3$ (0.46 – 0.08 ppm) for a sampling time of 148 min and 143 min, respectively. This company has reported that despite the low exposure to acetonitrile vapours, the operator was wearing an air-cap with pressurised air during filling operations.

Finland has reported data from a chemical company (1977-1991). The specific activity of this company is not known. Two kinds of activities have been monitored, the first involves laboratory work (3 exposed workers), and the second process work (22 exposed workers). The pattern of use has been reported as non-dispersive and the pattern of control as dilution ventilation. In both cases the arithmetic mean of measured concentrations is 10 ppm (standard

deviation for laboratory work is 8.48 and 16.46 for process work). Whether these data refer to short- or long-term exposures is not known. Type of sampling has not been reported either.

Near to 50% of the European acetonitrile production is used in pharmaceutical manufacturing processes. It can be used as a solvent or as raw material. In general, acetonitrile is handled in closed reaction and distillation section of the plant during the manufacturing process.

Table 4.6 shows long-term exposure data from some pharmaceutical companies.

Table 4.6 Long-term occupational exposure data

Company	Task	Year	Type of sampling	No of samples	Mean (ppm)	Range (ppm)	No of samples with (ppm)			
							<5	5-20	20-40	>40
Pharmaceutical company - 1 (Industry data)	No details	1980-1987	Personal	6	4	0.5 - 16.8	5	1	-	-
			Ambient	7	0.2	0 - 1.5	7	-	-	-
Pharmaceutical company – 2 (Industry data)	Process workers	1992	Personal	25	-	<5- 75.47	20	-	2	3
		1995	Personal	26	5.5	0 - 7.28	20	6	-	-
Plant manufacturing thiamine (HSE data)	Plant operators	1988	Personal	28	nd	nd	28	-	-	-
	Background	1988	Stationary	8	nd	nd	8	-	-	-

nd = Not detected

In the first company, only one value is higher than 5 ppm (16.8 ppm) and represents an exposure over three hours. Weighting it to eight hours would result in a value of 6.3 ppm.

Information from the 2nd company specifies that monitored workers were involved in general product processing. General processing duties include operating general pharmaceutical production equipment (reaction vessel, pipes and pumps). The activity related with the extreme case of 75.47 ppm measured in 1992 was solvent recovery involving feeding distillation column and sampling. Since 1992, this company has revised its personal monitoring programme. Workers wear a badge for the full shift. Any results greater than 25 % of the OEL (Action Level) are followed up to solve the issue, and it is determined whether exposures are due to procedural or engineering problems. Follow up includes engineering or procedural changes to rectify the matter. A record of these exposures is kept as well as their subsequent follow up. If exposures above the action level (25% OEL) are expected, PPE is mandatory and the operator is monitored. This company has reported that since this process was introduced, no non-conformances for acetonitrile occured. In fact, the higher level of acetonitrile monitored in 1995 was 7.28 ppm.

Data from Germany (MEGA database, BIA) collected in different companies (plastic and pharmaceuticals) and research laboratories between 1991 –1995 are summarised in **Table 4.7**.

Table 4.7 8h-TWA exposure data for acetonitrile

Type of company	No. of measurement data	No. of companies	50%-value (ppm)	90%-value (ppm)	95%-value (ppm)
All types of company/work areas	15	10	0.59	4.76	7.3

Samples were taken from drawing air through an activated carbon tube by means of a sampling pump. After elution with acetone, the quantification was carried out gas-chromatographically using a flame ionization detector. Detection limit reported was 0.1 mg/m^3.

Exposures in the range of the 90% value were determined in laboratories where acetonitrile is used as a mobile phase in chromatographic processes. In the same period, only one value of 10.1 ppm has been reported from a chemical industry as a short-term (<1h) exposure value.

Short-term exposure data have also been obtained from Industry and are presented in **Table 4.8**.

Table 4.8 Short-term* exposure data

Company	Year	Task	Type of sampling	No. of samples	Mean (ppm)	Range (ppm)
Chemical company	1990	Sampling, maintenance and cleaning	Personal	9	4.7	0.3 – 7.8
Pharmaceutical company - 1	1980 -1987	No details	Personal	4	18.17	2 - 42
Company purifying acetonitrile	1988	Drumming the first fraction	Stationary	1	------	35.7
	1990	---------	Stationary	2	24.9	14.7 – 35.1

*The sampling times have been reported. The measured periods were lower than 15 minutes in most cases but have been considered representatives over 15 minutes.

Seven percent of the acetonitrile produced is used in the manufacture of photographic film.

A company has reported uses of acetonitrile as a reactant and solvent in the manufacture of photographic chemicals:

- When used as a reactant, acetonitrile is introduced into a reaction vessel under vacuum from drums. Operators wear air masks, even if LEV is present. The charging time is no more than 15 minutes.

- When used as solvent in the crystallisation step, the reaction mixture (acetonitrile and isopropyl ether) is filtered on a nutsche at 0ºC, the cake is washed with another solvent previously refrigerated at 10ºC, pulled under vacuum and the product is dumped into fibre drums before loading into a dryer. Good general ventilation of 10 volumes per hour in the work area is installed with local exhaust ventilation on the nutsche, which has a cup that can be lowered during filling. During the different stages operators wear safety glasses with side shields, complete safety suit, impervious gloves and safety shoes.

The expected consumption of acetonitrile is 96,000 kg in 1999, which can be used for 180 batches in the process in which it is used as reactant and 60 batches in the process in which it is used as a solvent.

Personal monitoring exposure data have been obtained for the recrystallisation phase. Four samples were taken on four different batches (1 measurement per batch) during this process; the duration of exposures was 42 to 75 minutes. All short-term exposure values were within the range 9 ppm to 30 ppm.

Additional data have been obtained from a photographic company that uses a small amount of acetonitrile (14 tons/year), in the production of specific organic compounds in a batch process. Acetonitrile is used in reaction vessels with a capacity of 400 l to 1,000 l. In 1994, personal monitoring was carried out for periods of eight hours, and included work for the entire production process (filling and emptying reaction vessels, as well as the reaction process itself), except when full face filter masks were worn. The time-weighted exposure was between 7 ppm and 29 ppm. Neither the number of measurements nor individual values have been reported. The company has reported that when acetonitrile exposure was expected a face mask with filter was used in addition to protective clothing and gloves. In 1997, a completely closed system was introduced, under remote control from workers outside of the reaction vessel compartment. Emissions from the compartment were reduced by the use of scrubbers. Under these conditions, all measurements were below 0.06 ppm (limit of detection).

By analysing all measured exposure data together, a long-term exposure level of 7.3 ppm is estimated a reasonable worst-case situation and it will be used for risk characterisation purposes. This concentration is more than five times lower than the most representative exposure limit value of 40 ppm (8h-TWA).

A value of 35 ppm is considered representative of reasonable worst-case short-term exposures.

Literature data

NIOSH (1978) reported that in a plant using acetonitrile in a closed system, a single full-shift TWA concentration of 0.46 ppm was obtained.

Modelled data

In general, acetonitrile uses are in industrial processes (in closed reaction), so that exposure is likely in special tasks, which involve breaching the system. Therefore, exposure could be estimated by using the same approach as in the manufacturing section. Accordingly, the EASE model predicts that exposure will be in the range of 10 - 50 ppm (non-dispersive use with LEV). However, these concentrations will be received as brief and intermittent exposures rather than over a full workshift. In fact, short-term measured exposure data are in agreement with this range. Full-shift measured exposure data are close to the low part of this range.

In most companies, the same approach as in the manufacturing section is used. Accordingly, in the worst case, a worker could be dermally exposed to 84 mg acetonitrile/day (non-dispersive use, incidental contact, and 840 cm^2).

In companies that purify acetonitrile or distribute acetonitrile, filling operations involve run off acetonitrile into drums. For these operations intermittent contact can be assumed. A dermal exposure range of 0.1-1 mg/cm^2/day is estimated by EASE. For filling activities, it has been considered that 420 cm^2 of skin surface can be exposed. Therefore, in the worst case a worker could be dermally exposed to 420 mg acetonitrile/day.

Use of the substance in laboratories

Acetonitrile is mainly used in laboratories as a mobile phase in HPLC. Acetonitrile is fed by tube into the HPLC and the waste solvent is collected upon exiting the instrument, so that the likelihood of an exposure is very low. However, acetonitrile is usually transferred or filtered before entering the instrument and the likelihood of an exposure increases.

Measured data

Data from Germany (**Table 4.7**) show that exposures around 4.76 – 7.3 could be obtained in research laboratories in which acetonitrile is used in chromatography.

Modelled data

In general, laboratories are provided with hood with LEV. Therefore, the EASE model predicts that exposure will be in the range of 10-50 ppm (non-dispersive use with LEV).

Accounting for the generalised bad practice of not using the hood for short-time operations among laboratory workers, dilution ventilation can also be chosen as pattern of control. EASE exposure estimation will be in the range of 100-200 ppm. On the other hand, these activities involve small quantities and take only a few minutes and not a whole shift. Therefore, the quoted range is an overestimate.

Skin contact with acetonitrile is only likely when the mobile phase is prepared before entering the instrument. The frequency of this activity, in the worst case, is once per day. Assuming non-dispersive use, direct handling and incidental contact, EASE gives a range of exposure of 0-0.1 mg/cm^2/day. Considering that half of two hands (420 cm^2) could be exposed, an exposure level of up to 42 mg/day is estimated.

Literature data

No data were found in the literature about the levels of acetonitrile in laboratories.

Table 4.9 Summary of occupational exposure data

Exposure scenario	Activity	Inhalation exposure				Dermal exposure		
		Duration and frequency	Reasonable worst case		Method	Exposure level	Reasonable worst case	Method
			ppm	mg/m^3		($mg/cm^2/day$)	(mg/day)	
Manufacture	Sampling Filling Cleaning and maintenance	Full shift/daily Up to 25 days/year	0.1	0.17	Measured	0 – 0.1	84	EASE
Use as a solvent or chemical intermediate	Sampling Filling/emptying Cleaning and maintenance	Full shift/daily Up to 25 days/year	7.3	12.3	Measured	0.1-1	420	EASE
Use in laboratories (HPLC)	Preparation of the mobile phase	Full shift/daily	7.3	12.3	Measured	0 – 0.1	42	EASE

4.1.1.3 Consumer exposure

No quantitative data have been obtained for the evaluation of consumer exposure, neither from the chemical industry nor through literature reviewed.

Most of the acetonitrile produced is used in the synthesis of other substances within the chemical industry and does not reach consumers.

Looking at the accidental intoxication cases due to acetonitrile, it was concluded that acetonitrile could be present in nail remover. However, acetonitrile is not in the EC Cosmetics Inventory (96/335/EC) and is not approved for use in cosmetics in Europe. Therefore, it is not of concern for consumers.

4.1.1.4 Humans exposed via the environment

The human intake from indirect exposure in local and regional scenarios is presented in **Table 4.10**. The estimates were performed according to EUSES. The values for the local scenario are shown from five different release scenarios: Production, Pharmaceutical Industry, Butadiene Production, Other Uses and Laboratory Chemical.

Table 4.10 Estimated human intake of acetonitrile in mg/kg bw/day from local and regional scenarios of EUSES

Route	Local (mg/kg bw/day)					Regional (mg/kg bw/day)
	Production	Pharm. Industry	Butadiene Production	Other uses	Laboratory Chemical	
Drinking water	$7.27 \cdot 10^{-3}$	0.127	$1.52 \cdot 10^{-3}$	$2.42 \cdot 10^{-3}$	$2.45 \cdot 10^{-3}$	$6.89 \cdot 10^{-5}$
Fish	$1.25 \cdot 10^{-4}$	$1.62 \cdot 10^{-3}$	$1.11 \cdot 10^{-5}$	$3.6 \cdot 10^{-5}$	$9.89 \cdot 10^{-6}$	$1.19 \cdot 10^{-6}$
Leaf crops	$7.71 \cdot 10^{-4}$	$7.68 \cdot 10^{-4}$	$9.8 \cdot 10^{-5}$	$7.79 \cdot 10^{-6}$	$1.88 \cdot 10^{-5}$	$5.39 \cdot 10^{-6}$
Root crops	$7.66 \cdot 10^{-4}$	0.0228	$2.73 \cdot 10^{-4}$	$4.35 \cdot 10^{-4}$	$4.4 \cdot 10^{-4}$	$2.64 \cdot 10^{-6}$
Meat	$8.29 \cdot 10^{-8}$	$8.68 \cdot 10^{-7}$	$1.44 \cdot 10^{-8}$	$1.63 \cdot 10^{-8}$	$1.69 \cdot 10^{-8}$	$6.98 \cdot 10^{-10}$
Milk	$1.54 \cdot 10^{-6}$	$1.62 \cdot 10^{-5}$	$2.69 \cdot 10^{-7}$	$3.03 \cdot 10^{-7}$	$3.16 \cdot 10^{-7}$	$1.3 \cdot 10^{-8}$
Air	0.0127	0.0123	$1.6 \cdot 10^{-3}$	$1.2 \cdot 10^{-4}$	$3.03 \cdot 10^{-4}$	$8.85 \cdot 10^{-5}$
Total intake	0.0216	0.164	$3.5 \cdot 10^{-3}$	$3.02 \cdot 10^{-3}$	$3.22 \cdot 10^{-3}$	$1.67 \cdot 10^{-4}$

The estimates show that the most important human intake routes are via air and drinking water. According to the EUSES estimations the values for the total human intake of acetonitrile for the local scenario range from 0.00302 mg/kg bw/d to 0.164 mg/kg bw/d depending on the release/use category. Except production and pharmaceutical industry scenarios, which show the highest total human intake value, the other scenarios show very similar total human intake values.

4.1.2 Effects assessment: Hazard identification and Dose (concentration) – response (effect) assessment

4.1.2.1 Toxicokinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

In vivo studies

Although no quantitative analytical data are available, several pharmacokinetic and toxicity studies indicate that acetonitrile is readily absorbed from the lungs and gastrointestinal tract and through the skin, resulting in systemic toxicity. Most of the systemic toxic effects of acetonitrile and other nitriles are mediated through metabolism to cyanide, which is subsequently conjugated with thiosulfate to form thiocyanate and eliminated in the urine. Following the first observations of acetonitrile metabolism to cyanide and thiocyanate by Pozzani et al. (1959), many authors reported the same results in humans and in experimental animals both *in vitro* and *in vivo* (Amdur, 1959; Ohkawa et al., 1972; Willhite and Smith, 1981; Ahmed and Farooqui, 1982; Silver et al., 1982; Willhite, 1983; Tanii and Hashimoto, 1984a, 1984b, 1986; Freeman and Hayes, 1985a, 1985b; Ahmed et al., 1992). They all suggested a metabolic pathway in which acetonitrile is biotransformed by cytochrome P450 monooxygenase system initially. The proposed metabolic pathway involves the microsomal oxidation of the alpha carbon atom of acetonitrile and the formation of a reactive intermediate that may be a methylene cyanohydrin. The methylene cyanohydrin possibly undergoes further decomposition to cyanide ions and formaldehyde or undergoes covalent interactions with biological molecules.

The release of cyanide from acetonitrile and its subsequent metabolism to thiocyanate have been studied in various experimental conditions and in several animal species.

Oral route

Silver et al. (1982) reported that 11.8% of the dose administered orally to rats was excreted in the form of thiocyanate in the urine within 24 hours after ingestion.

Blood cyanide and serum acetonitrile concentrations were measured in groups consisting of eight rats, over a 72-hour period after p.o. administration of acetonitrile 1,470 or 4,300 mg/kg. Control animals received water at a dosage of 20 ml/kg. A second pharmacokinetic study was performed to further characterise the time course of acetonitrile metabolism to cyanide. The results showed maximum serum levels of acetonitrile after 7.5 hours; at 72 hours acetonitrile was barely detectable. Blood cyanide peaked to comparable levels for both doses after 7.5 hours and it had declined at 72 hours almost to base levels in the case of the lower dose (Freeman and Hayes, 1985). In the same study the authors found that acetone (an inducer of cytochromo P450) stimulates acetonitrile metabolism when the two compound are administered simultaneously. They suggested that acetone potentiates acetonitrile toxicity via a biphasic effect on acetonitrile metabolism, i.e. an initial inhibition followed by a stimulation of cyanide generation upon acetone elimination. Upon further characterisation of this inhibition, acetone was found to fit a competitive model of inhibition (Freeman and Hayes, 1987).

Willhite (1983) studied the *in vivo* biotransformation of acetonitrile in the golden hamsters. Non-pregnant female hamsters were given an oral dose or an intraperitoneal injection of 100, 200,

300 or 400 mg acetonitrile/kg body weight. The animals were killed 2 1/2 hours after dosing and the blood, brain, kidneys and liver levels of cyanide and thiocyanate were determined. Even within groups of hamsters to which the same dose of acetonitrile was given, there was a large measure of variation in the concentration of the metabolites. The liver and kidney contained greater concentrations of cyanide and thiocyanate than the brain. Whole blood, liver and kidney thiocyanate concentrations were up to 10 times those in brain and the blood thiocyanate concentrations more closely paralleled those in kidney than those in brain. Nevertheless, with an increase in the dose of acetonitrile, there was an increase in the concentrations of the two metabolites. The pattern of biotransformation was similar regardless of the route of administration or tissue studied. There was a consistent increase in the concentrations of cyanide and thiocyanate in all tissues studied following either oral or i.p. administration of 400 mg/kg acetonitrile when those concentrations were compared to the concentrations of the metabolites resulting from the other doses studied. The variations noted could be due to either differences in the individual rates of biotransformation of acetonitrile to cyanide in situ or differences in the individual rates of biotransformation of cyanide to thiocyanate or a combination of these factors. Elevated levels of cyanide and thiocyanate were detected in all studied tissues after both oral and intraperitoneal dosing.

Ahmed and Farooqui (1982) measured cyanide levels one hour after administration of LD_{50} doses of several saturated and unsaturated nitriles to male Sprague-Dawley rats. Animals in groups of six were fasted overnight and a LD_{50} of 2,460 mg/kg acetonitrile was administered orally. Control animals received equivalent volume of 0.9% NaCl. Following the treatment the animals were observed for one hour and then killed by decapitation. At the time of killing, blood was collected by completely draining the decapitated animals into heparinized tubes. Liver, kidney, brain and gastric content were collected, then frozen immediately in liquid nitrogen and stored at -30ºC until the time of analysis. The conversion of acetonitrile to cyanide proceeds at a slower rate than that of other nitriles. In fact, one hour after the administration of acetonitrile, the level of cyanide in blood was much lower than the level observed after acute doses of other nitriles. Peak concentrations of cyanide in blood were found 7.5 h after acetonitrile dosing and were comparable to those of other nitriles measured one hour after dosing. Brain cyanide concentration one hour after acetonitrile dosing was also lower than that measured after exposure to potassium cyanide (KCN) or other nitriles. Urinary excretion of thiocyanate after exposure to various nitriles indicated that in the case of acetonitrile the percentage of the dose excreted was lower than in the case of other nitriles even though the absolute given amount of acetonitrile, based on its oral LD_{50}, was much higher. These data indicate that the toxicity of acetonitrile is lower than that of cyanide and other nitriles as it can be seen comparing the oral LD_{50} value of acetonitrile (2,460 mg/kg body weight) to the oral LD_{50} of the other nitriles (i.e. 40 mg/kg for propionitrile or 90 mg/kg for acrylonitrile or 10 mg/kg for potassium cyanide 1 h after oral LD_{50}) (Ahmed & Farooqui, 1982). This is most probably due to the slower transformation of acetonitrile to cyanide and consequently, to the more efficient detoxification via thiocyanate excretion.

Inhalation route

Pozzani et al. (1959) first reported the presence of cyanide in the blood and thiocyanate in the urine following exposure to acetonitrile via inhalation. The studies were conducted in rats, monkeys and dogs under different experimental conditions. Fifteen male and fifteen female rats were exposed to acetonitrile vapour (166, 330 and 655 ppm) 7 hours per day, 5 days per week, for a total of 90 days. Some thiocyanate was excreted by the rats inhaling 330 and 166 ppm acetonitrile vapour, although the amount of thiocyanate excreted was not proportional to the

concentration of acetonitrile inhaled. Thiocyanate was not completely eliminated between daily exposures, but was almost completely excreted during the 2.5-day rest period over weekends. Repeated inhalation studies were also performed with monkeys and dogs exposed to 350 ppm of acetonitrile in air, in the same way as rats. The concentrations of thiocyanate in the urine of three dogs increased from 69 to 252 mg/l after the 5-day inhalation period and from 60 to 114 mg/l in the monkeys. Unlike the rats, dogs and monkeys continued to eliminate thiocyanate beyond the 2.5-day rest period over the weekend. Thiocyanate was also found in the urine of rabbits inhaling 2,000 and 4,000 ppm vapour for single 4-hour periods.

In order to know to how much cyanide is formed in the mammalian body after inhalation of lethal concentrations of acetonitrile, three beagle dogs were exposed for 4 hours to acetonitrile vapour at an air concentration of 16,000 ppm (27,000 mg / m^3). The animals were taken from the inhalation chambers for 3 minutes at approximately hourly intervals for bleeding. All animals died within 14 hours after terminating the inhalation. Considerable amounts of cyanide in blood were found in the dogs during the inhalation period. After one hour, concentrations of cyanide in blood were 33-53 μg/100 ml blood. Levels of cyanide in blood peaked after 3 hours (305-433 μg/100 ml blood) and were somewhat reduced at the end of the 4-hour exposure period (266-291 μg/100 ml blood). The authors did not discuss this pattern of absorption but noted one "analytical artefact" during the elimination phase of this study. Given the small number of animals used in this study and possible problems in the analytical technique, these data cannot be used to derive a rate coefficient for absorption. Nonetheless, the data indicate qualitatively that acetonitrile is absorbed rapidly via inhalation and suggest that the dogs may have been nearing steady- state blood concentrations at 3-4 hours after exposure.

In 1975, Haguenoer et al. reported their observations on the distribution and metabolic fate of acetonitrile in the rat after inhalation of acetonitrile at 2,800 or 25,000 ppm. At 25,000 ppm, all three rats died 30 minutes after the start of the exposure, following difficult breathing and cyanosis. Chemical analysis of various organs (heart, lungs, liver, spleen, kidneys, stomach, intestines, skin, muscle, brain and testes) was made. The mean concentration of acetonitrile ranged from 136 to 2,438 μg/100g of muscle and kidney, respectively, and that of free hydrogen cyanide from 27 to 402 μg/100 g of liver and spleen, respectively. The free hydrogen cyanide was more uniformly distributed, except in the spleen (402 μg/100g) and in the brain (129 μg/100g), where it was somewhat higher. The authors stated that the high concentrations of acetonitrile (2438μg/100g) found in the kidneys may have been due to either very high excretion of the acetonitrile or renal blockage. Acetonitrile concentrations in all the organs of rats exposed via inhalation (25,000 ppm) were up to 16 times those observed in a similar i.p. study (Haguenoer et al., 1975). In contrast to the i.p.study in which administration of acetonitrile was associated with a latency period of 3-12 hours between dosing and death, the rats in the present study died immediately after inhalation. In the second experiment, three rats inhaled 2,800 ppm acetonitrile 2 hours/day for up to 5 days. All showed laboured breathing, temporary anuria and diarrhoea. After the third exposure, one rat died with lung and brain haemorrhages. After the fourth exposure, the remaining two rats suffered paralysis and decreased urinary excretion. One died at the start of the fifth exposure, and the other died two hours after exposure was completed. Both rats had lost about 45% of their body weight during the 5 days of exposure. Autopsies of the rats revealed that all the organs examined contained concentrations of acetonitrile and free cyanide in the range of 96.0-286.9 and 53-990 μg/100g tissue, respectively. Concentrations of acetonitrile in the organs were high but variable in the three animals (highest in the kidneys 286.9 μg/100 g tissue). These values were lower than those noted for the 25,000 ppm exposure. The authors attributed this to a greater pulmonary elimination (exhaled air) of acetonitrile between exposures. By comparison, the average concentrations of free hydrocyanic acid in the

organs were slightly higher than those observed in the 25,000 ppm group, particularly in the spleen (990 μg/100 g tissue). However, the relative increase was greatest in the heart (4.9 times) and stomach (5.6 times) compared with only 2.4-fold in the spleen. The authors stated that the concentrations of hydrogen cyanide in the organs of the animals that died from inhaling acetonitrile were similar to those found in the animals that died from i.p. doses of acetonitrile. Additionally, the results implied that there was no quantitative relationship between the concentrations of the free hydrogen cyanide in the organs and exposures to acetonitrile. At either concentration of acetonitrile, a lengthy and persistent anuria was always observed as one of the effects. Such signs varied with the amount of acetonitrile inhaled and with the sensitivity of the animal.

Intraperitoneal route

Haguenoer et al. (1975) studied the distribution of acetonitrile and its metabolites in the tissues of Wistar rats after intraperitoneal administration of 2,340, 1,500 and 600 mg/kg of the compound. Two groups of four rats each and one group of three rats were given a single i.p. injection of 780 mg/rat (average weight 330 g). All the animals died within 3 to12 hours. The liver, lungs, spleen, kidneys, heart, brain muscle, intestines, stomach, testes and skin of each animal were analysed for acetonitrile, free hydrogen cyanide and its metabolic forms content. At 359 μg/100 g tissue, metabolites of hydrogen cyanide concentration was lowest in the liver; the concentrations in the spleen, stomach and skin were 1,317, 1,757 and 1,045 μg/100g of tissue, respectively. Free hydrogen cyanide found in the organs varied from 17μg/100g of tissue in the liver to 347 μg/100g in the spleen. Acetonitrile was found to be evenly distributed in various organs.

After a single intraperitoneal administration of acetonitrile (500 mg / rat), all animals died within 18 to 28 hours. Differents organs were analyzed and results showed acetonitrile in all organs. Spleen, heart and lungs were the organs with a highest quantity of acetonitrile, 221.1, 284.3 and 153.3 mg/100g, respectively. Concentrations of free hydrogen cyanide and its metabolites (thiocyanates, cyanohydrins and cyanocobalamine) found in the organs were higher than those found in the experiment with 2,340 mg/kg. The authors attributed this fact to a greater proportion of acetonitrile, which could have been hydrolysed as the evolution of the intoxication had been longer. The same observation can be made about the metabolites of hydrogen cyanide. If during the intoxication more hydrogen cyanide has been formed, the organism of the rats have had more time for detoxification, but not enough to avoid the death of the animals. It is for this reason that at lower doses of acetonitrile, a much longer latency period is observed between injection and death. This latency period can be long enough to allow a notable elimination of the toxic via the lungs and urine and a more important detoxification, allowing eventually the animals to live. This is what the authors wanted to confirm in the third experiment: Eight Wistar rats received a single dose of 200 mg acetonitrile per rat. They were split in two lots. The urinary elimination of acetonitrile and free hydrogen cyanide and its metabolites was followed during 11 days and compared to that observed in four rats of a witness lot. All rats survived with no apparent sign of toxicity and were killed for autopsy on the 11^{th} day. The heart, lungs, liver, spleen, kidneys, stomach, intestines, skin, muscle, brain and testes of each animal were examined for acetonitrile and for free hydrogen cyanide and its metabolites. Acetonitrile was not found. The free hydrogen cyanide was present only in traces in the kidneys of the intoxicated animals; the metabolites of hydrogen cyanide was found at similar levels in the witness animals. On each of the 11 days postexposure, urine was collected to measure free hydrogen cyanide and its metabolites and acetonitrile. On day 1, the urine contained an average of 92 μg free hydrogen cyanide, 5,391 μg of metabolites of hydrogen cyanide and 20.3 mg acetonitrile. No acetonitrile was measured after day 4 and free hydrogen cyanide excretion

averaged 5.3 μg/animal on day 11. Each control rat excreted from 1.5 to 5.2 μg of free hydrogen cyanide and from 9 to 40 μg of metabolites of hydrogen cyanide each day. No acetonitrile was found in the urine of control rats at any time. Tissue analyses at autopsy showed no important differences between the treated and the control rats. There was a dramatic decrease in the excretion of both forms of hydrogen cyanide after day 4 when acetonitrile was no longer present in the urine. The authors concluded that acetonitrile was low in toxicity and that the amount of the cyanide ion present was dependent on the rate of release of cyanide from the parent molecule. Also, they postulated that the large amounts of hydrogen cyanide liberated at the high doses (2,340 and 1,500 mg/kg) were responsible for the rat deaths.

In a study with male CD-1 mice treated with various nitrites including acetonitrile, Willhite and Smith (1981) showed that concentration of cyanide in tissues taken at death or sacrifice 2.5 h after injection of an i.p. LD_{50} dose of certain nitrites was elevated in all cases, but considerable variation was noted from nitrite to nitrite. The livers consistently had higher concentrations of cyanide than the brains. Even within groups of animals administered the same nitrite, there was considerable variation in the cyanide concentrations. The higher concentrations correlate with the more severe signs of intoxication. This large variation may be due to individual differences in the rate of cyanide liberation *in vivo*, to differences in the rate of cyanide biotransformation to thiocyanate or both. After intraperitoneal administration of 175 mg acetonitrile/kg, 47.8±36.1 and 13,4±4.8 μmol cyanide/kg were found in liver and brain, respectively. The male CD-1 mice treated died 2.5h after i.p. administration.

Silver et al. (1982) reported that urinary thiocyanate excretion for a 24h-period following i.p. administration of 30.8mg/Kg acetonitrile in SD rats was 2.2±0.2 mg/kg equivalent 4.4±0.5 % of the dose .

Intravenous route

In order to know how much of the formed thiocyanate would be excreted, three monkeys were injected intravenously with acetonitrile (0.1 ml/kg). After 4 to 8 weeks of this injection, they were injected with sodium thiocyanate (1.55 ml/kg of a 10% solution in saline). The percentages of the dose excreted as thiocyanate were 12% after acetonitrile injection and 55% after sodium thiocyanate injection. It appears that more than 12 % of the injected acetonitrile was converted into thiocyanate (Pozzani et al., 1959).

Ahmed et al. (1992) investigated the administration of acetonitrile and its metabolites using $^{14}CH_3CN$ molecule. In general, available information on metabolism of acetonitrile deals only with cyanide formation and suggests the metabolic pathway shown in **Figure 1**: the volatile compound, acetonitrile, is converted into non-volatile metabolite, namely formaldehyde cyanohydrin. The latter may undergo further metabolism to release cyanide and formaldehyde or it may covalently bind to tissue macromolecules via nucleophilic substitution on the electrophilic methylene carbon atom of the metabolite formaldehyde cyanohydrin. Furthermore, formaldehyde may also covalently bind to nucleophilic sites on tissue macromolecules via hydroxymethylene formation (Feeney et al., 1975), become incorporated in de novo synthesis of several endogenous compounds (Ntundulu et al., 1976) or undergo further metabolism to formic acid. Ahmed et al. (1992) did not seek the distribution of the cyanide group, but they investigated the distribution of either the whole acetonitrile molecule or metabolites with or without the cyanide groups as shown in **Figure 1**. The study showed that several tissues in addition to the liver have a capacity to metabolise and accumulate acetonitrile. Metabolism of acetonitrile is remarkably active in the liver and kidney as indicated by the rapid accumulation of non-volatile acetonitrile metabolites in these organs 5 min after administration of a single

intravenous dose (2.46 mg/kg) to male ICR mice (Sprague Dawley). However, accumulation of acetonitrile metabolites in the kidney may also be followed by rapid excretion of urinary metabolites. Both kidney and bladder contained high levels of radioactivity. While the radioactivity in the kidney declined with time, increased radioactivity in bladder contents was observed. This shift indicates that 2-^{14}C-acetonitrile and its metabolites were rapidly excreted from blood to kidney, urinary bladder and urine. High levels of radioactivity observed in liver, spleen, testes and skin indicated a fast transport rate from blood to tissues. At 24 and 48 h, radioactivity was still retained in the liver and gastrointestinal tract, and delayed accumulation and retention of 2-^{14}C acetonitrile metabolites was observed in the male reproductive organs and in the brain. The brain contained the least amount of non- volatile radioactivity at 5 min following 2-^{14}C acetonitrile administration, however, the levels of acetonitrile in the brain was almost equal to or slightly higher than that in the blood at 5 min after treatment. No 2-^{14}C acetonitrile was detectable in brain tissues after 24 h following treatment. The results of this study suggest that acetonitrile neurotoxicity may be due to the parent acetonitrile molecule rather than to any of its metabolites, which cannot penetrate the blood-brain barrier. Elimination rate constants of total 2-^{14}C-acetonitrile varied between tissues. Pharmacokinetics analysis of acetonitrile in tissues indicated that the distribution and excretion kinetics of the acetonitrile parent molecule in various tissues follow one comportment, first order kinetics. Meanwhile, the distribution of acetonitrile metabolites in the tissues follows a two-comportment model with elimination half-lives much longer than that for acetonitrile. The apparent fast elimination rates ($t_{1/2}\alpha$) were in the range of 0.08 h in the skin to 1.77 h in the eyes. The apparent slow elimination rates ($t_{1/2}\beta$) were between 8,60 h in the urinary bladder to 536,26 h in the smell intestinal tissues, The half-life of elimination of 2-^{14}C acetonitrile from blood and most tissues ranged from 5.52 h in the liver to 8.45 h in the blood. The liver, which is the major site for acetonitrile metabolism, has the shortest elimination half-life of 2-^{14}C-acetonitrile intact molecule. Although the total radioactivity in tissues declined over time the relative percentage of radioactivity covalently bound to lipids, proteins and nucleic acids increased. By 48 h following treatment with 2-^{14}C-acetonitrile, about 25-45% of the radioactivity present in the tissues was covalently bound to macromolecules and 30-50% was bound to lipids. Both hepatic and pancreatic tissues contained the highest macromolecular binding of radioactivity at 24 and 48 h after treatment. When the authors compared the whole body autoradiography distribution of acetonitrile described in the study to the distribution of the proposed metabolite, formaldehyde, described by Johansson and Tjälve (1979), they found that the hepatic uptake and retention of radioactivity derived from the two compounds were strikingly different. Much higher radioactivity was detected in the livers of animals treated with 2-^{14} C acetonitrile compared to those treated with $^{14}CH_2O$. Therefore, contrary to formaldehyde, the prolonged uptake and retention of the high radioactivity observed in the livers of animals treated with acetonitrile may indicate that this compound may undergo metabolic oxidation reactions and/or other metabolic reactions (such as conjugations) in the liver.

The persistence of radioactivity in the liver suggests a) the formation of reactive electrophilic intermediates that covalently bind to macromolecules of the hepatic cells and/or b) the incorporation of radioactivity, derived from acetonitrile, in the *de novo* synthesis of biological molecules through the one-carbon pool in hepatic tissues. Covalent binding studies indicated the irreversible interaction of radioactivity with macromolecules of most tissues, particularly the liver and gastrointestinal tract. On the other hand, the distribution of radioactivity from 2-^{14}C-acetonitrile in tissues with rapid cellular turnover such as blood forming organs, the lymphoid system and tissues with high rate of protein synthesis, such as the exocrine pancreas and salivary glands was similar to the distribution of radioactivity from $^{14}CH_2O$ (Johansson and Tjälve, 1979), Therefore,

this pattern may be explained on the basis of incorporation of radioactivity via CH_2O intermediate into one-carbon pool of the cell.

The authors concluded that acetonitrile undergoes bioactivation, mostly in the liver to a reactive metabolite that undergoes irreversible interaction with tissue macromolecules, particularly in the liver and gastrointestinal tissues and this reactive intermediate may be transformed into formaldehyde. Hence, the similarity of uptake, distribution and incorporation of radioactivity derived from 2-^{14}C-acetonitrile to that derived from $^{14}CH_2O$ in tissues of high cellular turn-over.

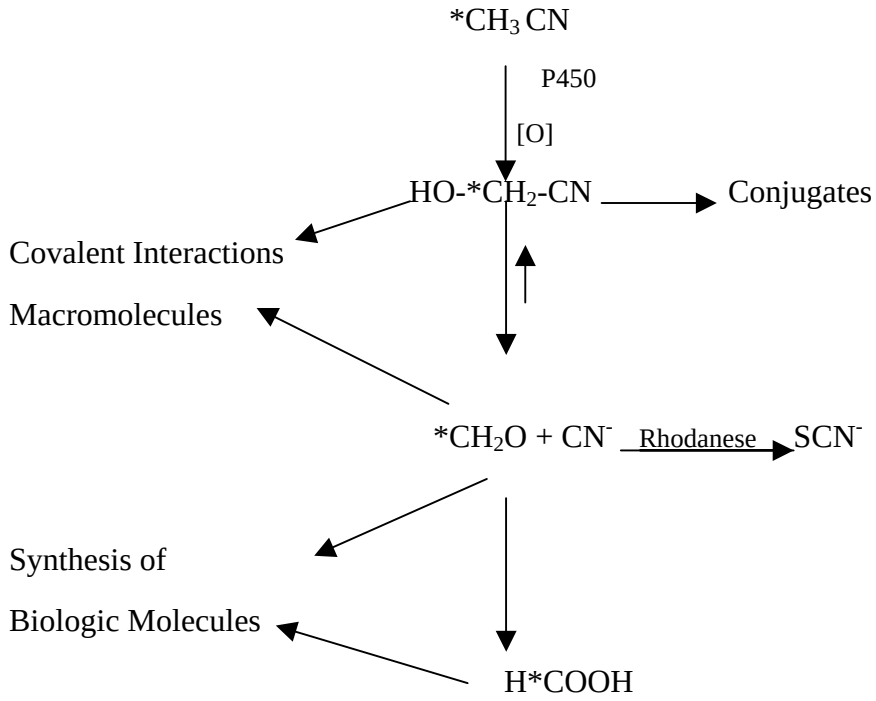

Figure. 1 Proposed metabolic pathway for acetonitrile

In vitro studies

The production of cyanide and thiocyanate from acetonitrile has also been demonstrated in a variety of "in vitro" studies (Ohkawa et al., 1972; Willhite, 1983; Tanii and Hashimoto, 1984; Freeman and Hayes, 1987), indicating involvement of P450 enzymes in the metabolism of acetonitrile.

Ohkawa et al. (1972) found that the amount of hydrogen cyanide released in mouse liver microsomal preparations was greatly increased by the addition of NADPH. Cyanide liberation was abolished when nitriles were incubated with CCl_4- pretreated mouse liver slices or microsomes (Willhite, 1979). After this result, it was studied *in vivo* by Willhite (1981) and the results were compared to those of previous studies. Pretreatment with a hepatotoxic dose of CCL_4 can protect mice against death from inhalation of lethal concentration of acetonitrile and other nitriles. The fact that CCl_4- induced hepatic dysfunction indicates that normal hepatic function is required in order to produce death as a consequence of nitrile exposure. Pretreatment with $Na_2S_2O_3$ or $NaNO_2$ also provides significant protection against the mortality associated with exposure to lethal concentration of acetonitrile.

Incubation of acetonitrile with hamster-liver slices was associated with a concentration-dependent increase in the generation of cyanide and thiocyanate. Since rhodanese activity is associated with the mitochondria, thiocyanate measurements were not attempted in experiments with hamster-liver microsomes. Incubation of acetonitrile with NADPH-fortified hamster-liver microsomes also resulted in a concentration-dependent increase in the liberation of free cyanide (Willhite, 1983).

Freeman and Hayes (1987) found that microsomal metabolism of acetonitrile to cyanide was found to be oxygen- and NADPH-dependent, and heat-inactivated tissue was unable to catalyse the reaction. NADH antaginized the NADPH-dependent metabolism of acetonitrile. The metabolism of acetonitrile to cyanide was linear with protein concentrations of 0-8 mg per incubation. Following a characteristic lag period of 10 min, the reaction was linear from 15 to 30 min. This metabolism was inhibited by carbon monoxide, metyrapone and SKF 525-A. Acetone pretreatment (24 h) *in vivo* increased the apparent V_{max} for acetonitrile metabolism without affecting the apparent K_m. When added *in vitro*, acetone competitively inhibited the metabolism of acetonitrile, with a K_1 of 0.41 mM. Dimethyl sulfoxide (K_1 = 0.51 mM) and ethanol (K_1 = 0.11 mM) were also competitive inhibitors of acetonitrile metabolism, and aniline HCL (K_1 = 4.77 μM) appeared to be a mixed inhibitor. These data are consistent with the hypothesis that the metabolism of acetonitrile to cyanide is mediated by a specific acetone-inducible isozyme of cytochrome P450 and the effects of acetone upon the acute toxicity and metabolism of acetonitrile appear to be related to the inhibition and induction of this enzyme.

The K_m and V_{max} values obtained from male ddY mouse microsomes were 4.19 mM and 14.3 ng cyanide formed in 15 min per mg protein, respectively (Tanii and Hashimoto, 1984).

Cobaltic protoporphyrin 9-chloride, which has been demonstrated to deplete hepatic cytochrome P450 content (Drummond and Kappas, 1982), markedly decreases the acetonitrile metabolism in isolated hepatocytes prepared from rats pretreated subcutaneously with cobalt-heme (90 μmol/kg) 48 h before killing, (Freeman and Hayes, 1987).

It is known that acetone as well as ethanol induces hepatic cytochrome P450 LM3a (LMeb) in rabbits. This isozyme is apparently similar to cytochrome P450 j which has been isolated from the hepatic microsomes of isoniazid-treated rats by Ryan et al. (1985). Since the metabolism of acetonitrile is increased in animals pretreated with acetone, Freeman & Hayes (1987) suggested that acetonitrile is likely metabolized by this cytochrome P450 isozyme, cytochrome P450 j (LM3a, LMeb). Agents (acetone, ethanol, dimethyl sulfoxide and aniline) that are substrates for or inhibitors of this isozyme inhibited the acetonitrile metabolism. Therefore, acetone and acetonitrile seem substrates for the same cytochrome P450 isozyme.

Dahl and Waruszewski (1989) studied the metabolism of acetonitrile to cyanide in male Fischer-344 rats nasal and liver tissues. They found that aliphatic nitrile was metabolised to cyanide by the ethmoturbinates as well as liver microsomes, although the rate of cyanide formation was lower for acetonitrile than for some other aliphatic nitriles. For acetonitrile, they found that the maximum cyanide production rates in nanomols cyanide/mg protein/minute by nasal maxilloturbinate and ethmoturbinate microsomes and liver microsomes for one millimolar initial substrate concentrations were 0, 0.9 and 0.098, respectively. High concentrations of rhodanese are present in the nasal respiratory and olfactory mucosa of the rat (Dahl, 1989). The authors conclude that detoxification of inhaled acetonitrile may occur to a substantial extent in the nasal cavity.

4.1.2.1.2 Studies in humans

Acetonitrile is well absorbed by all routes.

Quantitative data regarding the absorption of acetonitrile in humans after inhalation exposure are available (Dalhamn et al., 1968a, 1968b). In a group of 16 human subjects who were cigarette smokers, an average of 74% absorption of acetonitrile was measured when the smoke was held in the mouth for two seconds and was not inhaled. When the subjects were classified by the number of cigarettes smoked/day, a slight but statistically significant ($p<0.05$) inverse correlation was noted between the smoking rate and the extent of acetonitrile absorption (Dalhamn et al., 1968a). When the cigarette smoke was inhaled into the lungs, absorption of acetonitrile increased to 91% (Dalhamn et al., 1968b).

There are no studies of oral or dermal absorption in humans; however, human poisoning cases indicate that acetonitrile is well absorbed via both routes. Amdur (1959) reported the presence of cyanide in the blood, urine and tissues, and thiocyanate in serum of workers accidentally exposed to acetonitrile vapour. No blood cyanide was found in three volunteers inhaling 40, 80 and 160 ppm of acetonitrile vapour for four hours (Pozzani et al., 1959).

There is no specific human study describing acetonitrile biotransformation and elimination. However, accidental poisoning cases indicate that acetonitrile is biotransformed to cyanide and thiocyanate, which are then excreted from urine (IPCS, 1993).

In case of suicidal oral acetonitrile ingestion, elimination half-lives of 32 hours for acetonitrile and 15 hours for cyanide were calculated during the hospitalisation of the patient prior to death (Michaelis et al., 1991).

4.1.2.1.3 Summary of toxicokinetics, metabolism and distribution

Acetonitrile is well absorbed from the lungs, gastrointestinal tract and through the skin, although there are not available quantitative data.

Acetonitrile has a widespread distribution. It has been found in heart, lungs, liver, spleen, kidneys, stomach, intestines, skin, muscle, brain and testes. Free and conjugated hydrogen cyanide was also detected in all studied organs (Haguenoer et al., 1975). Following a single intravenous dose of 2-^{14}C-acetonitrile to mice, the highest levels of radioactivity occurred in the liver and kidney at 5 min and levels declined over time. At 24 and 48 hours, acetonitrile derived radioactivity was detected in the gastrointestinal, thymus, liver and male reproductive organs. Covalent binding studies at 24 and 48 hours after treatment indicated that 40-50% of total radioactivity present in the liver was bound to the macromolecular fractions of the tissues. The radioactivity contents of other organs were, in large part (40-50% of total), present in the lipid fraction of the tissue (Ahmed et al., 1992).

There are no indications that repeated administrations of acetonitrile result in its accumulation in animal tissues.

Acetonitrile is metabolised to cyanide via cytochrome P450. Firstly, a cyanohydrin intermediate is formed and spontaneously decomposes in liberating free cyanide and possible formaldehyde. Several studies have indicated that cyanide formed *in vivo* is subsequently conjugated with thiosulphate to form thiocyanate, which is eliminated in urine (Willhite, 1981; Willhite and Smith, 1981; Pozzani, 1959; Haguenoer et al., 1975; Silver et al., 1982; Ohkawa et al., 1972).

Cyanide is responsible for the acetonitrile toxicity. The conversion of acetonitrile to cyanide proceeds at a slower rate than that of other nitriles (Ahmed & Farooqui, 1982). This explains the lower toxicity of acetonitrile comparing with other nitriles. Moreover the more rapid rate at which cyanide is produced in the mouse appears to account for the much greater sensitivity of this species to the toxic effects of acetonitrile.

The microsomal metabolism of acetonitrile to cyanide was found to be oxygen- and NADPH-dependent, inactivated by heat and antagonised by NADH. Since the metabolism of acetonitrile is increased in animals pretreated with acetone, Freeman and Hayes (1987) suggested that acetonitrile is likely metabolised by the cytochrome P450j (LM3a, LMeb).

Elimination of acetonitrile occurs primarily through urinary excretion of the unchanged compound and free and bound hydrogen cyanide.

Pulmonary clearance of unchanged acetonitrile via exhalation is also an important pathway of elimination, especially at high exposure levels (Haguenoer et al., 1975).

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

In vivo studies

Several studies have been carried out with different species and by different routes. They are summarized in **Table 4.11**.

Oral

A large number of LD_{50} values for male and female Carworth Farms-Wistar or Nelson albino rats, in various assays over a 5-year interval, ranged from 1.7 to 8.5 ml/kg (1,327-6,762 mg/kg using a density value of 0.79 g/ml) by gastric intubation. The males appeared to be two to four times more susceptible than females to undiluted acetonitrile by this route, but this difference was not important when the animals were fasted overnight before intubation. In many instances delayed deaths occurred at or above the LD_{50} dosage level. However, the highest dosage levels caused rapid deaths.

Acetonitrile diluted in corn oil, water or 1% aqueous Tergitol 7 is apparently better absorbed from the intestine and in most assays is somewhat more toxic for both male and female rats than the undiluted compound (Pozzani et al., 1959). In the same study Pozzani obtained a LD_{50} 140 mg/kg for male guinea pigs by gastric route (fasted); acetonitrile was undiluted.

Significant differences in LD_{50} values were found between 14-day-old and adult rats, but not between young adults (80-160g body weight) and older adults (300-470 g body weight) (Kimura et al., 1971). Male Sprague-Dawley rats in groups of six were used for studies in the young and older adult rats, groups of 6-12 rats of both sexes were used for the new-born and 14-day-old rat toxicity studies. Acetonitrile was given orally via straight needle in undiluted form in nonfasted rats, and the animals were observed for a week following medication. A LD_{50}-value of 158 mg/kg was obtained using wealing (14-day) rats and a LD_{50} of 3,081 and 3,476 mg/kg using young and older adults, respectively.

Tanii and Hashimoto (1984) studied the mechanism of acute toxicity of nitriles in mice, using 4 animals per dose level and 4 different doses. The LD_{50} was 269 mg/kg, using male mouse strain ddY by oral route and the observation period was seven days.

Recently MPI Research (1998) has conducted an acute oral toxicity study of acetonitrile in mice in conformity to EPA/OECD Guidelines. Acetonitrile (HPLC Grade) was dosed once orally, by gavage, to six groups of 5 male and 5 female Crl:CD-1 (ICR) BR mice. The dosage levels were 300, 500, 650, 900, 1,200 and 2,000 mg/kg. The test article was administered as a weight/volume solution in distilled water and was dosed at a constant dosage volume of 10 ml/kg based on fasted body weight. Test article-related combined sex mortalities were 10, 30, 60, 80, 90, and 90% for dose levels 300, 500, 650, 900, 1,200 and 2,000 mg/kg of acetonitrile, respectively. With the exception of the 650 mg/kg group, mortalities were approximately equal for both sexes in the other groups. No mortalities occurred after study day 2 for any group. Significant clinical signs observed during the study included death, tremors, prostration, decreased activity, impaired righting reflex, laboured breathing, convulsions, gasping and increased salivation. All surviving animals were judged normal by study day 4, with the exception of a single animal in the 300 mg/kg group that exhibited increased salivation on study day 8. Body weight gains for survivors were similar for both sexes in all groups. The 300 and 500 mg/kg groups were the only groups with survivors from both sexes. Body weight gains were 2-3 grams for each sex. At necropsy, there were no test article-related findings in any animal. Based on the results of this study, the oral LD_{50} of acetonitrile was calculated to be 617 mg/kg for male and female mice combined (with 95% confidence limits of 450-787 mg/kg).

Inhalation

Pozzani et al. (1959) investigated the mammalian toxicity of acetonitrile. Groups of 1-3 male dogs were exposed to 2,000, 8,000, 16,000 and 32,000 ppm acetonitrile during 4 hours. There were no deaths in either of the lower two groups (1 and 2 dogs exposed). All those exposed in the upper two groups died (1 and 3 exposed).

Exposure for eight hours (12 male and 12 female Nelson rats at six concentrations, 32,000, 16,000, 8,000, 4,000, 2,000 and 1,000 ppm) generated LC_{50} values of 7,551 ppm (12,685 mg/m^3) and 12,435 ppm (20,890 mg/m^3) for males and females, respectively. This apparent difference in sensitivity between males and females is not evident in the 4-hour tests (LC_{50} value of 16,000 ppm, 26,880 mg/m^3). Prostration and convulsive seizures often preceded death. Marked to moderate pulmonary haemorrhage and congestion were evident both in survivors and in those which died. A LC_{50} value of 2,828 ppm. (4,751 mg/m^3) was established for rabbit exposed to 3 concentrations of acetonitrile during 4 hours; a LC_{50} value of 5,655 ppm (9,500 mg/m^3) was established for guinea pig, when groups of six (males and female) were exposed to 3 concentrations of acetonitrile during 4 hours.

Dogs appeared to be most resistant to vapour. The rabbit and guinea pig are somewhat more sensitive. There was a greater difference in individual susceptibility to vapour in rats than in the other three species tested.

Male CD-1 mice were exposed for 60 min to concentrations of acetonitrile ranging from 500 to 5,000 ppm (Willhite, 1981) and were followed for 14 days. The LC_{50} value for acetonitrile was 2,693 ppm (4,524 mg/m^3). Exposure to 5,000 ppm killed all mice within 2 hours. Toxic effects included intense dyspnea, gasping, tremors, convulsions and corneal opacity after 30-300 minutes. Gross autopsy of mice, which died, showed only particularly red livers in some cases.

Groups of six or 12 pregnant Syrian golden hamsters were exposed on day 8 of pregnancy to 1,800, 3,800, 5,000, 8,000 ppm acetonitrile during one hour (Willhite, 1983). Sacrifice occurred on day 14. There were no overt signs of toxicity in those exposed to the lowest concentration. One of six animals died in the 3,800 and 5,000 ppm groups and 3 out of 12 died in the highest concentration group. Overt effects included: Eye irritation, dyspnea, tremors, hypersalivation, ataxia, hypothermia, respiratory difficulty and coma. Histopathological examination of liver, kidneys and lung of affected animals did not reveal any abnormalities.

One female monkey exposed to 2,510 ppm acetonitrile vapour, appeared normal after the first inhalation day (7 h) but showed poor coordination followed by prostration and laboured breathing during the second inhalation day. Death occurred a few hours later. Engorgement of the dural capillaries and pleural effusion was found. Tissues were not examined microscopically because some autolysis occurred before autopsy (Pozzani et al., 1959).

MPI Research (1998), conforming to EPA/OECD Guidelines, conducted an acute inhalation toxicity study. Four groups of 5 male and 5 female Crl:CD-1 (ICR) BR mice were exposed for 4 hours, via whole-body exposure methods to nominal vapour concentrations of 3,203, 5,499, 4,653 and 3,747 ppm acetonitrile (groups 1 through 4, respectively). The respective mean analytical concentrations derived from infrared spectrometer analysis were 3,039, 5,000, 4,218 and 3,568 ppm for groups 1 through 4. Combined sex mortalities were 20, 80, 90 and 50% for groups 1 to 4, respectively. Male mortality was slight in each exposure group. All mortalities occurred on the day of exposure, except for a single male in group 1 that died on post-exposure day 1 (study day 2). Clinical signs observed during the exposure and up to 4 hours post-exposure included death, decreased activity, abnormal gait, loss of righting reflex, slow respiration, laboured breathing, rapid respiration, gasping, cold to touch limbs splayed, leaning to the right and yellow body surface staining. Surviving animals from groups 2 to 4 (5,000, 4,218 and 3,568 ppm) were judged normal by study day 2, therefore no toxic signs were recorded for these groups during the 14-day post-dose observation period. Clinical signs observed during the 14-day observation period for group 1 (3,039 ppm) included death, decreased activity and decreased defecation. Survivors from group 1 were judged normal by study day 5. Surviving animals body weights from groups 1 and 3 and the group 4 males remained at preexposure observation period. Of the two surviving females from group 2, one gained weight and one remained at the pre-exposure level during the first post-exposure week, but both lost weight (1 or 2 grams) during the second post-exposure week. Three of the 4 surviving females from group 4 gained weight (1g) during the first exposure week and regained their pre-exposure weight by the end of the second post-exposure week. At necropsy, no test article-related macroscopic findings were observed in male or female mice. All tissues were considered to be within normal limits. Based on the results of this study, the 4-hour LC_{50} of acetonitrile in mice (via whole-body exposure) was calculated to be 3,587 ppm (6,026 mg/m^3) with 95% confidence limits of 2,938-4,039 ppm.

Dermal

Acetonitrile has a LD_{50} by rabbit skin penetration of 987.5 (663.6 to 1,451.5) mg/kg of the undiluted compound applied under polyethylene sheeting. It is of interest to note that the toxicity of acetonitrile by skin penetration is increased when application is made as a 75% (v/v) aqueous solution. The LD_{50} is 395 (292.3 to 529.3) mg/kg of the 75% aqueous solution (Pozzani et al., 1959).

Neat acetonitrile was applied in covered contact to the clipped skin of six rabbits (Smyth and Carpenter, 1948). Contact was maintained for 4 days, followed by observation for 10 days or

until death (Smyth and Carpenter, 1944). The LD_{50} was given as 3,950 mg/kg; a density of 0.79 g/ml has been used.

MPI Research conducted a new acute dermal toxicity study in 1997, conforming to EPA/OECD Guidelines. Acetonitrile was dosed once dermally to a single group of 5 male and 5 female New Zealand white rabbits. The dosage level was 2,000 mg/kg. Acetonitrile was applied to the shaven intact skin on the back of each rabbit. The exposure period was approximately 24 hours. The dosage volume was 2.6 ml/kg based on a test article density of 0.777 g/ml. All animals survived to study termination. No signs of toxicity or ill health were observed in the males during the conduct of the study. With the exception of decreased defecation observed in three females for one day during the 14-day observation period (which is a probable test article-related change), no other signs of ill health or toxicity were observed in the females during the study. Mean group body weights for each sex increased at each observation interval. All animals gained weight at each observation interval with the exceptions of one male that lost 23 grams at study day 8 and another that lost two grams at study termination. At necropsy, no visible abnormalities were observed at the application site or other tissues. Based on the results of this study, the LD_{50} value of acetonitrile (HPLC Grade) is greater than 2000 mg/kg for male and female rabbits combined.

Other routes

Female rats (Wistar or Nelson albino) were given acetonitrile undiluted via i.p. route. Two extreme LD_{50} values of 7.96 and 0.85 ml/kg were found. A density value of 0.79 g/ml has been used to convert these to 6,288 and 672 mg/kg, respectively. When diluted in saline the LD_{50} range was 3,073-4,440 mg/kg. It is unclear why the range for neat acetonitrile should be so great (Pozzani et al., 1959).

Different values for the LD_{50} via i.p. route in mice (NMRI-SPF or CD-I strains) have been obtained by several authors using acetonitrile in water or saline: 175 mg/kg (Willhite and Smith, 1981); 198 mg/kg (Pozzani et al., 1959); 400 mg/kg(Zeller et al., 1969) and 521 mg/kg (Yoshikawa, 1968).

In the Willhite and Smith's study, 5 or 6 doses between 50-862 mg/kg of acetonitrile in water were administered i.p. to groups of 9 or 10 CD-I male mice. The animals were observed for the following seven days and the mean time to death was 423 ± 503 min. Intoxication syndrome ocurred within 1 to 5 hours, it included intense dyspnea, gasping, ataxia, corneal opacity, hypothermia and convulsions.

Cuny et al. (1932) reported a LD_{50} of 3,950 mg/kg for acetonitrile when it was injected subcutaneously into rats (cited in Pozzani et al., 1959).

There is only a reference with respect to route of administration intravenous in the IUCLID (Pozzani et al., 1959). An unstated number of male or female Wistar or albino rats, treated with neat acetonitrile, had LD_{50} of 1.68 ml/kg (1,327 mg/kg using a density value of 0.79 g/ml for conversion) in both cases.

Table 4.11 Summary of acute toxicity data

Route	Species	LD_{50} / LC_{50}	Reference
p.o.	Rat (Wistar-Nelson)	1,327-6,762 mg/Kg	Pozzani et al. (1959)
p.o	Guinea pig	140 mg/Kg	Pozzani et al. (1959)
p.o	Rat (SD) (14-day-old)	158 mg/Kg	Kimmura et al. (1971)
p.o.	Rat (SD) (Young adult)	3,081 mg/Kg	Kimmura et al. (1971)
p.o.	Rat (SD) (Older adult)	3,476 mg/Kg	Kimmura et al. (1971)
p.o	Mouse ddY	269 mg/Kg	Tanii and Hashimoto (1984)
p.o	Mouse (CD-1)	617 mg/Kg	MPI Research (1998)
Inhalation	Dog	13,440-26,880 mg/m^3 (4h)	Pozzani (1959)
Inhalation	Rat Nelson	26,880 mg/m^3 (4h)	Pozzani (1959)
Inhalation	Rat Nelson	12,685– 20,890 mg/m^3 (8h)	Pozzani (1959)
Inhalation	Rabbit	4,751 mg/m^3 (4h)	Pozzani (1959)
Inhalation	Guinea pig	9,500 mg/m^3 (4h)	Pozzani (1959)
Inhalation	Mouse CD-1	4,524 mg/m^3 (1h)	Willhite (1981)
Inhalation	Mouse CD-1	6,026 mg/m^3 (4h)	MPI Research (1998)
Dermal	Rabbit	987.5 mg/Kg undiluted	Pozzani et al. (1959)
Dermal	Rabbit	395 aqueous solution	Pozzani et al. (1959)
Dermal	Rabbit	3,950 mg/Kg	Smyth and Carpenter (1948)
Dermal	Rabbit	>2,000 mg/Kg	MPI Research (1997)
i.p.	Rat (Wistar or Nelson)	672-6,288 mg/Kg undiluted	Pozzani et al. (1959)
i.p.	Rat (Wistar or Nelson)	3,073-4,440 mg/Kg saline	Pozzani et al. (1959)
i.p.	Mouse (CD-1)	175 mg/Kg	Willhite and Smith (1981)
i.p.	Mouse	198 mg/Kg	Pozzani et al. (1959)
i.p.	Mouse (NMRI)	400 mg/Kg	Zeller et al. (1969)
i.p.	Mouse	521 mg/Kg	Yoshikawa (1968)
i.v.	Rat-Wistar or Nelson	1,327 mg/Kg	Pozzani et al. (1959)

4.1.2.2.2 Studies in humans

In vivo studies

Inhalation

In a human volunteer study, Pozzani et al. (1959) studied the acute inhalation toxicity of acetonitrile in three men aged from 31 to 47 years who inhaled 40 ppm for 4 hours. The two older subjects reported no adverse subjective response during and after the 4-hour inhalation period. There was no appreciable blood cyanide. The youngest subject reported no adverse subjective response during the inhalation period but he experienced a slight thightness in the chest that evening. The following morning he also reported a cooling sensation in the lung similar to that experienced when menthol is inhaled. This sensation persisted for approximately 24 hours. There was no detectable blood cyanide, but there was a slight increase in the urinary

thiocyanate level for this subject. All three subjects detected the odor of acetonitrile for the first 2 or 3 hours, after which they experienced some olfactory fatigue. The two older subjects inhaled 80 ppm acetonitrile vapour for 4 hours one week after the 40-ppm trial, with no symptom. No blood cyanide was detected in any of the samples taken after the inhalation period. Nine days later, these two subjects inhaled 160 ppm for 4 hours. One subject reported a slight transitory flushing of the face two hours after inhalation, and a slight feeling of bronchial tightness about 5 hours later. The blood cyanide and urinary thiocyanate levels of both subjects did not change significantly. The results of this study indicate that, with low-level exposure to acetonitrile, measurement of blood concentrations of cyanide or/and urine concentrations of thiocianate cannot be correlated with early symptoms.

Some cases of severe intoxications due to exposure to high concentrations of acetonitrile vapour have been reported:

Grabois (1955) reported on 16 workers at a chemical plant accidentally poisoned with acetonitrile vapour during the brush painting of the inside walls of a storage tank. A comprehensive discussion of this incident has been given by Amdur (1959). The paint contained 30-40% acetonitrile and the thinner contained 90-95% acetonitrile. Because of the viscosity of the paint, the tank was heated to 25°C and thinned on the second day before application. Ventilation of the tank was stopped.

One died after 2 days exposure, two were seriously ill and the remaining 13 workers were also affected. Symptoms included weakness, nausea and vomiting. Convulsive seizure and coma were reported prior to death. Post-mortem examination revealed cerebral, thyroid, liver, splenic and renal congestion, and a “peach pit” odour of all tissues. The blood and urine cyanide concentrations were 7,960 and 2,150 μg/litre, respectively. There was a trace of cyanide in the gastric fluid. Spleen, kidney and lung concentrations of cyanide were 3,180, 2,050 and 1,280 μg/kg tissue, respectively. No cyanide was detected in the liver.

The worker who died had worked inside the tank for about 12 hours. Of the two other seriously affected workers, one had worked inside the tank near three hours and the other had spent 12 hours painting around the ports from the outside of the tank but inside the last hour. Two other men, less severely affected, had painted inside the tank for no more than 2.5 hours each on the 2nd day.

Following the incident, the paint ingredients were no more heated, adequate ventilation was provided and the concentration of organic cyanide was kept below 17 ppm. There were no further incidents.

Dequidt et al. (1974) reported a heart failure and death occurred by acute acetonitrile poisoning in a 19-year-old male photographic laboratory worker. After handling acetonitrile for two days, he poured an unknown amount of acetonitrile and boiling water on the floor to clean it. Four hours after work he complained of epigastric pain and nausea and vomited repeatedly. The next day he became comatose and had convulsions. Large amounts of cyanide, thiocyanate and acetonitrile were found in the blood and urine. He died six days after the poisoning.

Dermal

Caravati and Litovitz (1988) reported a case of paediatric accidental exposure to an acetonitrile-containing cosmetic.

The exposure occurred both via the skin and by inhalation. Approximately 30 ml of a nail remover containing 98-100% acetonitrile spilled on a 2-year-old (12kg) previously healthy boy and his bed (amount of contact to the skin was not specified).

No symptoms were noted immediately after the exposure. Eight hours later, the boy was moaning, poorly responsive and vomited. He became lethargic and pale. Whole-blood cyanide levels were: 6mg/litre 12 hours after exposure, 60-70 μmol/litre from 24 to 48 hours and 15 μmol/litre after 60 hours. The patient was discharged three days later in good condition.

Oral

Caravati and Litoviz (1988) reported on a 16-month-old (11.8 kg) boy who ingested 15-30 ml of a nail remover (1-2g acetonitrile/kg body weight). The child vomited spontaneously about 20 min after ingestion. About 12 hours after ingestion he was found dead. Postmortem examination showed moderately severe pulmonary oedema, a blood cyanides level of 119 mg/kg and brain cyanide level of 0.2 mg/kg.

A case of acute acetonitrile intoxication in a 26-year-old man has been reported by Jaeger et al. (1977). The man ingested 40 g of acetonitrile in a suicide attempt. After a 3-hour latent period, he suffered from vomiting, convulsions, coma, acute respiratory insufficiency, severe metabolic acidosis and two cardiac arrests. Recovery occurred over the following three months after treatment. A dose of 570 mg/kg is estimated from this case of poisoning to be the dose that produced serious effects on human health without causing death.

A 22-year-old female ingested an unspecified amount of acetonitrile and acetone. She died about 30 hours later. Postmortem examination revealed pulmonary oedema and haemorrhage gastritis (Boggild et al., 1990).

Turchen et al. (1991) reported the case of a 39-year-old woman, who was found vomiting and confused seven hours after ingesting 59 ml of nail polish remover containing 99% acetonitrile. After 12 hours ingestion, she developed severe metabolic acidosis, seizures and shallow respiration. Eight hours after ingestion she had a whole blood cyanide level of 3,130 μg/litre. At 65 hours the serum cyanide level was 10 mg/l and the thiocyanate level was 120 mg/l, whereas at 77 hours they were 12 mg/l and 30 mg/l, respectively. She responded to the treatment with sodium nitrile and sodium thiosulphate. On the 5^{th} hospital day the cyanide level was 360 μg/l and thiocyanate level 30 mg/l. The patient was discharged on day six.

A case of acute acetonitrile poisoning of a 3-year-old (17.2 kg) child was reported by Geller et al. (1991). The child ingested 15-30 ml of a nail tip and glue remover containing acetonitrile. The amount of acetonitrile ingested was estimated to be 0.8-1.7 g/kg. Gastric lavage was performed. Three hours and 45 min after ingestion, the cyanide level in blood was 1.24 mg/l and the thiocyanate level 11 mg/l. Thirteen hours after ingestion the child vomited, was confused and developed seizures. The child was treated and discharged from hospital 42 hours after the ingestion.

Kurt et al. (1991) reported a case of a 15.8 kg 2-year-old girl who ingested 5-10 ml (0.25-0.5 g/kg) of a nail glue containing 84% acetonitrile. The following morning she was moaning, restless and vomiting. Toxic clonic seizures also appeared about 14hours after ingestion, she became comatose with hyperpnoea and tachycardia and with marked hypoxia and acidosis. She was treated and discharged from hospital after two days.

Vomiting occurred within six hours of a 23-month-old child ingesting 60 ml of a product containing at least 98% acetonitrile. He became unresponsive 24 hours after ingestion. He was treated and discharged from hospital on the 3rd day (Losek et al., 1991).

Michaelis et al. (1991) reported a case of suicidal oral acetonitrile ingestion in a previously healthy 30-year-old man. He ingested about 5 ml (64 mg/kg) of acetonitrile (98 %) and 30 min later, about one ml of ammonium and vomited once. Gastric lavage was performed 5.5 hours after ingestion. Peak serum acetonitrile and blood cyanide levels were 99.2 and 15.0 mg/l. Half-lives were calculated for acetonitrile and cyanide and found to be 32 and 15 hours, respectively.

Two fatal cases of a couple who ingested acetonitrile by mistake was reported by Jones et al. (1992). They were found dead with traces of vomit. Acetonitrile levels were 0.8 g/l in blood, 1.0 g/l in urine and 1.3 g/l in stomach contents. Blood inorganic cyanide levels were 4.5 mg/l (male) and 2.4 mg/l (female).

4.1.2.2.3 *In vitro* studies

Knox et al. (1986) studied cytotoxicity of acetonitrile in BCL-DL cells. The method used was dybinding and the harvest time 72 hours, the results were IC_{20}>24 mM, IC_{50}>24mM and IC_{80}>24mM.

Clothier and Hulme (1987) using Mouse 3T3-4 cells, FRAME Kenacid blue method and harvest time after 72 hours, obtained IC_{50} = 562 mM. Dierichx (1989) obtained IC_{50} = 494 mM using human hepatoma Hep G2, 24 hours harvest time and cellular protection content as method. The IC_{50} values in mouse neuroblastome cells and in rat glioma cells were 17.8 and > 20 mM, respectively.

4.1.2.2.4 Summary of acute toxicity

Different animal species and individuals of the same species varied widely in susceptibility to acetonitrile in single-dose toxicity studies by various routes.

The range of oral LD_{50} values acetonitrile in mammals is between 140 and 6,762 mg/kg body weight. Mouse and Guinea pig seem to be the most sensitive species. These studies were performed without GLP information.

A study showed that acetonitrile was more toxic to immature rats (14-day-old) than to older rats given oral doses of 160-3,500 mg/kg. Another study reported that using male or female Wistar or Nelson albino rats, the males were more susceptible than the females; 6,762 mg/kg for females and 1,327 mg/kg for males, the route was by gavage. In a well-conducted study in mice, the oral LD_{50} of acetonitrile was calculated to be 617 mg/kg.

The main symptoms in animals appear to be prostration followed by seizures and convulsions. Animals exposed to acetonitrile via different routes of dosing always showed respiratory symptoms: rapid and irregular respiration, laboured or difficult breathing and intense dyspnea.

In humans, ingestion of 1 to 2 g acetonitrile/kg causes occasionally death (in infants). A dose of 570 mg/kg was estimated from a case of poisoning in a 26-year-old man, to be the dose that produced serious effects on human health without causing death.

These data do not support the existing classification with R25; classification with R22 is proposed.

A LD_{50} >2000 mg/kg was obtained in a well-conducted acute dermal toxicity study in rabbits. This data do not support the existing classification with R24; classification with R21 is proposed based on human data which reported symptoms and levels of cyanide in blood as result of paediatric accidental exposure to an acetonitrile-containing cosmetic.

The 8-hour inhalation LC_{50} in male rats is 7,551 ppm (12,685 mg/m^3). Rabbits and mice were more sensitive than rats, with LC_{50} of 2,828 ppm (4,751 mg/m^3) and 2,693 ppm (4,524 mg/m^3), respectively and time of exposures of 4 hours for rabbits and 60 min for mice. A 4-hour exposure of dogs at concentrations up to and including 8,000 ppm (13,440 mg/m^3) produced no deaths, but deaths occured at concentrations of 16,000 and 32,000 ppm. (26,880 mg/m^3, 53,760 mg/m^3) Gross pathology indicated pulmonary haemorrhage and vascular congestion. In a well-conducted study of inhalation a LC_{50} 3,587 ppm (6,026 mg/m^3) was obtained in mice.

The determination of blood cyanide and urinary thiocyanate should not be relied upon as evidence of brief inhalation to low concentrations of acetonitrile vapour. No blood cyanide was found in human subjects inhaling 40, 80 and 160 ppm vapour, and there was no correlation between thiocyanate excretion and acetonitrile concentration. The variability of subjective responses of humans to 40, 80 and 160 ppm acetonitrile vapour suggests that, even if a concentration of the solvent vapour were selected that would not endanger the health of the majority of workers, it might cause discomfort to some of them.

Symptoms and signs of acute acetonitrile intoxication include chest pain, tightness in the chest, nausea, emesis, tachycardia, hypotension, short and shallow respiration, headache, and seizures. The systemic effects appear to be largely attributable to the conversion of acetonitrile into cyanide. Blood cyanide and thiocyanate levels are elevated during acute intoxication. Two deaths after exposure to acetonitrile vapour in the workplace have been reported. Elevated tissue cyanide concentrations were found in postmortem examination of these cases.

The animal data do not support the existing classification as T; R23. The levels causing toxicity in man are unknown but, probably, they are very high due to the detection of high levels of cyanide at post-mortem examination (7,960 μg/l blood), whereas no significant change was detected in the blood or urine of volunteers exposed to 0.27 mg/l (160 ppm) acetonitrile for 4 hours. Taking into account all available data, classification as harmful by the inhalation route (R20) is appropriate. Classification according to Annex 1 of Directive 65/548/EEC, see Chapter 1.

4.1.2.3 Irritation

4.1.2.3.1 Studies in animals

Skin

Data sheet IUCLID includes two studies about skin irritation. In one study a cotton pad (2.5 cm · 2.5 cm) saturated with acetonitrile is placed on the skin of white rabbits for 15 minutes or 20 hours. After the 15-minute application the treated area was first washed with undiluted polyethyleneglycol 400 and finally washed with 50 % aqueous polyethyleneglycol 400, after the 20-hour treatment however, the skin was not washed. The reaction was observed after 1, 3 and 8 days as well as

after removing the pad. No reaction was seen at any time, after either exposure period (Zeller et al., 1969).

Smyth and Carpenter (1948) describe the skin irritancy of acetonitrile similar to that of acetone. The method used in the determination is apparently the one used by Smyth and Carpenter (1944), in which 0.01 ml of the neat material was applied to the clipped belly of an albino rabbit. Observations were made after 24 hours. In one standard test (Clayton and Clayton, 1982) acetone is described as slightly irritant to the skin.

MPI Research has carried out a dermal irritation study of acetonitrile in rabbits in 1997. The study is conforming to EPE/OECD Guidelines. Acetonitrile was applied to the skin of six male New Zealand white rabbits. A dose of 0.5 ml of acetonitrile was applied to one intact skin site on the back of each rabbit. The test article remained in contact with the skin for 4 hours. The test sites were evaluated for dermal irritation approximately 0.5-1, 24, 48 and 72 hours following patch removal and scored based on the Draize method. All scores at each observation interval were 0 for each animal. No signs of ill health or test article-related effects were observed during the study.

Eye

In studies in five rabbits, one drop of neat acetonitrile was instilled into the eye. After 24 hours the effects were graded as 5 on a scale 1-10 (Smyth and Carpenter, 1948). The same authors (1944, 1946) gave details of the test and of data interpretation. The 1946 paper states that a score of ≥5 represents severe injury. Zeller et al. (1969) tested the acetonitrile, one drop of neat acetonitrile was applied to the conjunctiva sac of the rabbit's eye. The reaction was noted after 10 minutes, 1 and 24 hours, 3 and 8 days. An irritation score of 3, out of a possible maximum of six and strong vascular reaction with oedema or slight necrosis were recorded.

Exposure of pregnant hamsters to 1,800, 3,800, 5,000 and 8,000 ppm acetonitrile via inhalation for 60 min caused irritation to the eyes and the nose in 4 out of 12 in the top concentration group and irritation (without specification) to all exposed to 5,000 ppm. (Willhite, 1983). Corneal opacity has been observed after inhalation of acetonitrile in male mice (Willhite, 1981; Willhite and Smith, 1981).

MPI Research has carried out an eye irritation study of acetonitrile in rabbits in 1997. The study is conforming to EPA/OECD Guidelines. Acetonitrile (HPLC grade, 0.1 ml) was applied to the conjunctiva sac of six males New Zealand white rabbits without rinsing. The eye was held closed for one second and released, while the contralateral eye was treated in a similar way. The eyes were observed at 1, 24, 48 and 72 hours and 4, 7, 14 and 21 days after dosing, and scored by the Draize method. The mean scores for all animals over the period 24-72 hours were as follows: corneal opacity, 1.45; iris lesion, 0.83; conjunctiva redness, 3; conjunctiva oedema, 1.89. These effects were largely resolved in 21 days.

4.1.2.3.2 Studies in humans

Humans accidentally inhaling 500 ppm for brief periods, reported some nose and throat irritation; this dose has been estimated from the human poisoning cases (Admur,1959).

4.1.2.3.3 Summary of irritation

Two well-conducted irritation studies of acetonitrile in skin and eye indicated that acetonitrile is an eye irritant, but not a skin irritant.

Based on insufficient data no conclusions can be drawn regarding the respiratory irritating properties of acetonitrile.

Classification with R36 is proposed. Classification according to Annex I of Directive 67/548/EEC, see Chapter 1.

4.1.2.4 Corrosivity

The studies in animals and humans in Section 4.1.2.3. indicate that acetonitrile is not corrosive to the skin or the eyes.

4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

Skin

According to the OCDE Good Laboratory Practice, Hill Top Research Inc. (1997) has carried out a delayed contact hypersensitivity study with acetonitrile (HPLC grade) in guinea pigs (Buehler Test). In this study, male and female Hartley guinea pigs were exposed via the dermal route to undiluted acetonitrile (>99.9%). Out of the animals (10 male, 10 female) induced and challenged with undiluted acetonitrile, three showed slight, patchyerythema after 24 hours and only one continued to show this reaction after 48 hours. On the grading scale used for this test, the reaction is not considered as indicating sensitisation. The overall score for all animals was 0.1 at 24 hours and 0.03 at 48 hours. Negative control animals (5 male, 5 female) were exposed to distilled water. One animal showed slight, patchy eryteme after 24 hours and two showed this reaction after 48 hours. The overall score was 0.1 after both 24 and 48 hours challenge. The positive control material, α-hexylcinanmaldehyde (85%), caused sensitisation reactions after induction with a 2.5% solution in ethanol and subsequent challenge with dilutions of between 1% and 5% in acetone. Overall scores were between 0.8 and 1.6 for the 10 animals in this group.

4.1.2.5.2 Summary of sensitisation

Negative results were obtained in a well-conducted Buehler test. No classification for this effect is proposed.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in animals

Inhalation

Studies in rat

The National Toxicology Program (1994) has conducted a subchronic inhalation study, according to OCDE guideline No 413, in rats exposed to acetonitrile for 13 weeks.

Groups of 10 male and 10 female F344/N rats were exposed to 0, 100, 200, 400, 800 or 1,600 ppm (equivalent to 0, 168, 335, 670, 1,340 or 2,681 mg/m^3) acetonitrile via inhalation for six hours per day, 5 days per week for 13 weeks. Animals were approximately six weeks old on the 1st day of exposure.

Six male and three female rats exposed to concentrations of 1,600 ppm and one male exposed to 800 ppm died during the study; all but one of these deaths occurred during the first two weeks of the study. At exposure concentrations up to and including 800 ppm, the final mean body weights and body weight gains were generally similar to controls. At the 1,600 ppm concentration, body weight gains were lower than controls; the final mean body weights were 81% of the control value in males and 91% of the control value in females. Hypoactivity and ruffled fur were observed in 800 ppm males and 1,600 ppm males and females during the 1st week of the study. Additional clinical findings in 1,600 ppm males that died during week 1 were ataxia, abnormal posture, and clonic convulsions. No other treatment-related clinical findings were observed. Absolute and relative thymus weights of 800 and 1,600 ppm males and females were lower than those of the controls. Females exposed to 1,600 ppm had significantly greater absolute and relative heart, kidney and liver weights than those of the controls. No other organ weight differences were considered biologically significant.

An anaemia evidenced by decreases in red blood cell count, haemoglobin concentration, and haematocrit occurred in the 1,600 ppm males and females and in the 800 ppm female rats. The anaemia was characterised as nonresponsive, normocytic, and normochromic because the reticulocyte counts, mean erythrocyte volume, and mean erythrocyte haemoglobin concentrations in these groups were similar to control values. In the 1,600 ppm female rats, decreases in triiodothyronine (T_3) concentration occurred in the absence of alterations in thyroxine (T_4) and thyroidstimulating hormone (TSH) concentrations.

Minor, sporadic changes in other parameters were considered unrelated to treatment.

Gross and histopathologic changes were restricted to the 800 ppm male and 1,600 ppm male and female rats that died during the study; these included lung lesions consisting of congestion, oedema, and haemorrhage in alveoli.

A spectrum of lesions that included brain haemorrhage, cellular depletion of the bone marrow, thymus atrophy, lymphoid depletion of the spleen (females) and depletion of corpora lutea in the ovary were typically noted primarily in animals dying early.

In this study a NOAEL value of 400 ppm and a LOAEL value of 800 ppm were established.

In a well-conducted 2-year inhalation study carried out by the NTP in F344/N rats, exposure to acetonitrile to 0, 100, 200, and 400 ppm for 15 months or 2 years had no effect on body weight

gain or final mean body weights. Behaviour, general health and appearance of exposed male and female rats were similar to those of the controls throughout the study. There were no significant treatment-related effects on absolute or relative organ weights. At the 15-month interim evaluation, hematocrit values, haemoglobin concentration, erythrocyte count, mean erythrocyte volume, and mean erythrocyte haemoglobin in 400 ppm female rats were minimally lower than controls. Mean erythrocyte volume and mean erythrocyte haemoglobin in 400 ppm males were also minimally lower than controls; however, the erythrocyte count was slightly greater than that of controls. A NOAEL of 200 ppm can be established.

In a 92-day unpublished study (Coate, 1983) summarised in IUCLID and in an EPA report (Health Effects Assessment for Acetonitrile, 1987), acetonitrile was administered by inhalation to Fischer-344 rats at concentrations of 0, 25, 50, 100, 200 and 400 ppm, 6 hours / day, 5 days / week. All groups were composed of 10 animals of each sex. One male in the 400 ppm group died during the study. Parameters evaluated included body and organ weight, clinical chemistry, haematology, sperm count, motility and morphology, vaginal cytology, levels of serum T_3 or TSH or both, urinary cyanide levels and the histology of the major organs from the control group and the 400 ppm exposure group, the liver from the 100 and 200 ppm exposure groups and the nasal turbinates from all exposure groups. The only statistically significant effects observed in rats were slightly decreased mean leukocyte counts in males exposed to ≥100 ppm of acetonitrile and females exposed to 400 ppm of acetonitrile. Body weights were slightly increased during the study in females exposed to ≥100 ppm and males exposed to 400 ppm, but there were no statistically significant differences in terminal body weights between test and control rats. The biological significance of the decreased leukocyte counts is unclear. This effect was observed in mice too, but did not appear to indicate specific T-or B- lymphocyte dysfunction. Males exposed to 400 ppm of acetonitrile had slightly increased heart-to-body weight ratios, but no cardiac histopathological alterations. Hepatocyte vacuolisation was observed in all groups evaluated including control groups but the intensity of the vacuolisation was slightly greater in the female rats exposed to 400 ppm of acetonitrile. No other histopatological alterations were reported. Based on this study, EPA reported a NOAEL of 200 ppm.

Wang et al. (1964) reported that there was no change of iodine levels in the thyroid of Wistar rats exposed to 80 or 400 mg acetonitrile /m^3 (4 hours / day, 6 days / week) for 10 weeks (cited in IPCS).

In a published study from Pozzani et al. (1959a), Wistar rats were exposed to 0, 166, 330, and 655 ppm acetonitrile vapour, 7 hours / day, 5 days / week, during 90 days. All groups were composed of 15 animals of each sex; however, there were two sets of control groups (60 animals altogether). The body weight gains and the organ weights did not differ significantly from the controls. Furthermore, the few deaths that occured were attributable to extraneous infection, and their incidence was unrelated to concentration.

The tissues of the rats that survived the inhalation of acetonitrile for 90 days were examined microscopically. Out of the 28 rats having inhaled 166 ppm vapour, one had histiocyte clumps in the alveoli, and another had atelectasis. Only three cases of tissue abnormality were observed in the 26 rats having inhaled 330 ppm of vapour. These involved lung changes such as bronchitis, pneumonia, atelectasis, and histiocyte clumps in the alveoli. A small lung granuloma was found in one of the 29 rats, which served as controls for these two inhalation levels. After the inhalation of 655 ppm acetonitrile vapour, 10 out of 27 animals showed alveolar capillary congestion and/or focal oedema in the lug, often accompanied by bronchial inflammation,

desquamation and hypersecretion. Tubular cloudy swelling of the kidneys in eight rats and swelling of the livers of seven rats were observed.

Statistical analysis showed that these lung, kidney and liver effects occurred to a significantly greater degree than in the control animals with P-values of 0.001, 0.005 and 0.04, respectively. No lesions were found in the adrenals, pancreatic, spleens, testes and tracheas examined. Focal cerebral haemorrhage was observed in one of the five brains examined. The significance of the effects in the lower groups is unclear.

Some thiocyanate was excreted by the rats having inhaled 330 and 166 ppm acetonitrile vapour, although the amount of thiocyanate excreted was not proportional to the concentration of acetonitrile inhaled. The urinary thiocyanate apparently was not excreted entirely during the 17-hour rest period between inhalation periods but was virtually all excreted during the 2.5-day rest period over the weekend. The haematocrit and haemoglobin values of the five female rats having inhaled 655 ppm acetonitrile did not differ significantly from the controls.

A NOAEL <166 ppm and a LOAEL of 166 ppm were established.

Roloff et al. (1985, abstract) exposed groups of male and female rats (strain unspecified) to acetonitrile vapour: 0, 618, 1,847, 6,239 ppm (0, 1,038, 3,104, 10,485 mg/m^3) for one month (6 hours / day, 5 days / week). A citation of this summary states that more of 40 tissues were examined per animal (Johannsen and Levinskas, 1986). A full report of this study has apparently not yet been published. Death and reduced body weight gains were observed at the highest exposure level. Respiratory and/or ocular irritation were noted in animals exposed to 1,847 and 6,239 ppm. Mild anaemia was seen in animals exposed to acetonitrile; it is not clear at which concentration. A NOAEL of 618 ppm was established.

Studies in mice

The National Toxicology Program (1994) has conducted a subchronic inhalation study in mice exposed to acetonitrile for 13 weeks. Ten mice (B6C3F1) of each sex were exposed to acetonitrile vapour at 0, 100, 200, 400, 800 and 1,600 ppm, 6 hours / day, 5 days / week. All groups were composed of 10 mice of each sex.

All male and female mice in the 1,600 ppm groups died by week 3 of the study. Six additional animals, one female receiving 400 ppm and one male and four females having received 800 ppm also died before the end of the study. The final mean body weights and body weight gains of all female exposure groups that survived were similar to controls. The final mean body weight of all exposed male groups were slightly lower than that of the controls but was only statically significant at 800 ppm. Hypoactivity and a hunched, rigid posture were observed in 800 and 1,600 ppm mice during the first week of the study.

Nor chemical biochemistry nor haematology were carried out in this study.

Absolute liver weights were significantly greater than those of the controls in males exposed to concentrations of 200 ppm and above and in 800 ppm females; relative liver weights were significantly, greater in all exposed male groups and in females exposed to 400 ppm or higher.

Microscopic lesions were observed in the forestomach, liver, and adrenal gland. Grossly, focal or multifocal pale, dark brown or black lesions were consistently observed in the mucosa of the anterior forestomach of male and female mice exposed to concentrations of 200 ppm (7), 400 ppm (8), 800 ppm (8) and 1,600 ppm (5), in females and in males the incidence was 400 ppm (5), 800 ppm (6) and 1,600 ppm (1).

Microscopically, these lesions corresponded to focal or multifocal squamous epithelial hyperplasia. The average severity of these lesions was similar between exposure groups, with the exception of female mice exposed to 200 ppm in which the lesions were less prominent.

Focal ulcers associated with areas of epithelial hyperplasia occurred in one female exposed to 200 ppm and one male and five females exposed to 1,600 ppm. A high incidence of hepatocellular cytoplasmic vacuolation occurred in the male and female mice exposed to 400 ppm and 800 ppm. Vacuolation appeared to be a slight distension of preexisting cytoplasmic clear spaces and is considered to represent increased glycogen storage. The absence of such changes in hepatocytes of 1,600 ppm mice that died may be indicative of increased utilisation of hepatocyte glycogen stores.

Fatty degeneration was observed in the X-zone of the adrenal cortex in female control mice and female mice exposed to 200 ppm and to a lesser extent, in female mice exposed to 400 ppm. This change, which represents normal age-related regression or involution of this zone of the adrenal cortex, was absent in females exposed to 800 or 1,600 ppm. The absence of such a change in female mice exposed to 800 or 1,600 ppm may be an indication of stress- or exposure- related acceleration of this normal process.

Additional alterations that occurred only in mice that died during the study, including lymphoid depletion and lymphocytolysis in the thymus, spleen, and bone marrow and pulmonary congestion were considered to be non-specific changes typically observed in moribund animals.

In this study a NOAEL of 100 ppm for females and of 200 ppm for males were established.

In a 2-year study carried out by the NTP, B6C3F1 mice were exposed to 50, 100 and 200 ppm of acetonitrile, 6 hours / day, 5 days / week. Survival rates of exposed female mice and male mice exposed to 50 or 100 ppm were similar to those of the controls. Survival of male mice in the 200 ppm group was significantly greater than that of controls. Exposure to acetonitrile by inhalation for up to two years had no effect on body weight gain or final mean body weights of male and female mice. Clinical observations were not considered treatment-related. There were no significant treatment-related effects on absolute or relative organ weights. Neither chemical biochemistry nor haematology was carried out in this study. On the other hand, it was showed that the effect on the forestomach that appeared in the 13-week study is insufficient to attribute it to the chemical with any confidence.

In an unpublished study (Coat, 1983), sumarised in IUCLID and in an EPA report (Health Effects Assessment for acetonitrile, 1987), groups of 10 male and 10 female B6C3F1 mice were exposed to 0, 25, 50, 100, 200 or 400 ppm of acetonitrile vapour, 6 hours / day during 92 days (Coate, 1983). One male in each of the 50, 200 and 400 ppm groups died. There was an increase in body weight gain in all males exposed to 50, 100, 200 and 400 ppm, and in females from the 200 and 400 ppm groups; body weight gain was decreased by comparison with controls in the 25, 50 and 100 ppm female groups.

The statistically significant effects in mice included decreased blood urea nitrogen levels, red blood cell cholinesterase counts and haematocrits in females exposed to 200 or 400 ppm of acetonitrile. Hepatic vacuolisation and hypertrophy were observed at all dose levels evaluated including controls, but the intensity of the hepatic lesions appeared greater in male and female mice exposed to 200 or 400 ppm of acetonitrile. Liver/body weight ratio was increased in males in the 400 ppm group and in females in the 100, 200 and 400 ppm groups. Liver/brain weight ratio was increased in males in the 400 ppm and in females in the 100 and 400 ppm groups. A dose-related decrease in leukocyte count and serum Ig G levels occurred but did not appear to

indicate specific T- or B- lymphocyte dysfunction, and the biological significance of these findings is unclear.

A NOAEL value of 100 ppm was derived by EPA from this study, given that 200 ppm was associated with decreased blood urea nitrogen, erythrocyte counts and hematocrits females and increased intensity of hepatocytic vacuolisation and hypertrophy. No adverse effects in mice were noted at 100 ppm.

In an unpublished study cited in EPA (1987) and IUCLID, Immuquest Labs-Inc. (1984) exposed groups of unspecified numbers of female B6C3F1 mice to acetonitrile at 0, 100, 200 or 400 ppm, 6 hours / day, 5 days / week during 90 days. There were no effects on physical appearance, body weights or gross appearance at necropsy. Mice in the 200 and 400 ppm groups had atrophy of thymus, which was noted upon histopathological examination. Slight vacuolisation of hepatocytes accompanied by hydropic degeneration was also observed at 400 ppm. There were no treatment-related effects on selected clinical chemistries, but mice at 200 and 400 ppm had dose-related decreases in haematocrits, blood haemoglobin concentration, erythrocyte and leukocyte counts. Changes in haematological parameters were not significant at 100 ppm.

No significant effects on organ weights were observed, but the investigations noted a trend toward depressed thymus weights at 200 and 400 ppm.

After 14 days, Immuquest Labs evaluated immune function in mice exposed by the same protocol. A significant dose-related depression of serum concentration of Ig G was observed in all exposed mice, but no effects were observed on the Cunningham plaque-forming response to sheep erythrocytes, the lymphocyte blastogenesis test, delayed hypersensitivity response or susceptibility to challenge with PyB6 tumour cells. A NOAEL of 100 ppm was established given that 200 ppm was associated with decreased red blood cell cholinesterase, hematocrits, haemoglobin and white blood cell.

Study in rabbits, dog and monkeys

Degenerative changes in the epithelial cells of thyroid follicles were observed in rabbits exposed to 400 mg/m^3 (4 hours / day, 6 days / week) for 16 weeks (Wang et al., 1964, cited in IPCS).

Three male dogs inhaled 350 ppm acetonitrile (7 hours / day, 5 days / week) for 91 days (Pozzani et al., 1959). A statistically significant drop in the mean body weight was observed on the 3rd and 5th inhalation days. The haematocrit and haemoglobin values of the three dogs were depressed the 5th week of inhalation, but with the exception of one dog. There was a return to preinhalation values toward the end of the 91-day inhalation period. The depressions in those values were not accompanied by any significant deviation in the erythrocyte counts. No significant gross pathologic change was seen in the dogs. Histological examination of the tissues revealed some focal emphysema and proliferation of alveolar septa. LOAEL of 350 ppm.

Two studies have been carried out with Rhesus monkeys. Four Rhesus monkeys were involved in a 99-day inhalation study (7 hours / day, 5 days / week): one female was exposed to 2,510 ppm, two females to 660 ppm and one male to 330 ppm (Pozzani et al, 1959). None of the four monkeys experienced an appreciable weight loss during their respective inhalation periods. The monkey at the highest concentration 2,510 ppm, appeared normal after the 1st inhalation day but showed poor co-ordination followed by prostration and laboured breathing during the 2nd inhalation day. Death occurred a few hours later.

In the two monkeys exposed to 600 ppm there was also incoordination from the 2nd week. One monkey died on day 23 and the other on day 51. The monkey which inhaled 330 ppm exhibited over-extension reflexes and considerable excitability toward the end of its 99-day inhalation period and then was sacrificed. These three animals on gross examination showed focal dural or subdural haemorrhage in the parietal and/or occipital tissues immediately adjacent to the superior sagittal sinus. Engorgement of the dural capillaries and pleural effusion was found in the female, which died at the 2,510 ppm level. Tissues from monkey, which inhaled 2,510 ppm, were not examined microscopically because some autolysis occurred before autopsy. Examination of the tissues from monkeys, which inhaled 660 ppm, revealed focal areas of emphysema and atelectasis with occasional proliferation of alveolar septa. Focal cloudy swelling of the proximal and convoluted tubules of the kidneys was seen. The cause of death was not explained by the lesions observed in the tissues examined microscopically. The monkey, which had inhaled 330 ppm vapours, had chronic pneumonitis evidenced by diffuse proliferation of alveolar septa, monocytic infiltration, and pleural adhesions. The cause of death of the two others was not explained by the lesions observed in the tissues examined microscopically.

In another inhalation study (Pozzani et al, 1959), three male Rhesus monkeys were exposed to 350 ppm acetonitrile (7 hours / day, 5 days / week) for 91 days. A the end of the study the animals were sacrificed. At autopsy, slight to moderate haemorrhage of the superior and inferior sagittal sinuses were found in the brains of the three monkeys. Small discrete caseous nodules were seen in the lungs of two monkeys, as well as a pale liver in one of these two. Microscopic examination of tissues showed focal emphysema and diffuse proliferation of alveolar septa. Acute bronchitis was observed in one animal, and lung tissues from all three monkeys invariably contained focal accumulations of pigment-bearing macrophages.

Cloudy swelling of the convoluted kidney tubules, primarily proximal, was noted in two of the three monkeys.

NOAEL < 330 ppm, LOAEL = 330 ppm.

Oral and Dermal routes

Data regarding repeated dose toxicity of acetonitrile could not be located in the available literature.

Other routes

Marine et al. (1932a) studied the production of goiter and exophtalmos in prepubertal rabbits following s.c. administration of acetonitrile for up to 63 days. Male and female rabbits of Dutch and Belgian breeds, aged 3-5 months and weighing 1,184-1,911 g, were given daily injections of 79-118 mg/cc of acetonitrile. Exophthalmos developed as early as day 20 in the 3-month-old rabbits that had received daily injections of 79 mg of acetonitrile. According to the authors, this effect was seen only in the young Dutch rabbits and did not occur at all in the adult rabbits (6 months and older) of either strain. The authors noted a close relationship between exophtalmos and thyroid hyperplasia. Exophthalmos was absent in rabbits that showed little or no thyroid hyperplasia. When hyperplasia was more intense, exophthalmos appeared and was said to be proportional to the degree of hyperplasia. Exophthalmos is commonly a symptom of hyperthyroidism, but the authors did not speculate whether its occurrence was a direct or thyroid-mediated effect.

Marine et al. (1932b) investigated the production of thyroid hyperplasia in rats acetonitrile given in small doses. Twelve female albino rats, 6 litter mates aged 3 months and 6 litter mates aged 5 months were divided into three groups, 2 rats from each litter. The animals were fed a nongoitrogenic diet and received daily s.c. injections of acetonitrile in water at doses of 0.08 cc (62.4 mg), 0.04 cc (31.2 mg), and 0.02 cc (15.6 mg). At the end of 21 days, one rat from each group was killed. At autopsy all animals showed only slight thyroid hyperaemia. After 28 days of treatment, the rats showed definite thyroid hypertrophy. During the next eight days, the daily doses were gradually increased for the remaining nine rats until those initially on the smallest dose were receiving 0.05 cc (39 mg), and those initially on the largest dose were receiving as much as 0.15 cc (117 mg) of acetonitrile daily without any sign of cyanide poisoning. After 36 days of treatment, the thyroids were larger with increased hyperaemia. In general, these changes were proportional to dose.

A similar study in mice was carried out by Marine et al. (1932b). Twelve mice, 3.5 weeks old, weighing an average of 13 g and on the same diet as the rats, were divided into three groups to receive daily s.c. injections of acetonitrile at doses of 0.005 cc (3.9 mg), 0.0025 cc (1.95 mg) and 0.00125 cc (0.975 mg). After 11-34 days, only a slight thyroid reaction was produced. From these results, the authors concluded that, because thyroid reactions obtained in rats and mice exposed to acetonitrile was far less than those reported in rabbits receiving much smaller doses, rats and mice possess considerable resistance to goitrogenic substances.

4.1.2.6.2 Studies in humans

No data are available in humans.

4.1.2.6.3 Summary of repeated exposure studies

There is no information available on the effects of repeated exposure to acetonitrile in humans.

Mice appeared somewhat more susceptible to acetonitrile toxicity than rats. Mortality in the 13-week mouse study was complete at 1,600 ppm, and deaths of female extended down to the 400 ppm group.

In a 13-week study on rats, carried out by NTP, a NOAEL of 400 ppm is obtained given the haematology effects found in the superior doses. Later the NTP carried out an inhalation 2-year study in which it was established a 200 ppm NOAEL due to a slight anaemia observed in concentration of 400 ppm. Preliminary data from a not fully reported study (Coate, 1983, sumarized in EPA, 1987 and IUCLID) showed no adverse effects observed at 200 ppm.

In a 13-week study, carried out by NTP in mice, a NOAEL of 100 ppm was obtained due to the forestomach lesions. However, a 2-year study carried out later by NTP concluded that these findings established an effect of prolonged acetonitrile exposure on the forestomach of mice, but the magnitude of the neoplastic findings were insufficient to attribute them to the chemical with any confidence. In addition, other effects seen in the 13-week study such as increased ratio of liver weight / body weight and increased absolute liver weight, were not seen when evaluated at either 15 or 24 months in a life time study. In the 13-week study there were no other findings. However, this study as well as the 2-year study lacked of chemical biochemistry and haematology. Preliminary data from two unrelated not fully reported studies (Coate, 1983; Immuquest Labs-Inc., 1984, both summarised in EPA 1987 and IUCLID) showed a NOAEL of 100 ppm based on haematological data in B6C3F1 mice.

Dogs and monkeys exposed 7 hours / day, 3 days / week for 91 days at a mean acetonitrile vapour concentration of 350 ppm showed minor variations in body weight, haematocrit, and haemoglobin. Both, monkeys and dogs had pulmonary abnormalities.

Autopsy of monkeys noted cerebral haemorrhage and cloudy swelling of the kidney tubules was noted in two of the three exposed monkeys. Monkeys were more sensitive than dogs to the same dose of inhaled acetonitrile (350 ppm).

It has been noted that an intramuscular injection of 0.05 to 0.1 ml acetonitrile causes exophthalmia and thyroid hyperplasia in rabbits. The degree of exophthalmia could be related to the hyperplasia. Thyroid hyperplasia could also be induced by acetonitrile in rats and mice but did not develop phthalmia. Rats and mice showed posses considerable resistance to goitrogenic substances.

4.1.2.7 Mutagenicity

4.1.2.7.1 Studies in animals

In vitro studies

Several test methods for investigating the mutagenicity and genotoxicity of acetonitrile are available *in vitro*.

Bacterial studies

In a screening of tobacco smoke constituents for mutagenicity using the Ames test carried out by Florin et al. (1980), acetonitrile was assayed qualitatively using strains TA98, TA100, TA1535 and TA1537 with and without S-9 from aroclor-induced rats, at 3 μmol/plate and the results were negative.

Schlegelmilch et al. (1988) tested acetonitrile with the Ames test (*Salmonella*/microsome assay) performed with strains TA98 and TA100. The compound was tested with and without metabolic activation, using S9 preparations from phenobarbitone-pretreated and autoinduced rats. Autoinduction was performed by chronic (7 days) application of a dose equivalent to a 5 % oral LD_{50} value of acetonitrile. The concentrations used were 0.27, 1.35, 2.71, 13.6, 27.1, 136, 271 and 1350 mM with both strains in triplicate plates. Acetonitrile was not found to be mutagenic in the Ames/*Salmonella* assay with or without metabolic activation. The concentration 1350 mM results to be toxic with strain TA98 in the presence of S9-mix (phenobarbitone).

Mortelmans et al. (1986) tested acetonitrile in the *Salmonella* preincubation assay, strains TA1535, TA1537, TA97, TA98 and TA100 within a dose range up to 10 mg/plate and in the presence and absence of the metabolic activation systems prepared from Sprague-Dawley rats or Syriam hamster liver pretreated with Aroclor 1254. This study employed a 20-minute preincubation period at 37°C. All tests were repeated using either the same or different S9 concentrations (10% and 30%). Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of acetonitrile 100, 333, 1,000, 3,333 and 10,000 μg/plate. In the absence of toxicity 10,000 μg/plate was selected as the high dose. All assays were repeated. The test was performed in two different laboratories. Under the conditions of the study, acetonitrile did not cause a positive response, with or without metabolic activation, in any of the tested strains.

Yeast studies

Acetonitrile was tested for their genotoxic potential in an assay using *Saccharomyces cerevisiae* (strain D7), with and without metabolic activation (S9 preparations from phenobarbitone-pretreated and autoinduced rats). Ten concentration were used: 360, 405, 450, 495, 540, 585, 630, 675, 720 and 768 mM) in triplicate plates. No reversion from isoleucin auxotrophy to prototrophy exceeding the spontaneous rate could be detected in strain D7 after various doses of acetonitrile. There was no effect on mutation rate by the presence of a metabolic activation system. A weak recombinogenic effect was detectable, when phenobarbitone-induced S9 was added to the incubation mixtures, the number of trp^+ convertants on selective plates increased up to twice the spontaneous number although the corresponding doses were very high (675 and 720 mM). No convertogenic effect could be demonstrated when autoinduced S9 or no metabolic activation system was added to the incubation mixtures (Schlegelmilch et al., 1988).

Acetonitrile induced chromosome loss *Saccharomyces cerevisae* strain D61 M, with or without a cold interruption phase and in the absence of metabolic activation (Whittaker et al., 1989). The study was carried out in two laboratories with similar results. The following additions are made to 2ml of D61M culture: 1, 3, 5, 10, 20, 30, 50, 75 and 100 µl, to yield final concentrations of 0.05-4.76% (v/v), respectively. Positive and negative controls are also included. Cultures are incubated according of the two following protocols:

- Standard 16-h incubation at 30°C (tubes are shaken at 300 rpm at a 45° angle).
- Cold interruption (tubes are incubated at 30°C for 4 hours, transferred to an ice/water bath for 16 hours, vortex mixed, warmed for 2 min in a 30°C water bath and further incubated at 30° C for 4 hours).

Zimmerman et al. (1985) obtained similar results using 2.91% and 4.76% acetonitrile. All treatments started by pipetting the acetonitrile into a growing cell culture at a titer between 3 and 10 millions cells per ml, 4 hours of incubation at 28°C were followed by a storage of about 17 hours in ice. Cells were plated after 4-5 hours of additional incubation at 28°C. Acetonitrile strongly induced aneuploid but not recombination or point mutation.

Groschel-Stewart et al. (1985) demonstrated that the aneuploid-inducing activity of aprotic polar compounds in yeast was associated with their ability to block assembly *in vitro* of porcine brain tubulin. In the case of acetonitrile, this block could be achieved at concentrations much lower than those necessary to induce aneuploid in yeast.

Mammalian cells studies

A well-conducted gene mutation assay (HGPRT locus) in cultured Chinese hamster ovary (CHO) cells was carried out by Bioassay Systems Corporation (1984). This assay was conducted both in the absence and presence of Aroclor-1254-induced rat liver metabolic activation. The optimal dose levels for the mutation assay were selected following a preliminary toxicity test based on colony forming efficiency. Approximately 40% of the cells survived at 15.4 mg/ml while less than 0.03% survived at 77.1 mg/ml.

Based on those results concentrations ranging from 0.1 to 30 mg/ml were selected for the first nonactivated mutagenesis assays and 8 concentrations ranging from 1-25 mg/ml were tested in the 2^{nd} nonactivated mutagenesis assay. The exposure time was 16 hours. When the assay was carried out in the presence of 0.424 mg/ml of S9, CHO cells were exposed during 4 hours at

37°C to solvent ranging from 4-16 mg/ml in the 1st experiment and 8 concentrations ranging from 8-20 mg/ml in the 2nd experiment.

No significant differences (α = 0.05) between the mutation frequencies of acetonitrile and the negative control were found both in the absence and presence of S9.

Other gene mutation assay was carried out, in this case with L5178Y mouse lymphoma cells and in the presence and absence of metabolic activation system. Acetonitrile was neither mutagenic nor toxic at the maximum tested dose (5 μg/ml) (brief abstract Rudd et al., 1983).

Acetonitrile was tested in the Chromosome Aberrations test and Sister Chromatid Exchanges tests, using chinese hamster ovary cells (Galloway et al., 1987). Acetonitrile was tested both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix.

Each test consisted of concurrent solvent and positive controls and of at least three doses of acetonitrile. In the absence of toxicity, 5,000 μg/ml was selected as the high dose. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

In the Chromosomal Aberrations test, without S9, cells were incubated with acetonitrile for 12 hours; in the presence of S9, cells were treated with acetonitrile for 2 hours, after which the treatment medium was removed and the cells were incubated 12 hours in fresh medium. The concentrations tested were 500, 1,600 and 5,000 μg/ml both with and without S9. One hundred first-division metaphase cells were scored at each dose level. Result obtained showed a slight increase in the incidence of chromosome aberrations at 5,000 μg/ml ($P<0.05$) in the presence of S9. No induction of aberrations occurred in the absence of S9.

In the SCE test without S9, CHO cells were incubated for 26 hours with acetonitrile at 160, 500, 1,600 and 5,000 μg/ml doses. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. In the SCE test with S9, cells were incubated with acetonitrile (500, 1,600 and 5,000 μg/ml) and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and incubation proceeded for an additional 26 hours. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level. Acetonitrile produced a positive response without S9 at 5,000 μg/ml ($P<0.01$); with S9, no increase in SCE was noted. This result was considered to be weakly positive (increase at any single dose).

In vivo studies

Fix and Zeste genetic test systems employing female Drosophila melanogaster were performed by Osgood et al. (1991a, 1991b). Acetonitrile was found to induce chromosome loss as well as chromosome gain in both assays.

Acetonitrile was administered by feeding to either early third instar larvae (LF3), late third instar (LF4) in one experiment, or to young adult (AF) females. The concentrations tested were 2,000, 5,000, 20,000 and 50,000 ppm, the solvent system used was water.

With Fix, acetonitrile was strongly positive only after feeding to third instar larvae (LF3).

With Zeste, the chemical induced aneuploid after adult feeding (AF) with no discernable effect after LF3. Zeste larvae were substantially more sensitive to acetonitrile with 78% mortality at 20,000 ppm compared to 30% for Fix larvae. The magnitude of effect, the increment relative to the concurrent control was approximately 2-fold for Fix and 5-fold for Zeste (Osgood et al., 1991a).

The Drosophila Zeste system was used to monitor the induction of sex chromosome aneuploid following inhalation exposure of adult females to acetonitrile.

Acetonitrile was a highly effective aneuploidogens, inducing both chromosome loss and chromosome gain following short exposure to a concentration of 131 ppm (Osgood et al., 1991b). Adult females (2-3 days old) were used. Exposures to 0, 10, 30, 50 and 70 min were carried out. Statistically significant increases in the frequency of aneuploid offspring were detected following as little as 10-min exposure. After 30 and 50 min exposures, the combined frequency of aneuploid offspring was approximately 0.18% compared to a control frequency of 0.024%, a 7-fold increment.

No induction of unscheduled DNA synthesis was observed in rat hepatocytes exposed *in vivo* or *in vitro* to acetonitrile (Mirsalis et al., 1983, abstract).

Acetonitrile was tested in micronucleus test in NMRI mice (13 weeks old) (Schlegelmich et al., 1988). Two dose levels were tested amounting to a 40 and 60% LD_{50} (340 and 510 mg/kg) via i.p. The LD_{50} data were obtained from RTECS. The rat i.p. LD_{50} value presented in RTECS is 850 mg/kg. For each dose level and day, 4 male and 4 female NMRI-mice. The animals were killed 24, 48 and 72 hours after injection of a single dose. Bone marrow was obtained and the number of micronucleated polychromatic erythrocytes was counted by scoring 1,000 to 1,200 cells per animal from coded slides. In this study no assessment of bone marrow cytotoxicity neither control data for 48 hours and 72 hours time points were reported.

When the assay was carried out with autoinduction, the mice received daily a dose of the acetonitrile equivalent to 5% of the oral LD_{50} value for seven days. On the 8^{th} day i.p.injection of the high single dose followed.

Without autoinduction, the number of micronuclei induced by acetonitrile is higher than under any other condition, when a dose equivalent to a 60% LD_{50} value was administered and the animals were killed 24 hours after application of the compound. The frequency of micronuclei containing polychromatic erythrocytes under these conditions reached 4.26 per 1,000, compared to 1.68 per 1,000 in negative controls. However, autoinduced mice showed a peak nearly as high as this at 72 hours after administration of the compound, but no effect at 24 hours after application. This study did not include control micronucleus frequencies for comparison to the "induced" levels.

Peripheral blood samples were obtained from male and female B6C3F1 mice treated for 13 weeks with acetonitrile (100, 200, 400 and 800 ppm) via inhalation to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes in each of 10 animals per dose group.

Results with female mice were negative but in males, a small but significant increase ($P<0.006$) in micronucleated normochromatic erythrocytes was observed in the 400 ppm group. The incidence was lower in the highest exposure group (800 ppm). The survival rate was lower in the highest group, which may possibly account for the lower MN rate, but the association between exposure and micronuclei was weak in the absence of a strong dose-response relationship. In addition, no positive control group nor bone marrow cytotoxicity was reported (NTP, 1994).

A new study of Genetic Toxicology (Micronucleus test *in vivo*) has been recently conducted according to OCDE Guidelines. The study entitled "Acetonitrile: mouse bone marrow and peripheral blood micronucleus test" was carried out from Zeneca Central Toxicology (1998). In this study, male and female NMRI mice were exposed through the intraperitoneal route to undiluted acetonitrile (>99.9%). A single intraperitoneal dose was given to groups of 5 males at

a dose level of 100 mg/kg and to groups of 5 female mice at a dose level of 125 mg/kg. In each case the dose level used represents the maximum tolerated dose (MTD). Bone marrow samples were taken 18, 24 and 36 hours after dosing. Peripheral blood samples were taken prior to treatment (0 hour) and then at 24, 48, 72 and 96 hours after dosing. Effects upon bone marrow were evaluated by comparison with a control group at the same time point, whereas effects upon peripheral blood were evaluated by comparison both with a control group at the same time point and with the treatment group at the 0h time point.

No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values, were seen in the bone marrow of males at any of the sampling times investigated.

A small, but statistically significant increase in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control value, was observed in the bone marrow of females at the 36-hour sampling time. The value observed (0.7 MPE/1000 PE) was very close to the control value seen at the 24-hour sampling time (0.6 MPE/1000 PE). Therefore, it is not considered to be of biological significance. No statistically or biologically significant increases in the incidence of micronucleated polychromatic erythrocytes, over the vehicle control values or over the 0 hour control values, were seen in the peripheral blood of males or females at any of the sampling times investigated.

Comparison of the percentage of polychromatic erythrocytes showed a statistically significant increase in the bone marrow of males treated with acetonitrile at the 18 hour sampling time. Small increases in the percentage of polychromatic erythrocytes, over the 0-hour controls, were also observed in the peripheral blood of males at 48 and 72 hours after treatment and in females at 72 hours after treatment. These increases suggest that the bone marrow have been subjected to stress at maximally tolerated doses of acetonitrile.

4.1.2.7.2 Studies in humans

There is no information available.

4.1.2.7.3 Summary of mutagenicity

There are several data available on the genotoxicity of acetonitrile *in vitro* studies.

The information in bacteria indicates that acetonitrile is not mutagenic in Salmonella assay as well as when the assay is carried out employing a 20-minute preincubation period at 37°C. Acetonitrile induces mutations neither in chinese hamster ovary cells nor in mouse lymphoma L5178Y cells. These tests were conducted with and without S9 metabolic activation enzymes.

In cytogenetic tests with chinese hamster ovary cells, acetonitrile induced slight increase in sister chromatid exchanges without S9 and chromosomal aberrations with S9 for both endpoints; the increases were noted at the highest dose tested. In the SCE assay these increases were considered weak evidence of activity (increase at any single dose). In the chromosomal aberrations test the result was considered equivocal (absence of a statistically significant increase at any dose).

Positive results have been reported in assays, which measure the induction of aneuploid events.

Acetonitrile was found to be a potent inducer of aneuploid. No point mutations or recombination in a diploid strain of *Saccharomyces cerevisae* were found, even with relatively high concentrations.

Acetonitrile induced sex chromosomal aneuploid (both chromosome loss and chromosome gain) in oocytes of female *Drosophila melanogaster* fed an aqueous solution of the chemical either as larvae or as adults.

No induction of unscheduled DNA synthesis was observed in rat hepatocytes exposed *in vivo* or *in vitro* to acetonitrile.

In addition, weakly positive results were reported in a bone marrow micronucleus test with acetonitrile administered by intraperitoneal injection to male and female NMRI mice and a significant increase in micronucleated normochromatic erythrocytes was observed in peripheral blood samples from male mice treated with acetonitrile for 13 weeks; the frequency of micronucleated erythrocytes in female mice was not affected by exposure to acetonitrile. However, the interpretation of these findings is difficult, the i.p. study is the non-standard protocol and in the inhalation study, the association between exposure and micronuclei was weak in the absence of a strong dose-response relationship, in addition, no positive control group or bone marrow cytotoxicity was reported.

Negative results have been reported in a well-conducted Micronucleus test *in vivo* carried out on NMRI mice via intraperitoneal route.

In conclusion, acetonitrile did no induce gene mutations in bacteria, showed week clastogenic activity in cultured mammalian cells and was not clastogenic in a well-conducted *in vivo* micronucleus test. No classification for this effect is proposed.

4.1.2.8 Carcinogenicity

4.1.2.8.1 Studies in animals

The carcinogenicity of acetonitrile has been investigated in rats and mice using the inhalation route. These studies were carried out in compliance with the Good Laboratory Practice (NTP, 1994).

Rats

From the NTP subchronic rat inhalation study, the following acetonitrile exposure levels were selected for the 2-year inhalation study: 100, 200 and 400 ppm, based on reduced survival and gross and histopathologic lesions.

Groups of 56 male and 56 female Fischer 344 rats were exposed to acetonitrile by inhalation at concentrations of 0, 100, 200 or 400 ppm (equivalent to 0, 168, 335 or 670 mg/m^3) for 6 hours per day, 5 days per week for 103 weeks. Eight male and eight female rats from each exposure group were evaluated at 15 months.

The animals were observed twice daily for mortality and signs of toxicity or moribundity. Individual clinical observations were recorded every 4 weeks. The animals were weighed initially, weekly for the first 13 weeks, and at 4-week intervals thereafter. During the final 13 weeks of the study, body weights and clinical findings were recorded every two weeks.

Blood for haematology determinations was taken from the retro-orbital sinus of rats at the 15-month interim evaluation.

Necropsies were performed on all animals. Organs weighed at the 15-month interim evaluations were liver, lungs, and right kidney.

Complete histopathologic examinations were performed on all animals. In addition to gross lesions and tissue masses with regional lymph nodes, tissues examined included: adrenal gland, brain, bone and marrow, clitoral gland, oesophagus, heart, kidney, large intestine, larynx, liver lung, lymph nodes, mammary gland, nose, ovary, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder and uterus.

Survival rates of exposed rats were similar to those of the controls. Exposure to acetonitrile via inhalation for 15 months or 2 years had no effect on body weight gain or final mean body weights, and the behaviour, general health, and appearance of exposed male and female rats were similar to those of the controls throughout the study. There were no significant treatment-related effects on absolute or relative organ weights.

At the 15-month interim evaluation, haematocrit values, haemoglobin concentration, erythrocyte count, mean erythrocyte volume, and mean erythrocyte haemoglobin in 400 ppm female rats were minimally lower than controls. Mean erythrocyte volume and mean erythrocyte haemoglobin in 400 ppm males were also minimally lower than controls; however, the erythrocyte count was slightly greater than that of controls.

There was no evidence of significant exposure-related nonneoplastic lesions in male or female rats at 15 months or 2 years. The only questionable finding was the incidences of the hepatocellular adenoma (6%), carcinoma (6%) and adenoma or carcinoma (combined 10%) with a statically significant positive trend in male.

The incidences of hepatocellular adenoma were 0% at 0 ppm, 2% at 100 ppm, 2% at 200 ppm and 6% at 400 ppm. The incidences of hepatocellular carcinoma were 2% at 0 ppm; 0% at 100 ppm; 0% at 200 ppm and 6% at 400 ppm. And the incidences of hepatocellular adenoma or Carcinoma (combined) were 2% at 0 ppm, 2% at 200 ppm and 10% at 400 ppm.

The historical incidence of liver neoplasms in untreated male F 344/N rats were: range 0%-8% for hepatocellular adenoma, range 0%-4% for hepatocellular carcinoma and range 2%-8% for hepatocellular adenoma or carcinoma. Comparisons between exposure groups and controls were not significantly different. The incidence of hepatocellular carcinoma and the incidence of adenoma and carcinoma (combined) in the 400 ppm group of male was only slightly greater than the historical range for inhalation study controls, range 0%-4% and range 2%-8%, respectively.

The incidence of basophilic foci was greater in 200 ($P \leq 0.05$) and 400 ($P \leq 0.01$) ppm males than in controls and the incidences of eosinophilic and mixed cell foci we marginally greater in 400 ppm males than in controls.

The incidences of liver lesions in exposed female groups were similar to controls.

Mice

From the NTP subchronic mice inhalation study, the following acetonitrile exposure levels were selected for the 2-year inhalation study: 50, 100 and 200 ppm, based on reduced survival and gross and histopathologic lesions.

Groups of 60 male and 60 female mice B6C3F1, were exposed to acetonitrile via inhalation at concentrations of 0, 50, 100 or 200 ppm (equivalent to 0, 84, 168 or 335 mg/m^3) for 6 hours per day, 5 days per week for 103 weeks. Ten male and ten female mice from each exposure group were evaluated at 15 months.

Clinical examinations, a complete necropsy and microscopic examination were performed on all mice.

Survival rates of exposed female mice and male mice exposed to 50 or 100 ppm were similar to those of the controls. Survival of male mice in the 200 ppm group was significantly greater than that of controls.

Exposure to acetonitrile via inhalation for up to two years had no effect on body weight gain or final mean body weights of male and female mice. There were no significant treatment-related effects on absolute or relative organ weights.

There was an increased incidence of alveolar/bronchiolar adenoma in exposed male mice. The incidence was significantly increased in 200 ppm male (18/50, 36%) and was at the upper limit of the range of historical controls (range 6%-36%, 113/673, 16.8%).

The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) was similarly increased in exposed male mice 21/50 (42%), the range of historical controls was 10%-42% (150/673, 22.3%). In contrast the incidences of alveolar/bronchiolar adenoma and adenoma or carcinoma (combined) was inversely related to dose in female groups. The incidences in female controls were at the upper range of historical controls.

Proliferate lesions of the alveolar bronchiolar region were present as a continuum, with the distinction between hyperplasia, adenoma and carcinoma based on the size and morphologic characteristics of the lesion. Thus, a time chemical-related increased incidence in this neoplasm type would be expected to manifest as an increase in proliferate lesions of all three stages which was not apparent in the current study. Although the incidence of alveolar bronchiolar adenomas (18/50, 36%) in the 200 ppm male group is equal to the highest incidence seen in previous control groups for inhalation studies, the 86% survival of this group at 2 years was quite high and may have contributed to the apparent effect. For these reasons, the increased incidence of alveolar bronchiolar adenomas in the 200 ppm males was not attributed to acetonitrile exposure.

The incidence of hepatocellular carcinoma was greater than controls in males exposed to 100 ppm (13/49, 27%). The incidence of hepatocellular adenoma or carcinoma (combined) was similarly increased in 100 ppm males (30/49, 61%); this increase was significant and exceeded the range for hepatocellular adenoma o carcinoma (combined) of historical controls (range 11%-56%; 241/673, 35.8%). However, the incidence of hepatocellular neoplasms in the 200 ppm groups was less than that in the controls, and there was no indication of an effect of acetonitrile exposure on hepatocellular neoplasm in the females. The lack of evidence for a dose response in hepatocellular neoplasms in males coupled with an absence of confounding factors that would be expected to decrease the neoplasm response, such as the markedly lower body weight of 200 ppm males, suggests that this is a sporadic finding unrelated to acetonitrile exposure.

Effects on the forestomach were noted in exposed mice, to acetonitrile. At 15 month, the incidence of squamous hyperplasia of the forestomach was greater than controls in males and females exposed to 50 or 200 ppm; however, the increase was only significant in 200 ppm females ($P \leq 0.01$). At 2 years, the increase was dose-related in exposed groups; the increases were significant in male mice exposed to 200 ppm ($P \leq 0.05$) and female mice exposed to 100 or 200 ppm

(P≤0.01). Hyperplasia was generally focal and have minimal to marked severity. Minimal lesions were characterised by slight thickening of the stratum spinosum frequently accompanied by slight thickening of the overlying keratin layer and increased numbers of basal cells. Increasing severity was accompanied by progressive epithelial thickening and folding. In markedly severe lesions, folds of thickened epithelium projected above the mucosal surface. Focal ulcers and suppurative inflammation occurred in some severe lesions.

A few squamous cell papillomas were noted in mice exposed to 100 or 200 ppm, and one occurred in a control female. Papillomas were exophytic pedunculated and frond-like masses composed of hyperplastic, sometimes hyperkeratotic, squamous epithelium supported by a branched core of fibrous connective tissue stroma. The incidences of this benign tumour were not statistically significant in males or females and were within the ranges of historical controls.

The authors concluded that these findings established an effect of prolonged acetonitrile exposure on the forestomach of mice, but the magnitude of the neoplastic findings was insufficient to attribute them to the chemical with any confidence.

4.1.2.8.2 Studies in humans

Ott et al. (1989) researched lymphatic and haematopoietic tissue cancer in a chemical manufacturing environment. Exposure Odds Ratios (OR) for non-Hodgkins Lymphoma (52 cases), multiple myeloma (20 cases), non-lymphocytic leukaemia (39 cases) and lymphocytic leukaemia (18 cases) within a cohort of US workers from two chemical manufacturing facilities and a research and development centre between 1940-1978 were examined for 21 specific chemicals. For acetonitrile an OR of 5.2 was estimated for non-Hodgkins lymphoma (based on two cases), and 2.5 for non-lymphocytic leukaemia (based on one case).

There was a high likelihood of multiple, concurrent exposure to several chemicals in this investigation.

There was also a high correlation between individuals exposed to "nitriles" and individual exposed to “antioxidants”. Due the mixed and varied exposures, it is not possible to ascribe any increased tumour incidence solely to acetonitrile exposure.

4.1.2.8.3 Summary of carcinogenicity

There was an increase in liver adenomas and carcinomas separately and jointly in male rats at the highest test level (400 ppm). This result was named as equivocal by NTP. However, this was not significant when compared with the dedicated controls or historical control ranges.

There were no exposure-related liver lesions in female rats.

There were no increases in the incidences of lung and liver neoplasms in exposed groups of mice that were considered related to acetonitrile exposure. However, the incidence of squamous hyperplasia of the epithelium of the forestomach was significantly increased at 15 months in 200 ppm females. At 2 years, the increased incidence was dose-related in all exposed male and female groups. These findings establish an effect of prolonged acetonitrile exposure on forestomach of mice, but the magnitude of the neoplastic findings is insufficient to attributethem to the chemical with any confidence.

In summary, the results of the NTP bioassay on acetonitrile do not indicate that acetonitrile was carcinogenic in laboratory rats or mice. No classification for this effect is proposed.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Studies in animals

Effects on fertility

No studies specifically investigating effects on fertility are available. However, in a study of Morrissey et al. (1988), evaluation of rodent sperm, vaginal cytology and reproductive organ weight data from National Toxicology Program 13-week studies are showed.

An analysis was conducted of sperm morphology and vaginal cytology examination studies carried out at the end of fifty 13-week studies (25 for rats, 25 for mice) over a 3-year period. Reproductive organ weight (testis, epididymis, cauda epididymis) and sperm motility were the most statistically powerful endpoints evaluated, sperm head morphology may also be a sensitive endpoint for detecting reproductive toxicants.

Ten male and female Fischer 344 rats or B6C3F1 mice in 3 dose groups plus the control group were utilized. The 3 dose groups were selected after day 70 of the prechronic study. The objective was to select doses which were not causing overt toxic effects (mortality or severe depression of body weight or weight gain). The doses used were 100, 200 and 400 ppm and the route of administration was via inhalation. Male rats or mice showed no change in (absolute or relative) weight of the right cauda or right testis, and no effect on sperm motility. No data are provided on the effects of acetonitrile on the female reproductive system rats or mice.

Coate (1983), in a unpublished study, indicates that no adverse effects were seen on sperm motility or morphology or on vaginal cytology in rats or mice exposed during 92 days to 0, 25, 50, 100, 200 and 400 ppm acetonitrile via inhalation.

Developmental toxicity

Different developmental studies were carried out with rats, rabbits and hamsters exposed to acetonitrile via different routes.

Rats

The developmental toxicity of acetonitrile was studied in Sprague-Dawley rats after inhalation exposure for 6 hours / day, during days 6 to 20 of gestation, at dose levels of 0, 900, 1,200, 1,500 and 1,800 ppm. The number of animals exposed was 20 per dose.

Maternal lethality occurred at the highest concentration of acetonitrile (1,800 ppm) and resulted in the death of eight out of the 20 dams (40%). Maternal body weight gain during days 6 to 21 of gestation was significantly reduced at 1,800 ppm ($p<0.01$).

Maternal absolute weight gain was significantly reduced at 1,500 ppm ($p<0.05$) and was approximately 60% of that of the controls at 1,500 and 1,800 ppm. The incidence of pregnancy was not significantly altered by exposure to acetonitrile. Significant increases in the mean percentage of nonsurviving implants and early embryonic resorptions were observed at

1,800 ppm ($p<0.01$), concomitant with a nonsignificant decrease in the mean number of live foetuses per litter. One litter was completely resorbed at 1,800 ppm. Exposure to acetonitrile up to 1,800 ppm had no significant effect on the mean number of implantation sites, foetal sex ratio, or male and female foetal weights. One foetus from the control group exhibited exencephaly, unilateral exophtalmia and open eyelid. There were no significant changes in the incidences of any visceral variations (primarily dilated ureter) or skeletal variations (primaril-extra cervical and/or extra lumbar ribs and reduced ossification of sternebra) across exposure groups, even at maternally toxic doses.

For this study the NOAEL for maternal toxicity was 1,200 ppm. The NOAEL for developmental toxicity was 1500 ppm (Saillenfait et al., 1993).

An inhalation developmental toxicity study on acetonitrile was well conducted by Mast et al. (1994). Sprague-Dawley rats were exposed to 0, 100, 400 or 1,200 ppm acetonitrile, 6 hours / day, 7 days / week. Each of the four treatment groups consisted of 10 non-pregnant females, 10 positively mated females for a distribution study evaluating maternal blood for acetonitrile and cyanide and 33 positively mated females for evaluating developmental toxicity. Rats were exposed for 14 consecutive days (6-19 days of gestation for pregnant animals). Body weights were obtained throughout the study period and uterine and foetal body weights were obtained on the 20^{th} day of gestation. Live foetuses were sexed and examined for gross, visceral, skeletal and soft-tissue craniofacial defects. Acetonitrile and cyanide concentrations were determined in the maternal blood of the rats (6/group) on the 8^{th} and 18^{th} day of gestation. Exposure of rats of these concentrations of acetonitrile resulted in mortality in the 1,200 ppm group (2/33 pregnant females; 1/10 non-pregnant females) and the 400 ppm group (1/33 pregnant females). However, there were no treatment-related effects upon body weights or reproduction indices at any exposure level, nor was there a significant increase in the incidence of foetal malformations or variations. The only effect observed in the foetuses was a statically significant increase in the incidence of supernumerary ribs in the offspring at 100 ppm acetonitrile, there was no dose-response relationship and the incidence of this variation at both 400 and 1,200 ppm was not different from the control group.

Determination of acetonitrile and cyanide concentrations in maternal rat blood showed that acetonitrile concentration in the blood increased with exposure concentration for all exposed maternal rats. Detectable amounts of cyanide in the blood were found only in the rats exposed to 1,200 ppm acetonitrile (2 mg cyanide/ml of blood) on the 8^{th} day of gestation, the concentration declined to 0.8 mg/ml by the 18^{th} day of gestation while acetonitrile concentrations remained essentially constant over the exposure period. This decrease in the maternal blood cyanide level may have been due to induction of rhodanase, the enzyme thought to be responsible for the detoxification of cyanide (Klaasen et al., 1986).

In summary, the two highest exposure concentrations were maternally lethal to some rats; however, there was no reduction in body weights, body weight gains, or clinical signs of toxicity in surviving pregnant or non-pregnant rats. For this reason, the maternal NOAEL can be established in 1,200 ppm, the same value is considered with respect to developmental toxicity.

The developmental toxicity of acetonitrile was examined with an in vivo teratology screen adapted for use in the Long-Evans rat (Smith et al., 1987). The screen was extended to an evaluation of growth till postnatal days 41-42, and weight of several organs at sacrifice. Acetonitrile was dissolved in tricaprylin oil for administration at a volume of 0.1 ml/100 g body weight and administered by intubation from days 7 to 21 of gestation. The doses used for this study and number of rats were 0 (155), 50 (20), 150 (20), 300 (22) and 500 (20) mg/kg bw/day.

There was maternal toxicity in the two top dose groups (300, 500 mg/kg). At 300 mg/kg, 11 females were clearly moribund and therefore were sacrificed at midgestation, while at 500 mg/kg, 16 females died early in the treatment period; two deaths also occurred in the lowest dose group (50 mg/kg) compared to none of the 155 controls. Maternal weight gain was unaffected amongst those in the 50 and 150 mg/kg bw/day groups and those survivors in the 300 mg/kg bw/day group. In the 500 mg/kg bw/day group there was effect (non significant) on the number of viable litters and on the number of litters resorbed. No foetal abnormalities were reported. The only observations amongst the pups, which achieved statistical significance, were increased pup weight on day 4 (females 500 mg/kg) and increased organ weights on day 41-42; liver (females 300 mg/kg), lung (males 300 mg/kg) and spleen (males unstated group).

CD-1 rats were administered daily aqueous solutions of acetonitrile by gavage (125, 190 and 275 mg/kg) on gestation days 6-19. Although maternal effects occurred at 275 mg/kg bw/day; deaths (2/25), reduced weight gain (2/25), no other maternal effects were noted in any treated group. Embryotoxic effects, as shown by increases in early resorptions and postimplantation losses, were also noted in the high-dose group, statistically significant when compared with historic but not concurrent controls.

There was apparently no increase in the incidence of foetal abnormalities (no data presented) but a slight increase in unossified sternebra in all treated groups compared to concurrent, not historic, controls (IRDC, 1981).

Rabbits

A study conducted in compliance with FDA GLP was carried out by Argus Research Laboratories, INC (1984). The purpose of this study was to determine the embryo-foetal toxicity and the teratogenic potential of acetonitrile in the New Zealand White rabbit. Solutions of acetonitrile in reverse osmosis processed deionized water were administered by gavage on days 6 through 18 of presumed gestation to artificially inseminated rabbits. Acetonitrile was administered to rabbits (25 rabbits/group) at dose o (vehicle) 2, 15 and 30 mg/kg/day.

Animals given the highest dose showed anorexia and decreased body weight gain, death in 5 out of 25 rabbits and two abortions occurred at this level (30 mg/kg/day).

Average maternal body weight change was affected by administration of the middle and high dosages of acetonitrile, as compared with the vehicle. Average maternal body weight gain was decreased for high dosage group rabbits on days 6 to 19 of gestation; the decrease was significant ($P \leq 0.01$) on days 15 to 19 for this dosage group, as compared with values obtained for the control group. After completion of the agent-administration period, a significant increase in average maternal body weight was observed for middle ($P \leq 0.05$) and high ($P \leq 0.01$) dosage group rabbits on days 19 to 24, as compared with control values. The average maternal body weight gain for high dosage group rabbits remained higher than that observed for the control group for days 24 to 29 of gestation, but the difference for this period was not significant ($P > 0.05$). As compared with control values, the increase in average maternal body weight, which was observed for the middle and high dosage group rabbits after agent administration was stopped, represented a rebound effect. No effects were observed in the low dosage group rabbits.

With respect to the foetuses of the treated animals, a significant decrease ($P=0.011$) in the average number of live foetuses/litter and a slight (non-significant $P>0.05$) increase in resorptions was seen in the 30 mg/kg group.

Acetonitrile did not adversely affect the incidence of pregnancy or the average number of corpora lutea, implantations, average foetal body weight and sex ratio.

No gross external, soft tissue or skeletal malformations or developmental variations observed for foetuses were attributed to administration of acetonitrile.

As compared with control, a significantly increased (P=0.015) incidence of an extra ossification site in the parietal bones was observed for 4 foetuses in 2 high dosage group litters. The more valid parameter litter incidence did not demonstrate statistical significance (P>0.05). This minor variation is frequently observed in this strain of rabbit and was considered spontaneous.

The maternal NOAEL in this study was 15 mg/kg bw/day and the NOAEL with respect to developmental toxicity 30 mg/kg bw/day.

Hamsters

In a study by Willhite (1983), pregnant golden hamsters were exposed to acetonitrile via inhalation, ingestion or i.p. injection during the early primitive streak stage of embryogenesis.

In the study via inhalation, hamsters were exposed at 0, 1,800, 3,800, 5,000 or 8,000 ppm acetonitrile during one hour, on the 8th day of gestation. The number of animals exposed was 10, 6, 6, 6 and 12, respectively.

In order to determine if detoxification of the cyanide resulting from acetonitrile exposure would prevent developmental toxicity, a concurrent group was administered thiosulfate, 300 mg/kg by intraperitoneal injection, 20 min prior to the inhalation exposure. Thiosulfate injections were repeated at 2 hours intervals for the following 10 hours. To compare the inhalation, i.p, and oral route of administration, a second group was given a single i.p. injection of distilled water, 100, 200, 300 or 400 mg/kg acetonitrile on the morning of the 8^{th} day and a third group was given the same doses orally.

Maternal deaths occurred at 3,800, 5,000 and 8,000 ppm (1/6, 1/6 and 3/12, respectively). Resorptions were significantly increased (P<0.05) in the 5,00 ppm group. Foetal abnormalities were seen in the 5,000 ppm group (6, 11% of foetuses) and 8,000 ppm group (29; 25%). No abnormalities were seen in either of the other treated groups or in the controls. The most common abnormalities were exencephaly, encephelocoele and rib fusions. One foetus of the 8,000 ppm group had ectopia cordis. Average foetal weight was significantly reduced in the 8,000 ppm group.

In this study the maternal NOAEL is 1,800 ppm and NOAEL to developmental toxicity 3,800 ppm.

Concurrent treatment with thiosulfate prevented both maternal toxicity and subsequent developmental toxicity. Thiosulfate administration abolished the overt signs of acetonitrile poisoning in hamsters and reduced mortality to zero, but the animals were irritated by the vapour as evidenced by conjunctivitis and red mucous membranes. Multiple i.p. injections of thiosulfate resulted in a significant reduction in the number of malformed offsprinf following inhalation 8,000 ppm acetonitrile. In addition, the foetuses considered to be abnormal following thiosulfate prophylasis were less severely malformed than those recovered from dams exposed only to acetonitrile and the anomalies were limited to fusions of the ribs.

When pregnant golden hamsters were exposed to a single gavage or a single intraperitoneal injection of 0, 100, 200, 300 or 400 mg/kg on the 8^{th} day of gestation, maternal toxicity (deaths) occurred at a single gavage dose of 300 mg/kg (1/6) and 400 mg/kg (4/12). Resorptions were

significantly increased in the 200 and 400 mg/kg groups. The paper describes (no data presented) a significant reduction in body-weight gain associated with intubation of 100-300 mg/kg acetonitrile, but there was no significant difference in maternal body-weight gain associated with intubation of 400 mg/kg acetonitrile compared with intubation of an equivalent volume of water.

The incidence of malformations was significantly increased in the 300 and 400 mg/kg groups, 2 litters of the 100 mg/kg group also contained abnormal foetuses compared to none of those of the control group. Malformed foetuses were recovered from dams of the 300 and 400 mg/kg groups. Two of the five litters taken from dams treated with 400 mg/Kg acetonitrile either contained one or more malformed offspring or the entire litter was resorbed. The skeletal malformations seen included rib fusions and severe axial skeleton dysraphic disorders.

Like the inhalation group, administration of thiosulfate ameliorated the toxic effects.

Exposure of pregnant hamsters to acetonitrile by i.p. injection of 100-400 mg/kg, failed to result in a significant increase in either the number of malformed foetuses or the number of resorbed conception sites as compared with controls.

There was a significant increase in the average foetal body weight associated with i.p. injection of 200-400 mg/kg acetonitrile when compared to control, the relevance of this observation was not clear. There was no significant change in the average maternal body weight gain throughout days 8-14 of gestation.

A low incidence of abnormalities was seen in the 100 mg/kg group (2; 3% of foetuses), 200 mg/kg group (1; 2%) and 400 mg/kg group (4; 3%). There were no abnormalities in the 300 ppm group or in the litters of the six control hamsters.

Abnormalities occurred to the cranium (craniora chischisis), abdominal wall (gastroschisis). The statistical and biological significance of these findings is unclear.

In addition, there was no significant difference in the maternal body-weight changes following thiosulfate prophylaxis of dams given 400 mg/Kg acetonitrile as compared with those receiving acetonitrile alone.

4.1.2.9.2 Studies in humans

A case-control study of pregnancy outcome among Finnish laboratory workers revealed no association between exposure to acetonitrile and increased risk of spontaneous abortion in mothers, or malformation and birth weight in their children.

An odds ratio of 1.4 (95% Confidence Internal 0.4-4.7) for spontaneous abortion for exposure to acetonitrile was estimated when 206 cases were compared to 329 controls. No association between solvent exposure and risk of malformation was found in an analysis of 36 cases and 105 controls, or between solvent exposure and birth weight, in an analysis of 500 women (Taskinen et al., 1994).

4.1.2.9.3 Summary of toxicity for reproduction

In relation to fertility, there is no information available in humans and there are no animal studies specifically investigating such effects. However no changes were seen in (absolute or

relative) weight of the right cauda or right testis and no effect on sperm motility in rats or mice exposed for 13 weeks with 100, 200 and 400 ppm to acetonitrile.

On the other hand, several developmental studies are available in animals. In a well-conducted study, rats exposed by inhalation to acetonitrile did not result in significant foetal effects, even at concentrations, which were overtly toxic to the dam. In this study, a maternal NOAEL of 1,200 ppm and a NOAEL of 1,200 ppm with respect to developmental toxicity were established.

In another inhalation developmental toxicity study in Sprague-Dawley rats, a NOAEL of 1,200 ppm was obtained for maternal toxicity and a NOAEL of 1,500 ppm for developmental toxicity.

In two studies with pregnant rats that received acetonitrile by gavage no foetal abnormalities were reported.

In another study conducted in compliance with FDA GLP, pregnant rabbits administered 2, 15 or 30 mg/kg acetonitrile orally showed maternal toxicity at 15 and 30 mg/kg. The maternal toxicity at 15 mg/kg was expressed as a rebound effect on body weight gain after completion of the agent administration period. Embryotoxicity was observed only at the 30 mg/kg dose level. There were no treatment-related gross external, soft tissue or skeletal malformations or developmental variations.

Exposure of hamsters to 5,000 or 8,000 ppm acetonitrile on day 8 of gestation was shown to increase the incidences of resorptions and malformations including exencephaly encephalocele and rib fusions. Malformations identical to those noted following inhalation exposure occurred sporadically in a limited number of offspring after oral or intraperitoneal administration of 100-400 mg/kg acetonitrile.

In most of the available assays, teratogenicity was associated with maternal toxicity. However, in the case of exposure of pregnant hamsters to acetonitrile, the study is the non-standard protocol and the interpretation of the findings is difficult.

In human, no association between solvent exposure and risk of malformation, birth weight or spontaneous abortion has been observed. No classification is proposed.

4.1.3 Risk characterisation

4.1.3.1 General aspects

Acetonitrile is well absorbed from the lungs, gastrointestinal tract and through the skin, although quantitative data are not available.

Acetonitrile has a widespread distribution. It has been found in heart, lungs, liver, spleen, kidneys, stomach, intestines, skin, muscle, brain and testes. Free and conjugated hydrogen cyanide was also detected in all the studied organs.

There are no indications that repeated administrations of acetonitrile result in its accumulation in animal tissues.

Acetonitrile is metabolized to cyanide via cytochrome P450. Several studies have indicated that cyanide formed *in vivo* is subsequently conjugated with thiosulphate to form thiocyanate, which is eliminated in urine.

Cyanide is responsible for the acetonitrile toxicity. Normal hepatic function is required in order to produce toxicity as a consequence of nitrile exposure. The results obtained in experiments with CCl_4 pretreated mice suggest that there is little toxicity due to the parent nitriles in the absence of normal hepatic function. Pretreatment with $Na_2S_2O_3$ or $NaNO_2$ also provides significant protection against the mortality associated with exposure to acetonitrile. *In vitro*, inducers and inhibitors of cytochrom P450 increased or reduced the cyanide production from acetonitrile by isolated microsomes, respectively. The conversion of acetonitrile to cyanide proceeds at a slower rate than that of other nitriles. Interspecies variations in toxic response are probably related to the relative speed of cyanide formation from acetonitrile.

Elimination of acetonitrile occurs primarily through urinary excretion of the unchanged compound and free and bound hydrogen cyanide. Pulmonary clearance of unchanged acetonitrile via exhalation is also an important pathway of elimination, especially at high exposure levels.

The range of oral acetonitrile LD_{50} values in mammals is between 140 and 6,762 mg/kg body weight. Mouse and Guinea pig seem to be the most sensitive species. In a well-conducted study in mice, the oral LD_{50} of acetonitrile was calculated to be 617 mg/kg. In humans, a LD_{50} 570 mg/kg was estimated in case of acute acetonitrile intoxication. These data show the substance as harmful after oral administration.

A LD_{50}>2000 mg/kg was obtained in a well-conducted acute dermal toxicity study in rabbits. This data do not support classification of acetonitrile via dermal. However, classification as Harmful in contact with skin is proposed based in human data which reported symptoms and levels of cyanide in blood as result of paediatric accidental exposure to an acetonitrile-containing cosmetic.

All studies in animals, including a well-conducted study in mice given LC_{50}>2mg/l. In humans, the Pozzani study shows that there is a variability of subjective responses to 40, 80, 160 ppm of acetonitrile vapour, suggesting that, even if a concentration would not endanger the health of the majority of workers it might cause discomfort to some of them. Two deaths after exposure to acetonitrile vapour in the workplace have been reported. Elevated tissue cyanide concentrations were found in post-mortem examination of these cases. The levels causing toxicity in humans are unknown but, probably, very high due to the detection of high levels of cyanide at post-mortem examination.

Based on available information, acetonitrile is considered as harmful via the inhalation route.

Acetonitrile is not a skin or respiratory irritant.

Liquid acetonitrile is an eye irritant and did not cause sensitisation reactions in a well-conducted Buehler Test.

There is no information available on the effects of repeated exposure to acetonitrile in humans. In animals, mice are one of the most sensitive species to inhaled acetonitrile. A subcronic 13-week study carried out by NTP showed a 100 ppm NOAEL based on forestomach lesions observed and on an increase of liver weight. In a 2-year study carried out later by NTP it was observed that the lesions that appeared in the forestomach in the 13-week study were not related to the exposition to the substance and there was no correspondence either with other effects such as organ weight. Both studies lacked of chemical biochemistry and haematology. Therefore, a NOAEL from this study cannot be established. Preliminary data from two unrelated not fully reported studies (Coate, 1983; Immuquest Labs-Inc., 1984) showed a NOAEL of 100 ppm in mice based on haematological data.

In rats a NOAEL of 200 ppm was established based on a 2-year study, since the haematological effects were seen at 400 ppm. This NOAEL in rats is in accordance with the NOAEL of 100 ppm obtained in two not fully reported studies in mice, providing mice are a more sensitive species than rats.

Taken into account all considerations, a NOAEL of 100 ppm in mice is considered as meaningful and is used for risk characterisation.

Concerning repeated dose toxicity dermal and oral route no data have been found.

Acetonitrile did no induce gene mutations in bacteria, showed week clastogenic activity in cultured mammalian cells and was not clastogenic in a well-conducted *in vivo* micronucleus test. In consequence no classification is proposed.

The carcinogenicity of acetonitrile via inhalation has been investigated in rats and mice.

There was an increase in liver adenomas and carcinomas separately and jointly in male rats at the highest test level (400 ppm). However, this was not significant when compared with the dedicated controls or historical control ranges. There were no exposure-related liver lesions in female rats.

There were no chemical-related increases in malignant or benign neoplasms in male or female B6C3F1 mice exposed to 50, 100 or 200 ppm. However, exposure of male and female mice to acetonitrile via inhalation resulted in an increased incidence of squamous hyperplasia of the forestomach. These findings establish an effect of prolonged acetonitrile exposure on the forestomach of mice, but the magnitude of the neoplastic findings are insufficient to attribute them to the chemical with any confidence.

In summary, the results of the NTP bioassay on acetonitrile do not indicate that acetonitrile was carcinogenic in laboratory rats or mice.

In relation to fertility, there is no information available in humans and there are no animal studies specifically investigating such effects. However, no changes were seen in (absolute or relative) weight of the right cauda or right testis and no effect on sperm motility in rats or mice exposed for 13 weeks with 100, 200 and 400 ppm to acetonitrile.

On the other hand, several developmental studies are available in animals. In two inhalation developmental toxicity studies, one of them well conducted, carried out on rats, showing a NOAEL for maternal toxicity of 1,200 ppm, the NOAEL for developmental toxicity was 1,200 and 1,500 ppm.

In a study conducted in compliance with FDA GLP pregnant rabbits administered 2, 15 or 30 mg/Kg acetonitrile orally showed a maternal NOAEL of 15 mg/kg bw/day and the NOAEL with respect developmental toxicity of 30 mg/kg bw/day.

Exposure of hamsters to 5,000 or 8,000-ppm acetonitrile on the 8th day of gestation was shown to increase the incidences of resorptions and malformations including exencephaly encephalocele and rib fusions. Malformations identical to those noted following inhalation exposure occurred sporadically in a limited number of offspring after oral or intraperitoneal administration of 100-400 mg/kg acetonitrile.

In most of the available assays teratogenicity was associated with maternal toxicity. However, in the case of exposure of pregnant hamsters to acetonitrile, the study is the non-standard protocol and the interpretation of the findings is difficult.

The NOAEL of 1,200 ppm for maternal toxicity and the same NOAEL for developmental toxicity is clearly above the NOAEL of 100 ppm that will be used for risk characterisation.

To judge the magnitude of MOS the following considerations have been taken into account:

- An inhalative NOAEL of 100 ppm was obtained in mice having been shown one of more sensitive animal species to acetonitrile. This NOAEL is in accordance with the NOAEL of 200 ppm obtained in a 2-year inhalation study on rats providing mice are a more sensitive species than rats.

- There are no indications that repeated administration of acetonitrile result in its accumulation in animals tissues.

- The dose response curve is rather steep (LOAEL 200 ppm).

- Mild anaemia is observed at the LOAEL dose.

- Data from long- and short-term studies showed that there is a wide variation in inter and intraspecies susceptibility to acetonitrile.

4.1.3.2 Workers

Occupational exposure to acetonitrile may occur via inhalation and dermal route. Oral exposure would not be a significant route of exposure under normal working practices.

The following assumptions have been made:

- The body weight of the average worker is 70 kg and the worker breathes 10 m^3 of air during a 8-hour working day.
- In the absence of quantitative data on the bioavailability of acetonitrile, 100% absorption via inhalation and dermal routes has been assumed.
- A 0.03 kg bodyweight mouse breathes 0.027 litres of air per minute (USEPA, 1987).

The exposure estimations used in the workers risk characterisation are summarised in Section 4.1.1.2, **Table 4.9**.

4.1.3.2.1 Acute Toxicity

Comparing the estimated dermal exposure levels and inhalation short-term exposure levels with the LC_{50} in mice of 3,587 ppm and dermal LD_{50} in rabbits >2,000 mg/kg, it can be deduced that acute toxicity is not of concern for workers.

4.1.3.2.2 Irritation and Corrosivity

Acetonitrile is not considered as a skin or respiratory irritant: **conclusion (ii)**.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those that are being applied already.

Liquid acetonitrile is an eye irritant. Contact to the eyes should be rather uncommon regarding the industrial processes. However, in order to avoid the irritant effect of AN on eye, face shield and/or goggles are expected to be available for employee use in special task where a contact cannot be excluded: **conclusion (ii)**.

4.1.3.2.3 Sensitisation

Acetonitrile is not a sensitiser. Therefore, **conclusion (ii)** is reached.

4.1.3.2.4 Repeated-dose toxicity

Inhalation Exposure

The NOAEL of 100 ppm (168 mg/m^3), obtained via inhalation in mice is used for risk characterisation purposes. This respiratory NOAEL is considered reliable by reasons explained in the general part.

The Margins of Safety (MOS) between the inhalation exposure levels in all possible occupational scenarios and the NOAEL are showed in **Table 4.12**.

Table 4.12 Risk characterisation for repeated dose toxicity (inhalation exposure)

Exposure scenario	Estimated exposure		MOS	Conclusion
	ppm	mg/m^3		
Manufacture	0.1	0.17	988	(ii)
Use as a solvent or chemical intermediate	7.3	12.3	14	(ii)
Use in Laboratories	7.3	12.3	14	(ii)

Taking into account the toxicological considerations provided in the general part, only uncertainty regarding inter and intraspecies variability remains. The size of these margins of safety is considered sufficient to cover the uncertainty derived from this variability. Therefore, **conclusion (ii)** is reached for all scenarios.

Dermal Exposure

No dermal repeated-dose studies are available.

Acetonitrile is metabolised to cyanide by all routes, cyanide being the responsible of the acetonitrile toxicity. In fact, similar symptoms were seen in humans regardless of the route of administration indicating that the route of administration does not appear to be a major factor contributing to toxicity. Therefore, the inhalation NOAEL of 100 ppm (168 mg/m^3) can be converted to a dermal NOAEL of 54 mg/kg/day assuming that a 0.03 kg mouse breathes 0.027 litres of air per minute (USEPA, 1987), 6 hours exposure per day and 100% absorption.

Dermal exposure levels in mg/day can be converted to mg/kg/day assuming a 70kg worker.

The MOS between dermal exposure levels in all possible occupational scenarios and the dermal NOAEL are showed in **Table 4.13**.

Table 4.13 Risk characterisation for repeated dose toxicity (dermal exposure)

Exposure scenario	Estimated exposure		MOS	Conclusion
	mg/day	mg/kg/day		
Manufacture	84	1.2	45	(ii)
Use as a solvent or chemical intermediate	420	6	9	(iii)
Use in Laboratories	42	0.6	90	(ii)

The ratio of 9 obtained when acetonitrile is used as solvent or chemical intermediate seems low for protection of workers taking into account inter and intraspecies variability. Therefore, **conclusion (iii)** is reached for this scenario.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

The assessment has been carried out without taking into account the use of PPE. When PPE is used in accordance with Directive 89/656/EEC (this is, in fact, obligatory in the EU) dermal exposure will be reduced considerably and therefore, the MOS would increase accordingly.

Combined inhalation and dermal exposure

The respiratory NOAEL of 100 ppm (168 mg/m^3) is converted in an internal NOAEL of 54 mg/kg/day in the same way as in the precedent section. This internal NOAEL is compared with the combined exposure via both routes inhalation and dermal converted to mg/kg/day.

Table 4.14 Risk characterisation for repeated dose toxicity (combined exposure)

Exposure scenario	Inhalation exposure		Dermal exposure		Combined exposure	MOS	Conclusion
	mg/m^3	mg/kg/day	mg/day	mg/kg/day	mg/kg/day		
Manufacture	0.17	0.02	84	1.2	1.22	44	(ii)
Use as solvent or chemical intermediate	12.3	1.75	420	6	7.75	7	(iii)
Use in laboratories	12.3	1.75	42	0.6	2.35	23	(ii)

Taking into account inter and intraspecies variability, the size of MOS for the scenario "use as solvent or chemical intermediate" is not considered sufficient to protect workers from combined exposure to acetonitrile. Therefore, **conclusion (iii)** is reached for this scenario.

The assessment has been carried out without taking into account the use of PPE. When PPE is used in accordance with Directive 89/656/EEC (this is, in fact, obligatory in the EU) the contribution of dermal exposure to combined exposure will be reduced considerably and therefore, the MOS would increase accordingly.

4.1.3.2.5 Mutagenicity

Acetonitrile is not considered to be a genotoxic substance: **conclusion (ii)**.

4.1.3.2.6 Carcinogenicity

Acetonitrile is not considered as a carcinogen: **conclusion (ii)**.

4.1.3.2.7 Reproductive toxicity

Acetonitrile is not considered as a reproductive toxicant.

A NOAEL for maternal toxicity of 1,200 ppm has been considered. The comparison of this NOAEL with occupational exposure levels does not present any grounds for concern.

4.1.3.3 Consumers

Since acetonitrile is not present in the consumers products the **conclusion (ii)** is applied.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those that are being applied already.

4.1.3.4 Humans exposed via the environment

According to the EUSES estimations (see Section 4.1.1.4.) the values for the total human intake of acetonitrile for the local scenario range from 0.00302 mg/kg bw/d to 0.164 mg/kg bw/d depending on the release /use category.

A NOAEL of 39 mg/kg/day, derived from inhalation NOAEL, has been used to calculate the MOS for indirect exposure.

This NOAEL was calculated as follows:

168 mg/m^3 (100 ppm) · 0.039 m^3/24 h (mouse ventilatory volume, USEPA, 1987)·6 h / 24 h,·5 days /7 days : 0.03 kg = 39 mg/kg/day

Table 4.15 Estimated human intake of acetonitrile in mg/kg bw/d from local scenarios of EUSES

Scenario	Total intake	MOS
Production	0.0216	$1.81 \cdot 10^3$
Pharmaceutical Industry	0.164	238
Butadiene Production	$3.5 \cdot 10^{-3}$	$1.11 \cdot 10^4$
Other uses	$3.02 \cdot 10^{-3}$	$1.29 \cdot 10^4$
Laboratory Chemical	$3.22 \cdot 10^{-3}$	$1.21 \cdot 10^4$

The calculated MOS for total exposure of man via the environment is in the range from 238 to 12,900. For the regional scale the margin of safety is $2.34 \cdot 10^5$.

These MOS are considered acceptable, indicating no concern for human safety after indirect exposure.

The lowest MOS of 238 will be increased when additional measures will be applied taking into account that **conclusion (iii**) has been reached for environment when acetonitrile is use as a solvent in pharmaceutical industry.

Result

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Workers

Due to the fact that acetonitrile is a volatile, highly flammable liquid, it can rapidly lead to a dangerous concentration build up in air. Acetonitrile forms explosive mixtures with air and when

it is heated to decomposition it emits highly toxic cyanide. In the industrial setting, these risks are not of concern provided that adequate safety measures are taken. On the other hand, information is already provided on the label and in the safety data sheet.

4.2.1.2 Consumers

Acetonitrile has not been detected in consumer products.

4.2.2 Effects assessment: Hazard identification

4.2.2.1 Explosivity

Acetonitrile forms explosive mixtures with air. The lower explosive limit is 3.05% in volume and the upper explosive limit 17% in volume.

4.2.2.2 Flammability

Acetonitrile is a highly flammable liquid (flash point: 5ºC in open cup and 12.8ºC in closed cup) with an auto flammability temperature of 524ºC. It is a volatile liquid (vapour pressure 9.4-9.8 Kpa at 20ºC). The vapours can form a flammable mixture with air within the range of 4.4% to 16 volume%.

4.2.2.3 Oxidising potential

The test method (OECD-EEC) is not applicable to liquid substances.

4.2.3 Risk characterisation

Regarding its physico-chemical properties, flammability is a property of concern for acetonitrile since it is a volatile liquid, which is highly flammable. In the industry setting, the flammability risk is not of concern provided adequate safety measures are taken.

Information is provided on the label and in the safety data sheet.

Concerning use by consumers, acetonitrile is not present in the consumer products.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those that are being applied already.

5 RESULTS

5.1 ENVIRONMENT

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to atmosphere.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (iii) applies to the aquatic, terrestrial ecosystems and microorganisms in the sewage treatment plant as a consequence of exposure rising from the use in the pharmaceutical industry.

5.2 HUMAN HEALTH

5.2.1 Human health (toxicity)

Workers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (ii) applies to acute toxicity, irritation/corrosivity, sensitisation, mutagenicity, carcinogenicity and reproductive toxicity for all occupational scenarios.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (iii) applies to general systemic toxicity as a consequence of dermal exposure arising from use as a solvent and as an intermediate.

Consumers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion applies to workers for end uses scenarios .

5.2.2 Human health (risks from physico-chemical properties)

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

5.2.3 Unintentional sources

The risk assessment has identified other sources of exposure of the substance to humans and to the environment, in particular, the substance is produced during biomass burning and is present in automobile exhaust, which do not result from the life-cycle of the substance produced in or imported into the European Community. The assessment of the risks arising from these exposures are not part of the this risk assessment. The comprehensive Risk Assessment Reports as forwarded to the Commission by the Member State Rapporteur however provides information about these risks.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
bw	body weight / *Bw*, *b.w.*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 50 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]

EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 t/a)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient

Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration

pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum

UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)

European Commission

EUR 19839 EN - European Union Risk Assessment Report
Acetonitrile, Volume 18

Editors: B.G. Hansen, S.J. Munn, M.Luotamo, C. Musset, S. Pakalin, J. de Bruijn, F. Berthault, S. Vegro, G. Pellegrini, R. Allanou, S. Scheer.

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The report provides the comprehensive risk assessment of the substance acetonitrile. It has been prepared by Spain in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to man and the environment, laid down in Commission Regulation (EC) No. 1488/94.

The evaluation considers the emissions and the resulting exposure to the environment and the human populations in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined. For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The human health risk assessment for acetonitrile concludes that there is at present concern for workers. The environmental risk assessment for acetonitrile concludes that there is at present concern for aquatic ecosystem, terrestrial ecosystem and for microorganisms in the sewage treatment plant.

The conclusions of this report will lead to risk reduction measures to be proposed by the Commissions committee on risk reduction strategies set up in support of Council Regulation (EEC) No 793/93.

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