FOREWORD INTRODUCTION

ETHANOL

CAS N°: 64-17-5

SIDS Initial Assessment Report

For

SIAM 19

Berlin, Germany, 19 – 22 October 2004

1. Chemical Name:	ETHANOL
2. CAS Number:	64-17-5
3. Sponsor Country:	Czech Republic Contact Point: Ministry of Environment Contact Person: Karel Bláha, Ph.D. Director Department of Environmental Risks Prague
Co-sponsor Country:	Slovak Republic Contact Point: Centre for Chemical Substances and Preparations Contact Person: Peter Rusnak, Ph.D. Director Centre for Chemical Substances and Preparations Bratislava
4. Shared Partnership with:	CEFIC Ethyl Alcohol Group
5. Roles/Responsibilities of the Partners:	
• Name of industry sponsor /consortium	CEFIC Ethyl Alcohol Group (BP Chemicals Ltd, Sasol Gmbh, Sodes SA.) Contact Point: CEFIC Ethyl Alcohol Group Ave E van Nieuwenhuyse 4 B-1160 Brussels Belgium Graeme Wallace
• Process used	Documents drafted by industry consortia then peer reviewed by sponsor country experts.
6. Sponsorship History	
• How was the chemical or category brought into the OECD HPV Chemicals	This substance is an ICCA HPV chemical. Industry approached the sponsor country. The substance has not been part of any other assessment although it has been through the US HPV programme

Programme ?	sponsored by the US Renewable Fuels Association.
7. Review Process Prior to the SIAM:	The industry sponsor prepared the documents and the Czech and Slovak Competent Authorities reviewed the documents and provided edits and changes where necessary in an iterative process with the industry sponsor. All documents were subsequently updated, based on the comments from Competent Authorities on the submitted documents received through the CDG
8. Quality check process:	Data was reviewed against the OECD criteria as described in the SIDS manual. These criteria were used to select data for extraction into the SIDS dossier. Original data was sought wherever possible. Originally reported work was deemed reliable if sufficient information was reported (according to the manual) to judge it robust. Reviews were only judged reliable if reported by reputable organisations/authorities or if partners had been directly involved in their production.
9. Date of Submission:	23 July 2004
10. Date of last Update:	
11. Comments:	

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	64-17-5
Chemical Name	Ethanol
Structural Formula	CH_3-CH_2-OH

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

The assessment of the substance is focused on its use as industrial chemical. Ethanol consumption in alcoholic beverages is out of the scope of this report.

Ethanol is readily absorbed by the oral and inhalation routes and subsequently, metabolized and excreted in humans. At exposures relevant to occupational and consumer exposure during manufacture and use of ethanol containing products, the alcohol dehydrogenase metabolic route in the liver dominates and does not become saturated. This mechanism follows first order kinetics. The first step of the metabolic path is the rate-determining step; concentrations of the intermediate metabolite acetaldehyde are very low. Ethanol is not accumulated in the body. Dermal uptake of ethanol is very low.

Ethanol has a low order of acute toxicity by all routes of exposure. Lowest robust reported values are an inhalation LC_{50} of >60,000ppm (114,000 mg/m^3), 1 hour, mouse), and an oral LD_{50} of 8300mg/kg.bw (mouse). Ethanol is a moderate eye irritant but is neither a skin irritant nor a sensitizer.

For repeat dose effects, the lowest reported NOAEL is approximately 2400 mg/kg bw/day from a dietary study with rats. At higher doses, male rats showed minor changes to organ weights and haematology/biochemistry; female rats showed minor biochemistry changes and increased length of oestrus cycle along with liver nodules; adverse liver effects were observed at concentrations of 3600mg/kg.bw/day and above

The balance of evidence is that ethanol is not genotoxic. Negative results from a number of bacterial mutation assays appear to be reliable. Of the mammalian cell mutation assays a weak mutagenic effect in mouse lymphoma cells occurred only at very high ethanol concentrations. *In vivo* tests for chromosome aberrations in both rats and Chinese hamsters have given negative results. There is very little evidence to suggest that ethanol is genotoxic in somatic cells and it may have a very limited capacity to induce genetic changes *in vivo* but under very specific circumstances and at very high doses achievable in humans only by deliberate oral ingestion.

Evidence of the carcinogenicity of ethanol is confined to epidemiological studies assessing the impact of alcoholic beverage consumption. These do not indicate any such hazard exists from potential exposure to ethanol in the work place or from the use of ethanol in consumer products.

No fertility or developmental effects were seen at inhalation exposures up to 16000 ppm (30,400 mg/m^3). The lowest reported NOAEL for fertility by the oral route was 2000 mg/kg bw in rats, equivalent to a blood alcohol concentration of 1320 mg/l, although this was based on a significant increase in the number of small pups rather than a direct effect on fertility; such direct effects are not seen until much higher doses. Many studies exist examining the developmental end point for ethanol. However, most use very high doses and few are individually robust enough to allow a NOAEL to be established. However, the collective weight of evidence is that the NOAEL for developmental effects in animals is high, typically >=6400mg/kg bw, compared to maternally toxic effects at 3600 mg/kg bw. The potential for reproductive and developmental toxicity exists in humans from deliberate over-consumption of ethanol. Blood ethanol concentrations resulting from ethanol exposure by any other route are unlikely to produce reproductive or developmental effects.

Environment

The available physicochemical data are adequate to describe the properties of ethanol. The melting point for ethanol is -114°C, the boiling point is 78.3 °C and the log K_{ow} is –0.31. Ethanol has a measured vapour pressure of 57.3 hPa at 20°C. Ethanol has a specific gravity (density) of 0.7864 and a flashpoint of 14 °C. It is fully water miscible at ambient temperatures. Henry's Law constant is 0.000252.

Ethanol is stable to hydrolysis but is readily biodegradable (74% after 5 days) and is not likely to bioaccumulate (calculated logBCF=0.5). Ethanol is not persistent in the environment. Fugacity-based modelling shows that ethanol released into the environment will become distributed mainly into air and water. Relative distributions between compartments based on an emission pattern of 1000:100:10 were 57 % in air, 34 % in water, and 9 % in soil. These predictions are supported by the limited data available on prevailing concentrations, which shows that ethanol has been detected in outdoor air and in river water. The total tropospheric half life of ethanol is estimated to be 10-36 hours, with degradation due to hydroxyl, NOx and SOx radical-mediated photooxidation. As a volatile organic compound in the atmosphere, ethanol is a potential contributor to tropospheric ozone formation under certain conditions, however its photochemical ozone creation potential is considered to be moderate to low (40-45 relative to ethylene as 100).

The aquatic toxicity data in fish, invertebrates, and algae indicate a low order of acute toxicity with LC_{50}/EC_{50} values greater than 1000 mg/l. The most sensitive species were algae *Chlorella vulgaris* with a 96hr EC_{50} of 1000 mg/l and the invertebrate *Artemia Salina* with a 24hr LC_{50} of 1833 mg/l. Valid chronic toxicity data are available for two trophic levels. The lowest reported NOEC for invertebrates is 9.6 mg/l (10 day reproduction) whilst for plants it is 280mg/l (7 day study).

Exposure

Worldwide ethanol production was 25,000 kt in 2001. European production is 1,700 kt. Ethanol is manufactured either by fermentation of biomass or by the hydration of ethylene in a continuous, closed process; release from production facilities is low.

Ethanol use falls into four main categories: use as a solvent, in manufacture of chemicals, as a fuel additive and for potable drink manufacture. Solvent use is mainly in paint and ink manufacture and in pharmaceutical production. Ethanol is widely used in consumer products, mainly cosmetics, but also detergents, winter deicing and cleaning products, including detergents.

There is limited published data on exposure of workers to ethanol but what is available indicates that the vast majority of exposures are well below current occupational exposure limits (OELs). However, some scenarios were identified in one of the sponsor countries with the potential for high inhalation exposure (eg pharmaceutical manufacture). Personal protective equipment should be used in these situations to reduce actual exposure There is no data on consumer exposure from the use of products containing ethanol, but it is likely that the dominant source of consumer exposure to ethanol is through natural sources in foodstuffs and the consumption of alcoholic beverages.

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health: The chemical is currently of low priority for further work. The assessment of the substance is focused on its use as industrial chemical. Ethanol possesses properties that indicate a hazard for human health but these are manifest only at doses associated with consumption of alcoholic beverages. In the context of an industrial chemical, these hazards do not warrant further work as they are not likely to result from the manufacture and use of ethanol and ethanol containing products.

Environment: The chemical is currently of low priority for further work due to its low hazard profile.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 64-17-5
IUPAC Name: ethanol
Molecular Formula: C2H5OH
Structural Formula: CH3CH2OH
Molecular Weight: 46.07
Synonyms: Alcohol
Anhydrol
Ethyl alcohol
Ethyl hydrate
Ethyl hydroxide
Grain alcohol
Jaysol
Methyl carbinol
Potato alcohol
Spirit
Synasol
Tecsol

Substance type Alcohol
Physical description Colourless, clear
Degree of purity 95%-99.9%
Major impurities Up to 5% water
Optional additives Up to 5% denaturant or organoleptic modifier

1.2 Purity/Impurities/Additives

For Customs and Excise purposes, ethanol is often denatured with up to 1 to 5% of one or more denaturants to render it unsuitable for human consumption. Further small quantities of organoleptic modifiers or marker solvents may also be required. Typical denaturants are shown in the Table 1:

Table 1 Additives used for Customs and Excise purposes

Examples of commonly used denaturants	Methyl ethyl ketone Isopropanol Methanol Wood naptha Diethyl phthalate Ethyl acetate Toluene Cyclohexane	1-5% typical
Typical marker	Tertiary butyl alcohol	0.1%
Typical organoleptic modifier	Bitrex®	10 - 30ppm

1.3 Physico-Chemical properties

Table 2 Summary of physico-chemical properties

Property	Value	Comments/Reference
Physical state	Liquid	
Melting point	-114°C	Corcoran (1953)
Boiling point	78.3°C	Ambrose (1970)
Vapour pressure	57.3hPa at 19.6°C	Ambrose (1970)
Water solubility	Fully miscible	Howard (1990), Merck Index (1996)
Partition coefficient n-octanol/water (log value)	-0.31	Howard (1990)
Henry's law constant	0.000252 at 15°C	Kavanaugh (1999)
Flashpoint	14°C	BP Chemicals (1997)
Specific gravity	0.7864 at 20°C	Sakurai (1984)

There is no originally reported value available for the partition co-efficient. However, the text book from which the above value is obtained is a frequently cited source for solvent data. This source was deemed acceptable for the dossier submitted under the US HPV programme.

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes

Ethanol is one of the most ancient chemical substances, arising from the fermentation process by the action of yeast on sugars. The process is most commonly known as that by which beer or wine is produced but it is also why leavened bread has a small ethanol content.

Total world production was around 25 000 kt in 2001 (CEFIC, 2003). All potable alcohol, and a large proportion of industrial and fuel ethanol, is made by the fermentation process in which zymase, a yeast enzyme, changes simple sugars (e.g. as found in molasses) into ethanol and carbon dioxide. Biomass (sugarbeet/cane, molasses, cereals, rice, grain, cellulosics) are used as the feedstock for fermentation ethanol. Synthetic routes to ethanol also exist, and, although a process based on acetaldehyde arising from acetylene is quoted, the virtually universal synthetic route is via the hydration of ethylene to ethanol. The origin of the product, whether via the synthetic or fermentation route, does not in any way affect its physical, chemical or toxicological properties, although trace impurities can vary from route to route.

There are literally thousands of alcohol producers in Europe. These include those producing 'neutral' ethanol, which is used in both industrial applications and in beverages such as gin and vodka, and those producing spirit drinks containing ethanol (e.g. whisky and wine.) Figures for production in the Europe Union in 2001 are shown in Table 3. Synthetic ethanol represents about 30% of total production.

Table 3 Production of ethanol in European Union

Ethanol type	EU Production (ktpa)
Agricultural alcohol	790
Synthetic ethanol	500
Wine alcohol	230
Fuel alcohol*	180
TOTAL	1700

* subsidised agricultural ethanol designated for fuel use only

Source: CEFIC Ethyl Alcohol Group, 2003

Figures for the Czech Republic during the years 2000-2 are given in Table 4.

Table 4 Production of ethanol in the Czech Republic

2000	2001	2002
46.0 kt	41.1 kt	49.6 kt

Source: Sponsor country, 2004

US production is around 7 000 kt per year.

2.2 Use Pattern

Applications for ethanol industrially are split into solvent applications (both in industry and for consumer applications), use as a fuel and further processing. In Europe, the non-potable market is approximately 830 kt per year and has an approximate value of Euro 250 million (CEFIC, 2003). The split of end uses is shown in table 5 and then described in more detail in the following sub-sections:

Table 5 Non potable end uses of ethanol in Europe

Non potable end uses	(EU) ktpa
Solvent use	400
Intermediate manuf.	250
Fuel	180
TOTAL	830

Source: CEFIC, 2003. Data for 2001

Note that synthetic ethanol is used in non-potable applications only (non permitted by regulation). Fermentation alcohols are used in both industrial and potable applications

2.2.1 Use as a solvent

The use of ethanol as a solvent in Europe, includes both domestic and industrial usage (table 6). Industrially, ethanol is mainly used as a carrier solvent in inks (mainly flexographic) and coatings. Consumer applications include the use of ethanol in professional cosmetic formulations such as hair setting sprays and colorants, as well as in consumer cleaning and detergent preparations,

for example spray cleaners used in kitchens and bathrooms. Most perfumes consist of blends of predominantly natural essences in an ethanol base. The essences themselves are often extracted from flowers and barks using ethanol as the process solvent. Ethanol is also found in automotive deicing products. Ethanol is also used in pharmaceutical and personal care products. Preparations such as mouthwashes, and cough and cold medicines, are formulated with up to 30% ethanol. Ethanol is also used as an active biocidal product.

Table 6 European solvent uses of ethanol (CEFIC, 2003)

Industrial	ktpa	Domestic	ktpa
PAINT (industrial)	25	COSMETICS	145
INKS	70	DETERGENTS	25
PHARMACEUTICALS*	35	DEICING	40
FOOD*	5	CLEANING	15
FLAVOUR/FRAG	5	OTHER**	10
EXPLOSIVES	5		
OTHER**	20		
TOTALS	*165*		*235*
GRAND TOTAL			*400*

* use in manufacture (processing)

** includes use as a biocide

The Scandinavian product register (SPIN, 2003) records the following use of ethanol in registered preparations (table 7):

Table 7. SPIN database information

Country	Year	Number of preparations	Tonnage of preparations	In consumer preparations
FINLAND	2001	1075	86391	
NORWAY	2001	808	24754	X
DENMARK	2001	2470	16587	
NORWAY	2000	816	22782	
SWEDEN	2000	2260	60551	X
DENMARK	2000	2149	228769	
FINLAND	2000	1054	No data	
SWEDEN	1999	2265	51874	X

Solvent use of ethanol in the US, including consumer solvent applications, is approximately 500 ktpa.

2.2.2 Use as an intermediate for the production of synthetic chemicals

As a reactive chemical, ethanol in common with all alcohols reacts with acids to produce esters. Examples include ethyl acrylate, which is used as a reactive diluent in specialised coatings, and ethyl acetate, which is a widely used solvent in paint and coating formulations. Ethanol is used in the production of ethylamines, which in turn are reactive industrial chemicals used in downstream speciality applications including agrochemicals and pharmaceuticals. It can also be used to make ethoxypropanol, an increasingly used glycol ether solvent in coating formulations (CEFIC, 2003). This list should not be regarded as exhaustive.

350 ktpa of ethanol are used to produce downstream chemicals in the US. Ethyl acrylate production represents the largest application at just under 30% of the volume (CEFIC, 2003).

2.2.3 Use of ethanol in fuel

The use of ethanol as a component of automotive fuel varies widely throughout the world. In Europe, use is relatively limited. In the United States ethanol has a different profile. Because of the current statutory requirement to move to oxygenated gasoline fuels, ethanol is increasingly used as a gasoline additive. Total use is around 6 000 ktpa (85% of total US demand). There are more than 60 US producers, largely using fermentation processes. Total world consumption of ethanol in fuel applications is around 15 000 ktpa (CEFIC, 2003).

2.2.4 Ethanol use in potable products

The dominant use of ethanol in consumer ‘products’ is in potable products (alcoholic beverages and vinegar production – around 500 ktpa in Europe and around 900 ktpa in North America) (CEFIC, 2003). Consideration of such uses is outside the scope of this SIAR.

2.2.5 Marketing and distribution of product

Ethanol is distributed neat in closed systems such as marine vessels, tank cars (rail cars), and tank trucks. Smaller volumes, particularly from distributors, are supplied in closed containers varying from intermediate bulk containers (IBCs) to containers of 5 litres size. Products containing ethanol, such as paints, are supplied in closed containers varying from intermediate bulk containers (IBCs) to small cans and containers of 5 litres (1 gallon) or less (CEFIC, 2003).

2.3 Environmental Exposure and Fate

2.3.1 Sources of Environmental Exposure

Ethanol will enter the environment as emissions from its manufacture, use as a solvent and chemical intermediate, and release in fermentation and alcoholic beverage preparation. Natural emissions also occur as a plant volatile, from microbial degradation product of plant and animal wastes and in natural fermentation of carbohydrates (Howard, 1990). Concentrations of ethanol in some edible products are quite high. (EU, 1996; Howard, 1990; Lerici, 1996; Anonymous, 1986). Releases to the environment during production and industrial processing at larger production sites are minimized by the use of engineering controls and end-of-pipe abatement systems. Aqueous waste streams are routinely treated in biodegradation facilities. Any organic wastes from manufacture are typically incinerated on site or disposed of via specialist waste contractors (CEFIC, 2003). It is possible that small, farm scale fermentation manufactures may not have such extensive emission controls but by their nature, volumetric emissions will be low and dispersed.

The largest source of ethanol release to the environment is expected to be from use of ethanol containing products, including consumer products, where applications are open and engineering controls to recover and recycle solvent are not always used. The most likely environmental medium for ethanol release is the atmosphere although fugacity level 3 calculations (see section **Error! Reference source not found.**)show that distribution between compartments means that both the air and water compartments are significant regarding the fate of the substance.

There are no known regional specific factors that would make these potential sources of release unique. Therefore they should be regarded as common worldwide. However, the relative quantities of ethanol release from the use as a fuel additive may vary between regions due to the great variation in use in this application.

2.3.2 Photodegradation

Although ethanol can absorb radiation and is subject to direct photolysis, the principle mechanism for degradation is likely to be photochemical oxidation in the presence of atmospheric pollutants (photochemical sensitizers) of which the main ones in industrial regions are nitrogen oxides (NO_x) and sulfur oxides (SO_x). Pseudo first-order half-lives of 15.4 hrs and 13.8 hrs were calculated for nitrous oxide-mediated indirect photolysis (Yanagihara, 1997) and sulphur dioxide-mediated indirect photolysis (Hustert, 1978). Ethanol is therefore expected to degrade rapidly in NO_x and SO_x polluted atmospheres.

Using the EPA developed model AOPWIN (US EPA, 2002), secondary rate constants for hydroxyl radical mediated atmospheric photo-oxidation were calculated to be 3.58×10^{-12} cm^3/molecule-sec for ethanol. Using the standard assumptions of 1.5×10^6 hydroxyl radicals per cubic centimetre and 12hr/day sunlight, a pseudo first order half-life of around 3.0 days was calculated based on an estimated rate constant. In the presence of hydroxyl radicals, ethanol photodegradation half-life was 10 hours based on a measured rate constant (Campbell, 1976). These values are different because of significant differences in the underlying rate constants. In the absence of atmospheric contaminants and at light wavelengths typical of the troposphere (>290nm) ethanol was not degraded in the presence of oxygen and water. Photochemical degradation is the rate-limiting step governing the overall residence time of ethanol in air.

The photochemical tropospheric ozone creation potential (POCP) is 40-45% relative to ethylene at 100% (Anderson-Skold, 2000; Derwent 1996). The Maximum Incremental Reactivity (MIR) had an absolute value of 1.34 -1.69 g ozone/g volatile organic carbon (Carter, 2000). This indicates that ethanol would have a low to moderate contribute to tropospheric ozone creation.

2.3.3 Stability in Water

The octanol-water partition coefficient and Henry's Law value suggest that ethanol is unlikely to bioaccumulate and will volatize from surface waters, off-gas from groundwater and have a high vapour phase retardation. Volatilization from model rivers and lakes were calculated using EPIWIN (US EPA, 2002). Calculated half-lives of volatilization of ethanol from a model river or lake were 3.3 and 38.9 days respectively using a measured Henry's Law constant of 0.000252. Ethanol is considered moderately volatile and is stable to hydrolysis (Anbar, 1967; Lyman, 1990.)

2.3.4 Transport between Environmental Compartments

Based on a value of partition coefficient K_{ow} of –0.31, bioaccumulation of ethanol in aquatic organisms is not expected. Level III distribution modelling showed that most of the ethanol released to the environment would go primarily to air and water (see table 8), with the rest to the soil,

assuming emission ratios of substance to air, water, and soil are 1000:100:10 respectively (Mackay, 1996). The ration of the factors were chosen by a weighted summation of emission factors for each scenario, either using data from the EU Technical Guidance for Risk Assessment (EU, 2003) or by expert judgement, and then rounding to the nearest order of magnitude with the air emissions set to 1000kg.

Table 8 Environmental distribution of ethanol

Fugacity level III calculations Relative distributions between compartments based on an emission pattern of 1000:100:10	
Air	57%
Water	34%
Soil	9%

Wet deposition (rain-out) is expected to play an appreciable role in the atmospheric removal of ethanol (Howard, 1990)

2.3.5 Biodegradation

In aerobic conditions using adapted wastewater from domestic sewage, degradation was 74% after 5 days rising to 95% by day 15 and in similar conditions in synthetic seawater, ethanol was 45% degraded after 5 days rising to 75% by day 20 (Price, 1974). Biodegradation in a study to a MITI protocol showed degradation of 89% after 14 days (>70% after 10 days, CERI, 2004) and >90% within 10 days (Birch, 1991). Activated domestic sludges were capable of aerobically oxidizing ethanol, as measured by BOD, which was 37.3% of maximum after 1 day (Gerhold, 1966).

The rate of biodegradation in anaerobic conditions was calculated to be 17.9 ppm ethanol per day with a total methane recovery of 91% of the theoretical limit (Suflita, 1993).

Biodegradation is the main method of removal of ethanol from water. Ethanol is stable to hydrolysis. Reaction with hydroxyl radicals in aquatic media will not likely be a significant process (Anbar, 1969).

It can be concluded that ethanol meets the readily biodegradable criteria.

2.3.6 Bioaccumulation

The bioconcentration factor can be estimated using BCFWIN v2.15 (US EPA ,2002). The resultant LogBCF=0.5 indicates that ethanol is not likely to bioaccumulate.

2.3.7 Other Information on Environmental Fate

2.3.7.1 Stability in soil

The soil adsorption co-efficient can be predicted using PCKOCWIN v1.66 (US EPA, 2002). The resultant K_{oc} of 1 indicates that ethanol released to soil would move quickly through the soil.

2.3.7.2 Measured Environmental Concentrations

There is no recent environmental data on ethanol concentrations.

Ethanol and methanol were detected at Point Barrow, Alaska in 17 of 25 air samples at an average combined concentration of 0.52 ppb over 24 hours (Cavanagh, 1969). Ethanol was found in ground water suspected of leachate contamination at 190 ppb at one of 13 sites, and was detected at 58 ppb in landfill ground water where inorganic levels indicated good or unknown water quality (Sabel, 1983). Ethanol was also detected in surface water at a concentration of 4020 ppb in the Hayashida River in Japan near the site of a leather factory (Yasuhara, 1981).

2.4 Human Exposure

The assessment of the substance is focused on its use as industrial chemical. Ethanol consumption in alcoholic beverages is out of the scope of this report.

2.4.1 Occupational Exposure

Due to the volatile nature of ethanol, the most significant route of exposure is likely to be by inhalation. Ethanol manufacturing plants are continuous, enclosed processes with controlled occupational exposures. Potential exposures can occur during such operations as sample collection, maintenance of equipment, and loading of trucks and/or rail cars.

Occupational exposure limits in the USA and the main European countries of are in the range 500-1000 ppm (1900 mg/m^3) over 8 hours (see table 9).

Table 9 Occupational exposure limits

COUNTRY	8hr TWA Hygiene Limit	STEL
US (OSHA)	1900 mg/m^3 (1000ppm)	None
US (ACGIH)	1900 mg/m^3 (1000ppm)	None
Germany (MAK)*	960 mg/m^3 (500ppm)	Peak limit cat. II,1
UK (OES)	1920 mg/m^3 (1000ppm)	none
Slovak Republic	960 mg/m^3 (500ppm)	1920 mg/m^3 (1000ppm) (30 min, 4x per shift)
Czech Republic	1000 mg/m^3	3000 mg/m^3

Products containing ethanol, such as inks, lacquers, are manufactured in semi-enclosed batch processes, usually with extraction systems to control worker exposure. Similarly, processes using products containing ethanol are also usually semi-enclosed with extraction systems to control worker exposure. These processes can be continuous or batch in operation.

There is limited data available on measured exposure values, partly due to the perception that actual exposures are substantially lower than the relatively high OELs and also due to prioritisation of other more hazardous substances. The general data that is available (see appendix) confirms that the perception is probably correct in that for the industrial and occupational sectors for which data have been reported, daily time weighted average ethanol exposures are typically well below the prevailing regulatory exposure limits. Some detailed measurements from Slovakia confirm that most industrial exposures are low. Some scenarios were identified with the potential for high

inhalation exposure (eg pharmaceutical manufacture). Workers used PPE in these situations to limit actual exposure (Regional Authority of Public Health, Slovakia, 2004).

There is also the possibility of dermal contact with ethanol. It is common practice to recommend that workers when handling the material wear suitable protective gloves. The half life for the evaporation of ethanol from skin is 11.7 seconds (Pendlington, 2001) which implies that continuous immersion would be required for there to be any potential for dermal absorption.

2.4.2 Consumer Exposure

Ethanol is a natural component of many foods including beer, wines, distilled spirits, and a variety of fruits. Ethanol is also used in small quantities as a diluent in some medicinal products. Ethanol is also naturally present in a wide range of foods including fruits, yoghurt, ice cream, fruit juices and leavened bread (Anonymous, 1986; Greubet, 1997; Lerici, 1996;).

Consumers are also widely exposed to ethanol as a component of alcoholic beverages but discussion of this end use is beyond the scope of this review.

Non-food products containing ethanol include personal hygiene products, fragrances, cosmetics, adhesives, surface coatings, and inks. For Customs and Excise purposes ethanol spirit intended for non-beverage applications frequently contains an organoleptic modifiers, denaturants or marker solvents to render it unpalatable and/or traceable. These are used at levels from ppm up to a few percent. All routes of exposure (oral, dermal and inhalation) are feasible for these products as a whole but not all routes apply to all products.

Ethanol is unusual in that it also occurs naturally within the body. This natural burden is thought to be due to the metabolism of the intestinal microflora and produces blood alcohol concentration (BAC) levels of typically 0.062 to 0.73 mg/l (Sprung, 1981).

2.4.3 Indirect Exposure via the Environment

The measured environmental concentrations of ethanol are low, although the quantity of existing data is limited. Available information suggests that the greatest source of exposure to ethanol may be through their presence in alcoholic drinks, foodstuffs and medicines, which are regulated through different fora and are not considered further in this report.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

Following any route of intake resulting in an elevated blood ethanol level (BEL), metabolism proceeds in three basic steps. First, ethanol is oxidized within the cytosol of hepatocytes to acetaldehyde; second, acetaldehyde is rapidly converted to acetate, mainly in the mitochondria; and third, acetate produced in the liver is released into the blood and is oxidized by peripheral tissues to acetic acid and ultimately carbon dioxide, and water. The rapid conversion of the intermediate aldehyde means that concentrations are usually very low. However, polymorphism is seen in the acetaldehyde dehydrogenase enzyme meaning that some ethnic groupings are less well adapted to metabolise this intermediate with resultant higher concentrations, although they still remain low. At a concentration of 10mM (460 mg/l) ethanol, acetaldehyde levels are <2 µM (88 µg/l) indicating that the human aldehyde dehydrogenase enzyme has a very low Michaelis

Menton constant Km (Crabb, 1987), meaning that the reaction will proceed significantly at very low substrate concentrations. The main pathway for ethanol metabolism proceeds via alcohol dehydrogenase. However, other pathways for ethanol oxidation have been described including a microsomal ethanol-oxidizing system located in the endoplasmic reticulum and a catalase system located in the peroxisomes. The rate of hepatic metabolism of ethanol is concentration independent except at very low or very high concentrations. Blood ethanol in humans decreases more rapidly at concentrations over 300 mg/dl than at concentrations below this level, possibly due to oxidation by the microsomal ethanol oxidizing system. The maximum rate of metabolism is 100 - 125 mg/kg body weight/hour, although tolerant individuals may have higher metabolic rates (up to 175 mg/kg/hour) due to enzyme induction. Adults metabolize 7 - 10 g ethanol/hour reducing blood ethanol concentrations at a rate of 15 - 20 mg/100 ml/hour. Ethanol is metabolized more rapidly in chronic alcohol abusers (up to 40mg/100 ml/hour) and in children (up to 28 mg/100 ml/hour) (Ellenhorn, 1988). The kidneys and lungs excrete only 5 – 10 % of an absorbed dose of ethanol unchanged (Ellenhorn, 1988). The major route of excretion of ethanol is in the urine (Conibear, 1988). Ethanol is excreted in the un-metabolized form in urine, exhaled air and sweat. Its metabolic products are also excreted by exhalation and in the urine.

Studies in Animals

In vitro Studies

Ethanol penetration through pig's skin *in vitro* was greater in occluded cells than in non-occluded cells (2.19 mg/cm^2 and 0.10 mg/cm^2 in 24 hours respectively). At the maximum flux under occlusion, the amount of ethanol penetrating from a 1m^2 area of skin would give a blood alcohol level of about 40 mg/l in a 70 kg man. In a comparative human use study, none of the blood samples taken from sixteen human volunteers exhibited a detectable level of alcohol (Pendlington, 2001). Ethanol has a very low octanol:water partition coefficient and this is seen as contributing to the poor dermal uptake of ethanol in intact human skin. This study suggests that a systemic dose of ethanol is likely to be very low after the use of formulations delivering ethanol to the skin.

Studies in Humans

In a physiologically based pharmacokinetic (PBPK) model the parameters describing Michaelis-Menten metabolism of ethanol in the liver were varied using simulation and optimization software. For each exposure scenario the simulation was run for male workers in 'sitting awake' and 'light exercise' activity levels. Despite some limitations a reasonably good overall description of the data was obtained. The model predicted time courses of the mixed venous blood concentrations for the 12 exposure scenarios. The model predictions are that for men exposed to ethanol, at 0.942 and 1.88 mg/L for 8 hr and for the lower breathing rate in men exposed to 9.42 mg/L, the liver is able to metabolize ethanol at the rate it enters the body. However, for the higher breathing rate in men exposed to 9.42 mg/L and for men exposed to 37.6 or 63.6 mg/L the rate of ethanol delivery via breathing exceeds metabolic capacity and ethanol blood levels consequently rise for the duration of the exposure. Men exposed to 20 mg/L ethanol for 4 hr also showed a continued accumulation during exposure at the higher breathing rate but little or no accumulation at the lower breathing rate (Conolly, 1999).

Other work has shown a similarly good correlation between inhalation exposure and blood alcohol concentrations. A group of human volunteers (24 in one experiment, 16 in the second) were exposed to ethanol vapour concentrations up to 3610 mg/m^3 and resultant blood ethanol concentrations (BEC) measured of between 0.00066 and 0.0056 mg/cm^3. Regression analysis of the data shows that BEC = exposure (ppm) × 0.0029 (with a 7% error for 95% confidence). (Seeber, 1994). Around 60% of inhaled ethanol vapour is absorbed (Lester, 1951; Kruhoffer, 1983).

Ethanol is eliminated from the body mainly by metabolism in the liver and only minimally by urinary excretion and pulmonary exhalation. Other tissues such as kidney, stomach and intestines oxidize ethanol to a small extent. Once absorbed, alcohol is contained in the water compartment of the body. It is not stored or accumulated to any degree, so that the body burden at any point in time is a result of recent absorption, usually within the previous 12 hours (Conibear, 1988). Such a rate of elimination is supported by the data from Jones and the PBPK modeling work described above (Conolly, 1999; Jones 1993). In a study involving 130 patients that had abstained from alcohol intake for 24 hours, physiological ethanol concentrations were below 0.75 mg/ml with most values in the concentration range 0.1 and 0.2 mg/l (Sprung, 1981).

Conclusion

At exposures relevant to occupational and consumer exposure during manufacture and use of ethanol containing products (ie. Exposure via the inhalation route), the alcohol dehydrogenase metabolic route in the liver dominates and does not become saturated. This mechanism follows first order kinetics. The first step of the metabolic path is the rate-determining step; concentrations of the intermediate metabolite acetaldehyde are very low. Ethanol is not accumulated in the body. Dermal uptake of ethanol is very low.

3.1.2 Acute Toxicity

Studies in Animals

Inhalation

In acute inhalation studies, ethanol has shown a low order of acute toxicity. An LC_{50} value was not achieved at exposures of up to 60,000 ppm for 60 minutes in study in CD-1 mice (Moser, 1985). Mice in this study experienced moderate ataxia, which reversed after more than 4 hours recovery period at all exposure levels.

Dermal

No acute dermal toxicity was reported in a study in rabbits, LDL_0=20,000 mg/kg (Monick, 1968) and although this study is not experimentally robust, the result is consistent with the finding that ethanol uptake through intact skin is poor. No other dermal study or reported result has been identified.

Oral

Ethanol has a low order of toxicity in animals following single oral exposure. Robust figures are: LD_{50}=8300 mg/kg (oral, mouse) (Bartsch, 1976) and LD_{50}=15010 mg/kg (oral, rat) (Youssef, 1992). An age-related difference is reported (Wiberg, 1970) in which young rats (100 days old) were less sensitive than old rats (10-12 months old) with an LD_{50}=11,000 mg/kg versus 7,000 mg/kg. The main symptoms of acute exposure are those typical of substances which cause central nervous system depression e.g. inebriation, gait disturbance and dose-related decrease in response to painful stimuli, respiratory depression and coma. Deaths were due to cardio-respiratory failure.

Other Routes of Exposure

By other routes, ethanol has, again, shown low acute toxicity with LD_{50} = 9,710 mg/kg in male and LD_{50} = 9,450 mg/kg in female mice receiving i.p. ethanol (Schechter, 1995); LD_{50} = 9,200 mg/kg in male mice receiving i.p. ethanol (Ho, 1979) and LD_{50} = 6,710 mg/kg in young (100 days old) rats and LD_{50} = 5,100 mg/kg in older (10-12 months) rats receiving i.p. ethanol (Wiberg, 1970).

Studies in Humans

Oral

Oral consumption of ethanol containing beverages is known to produce symptoms of intoxication (e.g. drowsiness, loss of concentration). However, there is no evidence that such effects can be produced by inhalation or dermal routes of exposure.

Conclusion

Ethanol has a low order of acute toxicity by all routes of exposure.

3.1.3 Irritation

Skin Irritation

Studies in Animals

There is little evidence of skin irritancy in animal studies. A study conducted to OECD 404 standards in rabbits showed ethanol to be not irritating (Jacobs, 1992) which agrees with an earlier study (Phillips, 1972).

Studies in Humans

In the form of biocidally active surgical spirit (70-80% ethanol in water), there is a considerable history of dermal application of ethanol as an antiseptic with no concern for skin irritancy. Similarly, large amounts of ethanol are used in a variety of cosmetics, personal care and household cleaning products.

Eye Irritation

Studies in Animals

Available data from animal studies indicates that ethanol is moderately irritating to the eye. The most recent data indicates that, when assessed in an OECD 405 study, only mild redness and chemosis remained in by day 7 with all symptoms having disappeared by day 14 (ECETOC, 1998). An older study similarly concluded that ethanol is moderately irritating (Jacobs, 1987).

Studies in Humans

In humans, direct contact of liquid ethanol on the human eye causes an immediate sensation of burning and stinging, accompanied by reflex closure of the eye. The acute discomfort subsides rapidly, although foreign body type discomfort may be felt for a day or so. Recovery is complete.

Respiratory Tract Irritation

In humans, a concentration of 5000 ppm vapour is quoted as irritating and uncomfortable to breathe but tolerable (Lester, 1951). Much higher concentrations than this would induce lachrymation and coughing.

Conclusion

Ethanol is moderately irritating to the eyes but not irritating to skin. At high vapour concentrations, in air, ethanol is irritating to breathe.

3.1.4 Sensitisation

Studies in Animals

Skin

Ethanol (75 % v/v) was used as solvent in the induction phase of a Magnusson and Kligman sensitization test of a polyalkalene glycol. No skin reactions were evoked at challenge with the polyalkalene glycol in 75 % ethanol in either test or control group animals (BP Chemicals, 1984). No increase in ear thickness was recorded following challenge application of ethanol in a mouse ear swelling test (Descotes, 1988).

Studies in Humans

Skin

A literature review demonstrated that ethanol can be an allergen in immediate and delayed hypersensitivity by external or internal exposure and can produce subjective irritation, irritant contact dermatitis and non-immunologic contact urticaria (Ophaswongse, 1994). However, the widespread use of ethanol in cosmetics and in skin antiseptic formulations suggests that skin sensitization is not an end point of concern.

Conclusion

Ethanol is not considered to have sensitizing properties.

3.1.5 Repeated Dose Toxicity

There are many repeat dose studies in many species reported in the literature using ethanol. However, these are almost universally carried out to improve the understanding of the risks associated with the consumption of alcoholic beverages. Characteristically, these are carried out by the oral route and at high doses, well in excess of 1 g/kg, which limits their value in characterising the repeat dose toxicity of ethanol at doses relevant to occupational exposure and use of consumer products containing ethanol.

Studies in Animals

Oral

Studies (90 days duration) were carried out in rats and mice to assess whether 5% ethanol in drinking water would be an appropriate vehicle for a long-term toxicity and carcinogenicity study of urethane. Based on the water consumption data in the study and averaged body weights over the exposure period, this equated to doses of at least 4000 mg/kg in rats and 7500 mg/kg in mice. Data from this study yielded NOAEL values of >5%, for male rats (dose equivalent >4000 mg/kg) and female mice and <5% for male mice and female rats (dose equivalent <5000 mg/kg). Male rats showed minor changes to organ weights and haematology/biochemistry; female rats showed minor biochemistry changes and increased length of oestrus cycle along with liver nodules; male mice showed increased organ weights and some fatty changes to the liver and a decrease in sperm concentration at (NTP, 1996). In the ensuing long-term study (2 years duration) in mice, ethanol caused a marginal exposure-related increase in survival in males but had no effect on the survival of females. There was evidence of an ethanol-induced reduction in water consumption that was more marked in males than in females (NTP, 2002). A good quality supporting study in rats gave a NOAEL value of <5% , later refined to 2% (approximately 2400 mg/kg), for ethanol in oral feed ad libitum in rats (Holmberg, 1986). The LOAEL in this study was 3 % (approximately

3600 mg/kg) due to dose related hepatic yellowing and centrilobular steatosis. This appears to be the lowest robust oral NOAEL available.

Conclusion

Most data available on ethanol is via the oral route of exposure. Much is at high doses which limits its value to risk assessment of ethanol as a chemical substance. From the data available, it is possible to surmise that ethanol is of repeat dose low toxicity by the oral route, with a lowest reported NOAEL in animals of 2400 mg/kg for rats.

3.1.6 Mutagenicity

All discussion in this section is from in vitro data and in vivo data in animals.

In vitro Studies

Bacterial mutation assays

The results of bacterial mutagenicity assays (McCann, 1975; Blevins, 1982; Blevins, 1983, Hellmer 1992) have generally been negative for ethanol. A very weak positive effect of ethanol in a DNA repair test in *Escherichia coli*, but no effect in the Ames test with strains *Salmonella typhimurium* TA1535, 1537, 1538, 97, 98 and 100, was found by De Flora. (1984a). There was a weak but reproducible positive effect in *S. typhimurium* TA102 but only at ethanol concentrations of 160 and 240 mg/plate, which are concentrations considerably greater than the highest concentration specified for guideline testing (De Flora, 1984b). At least one positive result has been also reported but only at concentrations massively above the maximum normally recommended in guideline study protocols (Hayes 1985). Zeiger. (1992) found negative results at concentrations up to 10 mg/plate in *S. typhimurium* strains TA97, 98, 100, 104 and 1535 with or without Aroclor-induced S9, from both rats and Syrian hamsters, at two concentrations. Ethanol is not therefore considered to be mutagenic to *E. coli* and *S. typhimurium* bacteria.

Chromosome aberration tests

No chromosome aberrations were found in human lymphocyte cultures (Banduhn, 1985), or in human lymphoid cell lines at ethanol concentrations of 8 and 16 mg/ml (174 and 348 mM), (Brown, 1992). Negative results have also been reported with Chinese hamster (CHO) cells (Lin, 1989). Darroudi (1987) found chromosome aberrations induced by ethanol only in the presence an extract of the leaves of maize with an NADPH-generating cofactor mix, metabolising system, a system that induced chromosome aberrations in CHO cells as well as increasing the number of aberrations induced by 160 mM ethanol.

Many studies for this end point, including most of those above, are incomplete in design, as currently recommended for screening purposes (OECD, 1997) and individually cannot be regarded robust. In particular, they generally did not employ metabolic activation and, in some cases did not use a sufficiently wide dose range. However collectively and using a weight of evidence approach, there is little evidence that ethanol is clastogenic *in vitro.* In those cases where positive responses were recorded, concentrations were extremely high and it is possible that chromosome damage resulted from non-specific effects such as high osmotic pressure

Cell mutation assays

In a study designed to test the conditions under which false positive results may be generated by the mouse lymphoma assay, ethanol was one of 50 compounds tested (Wangenheim, 1988). This study showed a small but statistically significant increase in mutants at 4.2 and 34 mg/ml

without S9, and at 24 mg/ml with S9. However, in no case was the mutant frequency doubled and it was concluded that a maximum concentration of 20 mM is adequate to detect genotoxins and that higher concentrations may give false positive results. Ethanol is regarded negative in this assay. No increase in mutants was found following a 4 hr exposure of S49 mouse lymphoma cells to ethanol in the presence and absence of S9 (Friedrich, 1983). In the L5178Y mouse lymphoma assay, ethanol was negative at 35.9 mg/ml, a concentration well above the maximum recommended for this type of test (Amacher, 1980).

<u>In vivo Studies</u>

Micronucleus assays

When administered in drinking water to rats at 5% (about 4,000 mg/kg) for 10 to 30 days (Balansky,. 1993) or at 10% and 20% (about 7,850 and 15,700 mg/kg) for 3 or 7 weeks (Tates, 1980), or to mice at up to 40% (about 31,400 mg/kg) for 27 days (Chaubey., 1977), ethanol had no effect on micronucleus incidence in the bone marrow when administered in the drinking water. In the study delivering 40% ethanol there was some ethanol-related mortality, which was clearly above the maximum tolerated dose, recommended as the highest dose for micronucleus studies in the OECD Guideline No. 474 (OECD, 1997).

Baraona (1981) fed rats for 6 weeks with diet containing ethanol calculated to contribute 36% of the calorific intake, a dosage of about 12 to 16 g/kg/day. The incidence of micronucleated bone marrow erythrocytes was increased from 0.95 % to 1.30 % in polychromatic cells (PCEs) and from 0.67 % to 0.84 % in orthochromatic cells (OCEs), relative to pair-fed controls. The difference in PCEs was statistically significant, but only marginally ($p<0.05$), and was associated with a decrease in the number of nucleated cells and an increase in the proportion of the nucleated cells that were in mitosis in the bone marrow. The difference in OCEs was not statistically significant. An effect on the mitotic spindle may be responsible but the presence or absence of centromeres in the micronuclei induced by treatment was not investigated. Overall, there is no convincing evidence that ethanol induces micronuclei in the bone marrow of rodents.

Chromosome aberration tests

No chromosome aberrations were found in the bone marrow or peripheral blood lymphocytes of male Wistar rats given 10 or 20% (about 7,850 and 15,700 mg/kg) ethanol in the drinking water for 3 or 6 weeks (Tates, 1980). Similarly, in Chinese hamsters, no aberrations were found in the bone marrow after exposure to ethanol in the drinking water at 10% for 9 weeks (Korte, 1979) or at 20% for 12 weeks (Korte, 1981), and no aberrations were observed in the lymphocytes of Chinese hamsters after exposure to 10% ethanol in the drinking water for 46 weeks (Korte, 1981a). Acute *in vivo* studies of chromosome aberration have not been identified. These studies of high-dosage ethanol in chronic administration failed to demonstrate an effect attributable to ethanol treatment. Therefore it is deemed unlikely that an acute study would show any effect either.

Dominant lethal assay

A large collaborative and robust study involving three laboratories, in which mice were exposed by intubation to the maximum tolerated dose of ethanol (0.64 g/kg) and to 0.16 g/kg and mated for 8 weeks (James, 1982), failed to yield positive evidence of dominant lethalility for ethanol. The protocol design used was comprehensive and compliant with OECD test methods.

A high level of dominant lethality has been reported in one series of studies in mice (Badr, 1975; Badr, 1977). In one experiment, eight male mice, treated with ethanol (1.24 g/kg bodyweight) by intubation on three consecutive days were mated sequentially to one female mouse per week. An observed marked reduction in the mean litter size of the ethanol group, on the third week

of mating only, was taken as evidence of post-implantation loss due to dominant lethal mutations even though uterine contents were not examined. In another experiment in which mice were treated with either 1.24 or 1.86 g ethanol/kg, and the uterine contents examined on gestation day 13 to 15, there were significant increases in the frequency of dead implants and of the dominant lethal index at the second and third mating time-points on days 4 to 8 and 9 to 13. This result fails to correlate with the previous experiment in which the effect was seen only at the 14 to 17-day time-point. Because of the numerous errors and inconsistencies in the studies, they were assessed as invalid and not reliable.

In a study deliberately designed to reproduce the effects reported by Badr (1975 & 1977), but using i.p. injection rather than intubation, Rao (1994) provided evidence that ethanol did not have a significant dominant lethal effect. Some pre-implantation loss observed in this study was thought to be due to an effect on the fertilization capacity of sperm. The effects of ethanol (approximately 1.26 g/kg/day for 3 days) on the outcome of mating on days 1 to 4, 5 to 8 and 9 to 12 were investigated further in high numbers Swiss mice. There were markedly fewer pregnant females (by 34% and 30%) at the first two mating times in the treated group and a significant decrease in total and live implants in the second mating. There was no increase in dead implants from the first two mating occasions and only a small but statistically significant increase at the third mating. No dominant lethal effect was found in two other experiments using CBA and C57BL6 mice chronically exposed to ethanol in the drinking water.

No dominant lethal effect was found when ethanol was administered to mice included in a liquid diet as 20% (14 to 17 g/kg) or 30% (24 to 30 g/kg) of dietary calories for 4 weeks (Randall, 1982). Mean blood levels were 57 mg/dl and 80 mg/dl respectively. In a similar study using ethanol at 28% of dietary calories (22 to 25.5 g/kg/day), 5-week exposure of mice decreased testicular weight, reduced fertility and increased pre-implantation losses, foetal mortality and mutation index (Berryman, 1992).

In rats, 15 days of exposure to ethanol, increasing to 58% of dietary calories (estimated ethanol intake 7.2 to 14.4 g/kg/day), resulted in an increase in early abortions, considered to have be a possible dominant lethal effect (Klassen, 1976). However, only six pregnancies were examined in the ethanol treatment group and the males had been treated chronically with ethanol such that quality of the study was impaired. Increases in the frequency of dead implants were also found when male rats were treated with 20% ethanol in drinking water for 60 days prior to mating (Mankes, 1982). Histological examination revealed significant pathological changes in the testes of potential impact on litters. In contrast, Chauhan (1980) found no effect of exposure of rats via the drinking water (30% ethanol) for 5 weeks. These were studies of small group sizes (10 or less) and of low power for interpretation.

Many studies can be criticised on the grounds of inadequate numbers of animals or on the methods used to score or evaluate the incidence of early or late foetal deaths or distinguish between early and late deaths. Also, the very high ethanol doses used make interpretation of the effects difficult. The most satisfactory test is the inter-laboratory study performed to OECD guidelines (James, 1982), a study that gave a negative result in mice from which it is concluded that ethanol is negative in the dominant lethal assay in male mice.

Conclusion

Interpretation of the available data on the genotoxicity of ethanol is confounded by experimental inadequacies. Negative results from a number of bacterial mutation assays appears to be reliable but studies of chromosome aberration can be criticised for not including an exogenous metabolic activation system. Of the mammalian cell mutation assays a weak mutagenic effect in L5178Y mouse lymphoma cells occurred only at very high ethanol concentrations and, when possible

artefacts are considered, the results of this study, too, can be regarded as negative. *In vivo* tests for chromosome aberrations in both rats and Chinese hamsters have given negative results. The results of the micronucleus and dominant lethal assays are variable and negative only in more robust studies and the overall weight of evidence favours the conclusion that ethanol does not induce dominant lethality in assays using standard regulatory methodologies; interpretation of these studies is confounded by the effects of ethanol on the fertilizing capacity of sperm. There is therefore very little evidence to suggest that ethanol is genotoxic in somatic cells and it may have a very limited capacity to induce genetic changes *in vivo* but under very specific circumstances and at very high doses achievable in humans only by deliberate oral ingestion (Phillips, 2001).

3.1.7 Carcinogenicity

Many carcinogenicity studies in laboratory animals exist using ethanol as a test substance. However, these are almost universally carried out to improve the understanding of the risks associated with the consumption of alcoholic beverages. Characteristically, these are carried out by the oral route and at high doses, well in excess of 1g/kg, which means they are inadequately designed and provide too little data to characterise the carcinogenic potential of ethanol at doses relevant to occupational exposure and use of consumer products containing the substance.

In vivo Studies in Animals

Oral

A recent National Toxicology Program study conducted to GLP standards exposed mice to ethanol in drinking water at the relatively high doses of 2.5% and 5% for 2 years. It showed only equivocal evidence of carcinogenic activity of in males based on increased incidences of hepatocellular neoplasms. However, there was no evidence of carcinogenic activity of ethanol in female mice exposed to either concentration (NTP, 2002). There remains no robust evidence of carcinogenicity in laboratory animals at doses other than those associated with consumption of alcoholic beverages. This is consistent with the findings of IARC who concluded that there is inadequate evidence for the carcinogenicity of ethanol in experimental animals (IARC, 1988)

Studies in Humans

Although no epidemiological studies are available for ethanol *per se*, there are a large number of studies (retrospective cohort, prospective cohort and case-control studies) on the effects of alcoholic beverages, which contain ethanol and water as the two main components. These epidemiological studies clearly indicate that drinking alcoholic beverages is causally related to cancers of the oral cavity, pharynx (excluding the nasopharynx), larynx and oesophagus. The effect appears to be independent of beverage type. The aetiology is likely to proceed via a mechanism whereby frequent exposure to high local concentrations of liquid ethanol and its metabolites leads to persistent irritation, and eventually hyperplasia and finally tumor formation. (Greim H, 1999). For the oral cavity, pharynx, larynx and oesophagus, alcoholic beverage consumption in excess of 10-40 g ethanol per day is necessary before there is a convincing increase in the relative risk of cancer (Greim H, 1999; UK Dept of Health, 1995). Such a mechanism would not therefore be relevant to occupational exposure. Drinking alcoholic beverages is also likely to be causally linked to liver cancer. The liver is the primary site of metabolism and sees high concentrations of ethanol and its metabolites. Tumor formation is normally associated with cirrhosis, which is in turn normally seen only following chronic alcohol abuse in excess of 80g ethanol per day (Greim H, 1999; UK Dept of Health, 1995). Such a scenario is not relevant to occupational exposure to ethanol. Drinking alcoholic beverages is also possibly causally linked to cancer of the breast and large bowel. Should the causal link with breast cancer be proven, the mechanism is believed to be via disturbance of the hormonal system. There is little or no indication of a causal relation

with cancer of the stomach, pancreas, lung, urinary bladder, kidney, ovary, prostate, lymphatic or haematopoietic system. There is no convincing evidence that the carcinogenic effects of alcoholic beverages in humans occurs as a result of the mutagenic effect of ethanol, acetaldehyde or other beverage constituents (UK dept of Health, 1995). The International Agency for Research on Cancer has classified alcoholic beverages (but not ethanol) as Group 1 - carcinogenic to humans (IARC, 1988).

The International Life Sciences Institute (ILSI, 1999) has published an extensive review of the health issues relating to alcohol consumption. They concluded that ethanol is not a carcinogen by standard laboratory tests. Such tests are the normal measure for the assessment of industrial chemicals and any chemical that would be expected to present a carcinogenic hazard to workers or consumers during normal handling and use would be expected to show a positive result in one or more of such tests.

Conclusion

Taking into account the known information on uptake of ethanol by the inhalation and dermal routes and the lack of genotoxicity of ethanol, it can be concluded with some confidence that occupational exposure to ethanol and the use of ethanol in consumer products does not pose a carcinogenic hazard.

3.1.8 Toxicity for Reproduction

For a novel chemical produced in the quantity in which ethanol is manufactured, there would be requirements for testing by a relevant route of exposure according to OECD guidelines. Comparatively few published studies are useful for quantitative risk assessment and few precisely meet all the requirements of the specified regulatory guideline. Importantly there is only a small number that have used multiple dose levels in order to clearly demonstrate a lowest observed adverse effect level (LOAEL) and a no observed adverse effect level (NOAEL). Many do not meet the criteria for robustness required for inclusion but are nevertheless important for interpretative purposes and a weight of evidence approach.

Studies in Animals

Effects on Fertility

In laboratory animals there is a paucity of information on potential fertility effects in females. In one robust 2-generation study in mice, ethanol in drinking water at concentrations up to 15% (equivalent to 20.7 g/kg/day) had no demonstrable effect on fertility (George, 1985). Similarly, fertility over 7 weeks of treatment with ethanol at 10% or 25% of dietary calorie intake was not affected (Abel, 1989).

Few studies in males gave information on systemic ethanol exposure, which makes it difficult to estimate threshold levels for effects and few gave a range of exposures from which to estimate dose responses and 'no effect' levels. However, in one comprehensive study there was no effect on fertility in a group of 20 male rats given 3 g or 2 g/kg ethanol by oral intubation daily for nine weeks, achieving blood ethanol concentrations of 3380±150 and 1320±50 mg/l, respectively, (Abel, 1993). Although fertility was unaffected, this study did reveal higher incidences of runted pups in the resulting offspring at the highest exposure level (3g/kg). In another study (Abel, 1995), even with daily oral dosing of ethanol at 5 g/kg, there was no effect on male fertility.

In male rats exposed to ethanol by inhalation for 7 hours /day for 6 weeks in a combined fertility and developmental toxicity study there was no effect on fertility at 16,000 ppm (Nelson, 1985a, Nelson 1985b, Nelson 1988). All 19 males at this dosage successfully initiated a pregnancy.

The route of exposure would have resulted in the measured BEL (approximately 500 mg/l) being steadily maintained throughout the exposures.

An adverse effect on fertility was noted in male rats with administration of ethanol in the diet (10% ethanol derived calories) for 15 days prior to and throughout the mating period (Klassen, 1976). Only six pregnancies were initiated when the six exposed males were each paired with two untreated females. However, this study was confounded by general toxicity manifest as ataxia, lethargy and weight loss during the study period.

Reductions in ovary weight and reductions in oestradiol and progesterone in female rats receiving liquid diets containing 5% ethanol (36% EDC) for 49 days during the peri-pubertal period have been demonstrated (van Thiel, 1976). The reported BEL was relatively low (1100±90 mg/l) but the timing of the sample (taken 09.00 – 11.00 hours) was probably inappropriate to detect the peak likely at the usual time of feeding during the previous evening. Irregular cycles and longer oestrous cycles were noted in rats fed liquid diets containing 5% ethanol (36 % EDC) for 16 weeks but not after 8 weeks with 8 weeks recovery period (Krueger, 1982). Again, ovarian function was suppressed in rats that achieved blood alcohol levels of 2500 mg/l (Bo, 1982).

Few studies have given information on systemic exposure to ethanol, so it is difficult to determine the threshold for any adverse effects. One study demonstrated reduction in testis and epididymis weights related to effects on spermatogenesis in pubertal male mice given 5% ethanol containing liquid diets that achieved a BEL of 1600 mg/l (Anderson, 1985). Lower dose levels were not investigated, however, virtually all changes observed were found to be reversible.

Male rats exposed to ethanol vapour concentrations of 22, 23, 25 and 27mg/l (11,500 to 14,000ppm) continuously for 3-4 weeks achieved BELs of 94-187 mg/100ml (Rivier, 1983). BELs of ≥163 mg/mL were associated with inhibition of androgen secretion, but only in those animals that failed to grow. These results suggest that there is a threshold for adverse effects of around 130 mg/100ml (equivalent to NOAEC inhalation exposure of 23mg/l) by this relevant route of exposure.

Developmental Toxicity

Many of the published studies in laboratory animals have investigated the effects of high dose oral ethanol intake. High-dose studies are possible because of the low acute toxicity of ethanol, however, the use of high doses can cause difficulties when interpreting ethanol reprotoxicity data within the regulatory hazard assessment framework, because doses are often in excess of the maximum (1 $g.kg^{-1}.day^{-1}$) recommended in current chemical testing guidelines (e.g OECD 414, 416). Many rodent experimental studies are conducted using a 5% ethanol liquid diet, which provides 35-36% ethanol-derived calories. The achieved ethanol intake for a pregnant rat with this diet is approximately 10-12 $g.kg^{-1}.day^{-1}$ (i.e. >10 times the limit dose of a standard OECD 414 developmental toxicity study). A reduction in nutrient intake during a critical period of gestation would reduce foetal/pup weights and cause other postnatal effects.

<u>Assessment of ethanol by inhalation exposure</u>

Most relevant for occupational exposure hazard assessment are a series of inhalation developmental toxicity studies of ethanol conducted by Nelson *et al.,* (1985a, 1985b, 1988). In the first of these studies, the potential teratogenesis of inhalation exposure to ethanol was assessed conventionally (Nelson *et al.,* 1985a) as part of a more extensive study looking at a number of different alcohols, conducted by the US National Institute of Occupational Safety and Hygiene (NIOSH). Groups of 15 or 16 mated female Sprague-Dawley rats were exposed 7 hours/day throughout gestation (GD1-19, based on the presence of sperm on GD0) to ethanol concentrations of 0, 10000, 16000 or 20000 ppm. These resulted in average BELs of 0, 27, 420 and 1480 mg/l. Ethanol elicited severe maternal toxicity at 20 000 ppm but at the lower exposure levels, dams appeared hyperactive

after exposures. The authors reported male (but not female) foetal weights to be depressed at the 16 000 and 20 000ppm exposures, but the differences were small and not significant. There were also no significant differences in the incidences of external, visceral or skeletal malformations or variations. These results did not indicate teratogenicity at dose limiting maternal exposures.

Following on from the conventional teratogenesis study, groups of 15 mated female Sprague-Dawley rats were exposed similarly throughout gestation, but allowed to litter to assess potential behavioural effects in the offspring (Nelson, 1985b). Ethanol concentrations of 0, 10000 or 16000 ppm were used. There were no differences from controls in maternal weight gain or feed and water intake. Litter size and birth weights were unaffected, even at 16000 ppm, which would have been expected had the marginal differences in foetal weight at caesarean evaluation in the other study been a true treatment effect. Offspring survival and growth were unaffected and there were no postnatal 'behavioural' effects at the specified maternal exposures, establishing a developmental toxicity NOAEL at the highest dose tested (16000 ppm).

The results of inhalation studies showed that there was no indication of teratogenicity at dose limiting, maternally toxic concentrations. Apart from small non-significant differences in foetal weight of one sex, which were not apparent on pup birth weight, and small inconsistent differences in some aspects of neurochemical analysis, there was no evidence of developmental toxicity at 16 000 ppm, with an average steady state BEL of 420 mg/l.

Assessment of ethanol by oral exposure

In a robust study in mice, litter weight was not affected by ethanol-containing diets but malformations were significantly increased by maternal diets containing 25% or more of ethanol-derived calories. Grossly visible abnormalities, external, soft tissue and skeletal abnormalities affected the limb, eye, brain, heart, urinogenital tract and abdomen of foetuses (Randall, 1979). In rats treated with ethanol by gavage (12.5% v/v in distilled water daily throughout gestation and gestation plus lactation, learning was impaired in rats of both gender at 9 weeks relative to controls (Vaglenova, 1998). This remained evident in males, but not females, at 5 months. In offspring treated both pre- and post-natally with ethanol, 60% were poor learners compared with 33% in sucrose controls. Foetal weights were depressed and skeletal abnormalities occurred at 100% incidence in two strains of mice treated with ethanol at rates ranging 15 to 30% of calorie intake with a LOAEL of 15 % (Chernoff, 1977). These effects were primarily of the occipital bone but also affected the sternum and ribs. Visceral abnormalities affected 36 %, 100 % and 100 % of foetuses in the 3 ethanol treated groups. Dilated brain ventricles were the most frequent anomaly but open eyelids, exencephaly, gastroschisis and heart defects also occurred in the higher dose groups. in mice (Wier, 1987) is discussed further below.

'Effect' and 'no effect' levels for teratogenicity of orally administered ethanol were established in mice by Wier (1987) using an abbreviated evaluation of uterine contents at term (teratology probe) and a 'limited' postnatal study in which offspring were examined and weighed through to weaning on PND22. Ethanol dosages of 2.2, 3.6, 5.0, 6.4 and 7.8g.kg^{-1} were administered daily by gavage on GD8-14. Post-implantation losses were elicited at 5g.kg^{-1} and higher. In the postnatal component of the study, there were no significant effects on pup growth or survival at maternally toxic dose levels. A NOEL was established at 3.6g.kg^{-1} along with an embryotoxic LOAEL of 6400 mg/kg. However, the limited endpoints employed and the lack of BEL information reduced the value of this work for hazard assessment.

An important consideration in the interpretation of all oral developmental toxicity studies of ethanol is that pregnant animals exposed to alcohol consume less food than *ad libitum* non-alcohol subjects, whether the ethanol is in their drinking water, in a liquid diet or intubated. This means that malnutrition may be a confounding variable in such studies.

A series of older teratogenicity studies of ethanol are included since they followed relatively 'conventional' designs (Schwetz, 1978*)*. Pregnant CF-1 mice, Sprague-Dawley rats and New Zealand white rabbits were given 15% ethanol in their drinking water during the period of major organogenesis, (GD6-15 for mice and rats: GD6-18 for rabbits). BELs (measured in satellite groups of non-pregnant animals) were about 2000mg/l in mice and 250-500 mg/l in rats and rabbits. The values for rats and rabbits are considerably lower than expected from other studies administering such high ethanol concentrations and presumably are related to the observed and non-controlled decreased liquid intake and decreased maternal body weight in all species. Some skeletal variants in these studies were probably due to retarded fetal growth consequent to indirect maternal toxicity.

Conclusion

In extremis, ethanol can elicit adverse effects on the reproductive system and on fertility and fecundability in males and females and can trigger developmental toxicity in females. However, it is clear that this occurs at BELs that can only be achieved by the deliberate oral consumption of alcoholic beverages. In addition, the role of possible confounding factors is unclear. It can be concluded that blood ethanol concentrations resulting from ethanol exposure at doses relevant to occupational exposure and the use of consumer products containing ethanol are unlikely to produce reproductive or developmental toxic effects (Irvine, 2003).

3.2 Initial Assessment for Human Health

Human health hazards from exposure to ethanol are well known. However these are only evident at the very high doses associated with the consumption of alcoholic beverages. In the context of potential exposures resulting from occupational or consumer use of ethanol containing products (i.e. excluding use in beverages), ethanol appears to present a low human health hazard.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Valid acute aquatic toxicity data are available for fish, invertebrates, algae, and microorganisms.

Acute Toxicity Test Results

Toxicity to fish

Robust static (Johnson, 1980) and flow-through (Majewski, 1978) studies on freshwater fish gave LC_{50} values greater than 1100 mg/l. Values are shown in table 10. A robust static limit test showed a value >100mg/l (Ewell, 1986). A high reliable flow-through study with fathead minnows, conducted using EPA methodology, gave LC_{50} values at 1, 24, 48, 72 and 96 hours of 13,480 mg/l or greater than 18,000 mg/l (Mattson, 1976). There is good agreement between values for static and flow-through studies despite the fact that static tests are not optimal for studying a relatively volatile and readily biodegradable compound such as ethanol.

Table 10 Toxicity to fish

Species	Test period (hr)	LC_{50} (mg/l)
Salmo gairdneri	96	13,000
Salmo gairdneri	96	11,200
Pimephales promelas	96	>100
Pimephales promelas	96	14,200
Pimephales promelas	96	13,480

Toxicity to invertebrates

48-hour studies with the pelagic invertebrates *Daphnia magna* and *Cerodaphnia sp.* were conducted using method ASTM Standard E729-80 and gave reliable LC_{50} values of 12,340 mg/l and 5012 mg/l respectively (Takahashi, 1987). In similar studies a range of 24-h EC_{50} values in excess of 1000 mg/L have been reported (Bowman,1981; Calleja, 1994). A 24-h LC_{50} with the marine invertebrate *Artemia salina* was 1833 mg/L (Barahona-Gomariz, 1994). See table 11 for all robust values available.

Table 11 Toxicity to invertebrates

Species	Test and time period	Value (mg/l)
Ceriodaphnia	LC_{50} (48hr)	5,012
Daphnia magna	LC_{50} (48hr)	12,340
Artemia salina	LC_{50} (24hr)	1,833
Paramecium caudatum	LC_{50} (4hr)	5,980
Palaemonetes kadiakensis	EC_{50} (18hr)	1,000
Daphnia pulex	EC_{50} (18hr)	2,000
Hyallela azteca	EC_{50} (18hr)	1,000
Artemia salina	EC_{50} (24hr)	23,874

Toxicity to aquatic plants

Two robust studies of 96-hr duration each give NOEC values of <500 mg/l for the growth rate end point in *Chlorella vulgaris* and *Selenastrum capricornutum* (El Jay, 1996). Corresponding EC_{50} values were 1,000 mg/l and 10,000 mg/l. Another study conducted to a high standard in *Chlamydomonas eugametos* yielded a 48-hr EC_{50} of 2000mg/l (Hess, 1980) and a study on *Chlorella pyrenoidosa* and a variety of algal species all gave EC_{50} values greater than 1,000 mg/l (Cowgill, 1989; Hess, 1980; Stratton, 1987; Stratton, 1988). One study of 5 days duration in *Skeletonema costatum* gave a NOEC in the range 3,240 to 5,400 mg/l based on cell count and corresponding EC_{50} values of 10,943-11,619mg/l). The authors (Cowgill, 1989) remark that using EPA criteria, ethanol can be judged non-toxic by this test and that ethanol was used as a carbon source stimulating growth of the alga before inhibition began.

All results are shown in table 12.

Table 12 Toxicity to aquatic plants

Species	Test period (days)	EC_{50} (mg/l)
Chlorella vulgaris	4	1,000
Lemna gibba	7	4,432
Lemna minor	7	3,690
Selenastrum capricornatum	4	10,000
Chlamydomonas eugametos	2	2,000
Skeletonema costatum	4	10,943-11,619
Chlorella pyrenoidosa	10	1,180

Chronic Toxicity Test Results

Several chronic exposure studies have been conducted for periods ranging 4 to 21 days in a variety of freshwater and marine invertebrates. They consistently yield LC_{50} values in excess of 100 mg/l (Cowgill, 1991a; Rayburn, 1997). The lowest reported NOEC is 9.6mg/l for *Cerodaphnia sp* (Cowgill, 1991a).

Studies of 7 days exposure to ethanol in the higher (vascular) plants *Lemna gibba* and *L. minor*, conducted to EPA OTS 797.1160, gave EC_{50} values of 4432 mg/l respectively. Equivalent NOEC values were 280 and 778 mg/l respectively (Cowgill, 1991).

Toxicity to Microorganisms

Only one study has been found giving quantitative data for the bactericidal activity of ethanol. A study using *Pseudomonas putida* showed a 16-hour toxicity threshold of 6500 mg/L (Bringmann, 1980).

4.2 Terrestrial Effects

Studies on root growth in onions (Fiskesjo, 1985), germination of lettuce seeds (Reynolds, 1977) and coleoptile growth and respiration rate in maize (Nashed, 1958) have demonstrated inhibitory effects at ethanol concentrations in excess of 3000 mg/l. Studies of respiration in potato tuber tissue (Miller, 1935; Rychter, 1979) produced effects of both stimulation and inhibition of respiration at low concentrations and the toxicological significance of these findings is doubtful. The same is said for several studies in which plant growth was stimulated, perhaps with beneficial application, for example in oats (Mer, 1958), girasole (Reichhart, 1979), sugar cane (Clements, 1940) and potato (Guthrie, 1931). The toxicological relevance of these results is uncertain.

In the only study of the effect of ethanol on soil dwelling organisms found, the oligochaete worm *Eisenia foetida* showed a 48 hr LC_{50} of 0.1-1.0 mg/cm^2 loading on a filter paper test (Roberts, 1984). The 'dosage', presented in terms of concentration in a given area of substrate is difficult to interpret but would have equated to the worms being exposed to a theoretical vapour concentration in the range 200-2000 mg/l.

4.3 Initial Assessment for the Environment

Ethanol is expected to partition to the air and water compartments. It is readily biodegradable, is not expected to bioaccumulate. Ethanol is predicted to degrade rapidly in atmospheres where NO_X or SO_X are present. Valid acute aquatic toxicity data are available for fish, invertebrates, algae,

and microorganisms. The most sensitive species were algae *Chlorella vulgaris* with an EC50 of 1000 mg/l and the invertebrate *Artemia Salina* with an LC50 of 1833 mg/l. Chronic data is available for invertebrates and algae. In the long term tests the most sensitive species was clearly the invertebrate Ceriodaphnia with a NOAEC of 9.6mg/l. The data available on terrestrial species is difficult to extrapolate to a the context of a hazard assessment but does suggest that ethanol is of low toxicity.

5 RECOMMENDATIONS

It is recommended that ethanol be considered as low priority for further work because of very low toxicity to humans and the environment at conceivable exposures likely to result from the manufacture and use of ethanol and ethanol containing products.

6 REFERENCES

Abel EL. (1989) Duration of paternal alcohol consumption does not influence offspring growth and development. Growth Dev. Aging. 53(4):195-199

Abel EL. (1993). Rat offspring sired by males treated with alcohol. Alcohol. 10(3):237-242.

Abel EL. (1995) A surprising effect of paternal alcohol treatment on rat fetuses. Alcohol. 12(1):1-6.

Alexandersson, R. and Hedenstierna, G. (1988). Respiratory hazards associated with exposure to formaldehyde and solvents in acid-curing paints. Arch Environ Health 43(3):222-227.

Amacher, D.E. et al. (1980). Point mutations at the thymidinekinase locus in L5178Y mouse lymphoma cells. II. Test validation and interpretation. Mutation Res. 72, 447 - 474.

Ambrose D, Sprake CHS (1970), Thermodynamic properties of organic oxygen compounds. XXV. Vapour pressures and normal boiling temperatures of aliphatic alcohols. J Chem Thermodynam, 2, 631-45

Anbar, M., Netta, P. (1967). A compilation of specific bimolecular rate constants for the reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals with inorganic and organic compounds in aqueous solution. Int. J.Appl. Radiation & Isotopes 18, 493 - 523.

Anderson, R. A. Jr, Willis B.R. and Oswald, C. Spontaneous recovery from ethanol-induced male infertility. Alcohol 1985, 2: 479-484

Andersson-Skold, Y. and L. Holmberg. (2000) "Photochemical Ozone Creation Potentials (POCP) and Replacement of Solvents in Europe". Atmospheric Environment 34:3159-3169.

Anonymous (1986). Hearings before the Sub-Committee on Select Revenue Measures of the Committee of Ways and Means, US House of Representatives, 99th Congress, 2nd Session, May 12th & 9th, 1986.

Badr, F.M. & Badr, R.S. (1975). Induction of dominant lethal mutations in male mice by ethyl alcohol. Nature 253, 134 - 135.

Badr, F.M., Badr, R.S., Asker, R.L., Hussain, F.H. (1977). Evaluation of the mutagenic effectsof ethyl alcohol by different techniques. Adv. Exp. Med. Biol. 85A:25-46.

Balansky, R.M., Blagoeva, P.M., Mircheva, Z. I. and De Flora, S. (1993). Coclastogenicity of ethanol with cigarette smoke in rat erythroblasts and anticlastogencity in alveolar macrophages.Cancer Lett 72:183-189.

Banduhn, N. and Obe, G. (1985). Mutagenicity of methyl 2-benzimidazole, diethylstilboestrol and estradiol: Structural chromosomal aberrations, sister chromatid exchanges, C-mitoses, polyploidies and micronuclei. Mutat. Res. 1985;156:199-218.

Barahona-Gomariz, M.V., Sanz-Narrera, F., Sanchez-Fortun, S. (1994). Acute toxicity of organic solvents on Artemia salina. Bull Environ Contam Toxicol 52(5):766-771.

Baraona, E., Guerra, M., Lieber, C.S. (1981). Cytogenetic damage of bone marrow cells produced by chronic alcohol consumption. Life Sci. 29, 1797-1802.

Bartsch, W. et al. (1976) Acute toxicity of various solvents in the mouse and rat. Arzneim.-Forsch. 26, 1581 - 1583.

Berryman, S.H., Anderson Jr., R.A., Weis, J., Bartke, A. (1992). Evaluation of the co-mutagenicity of ethanol and delta9-tetrahydrocannabinol with Trenimon. Mutat. Res. 1992;278:47-60.

Birch, R.R., Fletcher, R.J. (1991) The application of dissolved inorganic carbon measurements to the study of aerobic biodegradability. Chemosphere 23(7):855-872.

Blevins, R.D., Shelton, M.S. (1983). Response of Salmonella typhimurium mutants to delta9-THC and in conjunction with known mutagens. J Environ Sci Health A18(3), 413-443.

Blevins, R.D., Taylor, D.E. (1982). Mutagencity screening of twenty five cosmetic ingredients with the Salmonella microsome test. J Environ Sci Health 1982;A17(2):217-239.

Bo, E.J., Krueger, W.A., Rudeen, P.K., Symmes, S.K. Ethanol-induced alterations in the morphology and function of the rat ovary. Anat Rec 1982; 202:235-260

Bowman, M., Olier, W., Cairns, T. (1981). Stressed bioassay systems for rapid screening of pesticide residues. Part 1: Evaluation of bioassay systems. Arch. Environ. Contam. Toxicol. 10:9-24.

BP Amoco Chemicals, internal data, Darton works, 1992.

BP Amoco Chemicals, internal data, Darton works, 1999.

BP Chemicals, (1997). Data provided by BP Chemicals. (Technical Services Publication.).

BP Chermicals Ltd (1984). Results of the Biological Investigations on Selected Materials of the Breox Polyalkylene Glycol Block Copolymer Range. BP Group Occupational Health Centre Toxicology Report 25-90-0150.

Bringmann, G. & Kuhn, R. (1980). Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test. Water Res. 14:231-241.

Brown, N.M., Trizna, Z., Pathak, S. (1992). Clastogenic interactions between lobeline sulfate and ethyl alcohol: A cytogenetic study. Anticancer Research 1992;12:1467-1470.

Calleja, M.C., Persoone, G., Geladi, P. (1994). Comparative acute toxicity of the firt 50 multicentre evaluation of in vitro cytotoxicity chemicals in aquatic non-vertebrates. Arch Environ Contam Toxicol 26(1):69-78.

Campbell, I.M. et al. (1976) Rate constants for reactions of hydroxyl radicals with alcohol vapours at 292 K. Chem. Phys.Letters 38, 362 - 364.

Carter, W.P.L. (2000), The SAPRC-99 Chemical Mechanism and Updated VOC Reactivity Scales URL http://www.cert.ucr.edu/~carter/reactdat.htm.

Cavanagh, L.A. et al. (1969) Atmospheric hydrocarbon and carbon monoxide measurements at Point Barrow, Alaska. Environ. Sci. Technol. 3:251-257.

CERI (2004) http://qsar.cerij.or.jp/cgi-bin/DEGACC/index.cgi?E

CEFIC (2003). Data provided by the Ethyl Alcohol Sector group of CEFIC.

Chaubey, R.C., Kavi, B.R., Chauhan, P.S., Sundaram, K. (1977). Evaluation of the effect of ethanol on the frequency of micronuclei in the bone marrow of Swiss mice. Mutation Res. 43:441-444.

Chauhan, P.S., Aravindakshan, M., Kumar, N.S., Sundaram, K. (1980). Failure of ethanol to induce dominant lethal mutations in Wistar male rats. Mutation Res. 79:263-275.

Chernoff, G. (1977). The fetal alcohol syndrome in mice: an animal model. Teratol. 15:223-230.

Clements, H.F., and E. Akamine. (1940) Root Stimulation in Sugar Cane (Saccharum officinarum) with Special Reference to the Efects of Ethyl Alcohol Am J Botany;27:482-487.

Coates Inks internal data, Farrington Gurney works, 2000.

Conibear, S.A. (1988). The absorption, metabolism, excretion, and health effects of industrially useful

Conolly, R. B. (1999). PBPK Modeling of Ethanol Pharmacokinetics in Human Males. BP-AMOCO internal report.

Corcoran, J., Kruse, H. and Skolnik, S. (1953). Thermal analysis of the systems hydrazine-methanol and hydrazine-ethanol. J. Phys. Chem. 57:435-437.

Cowgill, U., Milazzo, D., Landenberger, B. (1991). The sensitivity of Lemna gibba G-3 and four clones of Lemna minor to eight common chemicals using a 7-day test, Res. J. Water Pollut. Control Fed. 63:991-998.

Cowgill, U.M., Milazzo, D.P., Landenberger, B.D. (1989). Toxicity of nine benchmark chemicals to Skeletonema costatum, a marine diatom. Environ Toxicol Chem 8(5):451-455.

Cowgill, U.M., Milazzo, D.P. (1991a). The sensitivity of Ceriodaphnia dubia and Daphnia magna to Seven Chemicals Utilizing the Three Brood Test. Arch Environ Contam Toxicol 20(2):211-217

Crabb, D.W., Bosron, W.F., Li, T.-K. (1987). Ethanol metabolism. Pharmac Ther 34:59-73

Darroudi, F. & Natarajan, A.T. (1987). Induction of chromosomal aberrations and sister chromatid exchanges in CHO cells by mutagenic metabolites activated by plant microsomal extracts. Biologisches Zentralblatt 106:169-174.

De Flora, S., Zanacchi, P., Camoirano, A., Bennicelli, C, Badolati, G.S. (1984a). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutation Res.133;161-198.

De Flora, S., Camoirano, A., Zanacchi, P., Bennicelli, C. (1984b). Mutagenicity testing with TA97 and TA102 of 30 DNA-damaging compounds negative with other Salmonella strains. Mutat Res 134:159-165.

Derwent RG, Jenkin, ME, Saunders SM (1996). Photochemical ozone creation potentials for a large number of reactive hydrocarbons under European conditions. Atmosp. Env 30, 2, p181

Descotes, J. (1988). Identification of contact allergens: the mouse ear sensitization assay. J Toxicol -Cut & Ocular Toxicol 7:263-272.

ECETOC (1998). Eye irritation reference chemicals databank (second edition). Technical report no 48 (2), June 1988. European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels.

Ellenhorn, M.J., Barceloux, D.G.. Medical toxicology - Diagnosis and treatment of human poisoning. Elsevier, New York 1988. Pp 782-97.

El Jay, A. (1996) Toxic effects of organic solvents on the growth of Chlorella vulgaris and Selenastrum capricornutum. Bull. Environ. Contam. Toxicol. 57:191-198.

EU (1996). Code of practice for evaluation of fruit and vegetable juices", Association of the Industry of Juices and Nectors from Fruis and Vegetables of the European Economic Community, 1996, p 96/6

EU (2003). Technical guidance document (edition 2). http://ecb.jrc.it/existing-chemicals/

Ewell, W., Gorstch, J., Krnge, R. et al. (1986) Simultaneous evaluation of the acute effects of chemicals on seven aquatic species. Environ. Toxicol. Chem. 5:831-840.

Fiskesjo, G. (1985). The Allium Test as a Standard in Environmental Monitoring. Hereditas 102:99-112

Friedrich, U., Nass, G. (1983). Evaluation of a mutation test using S49 mouse lymphoma cells and monitoring simultaneously the induction of dexamethasone resistance, 6-thioguanine resistance and ouabain resistance. Mutat. Res. 1983;110:147-162.

Gerhold R, Malaney G (1966) Structural determinants in the oxidation of aliphatic compounds by activated sludge. J Water Poll Control Fed 38, 562-79

George, J., Myers, C., Reel, J. et al. (1985). Ethanol: Reproduction and fertility assessments in CD-2 mice when administered in the drinking water. NTP PB86144979.

Greubet, S. (1997). Versteckter Alkohol in Lebensmittel? Medizische Monatsschrift fur Pharmazeuten 20(8):218-219.

Greim, H (1999) Occupational Toxicants, Volume 12, Critical Data Evaluation for MAK Values and Classification of Carcinogens, 128-165. Wiley.

Guthrie, J.D. (1931) The Effect of Various Chemical Treatments of Dormant Potato Tubers on the Peroxidase, Catalase, pH, and Reducing Properties of the Expressed Juice Contr Boy T;3:499-508.

Hakim RB, Gray RH & Zacur H. (1998) Alcohol and caffeine consumption and decreased fertility. Fertility & Sterility. 70(4):632-637

Hayes S (1985) Ethanol induced genotoxicity. Mut Res 143, 23-27

Hellmer L, Bolcsfoldi G (1992) An evaluation of the E coli K-12 uvrB/recA DNA repair host mediated assay. Mut Res 272, 145-60

Hess, F. (1980). A Chlamydomonas algal bioassay for detecting growth inhibitor herbicides. Weed Sci. 28(5):515-520.

Ho, A and Ho, C. (1979). Toxic interaction of ethanol with other central depressants: antagonism by naloxone to narcosis and lethality. Pharmacol. Biochem. behav. 11:111-114.

Hollund, B.E. and Moen, B.E. (1998). Chemical exposure in hairdresser salons: effect of local exhaust ventilation. Ann Occup Hyg 42(4):277-281.

Holmberg, B., Kronevi, T., Ekner, A. (1986). Subchronic toxicity investigation of ethyl alcohol: a test for lowest effective dose (led) to be used in a long term bioassay for carcinogenicity. National Board of Occupational safety and Health, Solna, Sweden.

Howard, P (1990). Handbook of Environmental Fate and Exposure Data for Organic Chemicals, volume II. Solvents. Lewis Publishers: Chelsea, MI.

Hustert, K., Mansour, M., Korte, F. (1978) Reaktionen von essigester und aethanol in gegenwart von umweltkontaminanten (NO2 und SO2) unter simulierten troposphaerischen bedingungen. Chemosphere 1, 35-50.

IARC (1988). IARC monographs on the evaluation of carcinogenic risks to humans, Vol. 44. Alcohol drinking. IARC, Lyon, France.

ILSI. (1999). Health Issues Related to Alcohol Consumption. Ed. Macdonald, I. International Life Sciences Inst. 2nd edition. ISBN 1-57881-062-0.

Irvine, L. (2003) Relevance of the Developmental Toxicity of Ethanol in the Occupational Setting: A Review. Appl. Toxicol. 23, 289-99

Jacobs, G.A., Guido, A. (1987). OECD eye irritation tests on three alcohols. J. Coll. Am. Toxicol. part A, 9:56-57.

Jacobs, G.A. & Martens, M.A. (1992) OECD skin irritation tests on three alcohols. Acute toxicity data. J. Am. Coll.Toxicol. 11:733.

James, D.A. & Smith, D.M. (1982) Analysis of results from a collaborative study of the dominant lethal assay. Mutation Res. 97:303-314.

Johnson, W. and Finley, M. (1980). Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. U.S. Dept. of Interior, Fish and Wildlife Service, Washington, DC Resource Publication 137.

Jones AW, Andersson L (1993). Disappearance Rate of Ethanol from the Blood of Human Subjects: Implications in Forensic Toxicology. J Forensic Sci, JFSCA 1993;38(1):104-118.

Kavanaugh and Stocking (1999). A fate and transport of ethanol in the environment. EPA Blue Ribbon Panel. US EPA URL http://www.epa.gov/oar/caaac/mtbeethan.pdf.

Klassen, R.W. & Persaud, T.V.N. (1976). Experimental studies on the influence of male alcoholism on pregnancy and progeny. Exp. Pathol. 12;38-45.

Korte, A., Slacik-Erben, R., Obe, G. (1979). The influence of ethanol treatment on cytogenetic effects in bone marrow cells of Chinese hamsters by cyclophosphamide, aflatoxin B and patulin. Toxicology 12;53-61.

Korte, A., Wagner, H.M., Obe, G. (1981). Simultaneous exposure of Chinese hamsters to ethanol and cigarette smoke: cytogenetic aspects. Toxicology 20;237-246.

Korte, A., Obe, G. (1981a). Influence of chronic ethanol uptake and acute acetaldehyde treatment on the chromosomes of bone-marrow cells and peripheral lymphocytes of Chinese hamsters. Mutation Res. 88;389-395.

Krueger, W.A., Bo, W.J., Rudeen, P.K. (1982) Female reproduction during chronic ethanol consumption in rats. Pharmacol Biochem Behav;17:629-631.

Kruhøffer P (1983) Handling of inspired vaporized ethanol in the airways and lungs", Forensic Sci. Int, 21, 1-17.

Lerici, C.R., Nicoli, M.C. (1996). Chemical and physico-chemical properties affecting the quality and stability of bakery products. Adv. Food Sci 18(5/6):229-233.

Lester D. & Greenberg L.A. (1951). The Inhalation of Ethyl Alcohol by Man. Q. J Stud Alcohol 12:167-178.

Lin, Y-C., Ho, I-C., Lee, T-C. (1989). Ethanol and acetaldehyde potentiate the clastogenicity of ultraviolet light, methane methanesulfonate, mitomycin C and bleomycin in Chinese hamster ovary cells. Mutat. Res. 216:93-99.

Lyman, W., Reehl, W., Rosenblatt, D. (1990) Handbook of Chemical Property Estimation Methods: Environmental Behaviour of Organic Compounds. American Chemical Society, Washington, DC.

Majewski, H., Klaverkamp, J. and Scott, D (1978) Acute mortality and sub-lethal effects of acetone, ethanol and propylene glycol on the cardiovascular and respiratory systems of rainbow trout (Salmo gairdneri) Water Res. 13:217-221.

Mankes, R.F., LeFevre, R., Benitz, K.-F., Rosenblum, I. et al. (1982). Paternal effects of ethanol in the Long Evans rat. J. Toxicol. Envir. Hlth 10:871-878.

Mattson, V., Arthur, J. and Wallbridge, C. (1976). Acute toxicity of selected organic compounds to Fathead Minnows. U.S. EPA Environmental Reasearch Lab., Duluth, Minnesota, EPA 600/3-76-097.

McCann, J., Choi, E., Yamasaki, E., Ames, B.N. (1975) Detection of carcinogens as mutagens in the Salmonella/microsome terst: Assay of 300 chemicals. Proc Nat Acad Sci 1975;72(12):5135-5139.

Mackay, D. DiGuardo, A., Paterson, S., Cowan, C. (1996). Evaluating the environmental fate of a variety of types of chemicals using the EQC model. Env. Toxicol. Chem. 15(9):1627-1637.

Mer, C.L. (1958) Growth-Promoting Effect of Ethanol on Oat Seedlings Nature; 182:1812-1813.

Merck (1996) The Merck Index 12th edition, Merck & Co., Inc., Rahway, New Jersey.

Miller, L.P. (1935) Further Experiments on the Effect of Halogenated Aliphatic Compounds on the Respiration of Potato tubers. Contr Boy T;7:1-17

Moen, B.E. and Hollund, B.E. (2000). Exposure to organic solvents among car painters in Bergen, Norway. Ann Occup Hyg 44(3):185-189.

Monick, J.A. (1968) in Alcohols. Their Chemistry Properties and Manufacture. Reinhold 1969, LCCCN 68-23906:p75.

Moser, V; Balster (1985). Acute motor and lethal effects of inhaled toluene, 1,1,1-trichloroethane, halothane and ethanol in mice: effects of exposure duration. Toxicol Appl. Pharmacol 77:285-291.

Muiswinkel, W-J van, Kromhout, H., Onos, T. and Kersemaekers, W. (1997). Monitoring and modelling of exposure to ethanol in hairdressing salons. Ann Occup Hyg 41(2):235-247.

Nashed, R.B; R.E. Girton. (1958) Inhibition by Ethanol of the Growth and Respiration of Maize Roots and Coleoptiles. Am J Botany;45:190-193.

NTP (1996). National Toxicology Program Technical Report on Toxicity Studies on Urethane in Drinking water and Urethane in 5% Ethanol Administered to F344 Rats and B6C3F1 Mice. NTP, Research Triangle Park, NC, USA

NTP (2002) Toxicology and Carcinogenesis Studies of Urethane, Ethanol, and Urethane/Ethanol in B6C3F1 Mice (Drinking Water Studies). Draft National Toxicology Program Technical Report NTP TR 510 NIH Publication No. 02-4444, NTP, Research Triangle Park, NC.

Nelson, B., Brightwell, W., MacKenzie, D., Khan A, Wiegel W, Goad P. (1985a). Teratological assessment of methanol and ethanol at high inhalation level in rats. Fundam Appl Toxicol 5:727-736.

Nelson B, Brightwell W, Burg J. (1985b). Comparison of behavioral teratogenic effects of ethanol and n-propanol administered by inhalation to rats, Neurobehav. Toxicol. Teratol. 7(6): 779-783.

Nelson B, Brightwell W, MacKenzie-Taylor D, Burg J & Massari V. (1988). Neurochemical, but not behavioral deviations in the offspring of rats following prenatal or paternal inhalation exposure to ethanol, Neurotoxicol. Teratol. 10:15-22.

OECD (1997). Guidance for determining the quality of data for the SIDS dossiers (reliability, relevance and adequacy). Interim SIDS manual and Interim HPV chemicals manual.

Ophaswongse, S., Maibach, H.J. (1994). Alcohol dermatitis and contact dermatitis syndrome. A review. Contact Dermatitis 1994; 30:1-6.

Pendlington R.U. (2001). Fate of ethanol topically applied to the skin. Food Chem. Toxicol., 39, 169-174.

Phillips, L. II., Steinberg, M., Maibach, H.J., Akers, W.A. (1972). A comparison of rabbit and human skin response to certain irritants. Toxicol Appl Pharmacol 21:369-382.

Phillips P. J., Jenkinson, P. (2001). Is ethanol genotoxic? A review of the published data. Mutagenesis 16, 2 91-101.

Price, K.S. et al. (1974) Brine shrimp bioassay and seawater BOD of petrochemicals. J. Water Pollut. Control Fed. 46, 63 - 77.

Randall, C. and Taylor, W. (1979) Prenatal ethanol exposure in mice: teratogenic effects. Teratol. 19:305-312

Randall, C.L., Burling, T.A., Lochry, E.A., Sutker, P.B. (1982). The effect of paternal alcohol consumption on foetal development in mice. Drug and Alcohol Dependence 9;89-95.

Rayburn, J.R., Fisher, W.S. (1997). Developmental toxicity of three carrier solvents using embryos of the grass shrimp, Palaemonetes pugio. Arch Environ Contam Toxicol 33(2):217-221.

Rao, U.N., Aravindakshan, M., Chauhan, P.S. (1994). Studies on the effects of ethanol on dominant lethal mutations in Swiss, C57Bl6 and CBA mice. Mutat Res 1994;311:69-76.

Regional Authority of Public Health, Banská Bystrica (2004), Evaluation of the Occupational Exposure to Ethanol, 6 p.

Reichhart, D., Salaun, J.-P., Benveniste, I., F. Durst. (1979) Induction by Manganese, Ethanol, Phenobarbital and Herbicides of Microsomal Cytochrome P-450 in Higher Plant Tissues. Arch Bioch;196:301-303.

Reynolds, T. (1977) Comparative Effects of Aliphatic Compounds on Inhibition of Lettuce Fruit Germination Ann.Bot.;41:637-648.

Rivier C & Vale W. (1983) Influence of ethanol on reproductive functions of the adult male rat as a function of body weight. Alcohol Clin. Exp. Res. 7(2):210-212.

Roberts, B.L., H.W. Dorough (1984). Relative Toxicities of Chemicals to the Earthworm Eisenia foetida. Environ Toxicol Chem 3(1):67-78.

Rychter, A., H.W. Janes, C.-K. Chin, and C. Frenkel. (1979). Effect of Ethanol, Acetaldehyde, Acetic Acid and Ethylene on Changes in Respiration and Respiratory Metabolites in Potato (Solanum tuberosum) Tubers. Plant Physiol;64:108-111.

Sabel GV, Clark TP (1983) Volatile Organic Compounds as indicators of municipal solid waste leachate contamination. Ann Madison Conf Appl Res Pract Munic Ind Waste, p108.

Sakurai M, Nakagawa T (1984), Densities of dilute solutions of water in n-alkanols at 278.15, 288.15, 298.15, 308.15 and 318.15K. Partial molar volumes of water in n-alkanols. J Chem Thermodyn, 16, 171

Schechter, M. and Meehan, S. (1995). The lethal effects of ethanol and cocaine and their combination in mice: implications for cocaethylene formation. Pharmacol Biochem Behav 52(1):245-248.

Schwetz, B.A., Smith, F.A., Staples, R.E. (1978). Teratogenic potential of ethanol in mice, rats and rabbits. Teratology 18:385-392.

Seeber A, Blaszkewicz M, Kiesswetter E, Bandel T, Golka K, Heitmann P, Vangala R.R, Bolt H.M. (1994) Biomonitoring, Leistung und Befinden bei inhalitiver Ethanolexposition", Transactions of the German Soc, for Occ. & Env. Med. 34th annual meeting in Weisbaden, May 1994

SPIN database(2003) http://www.spin2000.net/spin.html

Sprung, R., Bonte, W., Rudell, E., Domke, M., Frauenrath, C. (1981). On the problem of endogeneous alcohol. Endogenous ethanol: Further investigations. Alcohol Drugs Behav. 18(2):65-70.

Stratton GW (1987) Toxic effects of organic solvents on the growth of blue-green algae. Bull. Environ. Contam. Toxicol. 38:1012-1019

Stratton GW; Smith TM (1988) Interaction of organic solvents with the green alga Chlorella pyrenoidosa. Bull. Environ. Contam. Toxicol. 40:736-742.

Suflita, J. and Mormile, M. (1993). Anaerobic biodegradation of known and potential gasoline oxygenates in the terrestrial subsurface. Environ. Sci. Technol. 27:976-978.

Sulik K. (1984). Critical periods for alcohol teratogenesis in mice, with special reference to the gastrulation stage of embryogenesis. Mechanisms of alcohol damage in utero. Ciba Found. Symp. 105:124-141.

Tabakoff, B., Grant, K.A., Hoffman, P.L., Little, H.J. (1999). Chapter 9: Alcohol and the Central Nervous System. In: Health Issues Related to Alcohol Consumption. Ed. Macdonald, I. International Life Sciences Inst. 2nd edition. ISBN 1-57881-062-0.

Takahashi, I.T., Cowgill, U.M., Murphy, P.G. (1987) Comparison of ethanol toxicity to Daphnia magna and Ceriodaphnia dubia tested at two different temperatures: Static acute toxicity test results. Bull. Environ. Contam. Toxicol. 39:229-236.

Tates, A.D. (1980) Cytogenetic effects in hepatocytes, bone-marrow cells and blood lymphocytes of rats exposed to ethanol in the drinking water. Mutation Res. 79, 285 - 288.

Testar X, Lopez D, Llobera M & Herrera E. (1986) Ethanol administration in the drinking fluid to pregnant rats as a model for the fetal alcohol syndrome. Pharmacol Biochem Behav. 24(3):625-30

UK Department of Health (1995). Sensible drinking. The report of an inter-departmental working group, Dec 1995.

US EPA (2002). http://www.epa.gov/oppt/p2framework/docs/epiwin.htm

Vaglenova, J., Petkov, V.V. (1998). Fetal alcohol effects in rats exposed pre- and postnatally to a low dose of ethanol. Alcohol: Clin and Exp Res 22(3):697-703.

Van Thiel, D.H., Gavaler, J.S., Lester, R. (1976) Alcohol-induced ovarian failure in the rat. J. Clin Invest 1976; 61:624-632.

Wangenheim, J. and Bolcsfoldi, G. (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. Mutagen. 3(3):193-205.

Wiberg, G., Trenholm, H., Coldwell, B. (1970). Increased ethanol toxicity in old rats: changes in LD50, in vivo and in vitro metabolism, and liver alcohol dehydrogenase activity. Toxicol. Appl. Pharmacol. 16:718-727.

Wier, P., Lewis, S., Traul, K. (1987) A comparison of developmental toxicity evident at term to postnatal growth and survival using ethylene glycol monoethyl ether, ethylene glycol monobutyl ether and ethanol. Teratogen Carcinogen Mutagen 7:55-64.

Wilsnack SC, Klassen AD & Wilsnack RW. (1984) Drinking and reproductive dysfunction among women in a 1981 national survey. Alcohol. Clin. Exp. Res. 8(5):451-458

Yanagihara, S., Shimada I., Shinoyama E., Chisaka, E., Saito, K. (1977) Photochemical reactivities of hydrocarbons. Proc. 4th Int. Clean Air Cong. 472 - 477.(See http://esc.syrres.com/efdb/Chemfate.htm)

Yasuhara, A. *et al.* (1981). Analysis of organic substance in highly polluted water by mass spectrometry. Environ. Sci. Technol. 15:570-3.

Youssef, A., Madkour, K., Cox, C., Weiss, B. (1992). Comparative lethality of methanol, ethanol and mixtures in female rats. J. Appl. Toxicol. 12(3):193-197.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T, Mortelamns, K. (1992). Salmonella mutagencity tests: V. Results from the testing of 311 chemicals. Environ Mol Mut 19;2-141

ANNEX

Summary of general occupational exposure values

Operation and location	Number of Samples	Average measurement	Range	Comments	Reference
Hairdressing salons, Netherlands	195	5.7ppm	0.05 – 30 ppm	28 salons in Wageningen and Rotterdam. 114 people sampled over two seasons	Muiswinkel, 1997 (Ann Occ Hyg 41(2)
Hairdressing salons, Norway	10	10ppm	2.1 – 19 ppm	6 salons in Bergen	Hollund, 1998 (Ann Occ Hyg 42(4)
Liquid ink manufacture, UK	3	30ppm	18 – 51 ppm	Open top manufacture typical of highest exposures likely to be seen	Coates Inks internal data, 2000
Flexographic printing, UK	6	52ppm	10 - 110 ppm	Greatest exposure in mixing/application areas	BP Amoco Chemicals, Darton, 1999
Flexographic printing, UK	15	69ppm	19 – 177 ppm	Greatest exposure in washoff area.	BP Amoco Chemicals, Darton, 1992
Vehicle refinishing, Norway	16	1.2ppm	0.8 - 8.1 ppm	Ethanol a small component of solvent mixtures. Ranges are averages of 3-6 individual measurements	Moen, 2000 (Ann Occ Hyg 44(3)
Wood coating, Sweden	38	17ppm	(3 – 70 ppm)	Study primarily looking at formaldehyde exposures. Range data only given for total solvent concentration.	Alexandersson, (Arch of Env Hlth, 1988)
Electro-technical company, Slovakia	3	3 ppm	1.2- 5.8 ppm	Operator - soldering, mechanical repair (personal sampling)	Regional Authority of Public Health, Slovakia, 2004
Wood processing company, Slovakia	1	104 ppm	86-162 ppm	Painter - spraying with spray gun under exhaust ventilation (personal sampling)	Regional Authority of Public Health, Slovakia, 2004
Bus Transport company, Slovakia	2	53 ppm	5-302 ppm	Painter - spraying of paint with spray gun (personal sampling)	Regional Authority of Public Health, Slovakia, 2004
Pharmaceutical company, Slovakia	16	591 ppm	5,9 - 3432 ppm	Chemist – operator (personal sampling)	Regional Authority of Public Health, Slovakia, 2004
Pharmaceutical company, Slovakia	2	1111 ppm	60-2441 ppm	Service engineer (personal sampling)	Regional Authority of Public Health, Slovakia, 2004
Pharmaceutical company, Slovakia	2	356 ppm	4,8-1260 ppm	Foreman (personal sampling)	Regional Authority of Public Health, Slovakia, 2004
Pharmaceutical company, Slovakia	8	127 ppm	1.5-851 ppm	background in the hall (stationary sampling)	Regional Authority of Public Health, Slovakia, 2004

*Values expressed as ppm have been converted

Note (data from measurements made in the Slovak Republic): Long-term stationary and personal measurements were applied. Time of sampling was >70% of the shift time, each measurement represents the whole shift exposure (Ref.: Regional Authority of Public Health, Slovakia, 2004.)

I U C L I D

Data Set

Existing Chemical	:	ID: 64-17-5
CAS No.	:	64-17-5
EINECS Name	:	Ethanol
EC No.	:	200-578-6
TSCA Name	:	Ethanol
Molecular Formula	:	C2H6O

Producer related part

Company	:	BP Chemicals Ltd.
Creation date	:	15.04.1994

Substance related part

Company	:	BP Chemicals Ltd.
Creation date	:	15.04.1994
Status	:	
Memo	:	
Printing date	:	19.11.2004
Revision date	:	15.04.1994
Date of last update	:	19.11.2004
Number of pages	:	340
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type :
Name : CEFIC
Contact person : Mr Graeme Wallace
Date :
Street : Av. E. van Nieuwenhuyse 4
Town : 1160 Bruxelles
Country : Belgium
Phone : +32 (0) 2 676 7410
Telefax : +32 (0) 2 676 7216
Telex :
Cedex :
Email : gwa@cefic.be
Homepage :

26.09.2003

Type : cooperating company
Name : BP Chemicals Ltd.
Contact person : Mr Jeff Kelsey
Date :
Street : Chertsey Road
Town : TW16 7LN London
Country : United Kingdom
Phone : +44 (0)1932 762577
Telefax : +44 (0)1932 764147
Telex :
Cedex :
Email : kelseyj@bp.com
Homepage :

26.09.2003

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

Remark : Ethanol is being notified under the biocides directive as a basic substance. It is in use in all EU Member States.

16.01.2004

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name : Ethanol
Smiles Code : CCO
Molecular formula : C_2H_6O
Molecular weight : 46.07

Petrol class :
08.01.2002

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type : typical for marketed substance
Substance type : Organic
Physical status : Liquid
Purity : ca. 95 - 99.9 % w/w
Colour : Colourless
Odour : Alcoholic

18.01.2002

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

Absolute ethanol

Alcohol

Anhydrol

30.04.2004

Ethyl alcohol

Ethyl hydrate

Ethyl hydroxide

Fermentation Alcohol

30.04.2004

Grain alcohol

Jaysol

30.04.2004

Methylcarbinol

Molasses Alcohol

30.04.2004

Potato Alcohol

30.04.2004

Spirit

30.04.2004

Synasol

30.04.2004

Tecsol

30.04.2004

1.3 IMPURITIES

Purity :
CAS-No : 7732-18-5
EC-No : 231-791-2
EINECS-Name : Water
Molecular formula : H_2O
Value : ca. .1 - 5 % w/w

18.01.2002

1.4 ADDITIVES

Purity type :
CAS-No :
EC-No :
EINECS-Name :
Molecular formula :
Value : ca. 1 - 5 % v/v
Function of additive : other: Denaturants or organoleptic modifiers

Remark : For customs and excise reasons, ethanol is usually denatured when supplied into non beverage applications. Permitted denaturants vary between EU member states and a large number of permitted formulations exist depending on end use and user preferences. There is no comprehensive list of denaturants authorised under EU legislation. Common denaturants include wood naptha, methanol, isopropanol, methyl ethyl ketone, ethyl acetate, cyclohexane and acetone, etc. Typical concentration ranges are 1 to 5%, however, quantities can be lower than 0.5% and exceed 20% without altering the essential characteristics of ethanol for non beverage applications. Bitrex is also often used at a concentrations of 10's ppm as an organoleptic modifier. This information should not be regarded as an exhaustive list of denaturants or concentrations used.
Reliability : (2) valid with restrictions

Flag : Critical study for SIDS endpoint
20.08.2003

1.5 TOTAL QUANTITY

Quantity : = 1700000 - tonnes produced in 2001

Remark : There are literally thousands of alcohol producers in Europe. These include those producing 'neutral' ethanol, which is used in both industrial applications and in beverages such as gin and vodka, and those producing spirit drinks containing ethanol (e.g. whisky and wine.) Figures for production in the Europe Union in 2001 are shown in this and subsequent records. Synthetic ethanol represents about 30% of total production.

Ethanol type	EU Production (tpa)
Agricultural alcohol	790 000
Synthetic ethanol	500 000
Wine alcohol	230 000
Fuel alcohol	180 000
TOTAL	1 700 000

Reliability : (1) valid without restriction
12.10.2004 (1)

Quantity : = 46000 - tonnes produced in 2000

Remark : Figures for production in Czech republic
Reliability : (1) valid without restriction
12.10.2004 (2)

Quantity : = 41100 - tonnes produced in 2001

Remark : Figures for production in Czech republic
Reliability : (1) valid without restriction
12.10.2004 (2)

Quantity : = 49600 - tonnes produced in 2002

Remark : Figures for production in Czech republic
Reliability : (1) valid without restriction
12.10.2004 (2)

Quantity : = 7000000 - tonnes produced in 2001

Remark : Figures for production in the USA
Reliability : (2) valid with restrictions
12.10.2004 (1)

Quantity : = 25000000 - tonnes produced in 2001

Remark : Worldwide production
Reliability : (2) valid with restrictions
12.10.2004 (1)

1.6.1 LABELLING

Labelling : as in Directive 67/548/EEC
Specific limits : No
Symbols : F, , ,
Nota : , ,
R-Phrases : (11) Highly flammable
S-Phrases : (2) Keep out of reach of children
(7) Keep container tightly closed
(16) Keep away from sources of ignition - No smoking

Reliability : (1) valid without restriction
20.08.2003

1.6.2 CLASSIFICATION

Classified : as in Directive 67/548/EEC
Class of danger : highly flammable
R-Phrases : (11) Highly flammable
Specific limits :

Reliability : (1) valid without restriction
20.08.2003

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : Type
Category : Use in closed system

12.10.2004 (1)

Type of use : Type
Category : Non dispersive use

12.10.2004 (1)

Type of use : Industrial
Category : Personal and domestic use

12.10.2004 (1)

Type of use : Type
Category : Wide dispersive use

12.10.2004 (1)

Type of use : Industrial
Category : Basic industry: basic chemicals

12.10.2004 (1)

Type of use : Industrial
Category : Chemical industry: used in synthesis

12.10.2004 (1)

Type of use : Industrial
Category : Fuel industry

12.10.2004 (1)

Type of use : Industrial
Category : Paints, lacquers and varnishes industry

12.10.2004 (1)

Type of use : Industrial
Category : Public domain

12.10.2004 (1)

Type of use : Use
Category : Anti-freezing agents

12.10.2004 (1)

Type of use : Use
Category : Cosmetics

12.10.2004 (1)

Type of use : Use
Category : Fuel

12.10.2004 (1)

Type of use : Use
Category : Intermediates

12.10.2004 (1)

Type of use : Use
Category : Solvents

12.10.2004 (1)

Type of use : Use
Category : Biocide

12.10.2004 (1)

Type of use : Use
Category :

Remark : Beverages.
12.10.2004 (1)

1.7.1 DETAILED USE PATTERN

Industry category : 5 Personal / domestic use
Use category : 5 Anti-freezing agents
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : Available
Product type/subgroup :
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

12.10.2004 (1)

Industry category : 5 Personal / domestic use
Use category : 15 Cosmetics
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : Available
Product type/subgroup :
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

12.10.2004 (1)

Industry category : 5 Personal / domestic use
Use category : 48 Solvents
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : Available
Product type/subgroup :
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

12.10.2004 (1)

Industry category : 5 Personal / domestic use
Use category : 39 Biocides, non-agricultural
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : Available

Product type/subgroup : 01 Human hygiene biocidal products
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

12.10.2004 (1)

Industry category : 6 Public domain
Use category : 39 Biocides, non-agricultural
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : Available
Product type/subgroup : 02 Private area and public health area disinfectants and other biocidal products
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

12.10.2004 (1)

Industry category : 6 Public domain
Use category : 39 Biocides, non-agricultural
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : Available
Product type/subgroup : 03 Veterinary hygiene biocidal products
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

12.10.2004 (1)

Industry category : 6 Public domain
Use category : 39 Biocides, non-agricultural
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : Available
Product type/subgroup : 04 Food and feed area disinfectants
Tonnage for Application :
Year :
Fraction of tonnage for application :

Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

12.10.2004 (1)

Industry category : 14 Paints, lacquers and varnishes industry
Use category : 48 Solvents
Extra details on use category : Solvent based

Emission scenario document : Available
Product type/subgroup :
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

12.10.2004 (1)

Industry category : 3 Chemical industry: chemicals used in synthesis
Use category : 48 Solvents
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : Available
Product type/subgroup :
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : yes:
Private use :
Recovery :

Remark : Pharmaceutical processing
12.10.2004 (1)

Industry category : 15/0 other
Use category : 18 Explosives
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : not available
Product type/subgroup :
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :

Private use :
Recovery :

Remark : Damping agent
12.10.2004 (1)

Industry category : 15/0 other
Use category : 26 Food/feedstuff additives
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : not available
Product type/subgroup :
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

Remark : Food flavourings, fragrances, beverages
12.10.2004 (1)

Industry category : 15/0 other
Use category : 27 Fuels
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : not available
Product type/subgroup :
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

12.10.2004 (1)

Industry category : 2 Chemical industry: basic chemicals
Use category : 55/0 other
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : not available
Product type/subgroup :
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

Remark : Raw material
12.10.2004 (1)

1.7.2 METHODS OF MANUFACTURE

Origin of substance : Synthesis
Type : Production

Remark : All potable alcohol, and a large proportion of industrial and fuel ethanol, is made by the fermentation process in which zymase, a yeast enzyme, changes simple sugars (e.g. as found in molasses) into ethanol and carbon dioxide. Biomass (sugarbeet/cane, molasses, cereals, rice, grain, cellulosics) are used as the feedstock for fermentation ethanol. Synthetic routes to ethanol also exist, and, although a process based on acetaldehyde arising from acetylene is quoted, the virtually universal synthetic route is via the hydration of ethylene to ethanol.
12.10.2004 (1)

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : OES (UK)
Limit value : 1920 mg/m3

Reliability : (1) valid without restriction
12.10.2004 (3)

Type of limit : MAK (DE)
Limit value : 960 mg/m3

Remark : Peak limit category II,1
Carcinogen category 5
Pregnancy group C
Mutagen group 2
Reliability : (1) valid without restriction
12.10.2004 (4)

Type of limit : other: ACGIH PEL
Limit value : 1900 mg/m3

Reliability : (2) valid with restrictions
12.10.2004 (5)

Type of limit : other: Czech republic
Limit value : 1000 mg/m3
Short term exposure limit value
Limit value : 3000 mg/m3
Time schedule :
Frequency : Times

Reliability : (1) valid without restriction
12.10.2004 (2)

Type of limit : other: NIOSH PEL
Limit value : 1900 mg/m3

Reliability : (2) valid with restrictions
12.10.2004 (5)

Type of limit : other: OSHA PEL
Limit value : 1900 mg/m3
Short term exposure limit value
Limit value : 0
Time schedule :
Frequency : Times

Reliability : (2) valid with restrictions
12.10.2004 (5)

Type of limit : other: Slovak republic
Limit value : 960 mg/m3
Short term exposure limit value
Limit value : 1920 mg/m3
Time schedule : 30 minute(s)
Frequency : 4 times

Reliability : (1) valid without restriction
12.10.2004 (6)

1.8.2 ACCEPTABLE RESIDUES LEVELS

Proposed residues level : None
Maximum residue level : mg/kg

Remark : Ethanol occurs naturally and is endogenously produced. Human consumption of ethanol in alcoholic beverage can be high. A meaningful and controllable ARL may therefore be impossible to establish.
Reliability : (4) not assignable
08.08.2003

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

Classified by : other: US EPA
Labelled by :
Number :
Class of danger : other: not listed as toxic

Remark : No entry for ethanol in Hazardous Air Pollutants list, Air Toxics Website
Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint
12.10.2004 (7)

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

Type : EINECS
Additional information : Listed as ethanol, 2005786

17.01.2002

Type : TSCA
Additional information : Listed as ethanol, molecular formula C2H6O

22.09.2003

Type : Annex I, Council Regulation (EEC) No. 793/93
Additional information : Listed as ethanol, ethyl alcohol, Notes r59

17.01.2002

Type : Council Directive (EEC) No. 76/769
Additional information : Listed as ethanol, ethyl alcohol, Notes r59

17.01.2002

Type : IARC
Additional information : Listed as ethanol.

26.09.2003 (8)

Type : other: Canada, Transportation Dangerous Goods, Schedule II
Additional information : Listed as ethanol or ethanol solutions, No. UN 1987

17.01.2002

Type : other: DOT UN/NA
Additional information : NA1987, Hazard Class 3.

17.01.2002

Type : Annex I, Council Regulation (EEC) No. 793/93
Additional information : Index number 603-002-00-5. Risk Phrases 11, Symbol F

17.01.2002

Type : other: FDA Direct Food Additives
Additional information : Se 21 CFR 184.1293.

17.01.2002

Type : other: FDA Everything Added to Food in the US
Additional information : EAFUS document 421

17.01.2002

Type : other: FEMA Generally Recognised as Safe List

Additional information : Listed as Ethanol. TRGS 900 Lim value 1000 ml/m3 (ppm) = 1900 mg/m3.
FEMA no. 2419

26.09.2003

Type : other: HPV Chemicals
Additional information : Fully sponsored

17.01.2002

Type : other: Japan - Examined Substances/National Inventory
Additional information : Listed as ethanol. ENCS No. (2)-202

17.01.2002

Type : other: Korea, National Inventory (KECI)
Additional information : Listed as ethanol, ethyl alcohol No. KE-13217
ECL 2-858

26.09.2003

Type : other: NIOSH Health effects
Additional information : Listed as ethyl alcohol (ethanol). Health effects nihe267

22.09.2003

Type : other: NIOSH OELs
Additional information : Listed as ethyl alcohol, ni11.

17.01.2002

Type : other: NTP
Additional information : Listed as ethanol for toxicological testing.

17.01.2002

Type : other: OSHA
Additional information : OELs see 29 CFR 1910.1000 (Subpart Z)

17.01.2002

Type : other: UK HSE EH40
Additional information : Listed as ethanol. OELs

17.01.2002

Type : AICS
Additional information : Registered under CAS number

26.09.2003

Type : other: Norway
Additional information : YL number = 1163

26.09.2003

Type : CHINA
Additional information : Listed

26.09.2003

Type : PICCS
Additional information : Listed as CAS number

26.09.2003

Type : other: Switzerland
Additional information : BAG number = G-1158, list category 1/not listed

26.09.2003

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

Type : degradation product
CAS-No : 64-19-7
EC-No : 200-580-7
EINECS-Name : acetic acid
IUCLID Chapter :

Remark : By oxidation.
17.01.2002

Type : combustion products
CAS-No :
EC-No :
EINECS-Name : Oxides of carbon
IUCLID Chapter :

17.01.2002

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

Source of exposure : other: endogenous ethanol
Exposure to the : Substance

Method : Blood samples were collected from 130 subjects that had not consumed alcohol over the previous 24 hours, from 10 test subjects on 4 different days to ensure abstinence from ethanol consumption and from a total of 30 patients receiving treatment for metabolic illnesses, in some cases receiving treatment for withdrawal from alcohol.

Samples taken with a 'Koller venule' with the addition of NaF. Blood alcohol levels were determined with a 2-column chromatograph and headspace technique - details given in a separate reference (Bonte, 1981).
Remark : All of the individual measurement values are presented in the reference. From these it is possible to estimate that the endogenous ethanol range covering 95% of the population is 0.062 to 0.73mg/l.
Result : In the first group, physiological ethanol concentrations were all below 0.75 mg/ml with most values in the concentration range 0.1 and 0.2 mg/l.

In the second and third group, values were essentially the same as in

group 1.
Reliability : (2) valid with restrictions
12.10.2004 (9)

Source of exposure : Human: exposure of the consumer/bystander
Exposure to the : Substance

Remark : This paper is primarily about the role of ethanol produced during dough fermentation in the production of Maillard reaction products and their role as antioxidants. Ethanol production during bread making is discussed.
Result : About 1.6 to 2.8 g ethanol is produced per 100 g flour. Some is utilized in the Maillard reaction therefore baked bread has a lower ethanol content than this.
Reliability : (2) valid with restrictions
12.10.2004 (10)

Source of exposure : Human: exposure of the consumer/bystander
Exposure to the : Substance

Remark : Human exposure to alcohol from different foods and drinks is tabulated:

	Level (g/100g)
Alcohol-free beer	0.2-0.33
Malt beer	1.16-1.28
Apple juice	0.14
Grape juice	0.17
Kefir	0.5
Black Forest gateaux	0.5-1.0
Praline with alcohol filling	6.95
Ice-cream (alcohol/fruit)	0.74-2.44
Tiramisu	1.0
Wine saurkraut	0.1-0.8
Worcestersauce	0.79

Reliability : (4) not assignable
12.10.2004 (11)

Source of exposure : Human: exposure of the consumer/bystander
Exposure to the : Substance

Result : Alcohol content of food products:

Product:	%vol
A-1 sauce	0.04
Canada Dry Ginger Ale	0.08
Corr's Grapefruit Soda	0.17
Corr's Lemon Tangerine soda	0.21
Dr Pepper	0.03
Gerstel Brau (sample 1)	0.53
Gerstel Brau (sample 2)	0.51
Grey Poupon Mustard	0.06
Haagan-Daz Rum-Raisin icecream	0.35
Haagan-Daz vanilla icecream	0.48
Heinz catsup	0.04
Hunt's Snack Pak Vanilla Pudding	0.04
Kikkoman Soy Sauce	2.3
Kikkoman Teriyaki	3.0
Knott's Berry Farm Strawberry Pres.	n/d
Knudsen Black Cherry Juice	0.10
Knudsen Cherry cooler	0.19

Knudsen Cider and Spice	n/d
Knudsen Hibiscus Cooler	0.06
Kroger Vanilla extract	35.7
Lea and Perrins Worcestershire Sauce	0.16
Martinelli Sparkling Cida	0.05
S&W Ripe Olives	n/d
S&W Sauerkraut	0.15
Schweppes Tonic	0.08
Scope Mouthwash	18.9%
Seven-Up	0.07
Smucker's Strawberry Jelly	n/d
Spice Islands Red Wine Vinegar	0.24
Spice Islands White Vinegar	0.24
Sprite	0.06
Texas Select malt Beverage	0.24
Vick's NyQuil (25% label)	25.1
Vlasic Kosher Dill Pickles	0.04

Reliability : (4) not assignable
12.10.2004 (12)

Source of exposure : Human: exposure of the consumer/bystander
Exposure to the : Substance

Remark : Quality requirement for all orange juices: ethanol less than 3g/l.
Reliability : (4) not assignable
12.10.2004 (13)

Source of exposure : Human: exposure of the consumer/bystander
Exposure to the : Substance

Remark : ı the SPIN product register of the Scandinavian countries (Finland, Sweden, Denmark) showing the number of registered products for the year 2001 in Use (NACE). Only products with total registration >10te shown.

Country	Category of use	Number of products	Preparation quantity (tonnes)
FIN	Pulp, paper and paper products	14	42261.0
FIN	Chemicals and chemical products	13	16956.1
N	Chemicals and chemical products	88	12272.3
FIN	Radio, television and communication equipment	19	12239.8
DK	Chemicals and chemical products	115	11342.1
DK	Publishing, printing and reproduction media	123	6814.7
N	Construction	159	3414.4
DK	Construction	272	3025.3
FIN	Research and development	30	2797.5
FIN	Manufacture of food products and beverages	15	2515.0
FIN	Supporting transport activities	6	2500.0
FIN	Sale, maint. repair of motor vehicles	78	2418.1
N	Health and social work	8	2367.7
DK	Private households with employed persons	186	2216.7
FIN	Publishing, printing and reproduction media	121	1528.9
FIN	Wood and products except furniture	18	1422.1
DK	Manufacture of furniture	375	1314.1
FIN	Health and social work	31	1193.1
N	Land transport; transport via pipelines	10	1114.0
DK	Other business activities	119	1010.7
DK	Manufacture of wood prods. except furniture	285	934.6
DK	Sewage and refuse disposal	5	607.9
FIN	Land transport; transport via pipelines	7	476.0
DK	Health and social work	79	443.2
FIN	Construction	70	406.4
N	Manufacture of wood prods. except furniture	52	367.0
DK	Sale, maintenance, repair of motor vehicles	310	347.5
DK	Manufacture of rubber and plastic products	183	339.5
DK	Fabricated metal products, except machinery	339	304.1
DK	Manufacture of food products and beverages	426	258.0
N	Sale, maintenance, repair of motor vehicles	90	248.5

N	Publishing, printing, reproduction media	24	189.9
FIN	Forestry	4	186.1
N	Manufacture of furniture; manufacturing n.e.c.	82	180.2
FIN	Fabricated metal products, except machinery	19	174.1
DK	Manufacture of other transport equipment n.e.c	239	172.9
FIN	Other business activities	41	156.8
DK	Manufacture of basic metals	25	155.3
FIN	Manufacture of furniture; manufacturing n.e.c.	37	132.3
N	Retail trade, except of motor vehicles	11	131.7
N	Manufacture of other transport equipment n.e.c	144	110.4
DK	Medical, precision and optical instruments etc	19	98.3
DK	Retail trade, except of motor vehicles	43	90.5
DK	All kinds of activities	48	87.9
DK	Public administration and defence	34	84.1
N	Manufacture of food products and beverages	20	78.1
DK	Manufacture of machinery and equipment	61	65.0
FIN	Extra-territorial organisations and bodies	26	63.0
N	Education	4	58.7
DK	Agriculture and horticulture	14	40.1
FIN	Non-metallic mineral products	7	35.5
DK	Motor vehicles, trailers and semi-trailers	56	28.7
DK	Pulp, paper and paper products	18	20.2
DK	Wholesale trade and commission trade	9	17.2
FIN	Motor vehicles, trailers and semi-trailers	17	16.0
DK	Radio, television and communication equipment	21	15.3
DK	Manufacture of elec. machinery and apparatus	77	14.1
N	Fabricated metal prods, except machinery and eq.	47	12.8
N	Other service activities	21	10.7

Reliability : (4) not assignable
12.10.2004 (14)

Source of exposure : Human: exposure of the consumer/bystander
Exposure to the : Substance

Remark : Data from the SPIN product register of the Scandinavian countries (Finland, Sweden, Norway, Denmark) showing the number of registered products for the year 2001 in Industrial Use (national) category. Only products with a tonnage >10 are shown.

Country	Product type	Number of products	Tonnage of product
DK	Paints, varnishes and ink	61	5448.0
DK	Printing of books and offset printing	9	3299.1
DK	Private households with employed persons	186	2216.7
DK	Other printing works n.e.c.	20	1197.4
DK	Manufacture of pharmaceuticals	6	1123.7
DK	Printing and service activities	35	1105.0
DK	General construction of buildings	48	1028.4
DK	Bricklaying contractors	30	1019.4
DK	Industrial cleaning	42	770.5
DK	Construction	10	677.6
DK	Chemicals and chemical products	19	524.0
DK	Manufacture of furniture	76	446.9
DK	Perfumes and toilet preparations	14	406.2
DK	Varnishing/acid washing of furniture, etc.	191	302.1
DK	Manufacture of chairs and seats	74	273.7
DK	Publishing	12	270.2
DK	Manufacture of wood products, ex. furniture	45	238.7
DK	General repair shops	127	218.2
DK	Manufacture of plastic packing goods	40	200.5
DK	Printing of newspapers	9	193.8
DK	Publishing, printing and reproduction media	40	178.9
DK	Other printing works	11	175.7
DK	Floor and wall covering and floor planing	27	175.2
DK	Manufacture of soap and detergents	4	174.5
DK	Manufacture of other products of wood n.e.c	28	170.3
DK	Manufacture of other chemical products n.e.c	6	151.4
DK	Hospital activities	21	134.1
DK	Building and repairing of ships and boats	41	129.0
DK	Manufacture of plastic products	126	120.6

DK	Treatment and coating of metals	228	116.7
DK	General cleaning activities	70	108.3
DK	Painting	133	100.2
DK	Manufacture of other kitchen furniture	95	97.6
DK	Specialized cleaning activities	38	93.0
DK	All kinds of activities	48	87.9
DK	Office and shop furniture except of chairs	63	85.3
DK	Household furniture/varnishing of furniture	60	79.6
DK	Dispensing chemists	4	77.6
DK	Dentists	7	77.5
DK	Motor vehicle painters	130	67.5
DK	Production of mineral waters and drinks	118	53.2
DK	Manufacture of agricultural machinery	4	52.2
DK	Treatment and coating of metals	10	51.8
DK	Central heating radiators and boilers	9	51.8
DK	Manufacture of other fabricated metal prods	9	51.8
DK	Manufacture of builders carpentry/joinery	137	51.4
DK	Manufacture of other products of wood	9	47.7
DK	Casting of iron	6	47.0
DK	Manufacture of ice cream	96	33.8
DK	Administration of the state	9	32.3
DK	Fire service activities	8	32.1
DK	Home nursing activities/general health care	7	32.1
DK	Home help	7	32.1
DK	Manufacture of electronic equipment	28	30.2
DK	Residential nursing homes/sheltered homes	7	29.7
DK	Day institutions for elderly people	5	29.4
DK	Manufacture of food products and beverages	32	28.0
DK	Basic iron and steel and of ferro alloys	9	27.2
DK	Manufacture of cast iron tubes	5	27.0
DK	Casting of steel	5	27.0
DK	Manufacture of cocoa; chocolate and sugar	102	25.6
DK	Retail sale of automotive fuel	19	21.5
DK	Other motor vehicle services	24	21.3
DK	Human and health activities	22	20.7
DK	Fabricated metal prods, except machinery	96	20.2
DK	Manufacture of chairs except of upholstery	15	19.9
DK	Building and repairing of ships	113	18.9
DK	Corrugated paper and paperboard etc.	14	17.4
DK	Serigraphic printing	16	17.2
DK	Plastic plates/sheets/hoses/film/tubes etc.	4	16.3
DK	Building/repairing of boats	80	15.9
DK	Joinery installation	46	12.6
DK	Motor vehicles, trailers and semi-trailers	34	12.2
DK	Manufacture of machinery and equipment	46	11.5

Reliability : (4) not assignable
12.10.2004 (14)

Source of exposure : Human: exposure of the consumer/bystander
Exposure to the : Substance

Remark : Data from the SPIN product register of the Scandinavian countries (Finland, Sweden, Norway, Denmark) showing the number of registered products for the year 2001 in Use category UC62. Only products with total registration >10te shown.

Country	Application	Number of prods reg.	Tonnage of prods reg.
N	Solvents	47	16323
DK	Solvents	158	10174
DK	Cleaning/washing agents	306	3820
N	Non-agri. pesticides/preservatives	11	3605
N	Cleaning/washing agents	127	2102
DK	Reprographic agents	130	2024
DK	Non-agri. pesticides/preservatives	58	1031
DK	Paints, laquers and varnishes	700	891
DK	Process regulators	84	867
DK	Anti-freezing agents	16	865
DK	Surface treatment	99	661
N	Paints, laquers and varnishes	392	364
N	Reprographic agents	29	193
N	Surface treatment	20	177
N	Process regulators	21	146

DK	Adhesives, binding agents	136	139
DK	Surface-active agents	18	134
N	Adhesives, binding agents	54	90
DK	Others	15	81
DK	Food/feedstuff flavourings/nutrients	331	50
DK	Impregnation materials	18	38
DK	Anti-set-off and anti-stick agents	11	23
DK	Cosmetics	31	19

Reliability : (4) not assignable
12.10.2004 (14)

1.11 ADDITIONAL REMARKS

Memo : Conversion factors

Remark : 1 mg/m^3 = 0.52 ppm
1 ppm = 1.92 mg/m^3
26.09.2003 (15)

Memo : Threshold Odour Concentration (Air)

Remark : 50% of threshold odour concentrations are below 100 mg/m^3
Reliability : (4) not assignable
22.06.2004 (15)

Memo : Threshold Odour Concentration (Water)

Remark : 50% of Threshold Odour Concentrations are at approximately 800 mg/l.
Reliability : (4) not assignable
22.06.2004 (15)

1.12 LAST LITERATURE SEARCH

Type of search : External
Chapters covered : 4
Date of search : 22.11.2002

08.01.2002 (16)

Type of search : External
Chapters covered : 4
Date of search : 09.01.2002

11.01.2002 (17)

Type of search : External
Chapters covered : 5
Date of search : 18.01.2002

18.01.2002

1.13 REVIEWS

Memo : Fertility

Remark : Studies in mice and rats generally have not shown an effect on reproductive performance.

When female C57Bl/Crgl mice were given 10% ethanol (v/v) in water as the drinking fluid before mating, throughout gestation and lactation, no significant effect on reproductive capacity was seen (Thiessen D.D. et al. 1966. Q. J. Stud. Alcohol 27, 591, cited in IARC, 1988).

When female Wistar rats were given 20-25% of the calories consumed as 12% ethanol in a sucrose solution as the drinking fluid before mating and throughout gestation and lactation, there was no effect on reproductive performance (Oisund J.F. et al. 1978. Acta pharmacol. toxicol. 43, 145, cited in IARC, 1988).

Exposure of male rats [strain unspecified] to ethanol in utero or as neonates by administration of a liquid diet containing ethanol (36% of total calories) resulted in adverse effects on gonadal growth and development and disturbances in their sexual behaviour and performance when adult (Parker S. et al. 1984. Neurobehav. Toxicol. Teratol.6, 289, cited in IARC, 1988).

Mating of female Holtzman rats fed a liquid diet containing 5% ethanol for 16 weeks with untreated males resulted in no adverse effect on fertility, litter size or neonatal body weight (Krueger W.A. et al. 1982. Pharmacol. Biochem. Behav. 17, 629, cited in IARC, 1988).

Studies in mice and rats have shown effects on the testis and on other reproductive tissues.

In a study in which male C57BL/6J mice were given 5 or 6% (v/v) ethanol in a liquid diet for 70 days or 35 days, respectively, there was a significant decrease in testicularweight and in seminal vesicle/prostate weight, an increase in the frequency of germ-cell desquamation, inactive seminiferous tubules, inhibition of in-vitro fertilisation of mouse oocytes by epididymal spermatozoa, as well as a significant decrease in the total number of motile sperm. During a ten-week recovery period, improvement was greater in the group given 5% than in those given 6% ethanol (Anderson R.A. et al. 1985. Alcohol 2,
479, cited in IARC, 1988). Preparation of sperm from the cauda epididymis five weeks after oral administration of ethanol (1, 2 or 4 ml/kg bw) to male (CBA x Balb/c)F1 mice
five times daily did not show sperm anomalies (Topham J.C. 1980. M. Res. 69, 149, cited in IARC, 1988). Addition of ethanol to ram spermatozoa (0.62M; 15 µl in 0.4 ml semen
samples containing2-dioxy-D-glucose) inhibited sperm motility (Mayevsky A. et al. 1983. Archs Toxicol. Suppl.6, 295, cited in IARC, 1988).

There is evidence in vitro and in vivo that ethanol is toxic to animal and human Leydig cells and seminiferous tubules (Gavaler J.S. & Van Thiel D.H. 1987. M. Res. 186,
269; Van Thiel D.H. et al. 1983. Pharmacol. Biochem. Behav. 18, 317,both cited in IARC, 1988). Male Sprague- Dawley rats maintained on a liquid diet containing 6% ethanol (95%
v/v) for one week followed by four weeks on a 10% ethanol liquid diet showed adverse effects on sex organs (testes, seminal vesicles, ductules) as well as a significant
decrease in serum testosterone levels (Klassen R.W. & Persaud T.V.N. 1978. Int. J. Fertility 23, 176, cited in IARC, 1988). Male Sprague-Dawley rats that received an intraperitoneal injection of 2.5 g/kg bw ethanol

showed a significant decrease in the levels of luteinizing hormone and testosterone and marked attenuation of testicular steroidogenesis (Cicero T.J. et al. 1979. J. Pharmacol. exp. Ther. 208, 210, cited in IARC, 1988.)

Exposure of male rats [strain unspecified] to ethanol in utero or as neonates by administration of a liquid diet containing ethanol (36% of total calories) resulted in adverse effects on gonadal growth and development and disturbances in their sexual behaviour and performance when adult (Parker S. et al. 1984. Neurobehav. Toxicol.Teratol.6, 289, cited in IARC, 1988). Subcutaneous administration of 7.9 g/kg bw ethanol to female CD rats inhibited ovulation, primarily by blocking ovulatory surges of luteinizing hormone (Kieffer J.D. & Ketchel M.M. 1970. Acta endocrinol. 65, 117, cited in IARC, 1988). Blood levels of luteinizing hormone varied with dose and timing of treatment, but ethanol administered by intraperitoneal injection increased the secretion of prolactin by female Wistar rats (Alfonso M. et al. 1985. Gen. Pharmacol. 16, 43, cited in IARC, 1988). Exposure of Sprague-Dawley rats to ethanol (average, 11.6 g/kg bw) in utero altered the adult patterns of luteinizing hormone secretion in male and female offspring, indicating an effect on the central mechanisms that control secretion of pituitary luteinizing hormone (Handa R.J. et al. 1985. Life Sci. 37, 1683, cited in IARC, 1988). Administration of 5% ethanol (36% of total calories) in a liquid diet to female Wistar rats for 49 days decreased ovarian weight by 60% and significantly
decreased plasma oestradiol-17ß levels and the development of oestrogen target organs (Van Thiel D.H. et al. 1978. J. clin. Invest. 61, 624, cited in IARC, 1988).

Ovarian function in female Holtzman rats, 20 days of age, was suppressed by feeding of liquid diets containing 5% ethanol (36% of total caloric intake) for up to 55 days, in which blood ethanol concentrations averaged 2.5 g/l, but not by 2.5% ethanol (Bo W.J. et al. 1982. Anat. Rec. 202, 255, cited in IARC, 1988).

Vaginal opening was delayed in female Holtzman rats fed a liquid diet containing 5% ethanol for eight or 16 weeks. Among rats treated for 16 weeks, irregular oestrous cycles and cycles longer than those in control were observed. Mating of these females with untreated males resulted in no adverse effect on fertility, litter size or neonatal body weight (Krueger W.A. et al. 1982. Pharmacol. Biochem. Behav. 17, 629, cited in IARC, 1988).

In female macaque monkeys that administered ethanol to themselves intravenously on a schedule of reinforcement used for food acquisition, providing 2.9-4.4 g ethanol/kg
bw/day for 3-6.5 months, amenorrhoea, atrophy of the uterus, decreased ovarian mass and significant decreases in luteinizing hormone levels were observed (Mello N.K. et al. 1983. Science 221, 677, cited in IARC, 1988). In female rhesus monkeys infused intravenously with 2-4 g/kg bw ethanol after spontaneous onset of labour or following the induction of labour by infusion of oxytocin, partial suppression of labour was observed only in preterm animals with irregular uterine contractions (Horiguchi T. et al. 1971. Am. J. Obstet. Gynecol. 109, 910, cited in IARC, 1988).

Reliability : (2) valid with restrictions
22.06.2004 (18)

Memo : Developmental effects.

Method : A large number of literature references were reviewed. These are listed below as 'sources'. The overall summary of this review is presented in the Remarks paragraphs.

Result : The purpose of this document is to review recent (generally post 1990) scientific literature on the developmental effects of ethanol exposure and the research on potential mechanisms of adverse effects. The review focuses on the confounding influences, such as nutritional defects, and also examines the evidence for thresholds for adverse effects of ethanol exposure on development. Consideration of thresholds is of particular importance, to place the observed effects related to high ethanol exposures from excessive beverage consumption in context with the low exposures encountered with normal handling and use of ethanol in the occupational setting.

Ethanol is a commonly used industrial solvent, reactant and intermediary product, potential exposure to which in the occupational setting is by the inhalation and dermal routes. It is unique amongst chemicals in that it is present in many consumer goods and pharmaceutical preparations and it has been consumed by humans for millennia as a beverage and as a constituent of foods.

Excessive consumption of ethanol containing beverages increases the risk of adverse health effects, can be addictive and is associated with widespread social problems. A range of specific congenital malformations, known as the foetal alcohol syndrome (FAS), has been identified in a low percentage of children born of 'heavy drinking' / alcoholic mothers. Numerous animal studies and human epidemiology studies have confirmed an association with ingestion of high levels of ethanol during pregnancy and FAS, and also of lesser alcohol related birth defects (ARBDs) and alcohol related neurodevelopment disorders (ARNDs). There are conflicting results in human epidemiology studies as to threshold levels of ethanol consumption in relation to adverse pregnancy outcomes, reflecting confounding influences such as smoking history, nutritional status and socioeconomic status. Other factors also contribute to the variability of these studies, including difficulty inverifying intake of ethanol, different patterns of consumption and polymorphism in ethanol metabolism. There is general agreement that full expression of FAS occurs only with the chronic maternal ingestion of at least 2g/kg per day ethanol (approximately 3-4oz/ 90-120g ethanol per day for an average sized woman). his would be achieved by drinking about 10-12 'UK' drinks or 6-8 'US standard' drinks and is associated with a blood alcohol level (BAL) of around 150-200mg/dL. There is, however, less agreement as to the threshold for other effects (ARBDs and ARNDs). In recent concerted European epidemiology studies (EUROMAC), no cases of FAS were identified amongst 8448 pregnancies where the mothers consumed 'moderate' amounts of ethanol. Although 'binge' drinking (i.e. 5 or more 'standard' drinks per occasion) at critical stages of intrauterine development may be related to adverse effects, the studies found an ethanol intake of around 120g per week (averaged at 2 'standard' drinks per day) to be generally without adverse effect.

Some adverse effects on children's cognitive function at up to 14 years of age were observed in the 'Seattle' longitudinal epidemiology studies at lower average weekly maternal ethanol consumption. However, these averages included 'binge' drinkers, who constituted 25% of the participating mothers. Differentiation of results according to pattern of intake is critical, since it is the number of drinks per occasion and the attendant higher peak BAL, rather than a relatively constant lower BAL, which appears to be the major risk factor for ARBDs. There is also evidence of a threshold for adverse effects in animal studies. In a recently reported study of pregnant rats, carefully controlled for nutritional deficiencies, the effects of gastric intubation during pregnancy of 'moderate' levels of ethanol were estimated. The authors found that doses of 0.15 and 0.30g/kg ethanol (achieving BALs of up to 150mg/dL) were below the threshold for adverse effects on neonatal birth weight or on subsequent learning of weaned pups in passive avoidance tests.

In an earlier primate study in pregnant Macaques, it was found the BAL must be greater than 140mg/dL in order to produce neurological impairment or dysmorphology in offspring. This was achieved with dosages of at least 1.2g/kg/day. The authors also concluded that a dose of 0.6g/kg/day to the Macaque, which achieved a BAL of 51-71mg/dL, was without developmental toxicity. Although there have been occasional studies in animals reporting effects at lower ethanol exposure levels, usually there are questions about the validity of the BAL. It is generally found that BALs of around 100mg/dL during pregnancy in laboratory animals are without significant changes in offspring behaviour.
One of the more important findings from animal research is that nutritional aspects of prenatal ethanol exposure are inseparable from ethanol's teratogenic effects. Research inanimals has demonstrated that inadequate maternal diets can exacerbate the effects of ethanol and has confirmed that ethanol can directly and indirectly compromise nutritional status. However, although reducing nutrient deficiencies appears to mitigate some effects of lower doses of ethanol, foetal alcohol effects do not appear to be eliminated by dietary supplement beyond nominally adequate diets. The underlying basic mechanism by which ethanol consumption leads to FAS and ARBDs remains unknown. However, since ethanol apparently affects CNS development at all stages, itis highly unlikely that a single mechanism could be responsible for all of the varied effects that have been observed. Ethanol affects several physiological reactions that potentially can alter embryogenesis. These include altered metabolism with consequent functional changes of various nutrients in the foetus, hypoxia with interrupted blood supply to foetal organs, free radical production and a host of other actions. It is also possible that ethanol produces FAS through an interaction with certain nutrients, including vitamins and minerals. Research into the elucidation of mechanisms of effect continues and will ultimately refine the process of risk assessment. However for the present time, animal studies using the inhalation route of administration are the most relevant for occupational risk assessment. In conventional rodent developmental toxicity studies, investigating the effects of inhaled ethanol during pregnancy, there was no significant developmental toxicity, even at high doses, which induced maternal intoxication (narcosis), and with which BALs of 148-193mg/dL were achieved. Small quantities of ethanol are rapidly metabolised. It isonly after the ingestion of large quantities, such as from beverages, that metabolism is saturated and the high BALs, associated with toxicity, are achieved. Ethanol absorbed by inhalation is metabolised similarly and, unless exposure is significant, no noticeable effects are observed. In a recently reported human pharmacokinetic modelling study, with normal breathing of ethanol vapours of up to 5,000ppm (10 times the maximum workplace concentration [MAK]), the liver was able to metabolise ethanol at the rate it entered the body.
The BALs achieved through abuse of alcoholic beverages, and associated with developmental toxicity, would not be reached in the occupational setting. Nor is the abuse situation of relevance in the directed use of any other consumer product. For example a 30mL dose of a cold medicine which, for a consumer product contains a high (25%) ethanol concentration, would only be expected to give a peak level of 15-18mg/dL, even if all of it was absorbed instantaneously.

Conclusion : Based on abuse of alcoholic beverages,
ethanol appears to be a human developmental toxicant at high intake levels and to elicit adverse health outcomes on the mother. However, the role of possible confounding factors such as nutrition and socioeconomic status is unclear. Furthermore, the threshold at which adverse effects are achieved is not exceeded by a significant margin in the occupational setting or in consumers using ethanol containing non-beverage products.

Reliability : (2) valid with restrictions
10.08.2003 (19)

Memo : Mutagenicity

Method : Mutagenicity studies in vitro and in vivo were studied in the context of both industrial exposure to ethanol and exposure through consumption of alcoholic beverage. The main findings of the review are presented in the following remarks.

Remark : The available data on ethanol from standard genotoxicity test methods are incomplete. There is clear evidence that ethanol is not a bacterial or mammalian cell mutagen but in vitro assays for chromosome aberration, although mostly negative, have generally not included exogenous metabolic activation. Evidence from the use of ethanol as a vehicle control suggests that it is not mutagenic or clastogenic in vitro.

Reported tests for chromosome aberration induction in vivo are all negative and only a minority of micronucleus tests are positive. Conflicting results have been reported for the dominant lethal assay, although an interlaboratory study performed to OECD guidelines was negative. There is some evidence that ethanol induces SCE in vivo and can also act as an aneugen at high doses. Many in vivo studies were designed to model alcoholism and used very high doses, sometimes for long periods. Outcomes may have been affected by disturbances of metabolism giving rise to secondary effects.

Conclusion : It is concluded that there is no robust evidence that ethanol is a genotoxic hazard according to the criteria normally applied for the purpose of classification and labeling of industrial chemicals. Some degree of genotoxicity may result from excessive alcohol drinking, butthis is not considered relevant to any conceivable exposureobtainable by either inhalation or dermal exposure in the workplace.

Reliability : (2) valid with restrictions
25.08.2003 (20)

Memo : Carcinogenicity

Remark : Drinking of alcoholic beverages:
Although no studies are available for ethanol per se, there are a large number of epidemiological studies (retrospective cohort, prospective cohort and case-control studies) on the effects of alcoholic beverages, which contain ethanol and water as the two main components.
These epidemiological studies clearly indicate that drinking alcoholic beverages is causally related to cancers of the oral cavity, pharynx (excluding the nasopharynx), larynx and oesophagus. The effect appears to be independent of beverage type. Drinking alcoholic beverages is also likely to be causally linked to liver cancer and possibly to cancer of the breast and large bowel. There is little or no indication of a causal relation with cancer of the stomach, pancreas, lung, urinary bladder, kidney, ovary, prostate, lymphatic or haematopoietic system.

The International Agency for Research on Cancer has classified alcoholic beverages as Group 1 - carcinogenic to humans.

Reliability : (2) valid with restrictions
26.09.2003 (18)

Memo : Allergic contact dermatitis

Remark : Literature review demonstrates that ethanol can be an allergen in immediate and delayed hypersensitivity by external or internal exposure and can produce subjective irritation, irritant contact dermatitis and non-immunologic contact urticaria.

Allergic contact dermatitis was confirmed by positive patch testing at low (1%) concentration of challenge. Ethanol can also produce subjective irritation contact dermatitis but neither phototoxiciuty nor photoallergy has been documented. Contact dermatitis caused by ethanol may be misdiagnosed or overlooked.

Reliability : (4) not assignable

26.09.2003 (21)

Memo : Sensible Drinking - Health Effects

Remark : This Report of a Department of Health Inter-Departmental Working Group looks at the beneficial long term effects of alcohol, the harmful effects of alcohol including its influence on all cause mortality and examines the case of women and alcohol consumption. It gives general public health advice and provides recommendations for future action.

Conclusion : This is an excellent source of material examining the implications of non occupational exposures to alcohol.

Reliability : (2) valid with restrictions

10.08.2003 (22)

Memo : Health Issues Related to Alcohol Consumption

Remark : A comprehensive review of the literature to 1999 covering moderate drinking as a concept, assessment of alcohol consumption, and effects of alcohol on genetics, body weight, cardiovascular system, pregnancy, breast cancer, bone, CNS, cancers of the digestive tract and larynx and the liver.

Reliability : (2) valid with restrictions

16.01.2004 (23)

Memo : Moderate Drinking: Concepts, definitions and public health significance

Remark : The concept of moderate drinking is reviewed with consideration of moderate as non-intoxicating, as statistically normal, as non-injurious, as problem-free, and as optimal. Many different quantitative definitions are considered, all converted to similar units of g/day. For those sources which carry definitions of light, moderate and heavy drinking, the range quoted from multiple references and multiple national definitions for moderate is 4.5 to 50 g/day at the lower end and 24-80g/day at the upper end. The recommended level of alcohol intake for 'problem free' drinking is recommended as 24-60g/day for men and 12-36g/day for women. For the average man, the optimal level of ethanol consumption is 10-19g/day whilst a non-injurious level is 30-40g/day. For women, these levels are halved and do not apply in cases of pregnancy where abstinence is recommended as the safest choice (whilst accepting that occasional light drinking 1-2 drinks/week may have no adverse effects.)

Reliability : (2) valid with restrictions

12.10.2004 (24)

2.1 MELTING POINT

Value : = -114 °C
Sublimation :
Method :
Year : 1953
GLP : no data
Test substance : other TS:USI absolute

Method : Freezing and melting points determined in a stirred cell designed to protect contents from contact with atmosphere. The cell was surrounded with a clear glass dewar flask which provided uniform changes in temperature when the assembly was immersed in cooling or warming baths. The temperature in the cell was measured with a copper-constantan thermocouple inserted into a thermoouple well which contained n-propyl alcohol as a thermal conducting medium. The thermocouple was calibrated by measuring the freezing points of purified materials. Freezing points of benzene, water, carbon tctrachloride, mercury, chloroform and toluene were determined and a correction curve plotted. From this curve a correction was applied to the freezing points of the mixtures being studied. Cooling was using liquid nitrogen.
Remark : The authors noted that ethanol was prone to supercooling.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
11.09.2002 (25)

Value : = -114.1 °C
Decomposition : no, at °C
Sublimation : No
Method : other
Year :
GLP :
Test substance :

Remark : Method not specified.
Value after Corcoran, J., Kruse, H. and Skolnik, S. (1953) Thermal analysis of the systems hydrazine-methanol and hydrazine-ethanol. J. Phys. Chgem. 57:435-437.
Reliability : (2) valid with restrictions
16.01.2004 (26)

2.2 BOILING POINT

Value : = 78.3 °C at 1013.25 hPa
Decomposition : No
Method : other
Year : 1970
GLP : no data
Test substance : other TS

Method : Comparative ebulliometry. The apparatus used is described in detail in one of the references along with a detailed description of its operation. The two boilers (reference plus test substance) are connected by a common pressure line. Platinum resistance thermometers are used to measure the temperature using a Mueller bridge. Water was used as the reference substance. The method is reported to be repeatable to within a few thousandths of a degree.

Result : The results consist of two sets of readings for the thermometers, one for the sample and one for the standard. The data was processed by computer to establish the best fit Antoine and Kirchoff equations. The source of the reference data for water is quoted and any necessary corrections are described.

The result is quoted as 351.443K and is corrected for the freezing point of water of 273.15K
Source : Riddick JA, Bunger WB, Sakano TK (1986), Techniques of Chemistry, Vol II, Organic Solvents, Physical Properties and Methods of Purification, Wiley
Test substance : Samples purified before use, including drying in vapour phase. Fraction molarity purity 0.9995
Reliability : (2) valid with restrictions
Whilst old and not to an OECD protocol, the method and technique is well reported.
Flag : Critical study for SIDS endpoint
09.11.2004 (27) (28)

Value : = 78 °C at 760 hPa
Decomposition : No
Method : other
Year : 1951
GLP : no data
Test substance : as prescribed by 1.1 – 1.4

Source : Budavari, S., (ed.) (1996). The Merck Index. 12th Ed.
Merck&Co: Whitehouse Station, NJ.
Reliability : (2) valid with restrictions
09.11.2004 (29)

Value : = 78.5 °C at
Decomposition :
Method : other: IP71 section 1/97
Year : 1997
GLP :
Test substance : other TS: double rectified absolute alcohol

Remark : Data generated by SG Redwood (UK) Ltd, ISO9002 No Q4856
Reliability : (2) valid with restrictions
09.11.2004 (30)

Value : = 78.2 °C at
Decomposition : No
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Method not specified.
Reliability : (4) not assignable
29.09.2003 (31)

2.3 DENSITY

Type : Density
Value : = .7864 g/cm? at 25 °C
Method : other
Year : 1984

GLP : no data
Test substance : other TS

Method : Density determined using an oscillating tube densitometer DMA 60/61 (from Paar). The density is determined by measuring the natural vibration frequency of a U shaped glass tube filled with the test fluid. The density is related to the period of oscillation of the instrument but can be calculated relative to a known substance, in this case water, by the equation:

Ds - Dw = A x (Ts2 - Tw2)

where Ds and Dw are densities of substance and water, and Ts2 and Tw2 are the oscillation periods to the power of 2 of the test substance and water respectively. The constant A can be determined by making measurements with water and air. (Density values for water from reference Kell (1975)

The temperature was maintained within 0.002K and measured using a calibrated quartz thermometer. Water content was checked using a Karl Fisher titration apparatus.
Result : Results available at 5, 15, 25, 35 and 45C to an accuracy of 0.01C and 2E-6 for density.
Source : Riddick JA, Bunger WB, Sakano TK (1986), Techniques of Chemistry, Vol II, Organic Solvents, Physical Properties and Methods of Purification, Wiley
Test substance : Ethanol dried over a molecular sieve 3A then fractionally distilled. Water reference deionised then distilled using a quartz still.
Reliability : (2) valid with restrictions
Not to a standard OECD protocol but reported in detail and considered reliable
Flag : Critical study for SIDS endpoint
11.11.2004 (32) (33)

Type : relative density
Value : = .7896 at 20 °C
Method : other: IP365/97
Year : 1997
GLP :
Test substance : other TS: double rectified absolute alcohol

Remark : Test performed by SG Redwood (UK) Ltd. ISO9002, no Q4856
Reliability : (2) valid with restrictions
11.11.2004 (30)

Type : relative density
Value : = .789 at 20 °C
Method : other
Year :
GLP :
Test substance :

Reliability : (4) not assignable
29.09.2003 (31)

Type : Density
Value : = .7892 - .7896 at 20 °C
Method : other: ASTM D4052
Year : 2003
GLP :
Test substance : other TS

Remark : Sales specification for ethanol.
Test substance : >99.9% ethanol
Reliability : (2) valid with restrictions
Routine property measured to standard method.
18.10.2004 (34)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : = 57.26 hPa at 19.6 °C
Decomposition : No
Method : other (measured)
Year : 1970
GLP : no data
Test substance : other TS

Method : Comparative ebulliometry. The apparatus used is described in detail in one of the references along with a detailed description of its operation. The two boilers (reference plus test substance) are connected by a common pressure line. Platinum resistance thermometers are used to measure the temperature using a Mueller bridge. Water was used as the reference substance. The method is reported to be repeatable to within a few thousandths of a degree. The substance was first boiled at a pressure of around 15kNm-2 to check for consistency in boiling temperature.
Result : Multiple measurements of vapour pressure at temperatures between approximately 20C and 93C (i.e. above the boiling point).
Test substance : Samples purified before use, including drying in vapour phase. Fraction molarity purity 0.9995
Reliability : (2) valid with restrictions
Whilst old and not to an OECD protocol, the method and technique is well reported.
Flag : Critical study for SIDS endpoint
11.11.2004 (27) (28)

Value : = 78.7 hPa at 25 °C
Decomposition :
Method : other (measured)
Year : 1948
GLP : no data
Test substance : other TS: Commercial absolute

Method : Scatchard equilibrium still. Ethanol was fractionated in a 5 foot column packed with glass helices and then treated with magnesium ethylate. The final product of d(sup 25)(sub 4) 0.78506 was kept under its own vapour pressure in a sealed container over magnesium ethylate and samples were withdrawn by vacuum distillation.

Vapour pressure was measured using an inverted U-tube manometer and 12 mm diameter tubing read with a M901 Gaertner cathetometer at a distance of 250 m.

Static measurements were made by vapour pressure cell connected directly to the manometer. Agreement between
methods was within 0.2 mmHg.
Result : Value was recorded as 59.03 mmHg and converted.

Source : U.S. Environment Protection Agency High Production Volume, Chemical Right to Know Program.
Reliability : (2) valid with restrictions
11.11.2004 (35)

Value : = 66.3 hPa at 21.2 °C
Decomposition :
Method : other (measured): see remark
Year :
GLP :
Test substance :

Remark : Test performed at Sheffield Hallam university using and isoteniscope and internal method QP58
Result : Vapour pressure 76.0 hPa @ 24.4 deg C
Reliability : (2) valid with restrictions
11.11.2004 (30)

Value : = 66.66 hPa at 25 °C
Decomposition :
Method : other (measured)
Year :
GLP : no data
Test substance :

Remark : Method not specified.
Reliability : (4) not assignable
11.11.2004 (36)

Value : = 18 at 38 °C
Decomposition :
Method :
Year :
GLP : no data
Test substance :

Remark : Value is Reid vapour pressure in psi.
Reliability : (4) not assignable
11.11.2004 (37)

Value : = 49 - 56 at °C
Decomposition :
Method :
Year : 1999
GLP : no data
Test substance :

Remark : Value is mmHg.
Reliability : (4) not assignable
11.11.2004 (37)

Value : = 179.35 hPa at 40 °C
Decomposition : No
Method : other (measured)
Year : 1979
GLP : no data
Test substance : other TS

Method : Method designed to pressure isotherm data for binary mixtures.

		Temperatures measured to 0.01C.
Reliability	:	(4) not assignable
11.11.2004		(38)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	= -.31 at 25 °C
pH value	:	
Method	:	other (measured)
Year	:	1985
GLP	:	no data
Test substance	:	no data
Method	:	Test method and date are not known. There is no mention of surface activity, dissociative properties or of water solubility.
Reliability	:	(2) valid with restrictions This value has been accepted by U.S. Environment Protection Agency High Production Volume, Chemical Right to Know Program.
Flag	:	Critical study for SIDS endpoint
05.10.2003		(35)
Partition coefficient	:	octanol-water
Log pow	:	at °C
pH value	:	
Remark	:	QC reviewed value
Reliability	:	(2) valid with restrictions
19.10.2004		(39)
Partition coefficient	:	octanol-water
Log pow	:	= -.16 - -.32 at °C
pH value	:	
Remark	:	Value expressed as log Kow and presumed calculated.
Reliability	:	(4) not assignable Secondary source
29.09.2003		(40)
Partition coefficient	:	octanol-water
Log pow	:	= -.32 at 25 °C
pH value	:	
Remark	:	No details of method available.
Reliability	:	(4) not assignable
19.10.2004		(41)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	> 10000 mg/l at 25 °C
pH value	:	= 0
concentration	:	at °C
Temperature effects	:	

Examine different pol. :
pKa : 16 at 25 °C
Description :
Stable :
Deg. product :
Method : Other
Year : 1900
GLP : no data
Test substance : no data

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
19.10.2002 (35)

Solubility in : Water
Value : at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description : Miscible
Stable :

Reliability : (2) valid with restrictions
16.01.2004 (26)

Solubility in : Water
Value : at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description : Miscible
Stable :

Reliability : (4) not assignable
29.09.2003 (42)

Solubility in : Water
Value : at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : 0 at 25 °C
Description :
Stable :

Remark : Ethanol is stable in water. pKa is irrelevant.
Reliability : (4) not assignable
29.09.2003 (1)

Solubility in : other: ether, acetone, benzene
Value : at °C
pH value :
concentration : at °C
Temperature effects :

Examine different pol. :
pKa : at 25 °C
Description : miscible
Stable :

Reliability : (4) not assignable
29.09.2003 (5)

Solubility in :
Value : at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description : Miscible
Stable :

Remark : Described as infinite solubility of ethanol in water and water in ethanol.
Source : Riddick JA, Bunger WB, Sakano TK (1986), Techniques of Chemistry, Vol II, Organic Solvents, Physical Properties and Methods of Purification, Wiley
Reliability : (4) not assignable
11.11.2004 (43)

2.6.2 SURFACE TENSION

Test type : Ring method
Value : = 24.5 mN/m at 20 °C
Concentration :
Method : other: See remark
Year : 1997
GLP :
Test substance : other TS: double rectified absolute alcohol

Remark : Test performed by Sheffield Hallam University using a torsion balance and internal method QP55.
Reliability : (2) valid with restrictions
29.09.2003 (30)

2.7 FLASH POINT

Value : = 14 °C
Type : closed cup
Method : other: Abel closed cup. IP170/95
Year : 1997
GLP :
Test substance : other TS: double rectified absolute alcohol

Remark : Test performed by SG Redwood (UK) Ltd. ISO9002. no Q4856
Reliability : (2) valid with restrictions
16.10.2003 (30)

Value : = 13 °C
Type : closed cup
Method : other
Year :

GLP : no data
Test substance :

Reliability : (4) not assignable
16.10.2003 (31)

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

Result : highly flammable
Method : other
Year :
GLP : no data
Test substance :

Remark : Based on flashpoint and Dangerous Substances Directive AnnexI classification.
Reliability : (2) valid with restrictions
29.09.2003 (44)

2.10 EXPLOSIVE PROPERTIES

Result : Other

Remark : Explosivity range 3.3 - 19% in air by volume
Reliability : (4) not assignable
29.09.2003 (5)

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

Acid-base constant : pKa = 15.9 @ 20 deg C

Reliability : (4) not assignable
29.09.2003 (5)

2.13 VISCOSITY

Test type : other: not specified
Test procedure :
Value : = 1.22 - mPa s (dynamic) at 20 °C
Result :
Method : other: IP71 1/97
Year : 1997
GLP :
Test substance : other TS: double rectified absolute alcohol

Remark : Test performed by SG Redwood (UK) Ltd. ISO9002, no. Q4856.
Reliability : (2) valid with restrictions
29.09.2003 (30)

2.14 ADDITIONAL REMARKS

Memo : Henry's Law Constant

Remark : Henry's constant = 0.000252 at 15C.

Substances with values for Henry's Constant <0.05 are:

1. unlikely to volatize from surface waters,
2. unlikely to off-gas from groundwater,
3. have a high vapour phase retardation.

Source : U.S. EPA (1999) URL http://www.epa.gov/oar/caaac/mtbeethan.pdf.
Reliability : (4) not assignable
No reference to published experimental work is given so the reliability of this value cannot be evaluated. However, the validity of this study, published by the U.S. EPA, is highly dependent upon this value for Henry's Law Constant.
19.10.2004 (40)

Memo : Henry's Law Constant

Remark : Calculated value from HENRYWIN (EPIWIN suite)

```
----------+-------------------------------------------+---------+----------
  CLASS  |    BOND CONTRIBUTION DESCRIPTION          | COMMENT
|  VALUE
----------+-------------------------------------------+---------+----------
 HYDROGEN |   5  Hydrogen to Carbon (aliphatic) Bonds  |         | -0.5984
 HYDROGEN |   1  Hydrogen to Oxygen Bonds              |         |  3.2318
 FRAGMENT |   1  C-C                                   |         |  0.1163
 FRAGMENT |   1  C-O                                   |         |  1.0855
 FACTOR   |   *  Non-cyclic alkyl or olefinic alcohol  |         |  -.2000
----------+-------------------------------------------+---------+----------
 RESULT   |   BOND ESTIMATION METHOD for LWAPC VALUE   |
TOTAL  |  3.635
----------+-------------------------------------------+---------+----------
HENRYs LAW CONSTANT at 25 deg C = 5.67E-006 atm-m3/mole
                                = 2.32E-004 unitless

--------+---------------------------------------------+------------+--------
        |      GROUP CONTRIBUTION DESCRIPTION         |  COMMENT  |
VALUE
--------+---------------------------------------------+------------+--------
        |        1  CH3 (X)                           |            | -0.62
        |        1  CH2 (C)(O)                        |            | -0.13
        |        1  O-H (C)                           |            |  4.45
--------+---------------------------------------------+------------+--------
 RESULT |  GROUP ESTIMATION METHOD for LOG GAMMA VALUE  |
TOTAL   |  3.70
--------+---------------------------------------------+------------+--------
HENRYs LAW CONSTANT at 25 deg C = 4.88E-006 atm-m3/mole
                                = 2.00E-004 unitless
```

Reliability : (2) valid with restrictions
05.01.2004 (45)

Memo : Henry's Law Constant

Remark : Reference cites 3 values: an experimental log value of -3.59 (with no reference) and two calculated values using group and bond contribution methods described in the reference. These are 3.70 and 3.72 respectively.

Reliability : (4) not assignable

19.11.2004 (46)

3.1.1 PHOTODEGRADATION

Type : Air
Light source : Other
Light spectrum : ca. 345 - 355 nm
Relative intensity : based on intensity of sunlight
Conc. of substance : 2 mg/l at 30 °C
INDIRECT PHOTOLYSIS
Sensitizer : other: Nox
Conc. of sensitizer : 1 mg/l
Rate constant : = .045 cm³/(molecule*sec)
Degradation : = 20 % after 5 hour(s)
Deg. product :
Method : other (measured): Photodegradation Test
Year : 1977
GLP : no data
Test substance : no data

Method : Light source: UV fluorescent lamps.
Analytical methods: GC and UV spectroscopy.
Controls: unclear.
Light intensity 700 muW/cm^2.
Ethanol was irradiated for 5 - 6 hours in a 12 cubic metre "smog chamber" at 55% relative humidity. The amount of ethanol present was measured by GC.
Result : Indirect photolysis; t1/2 15.4 hours. % degradation results other than half-life: A 20% decrease in ethanol concentration was observed after 2 hr. Rate constant calculated (1st Order assumed) 0.045 hr^-1 and half life 15.4 h. Ethanol ranked low on authors reactivity scale.
NO depletion rate: 2.3ppb/min
Reliability : (2) valid with restrictions
No data on hydroxy radical concentrations given.
Flag : Critical study for SIDS endpoint
19.10.2004 (47)

Type : Air
Light source : other: High pressure mercury lamp with water cooled pyrex filter
Light spectrum : > 290 nm
Relative intensity : = 125 based on intensity of sunlight
Spectrum of substance : lambda (max, >295nm) : 182 nm
epsilon (max) :
epsilon (295) :
Conc. of substance : at 25 °C
INDIRECT PHOTOLYSIS
Sensitizer : other: O2, NOx, water
Conc. of sensitizer :
Rate constant : cm³/(molecule*sec)
Degradation : % after
Deg. product :
Method : other (measured)
Year : 1978
GLP : no data
Test substance : other TS: analytically pure

Remark : Ethanol was irradiated in a 4 litre or 20 litre reactor in the presence of corresponding amounts of water, nitrogen dioxide and sulphur dioxide in synthetic air. The test concentrations used were 100, 500 and 1000 ppm (mg/l). Irradiation was for up to 10 hours. Samples were taken for analysis

every hour and the reduction in ethanol concentration was measured by GC. Light intensity 125W; 25-30 degree C; samples were taken hourly.

Concentration of substance: 100, 500, 1000ppm.
Relative intensity in Watts relative to sunlight.

Original paper in German.

Result : No degradation of ethanol occurred in the presence of oxygen after irradiation at >290 nm, but degradation was evident at >230 nm.

After irradiation for 4 hours in the presence of nitrogen dioxide, 30% degradation occurred at 100 ppm, 55% at 500 ppm and 80% at 1000 ppm. Half life calculated to be 13.8 hours.

No degradation was seen in the presence of water at >290 nm.

Conclusion : Ethanol is not degraded in the troposphere by photodegradation in the absence of sensitisers to promote indirect degradation.

Reliability : (2) valid with restrictions
No data on hydroxy radical concentrations given. No data available on concentration of sensitisers used.

19.10.2004 (48)

Type : Air
Light source :
Light spectrum : Nm
Relative intensity : based on intensity of sunlight
Conc. of substance : at 19 °C
INDIRECT PHOTOLYSIS
Sensitizer : OH
Conc. of sensitizer :
Rate constant : = .0000018 cm^3/(molecule*sec)
Degradation : % after
Deg. product :
Method : other (calculated)
Year : 1976
GLP : no data
Test substance : no data

Result : A values for the half-life in a typical urban sunlit atmosphere was calculated from the rate constant for the reaction of the hydroxyl radical with ethanol vapour, assuming hydroxyl radical concentrations in such atmospheres to the of the order of 10E-14 mol.dm-3. The calculated halflife was 10 hours.

The rate constant was 1.8 +/- 0.2 x 10^-9 dm^3mol^-1s^-1

Test condition : Hydroxyl radicals were generated in a reaction vessel in a "dark system" by chain reaction in a hydrogen peroxide/nitrogen dioxide/carbon monoxide substrate mixture. No light source is used in this system.

Reliability : (2) valid with restrictions
No detailed methodology provided.

19.10.2004 (49)

Type : Air
Light source :
Light spectrum : Nm
Relative intensity : based on intensity of sunlight

Remark : This is a critical review of the available photodegradation data. Values quoted are:

Rate constant k (E-12 cm3.mol-1.s-1)[temperature(K)]
3.2+/-0.4 [292] Campbell et al
3.74+/-0.37 [296+/-2] Overend et al
2.62+/-0.36 [298] Ravishankara et al
3.5+/-0.6 [295+/-2] Cox et al
2.07 [300] Meier et al
3.0 +/-0.6 [298] Lorenz et al
3.66 +/-0.42 [303+/-2] Kerr et al
3.4 +/-0.17 [293] Grenhill et al
3.33 +/-0.23 [296] Wallington et al
3.26 +/-0.14 [293] Hess et al

Conclusion : Following a critical review of the available data, the author concluded that the study by Hess et al (1988) was the preferred value for recommendation. This study measured rate constants over the temperature range 293-750K. An overall rate constant was calculated from the Hess data using a unit weighted least squares regression, yielding a rate constant of 3.27E-12 at 298K with an estimated uncertainty of +/-20%.

Reliability : (2) valid with restrictions
Whilst this is a secondary source, the data within it are critically evaluated. The data reviewed presents a consistent picture and a weight of evidence approach also confirms the conclusions reached.

18.10.2004 (50)

Type : Air
Light source :
Light spectrum : Nm
Relative intensity : based on intensity of sunlight
Deg. product :
Method : other (calculated)
Year : 2000
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : SAPRC-99 chemical mechanism linked with EKMA box model descriptions of air pollution episodes in 39 urban locations. The EKMA approach involves use of single-cell box models to simulate how ozone formation in one day episodes is affected by changes in ROG and NOx inputs. Such single-cell models cannot represent realistic pollution episodes in great detail but they can represent dynamic injection of pollutants, time-varying changes of inversion heights with entrainment of pollutants from aloft as the inversion height increases throughout the day, and time-varying photolysis rates, temperatures, and humidities. Thus, they can be used to simulate a wide range of the chemical conditions which affect ozone formation from reactive VOCs and NOx. These are the same as those affecting VOC reactivity. The incremental reactivity is the change in ozone formed caused by adding, in this case, ethanol to the initial and emitted base reactive organic gas mixture in a scenario, divided by the amount of VOC added.

Remark : Type: VOC reactivity scale.

This is an update of the SPARC-90 mechanism of Carter (1990) and incorporates recent reactivity data on the Maximum Incremental Reactivity (MIR) Scale from a wide variety of VOCs.

Result : Absolute Value: 1.34 - 1.69 gm ozone/g VOC
Reliability : (4) not assignable

18.10.2004 (51) (52)

Type : Air
Light source :
Light spectrum : Nm

Relative intensity : based on intensity of sunlight
Deg. product : Yes
Method : other (calculated)
Year : 2000
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Calculated using the Harwell Photochemical Trajectory Model using the updated IVL photochemical scheme, updated using the oxidation mechanism of the acetates, as described in the Master Chemical Mechanism oxidation mechanism. The model follows chemical development in air parcels as they travel across from continental Europe to the UK. Parcel dimensions are 10km squares to the top of the boundary layer, the latter being 300m at 06:00 rising to 1300m by 14:00, falling from early evening back to 300m. The chemical inventory of the parcel is stated in the reference (95 hydrocarbons plus methane.) The model contains 771 thermal chemical reactions, with rate constants obtained from Atkinson (1989, 1990, 1992, 1994) or calculated using SAR (Atkinson(1986, 1987).
Remark : Type: Tropospheric ozone creation potential.

The photochemical ozone creation potential (POCP) concept can be regarded as useful in locations with high NOx concentrations where the rate limiting step in ozone creation is the VOC composition and reactivity.
Result : Photochemical Ozone creation potential = 39.9% (Andersson-Skold) or 44.6 (Derwent) relative to ethylene at 100%, with ethanol representing 4.28% of total VOC emissions. POCP figures are considered to be to an accuracy of +/-5. Ethanol is considered to degrade by reaction with OH radicals and via carbon/carbon scission.
Reliability : (4) not assignable
18.10.2004 (53) (54)

Type : Air
Light source :
Light spectrum : Nm
Relative intensity : based on intensity of sunlight

Remark : The estimated half-life of ethanol in the atmosphere ranges from 4 to 5.9 days, based on a hydroxyl radical concentration of 800,000 molecules/cubic centimeter.
Reliability : (4) not assignable
18.10.2004 (35)

Type : Air
Light source :
Light spectrum : Nm
Relative intensity : based on intensity of sunlight
DIRECT PHOTOLYSIS
Halflife t1/2 : = 3 day(s)
Degradation : % after
Quantum yield :
INDIRECT PHOTOLYSIS
Sensitizer :
Conc. of sensitizer :
Rate constant : = .0000000000035763 cm^3/(molecule*sec)
Degradation : % after
Deg. product :
Method : other (calculated)
Year :
GLP :

Test substance :

Remark : Note: Half life based on 12 hour day.
Calculated value.
Result : SUMMARY (AOP v1.90): HYDROXYL RADICALS

Hydrogen Abstraction = 3.4363 E-12 cm3/mol-sec
Reaction with N, S and -OH = 0.1400 E-12 cm3/mol-sec
Addition to Triple Bonds = 0.0000 E-12 cm3/mol-sec
Addition to Olefinic Bonds = 0.0000 E-12 cm3/mol-sec
Addition to Aromatic Rings = 0.0000 E-12 cm3/mol-sec
Addition to Fused Rings = 0.0000 E-12 cm3/mol-sec

OVERALL OH Rate Constant = 3.5763 E-12 cm3/mol-sec
Reliability : (2) valid with restrictions
12.11.2004 (45)

3.1.2 STABILITY IN WATER

Type : Abiotic
t1/2 pH4 : at °C
t1/2 pH7 : at °C
t1/2 pH9 : at °C
Deg. product :
Method : other (calculated)
Year : 1990
GLP : no data
Test substance : other TS: 100% ethanol

Remark : Duration (days) of test: Not relevant.
Positive/negative controls: Not relevant.
Analytical procedures used to measure test substance loss:
Not relevant.
Reference is to method. According to Lyman et al. both
alkanes and alcohols are resistant to hydrolysis.
Result : Ethanol is not expected to undergo hydrolysis.

(This is deduced from basics according to Lyman et al.
(1990)).
Reliability : (2) valid with restrictions
19.10.2004 (55)

Type : Abiotic
t1/2 pH4 : at °C
t1/2 pH7 : = 2 year at °C
t1/2 pH9 : at °C
Deg. product :
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Ethanol is stable in water and is not hydrolysed. Hydrolysis
as a function of pH is, therefore, an irrelevant endpoint.
Half-life calculated from the rate constant for the
reaction with hydroxyl radicals (1.1 x 10sup9) at room
temperature (15 - 25 °C). Actual figure for half-life given
in ENVIROFATE database (Environmental Fate. SilverPlatter,

Chem-Bank, June1993).

Half-lives of between 334 days and 36.6 years have been calculated for photooxidation in water based on the same rate constant (Handbook of Environmental Degradation Rates (1991) Eds Howard, P.H. et al. Lewis Publishers, Michigan).

Reliability : (2) valid with restrictions

19.10.2004 (56)

3.1.3 STABILITY IN SOIL

Type : Laboratory
Radiolabel :
Concentration : 32249 mg/kg
Soil temperature : 25 °C
Soil humidity :
Soil classification : Other
Year :
Deg. product :
Method :
Year :
GLP : no data
Test substance :

Result : Addition of ethanol to soil caused an immediate respiration increase. When 14 mumoles of ethanol were added to soil, 12 mumoles of oxygen were consumed before the soil respiration dropped to the level of untreated soil. 81-92% of CO_2 respired from the soil (in excess of that respired from control soil) was derived from the radiolabelled ethanol substrate.

Test condition : Soil tested was Drummer silty clay loam soil. Soil (20 g) in a glass dish was placed in a Warbiurg flask containing 2 ml sodium hydroxide solution for absorbing carbon dioxide and 2 ml aqueous radiolabelled ethanol solution, which was added directly to the soil.

Respiratory rate over 4 hr was evaluated by measuring O_2 used and CO_2 released. The amount of ethanol added was either 14 or 40 mumoles.

Reliability : (4) not assignable

19.10.2004 (57)

3.2.1 MONITORING DATA

Type of measurement : concentration at contaminated site
Media : surface water
Concentration :
Method :

Method : Water sampled from Matsubara region of Tatsuno City. Samples divided into 2, half (1.7l steam distilled with diethyl ether (100ml) for 3 days. Ether then dried with anyhydrous Na_2SO_4 then concentrated. Remaining 2l vacuum distilled at 10-4torr after freezing and receiver cooled at -80C. Trapped water extracted with diethyl ether (300ml) for 24 hours, then concentrated.

Concentrates analysed by GC-MS. Column packed with Thermon-1500 (5% polyeters, 5% starch, 0.3% phenolic resin on acid washed, DMCS treated Chromasorb W). Temp 50C for 2 min then ramped to 210C at 4C/min. Injector temp 240C, flow rate (helium carrier) 40ml/min.

Remark : Measurements made in the Hayashida River in Tatsumo city, Japan (site of leather industry). River recognised as one of the most polluted in Japan. (typical COD 200ppm, BOD 380ppm).

Result : Ethanol was detected (GC-MS) at a concentration of 4020 ppb.

Reliability : (2) valid with restrictions

19.10.2004 (58)

Type of measurement : background concentration

Media : Air

Concentration : ca. .001 mg/l

Method :

Method : Analysis in of the atmosphere of Point Barrow, Alaska.

200ml sample of air taken by gas tight syringe from a windward location. 4 fillings of a 50ml syringe taken over a period of 2 mins and injected into the inlet system of analyser. 25 measurements taken at hourly intervals over a 24 hour period on 2nd/3rd Sept 1967.

Cryogenic condensation trap used to concentrate organic components from the air, packed with Carbowax 20M, prior to injection system of GC analyser (6ft, 20% Carbowax column, 78-82C, He carrier @ 22ml/min). Trap release using water at 95C. Detector calibrated using acetone.

Remark : The authors postulated that as ethanol is a product of fermentation, the likely source of the measured ethanol is natural.

Result : Ethanol/methanol was detected in 17 of 25 samples. The average concentration over 24 hours was 0.52 ppb (range 0 - 1.2 ppb). Using a conversion of 1.9ug/l=1ppb would imply a concentration of 1.0ug/l.

Reliability : (2) valid with restrictions

19.10.2004 (59)

Type of measurement : concentration at contaminated site

Media : ground water

Concentration :

Method :

Method : VOC data were obtained from 20 Minnesota Pollution Control Agency permitted municipal solid waste landfills and two unauthorised dumps. The following wre taken: 6 leachate samples, 45 groundwater wells at 13 landfills with suspected contamination, 21 groundwater wells at 8 landfills plus the unauthorised landfills where water quality was believed good. Samples were collected in 40ml glass vials, no headspace, sealed with teflon lined caps. 40mg sodium thiosulphate added to each sample to remove residual chlorine. Quadruplicate samples used plus field blanks as controls. Collection methodology described (either direct transfer to sample bottles or stainless steel ladle used.) The authors recognised the potential for contamination from the plastic materials used in well construction.

Result : Ethanol was found in ground water suspected of leachate contamination (based on levels of inorganics) at 190ug/l at one of 13 sites, and was detected at 58ug/l in 1 out of 7 landfill ground water samples where inorganic levels indicated good or unknown water quality. It was found in 2 of 6 landfill leachates at concentrations in the range 23-110mg/l.

Reliability : (4) not assignable
No data given on analytical methods which makes reliability assignment not possible.
19.10.2004 (60)

Type of measurement : background concentration
Media : drinking water
Concentration :
Method :

Result : Ethanol has been detected (not quantified) in U.S. city water supplies, hotel drinking water (one of one tested) and water treatment plants (one of three tested).
Reliability : (4) not assignable
19.10.2004 (35)

Type of measurement : background concentration
Media : ground water
Concentration :
Method :

Remark : Sources include animal wastes, plants, insects, forest fires, microbes, and volcanoes.
Reliability : (4) not assignable
19.10.2004 (61)

Type of measurement : background concentration
Media : Food
Concentration :
Method :

Result : Values for ethanol concentrations detected in foods are as follows:
Lima, common, mung and soy beans - 1500 - 7900 ppm (average 4200 ppm)
Split peas - 3600 ppm
Lentils - 4400 ppm
Fried bacon - identified, not quantified as volatile flavourcomponent

Mountain Beaufort Cheese (French Alps) - identified, not quantified
Reliability : (4) not assignable
19.10.2004 (35)

Type of measurement : concentration at contaminated site
Media : Air
Concentration :
Method :

Result : Ethanol was detected (cryogenic trap, GC-FID) at concentrations of 29 - 57 ppb (air pollution peak in Japan).
Reliability : (4) not assignable
19.10.2004 (62)

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III
Media :
Air : 56.6 % (Fugacity Model Level I)
Water : 34.3 % (Fugacity Model Level I)
Soil : 9.1 % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method :
Year :

Remark : Fugacity calculations based on the following emissions scenario:
Air 1000kg/hr
Water 100kg/hr
Soil 10kg/hr.

These are calculated as follows:

	Tonnes	Fraction Release		
	ktpa	air	water	soil
Paint	25	0.8	0.15	0.01
Ink	70	0.95	0.05	0.01
Pharma	35	0.05	0.007	0.0001
Food	5	0.01	0.0005	0.001
Flavour	2.5	0.01	0.0005	0.001
Fragrances	2.5	1	0	0
Explosive	5	0.01	0.0005	0.001
Other	20	0.5	0.01	0
Cosmetic	145	1	0	0
Detergent	25	0.175	0.6	0.002
Deicing	40	1	0	0
Cleaning	15	0.175	0.6	0.002
Other	10	0.175	0.6	0
Intermed.	250	0.05	0.007	0.0001
Fuel	180	0.01	0.0005	0.001

Notes: Emission fractions either from EU technical guidance for risk assessment or, where not available, by expert judgement. Explosive emissions deemed similar to fuel use Food/flavour use - assumptions based on consumption - little emitted - fuel factors used. Fragrances as cosmetics. Split flavour/fragrance tonnage 50/50. To obtain overall emission rations, total emissions per compartment summed then ration calculated and rounded to nearest power of 10.

Physicochemical data used:

Melting point: -114C
Boiling point: 78.2
LogKow: -0.31
water solubility: 1000g/l
vapour pressure: 59.3mmHg @ 25C
Henry's coefficient: 5E-6 atmm-3mol-1 @ 25C
pKa: 5.9 @ 25C

Result : Total persistence time estimated as 80.3 hrs
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
19.10.2004 (63)

Type : Volatility
Media : soil - air
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method :
Year :

Remark : Ethanol is relatively volatile and would therefore readily evaporate from soil at the soil/air interface.
Reliability : (4) not assignable
19.10.2004 (5)

Type : Volatility
Media : water – air
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method :
Year :

Remark : The estimated half life for evaporation of ethanol from water 1 m deep with a 1 m/sec current and 3 m/sec wind is 6.1 days.
Reliability : (4) not assignable
19.10.2004 (5)

Type : Other
Media : water – air
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method :
Year :

Remark : Volatilization from Water:
Henry LC: 5E-006 atm-m3/mole (Henry experimental database)
Half-Life from Model River: 80.17 hours (3.34 days)
Half-Life from Model Lake : 931.5 hours (38.81 days)
Reliability : (4) not assignable
19.10.2004 (64)

Type : Other
Media : water – air
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method :
Year :

Remark : When released to the atmosphere, etheanol will photodegrade with a half life ranging from hours in polluted urban areas to approximately 6 days in the atmosphere. Due to its solubility in water, rainout may be an important process.
Reliability : (4) not assignable
19.10.2004 (35)

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level III
Year : 2001

Remark : Adsorption coefficient: Not given.
Desorption: Not given.
Volatility: Not given.
Model used: EQC model of Mackay et al. (1996).
Version 1.01
Date: 1997.

The following input parameters were used:

MWt 46.09 g/mol
Temp. 25 degC
Water solubility 716,000 g/m^3 calculated from vapour pressure and Henry's law constant of 5e-06 atm.m^3/mol (Gaffney, 1987)
Vapour Pressure 7870 Pa (59.03 mm)
Log Kow -0.31
Melting point -114 deg C
t1/2 air 203 hr (Graedel, 1978)
t1/2 water 182 hr (from biodegradation data)
t1/2 sediment 210 hr (from biodegradation data)
Environmental conditions: left at the default values of the model.

Result : Air 13.0% 1.60e-8 mol/m^3 (738 ng/m^3)
Water 44.8% 2.75e-5 mol/m^3 (1271 ng/l)
Soil 42.1% 2.88e-4 mol/m^3 (8.3 ng/g)
Sediment 0.039% 9.50e-6 mol/m^3 (0.34 ng/g)

Adsorption coefficient, desorption and volatility not given.

At steady state 67% of additional inputs of ethanol are lost through reactions and 33% are lost through advection.
Reliability : (2) valid with restrictions
19.10.2004 (65) (66)

Media : Other
Method :
Year :

Remark : PCKOCWIN v1.66 Results

First Order Molecular Connectivity Index ....: 1.414
Non-Corrected Log Koc ....: 1.3755
Fragment Correction(s):
1 Aliphatic Alcohol (-C-OH) ....: -1.5193

Corrected Log Koc ....: -0.1438
Over Correction Adjustment to Lower Limit Log Koc: 0.0000

Estimated Koc: 1

19.10.2004 (64)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : Aerobic
Inoculum : other: wastewater from domestic sewage
Contact time :
Degradation : = 74 (±) % after 5 day(s)
Result : readily biodegradable
Kinetic of testsubst. : 5 day(s) = 74 %
10 day(s) = 74 %
15 day(s) = 95 %
20 day(s) = 84 %
%
Deg. product :
Method : other: BOD protocol
Year : 1974
GLP : no data
Test substance : no data

Method : Biodegradability was measured in fresh water according to "standard methods for the examination of water and waste water" 13th Ed. American Public Health Association, New
York, 1971.
Remark : Three concentrations tested (3, 7 and 10 mg/l) with at least two in duplicate. Tested concentrations gave a BOD of 3 to 30mg/l. Dissolved oxygen measured periodically (5 times in 20 days).

Inoculum: wastewater from domestic sewage (assumed not adapted).

COD measured.
Result : Measured COD was 1.99 mg O2/mg. Theoretical oxygen demand 2.1 mg/mg.
Reliability : (2) valid with restrictions
Well reported study. Not to GLP and no test substance purity or source quoted.
Flag : Critical study for SIDS endpoint
19.10.2004 (67)

Type : Aerobic
Inoculum : other: Filtered, settled domestic wastewater as seed in synthetic seawater
Contact time :
Degradation : = 75 (±) % after 20 day(s)
Result : readily biodegradable
Kinetic of testsubst. : 5 day(s) = 45 %
10 day(s) = 68 %
15 day(s) = 72 %
20 day(s) = 75 %
%
Deg. product :

Method : other: BOD protocol
Year : 1974
GLP : no data
Test substance : no data

Remark : Biodegradability measured in synthetic salt water according to "standard methods for the examination of water and waste water" 13th Ed. American Public Health Association, New York, 1971. Three concentrations tested (3, 7 and 10 mg/l). COD measured.
Inoculum (other): Seed used was developed in seawater taken from Lavaca Bay, Texas. The seed was maintained by adding small amounts of settled raw wastewater periodically as a source of substrate. No further information available.
Concentration of test chemical, vehicle, pre-acclimation conditions: 3, 7 and 10 mg/l ethanol was added using 0.1% stock solution.
Temperature of incubation: Not specified.
Dosing procedure: Not described. Domestic wastewater was placed in bottles to which was added aerated dilution water containing ethanol.
Sampling frequency: BOD every 5 days; ethanol concentration was not monitored.
Were appropriate controls and blank system used? Yes.
Analytical method: Cumulative oxygen uptake in ethanol-amended and control samples was measured with dissolved O2 meter.
Method of calculating measured concentrations: Degradation rate was calculated as the % of theoretical oxygen demand utilized.
Lag time: Not measured.
Observed inhibition: Not measured.
Excessive biodegradation: Not discussed.
Excessive standard deviation: Not discussed.
Time required for 10% degradation: Not discussed. At 5 days, 74% of ethanol had been degraded
Total degradation at end of test: 84% .
Reliability : (2) valid with restrictions
Well reported study. Not to GLP and no test substance purity or source quoted.
19.10.2004 (67)

Type : Aerobic
Inoculum : activated sludge, domestic
Concentration : 500 mg/l related to
related to
Contact time :
Degradation : = 37 (±) % after 1 day(s)
Result : readily biodegradable
Deg. product :
Method : other
Year : 1966
GLP : no data
Test substance : other TS: Analytical grade

Remark : All sludges were capable of oxidizing ethanol as measured by BOD which was 37.3% of maximum and similar to that for other short-chain alcohols.
Inoculum (other): Fresh activated sludge: activated sludges

were obtained from municipal treatment plants in Columbus, Hilliard and Linworth, Ohio.
Concentration of test chemical, vehicle, pre-acclimation conditions: 500 mg/l ethanol was added to 125 ml flasks containing 20 ml blended sludge with a concentration of 2500 mg/l suspended solids.
Temperature of incubation: 20 deg C.
Dosing procedure: see above.
Sampling frequency: BOD 6, 12 and 24 h after inoculation. Ethanol concentrations were not measured.
Were appropriate controls and blank system used? Yes.
Analytical method: Oxygen uptake measured by Warburg respirometer.
Method of calculating measured concentrations: Not discussed.
Lag time: Not discussed.
Observed inhibition: Not measured.
Excessive biodegradation: Not discussed.
Excessive standard deviation: Not discussed.
Time required for 10% degradation: Not discussed. At 6 hours oxygen demand was 12.9% of theoretical.
Total degradation at end of test: 37.3% at 24 hrs.

Reliability : (2) valid with restrictions
12.11.2004 (68)

Type : Anaerobic
Inoculum : other: sediment and groundwater from methanogenic portion of shallow anoxic aquifer contaminated by landfill leachate
Concentration : 50 mg/l related to Test substance
related to
Contact time : 30 day(s)
Degradation : = 91 (±) % after 30 day(s)
Result : readily biodegradable
Deg. product :
Method : other
Year : 1993
GLP : no data
Test substance : no data

Method : Production of methane by ethanol-containing sediment was monitored by an automated pressure transducer system.

The acclimation period was 25-30 days.

Remark : Inoculum (other): Sediment and groundwater from a methanogenic portion of a shallow anoxic aquifer contaminated by landfill leachate.
Concentration of test chemical, vehicle, pre-acclimation conditions: 50 ppm C as ethanol. Ethanol was added to slurries of 50 g sediment and 75 ml groundwater in 160 ml bottles.
Temperature of incubation: Room temperature.
Dosing procedure: Not described.
Sampling frequency: Not described.
Were appropriate controls and blank system used? Yes, autoclaved controls.
Methane measurement: GC with flame ionization detector.
Method of calculating measured concentrations: Degradation rate was calculated as the mean of three tests.
Lag time: Acclimation period was 25-30 days.
Observed inhibition: Not discussed.

Excessive biodegradation: Not discussed.
Excessive standard deviation: Not discussed.
Time required for 10% degradation: Not discussed. (Rate calculated as 17.9 ppm C/day.
Total degradation at end of test: 91% of theoretical CH_4 production was recovered.
The rapidity and completeness of ethanol biodegradation is supported by the work of Corseuil et al., 1998 and by Yeh and Novak (1994) in both of which complete mineralization was achieved in aerobic or anaerobic conditions.

Result : The rate of biodegradation was calculated to be 17.9 ppm C/day and total methane recovery was 91% of the theoretical limit.

Reliability : (2) valid with restrictions
No detailed results available, only final result reported. Not GLP and no substance source or purity information.

19.10.2004 (69)

Type : Aerobic
Inoculum : activated sludge
Contact time :
Degradation : = 96.8 (±2.4) % after 15 day(s)
Result : readily biodegradable
Kinetic of testsubst. : 4 day(s) ca. 80 %
8 day(s) ca. 88 %
11 day(s) ca. 100 %
15 day(s) ca. 92 %
20 day(s) ca. 98 %
Control substance : Benzoic acid, sodium salt
Kinetic : 1 day(s) ca. 10 %
5 day(s) ca. 70 %
Deg. product :
Method : OECD Guide-line 301 B "Ready Biodegradability: Modified Sturm Test (CO_2 evolution)"
Year : 1991
GLP : no data
Test substance : other TS

Method : The mineral medium used was adopted from that recommended in the 1988 OECD Ring Test of Ready Biodegradability (OECD 301). Stock solutions based on demineralised water. Stock solution (d) used EDTA as a preservative.

Since only about 1% of cells in activated sludge are active it was considered that a 'cleaner' inoculum of similar activity could be obtained using secondary effluent from an activated sludge plant. The level of organic carbon introduced when using 10% by volume of secondary effluent to inoculate the test is only about 1-2 mg/litre. The test was therefore inoculated with secondary effluent from an activated sludge plant treating domestic sewage. The collected effluent was first passed through a coarse filter to remove gross particulate matter. The level of inorganic carbon in the inoculum was reduced before use by sparging with carbon dioxide-free air for about one hour while maintaining the pH at 6.5.

The CO_2 produced in the control vessels, using the maximum inoculum concentration of 10% secondary effluent, was in the range 0.4 - 1.3 mg/litre. Hence, in cases where positive results were obtained less than 10% of the carbon dioxide produced was derived from the control. The inoculum was not pre-adapted.

Using suitable volumetric apparatus 100±1 ml of the mineral salts media is dispensed into '125 ml' Hypo-Vial [Pierce Warriner (UK) Ltdl. The media is prepared so as to contain 0.5 to 10% by volume of inoculum and 2 to 10 mg/litre of test substance as organic carbon. Controls containing the same inoculum concentration but no test compound are also prepared. The vials are sealed with butyl rubber septa and aluminium crimp seals and placed on an orbital shaker in a temperature controlled environment. To follow the course of biodegradation and to statistically evaluate the extent of biodegradation on the final day of the test a minimum of 12 vessels is required per test substance. This provides for a data point every fourth day and 4 replicates for the assessment of the final extent of biodegradation on the 28th day of the test.

A vessel is removed from the shaker as required, a sample of the headspace gas withdrawn using a gas syringe and the concentration of carbon dioxide determined. The seal is then broken and the concentration of dissolved inorganic carbon (DIG) in the solution is measured immediately. Similar determinations are made for a control vessel which does not contain the test substance. The difference in the total inorganic carbon found in the test and control vessels allows the quantity of carbon dioxide produced from the test compound to be ascertained.

Positive control: sodium benzoate.

The determination of carbon dioxide in both gaseous and aqueous samples was performed using a modified lonics 555 TC-T0C Analyser. Carbon dioxide is released from aqueous samples of carbonate/bicarbonate by direct injection using a 0-200 ~tl Hamilton constant rate syringe onto an inert support loaded with phosphoric acid. The temperature in the reaction chamber is controlled at 150"C and pure nitrogen is used as the carrier gas. The detection system is a high sensitivity non-dispersive infra-red analyser. Gaseous samples are injected using a good quality gas-tight syringe.

The analyser is calibrated for the analysis of gaseous samples by injecting suitable volumes of a 0.25% v/v mixture of carbon dioxide in nitrogen. For liquid samples the instrument is calibrated using standard solutions of sodium hydrogen carbonate in the range 0 - 20 mg/I as DCC.

Remark : Ethanol used as a comparator volatile compound in a study of the applicability of a modified form of the CO2 production test for assessing ultimate biodegradability under aerobic conditions.

The test substance in a dilute mineral salts solution is incubated in seated vessels with appropriate micro-organisms for a period of up to 28 days. Only about two thirds of the volume of the vessel is filled with liquid. At the test concentrations used only about 15% of the available oxygen in the headspace gas is required for the complete oxidation of all test compound carbon to carbon dioxide.

Any carbon dioxide produced by the breakdown of the test material is distributed between the liquid and gaseous phases. Periodically a vessel is taken, a sample of the headspace gas withdrawn using a gas syringe and the concentration of carbon dioxide in the headspace gas determined. The seal is then broken and the concentration of dissolved inorganic carbon (DCC) in the solution is measured. Similar determinations are made for a control vessel which does not contain the test substance. The difference in the total inorganic carbon found in the test and control vessels allows the quantity of carbon dioxide produced from the test compound to be ascertained. From a knowledge of the quantity of test material added and

its carbon content the extent of mineralisation can be calculated.

Result : BOD28 Mean = 96.8%. SD = 2.4
The method is shown to be compatible with existing techniques and is applicable to the testing of insoluble and volatile compounds.

Test substance : Reagent grade

Reliability : (2) valid with restrictions
No temperature quoted for study and data only available in graphical form, otherwise well reported.

19.11.2004 (70)

Type : Aerobic
Inoculum : predominantly domestic sewage
Concentration : 2 g/l related to Test substance
related to
Contact time : 5 day(s)
Degradation : (±) % after
Result :

Remark : Ethanol was used as a comparator substance in the description of a new potential test.
Oxygen utilization was immediate and attained a high rate early in the test period. After 5 days the O2 uptake had passed the optimum rate and the slope of the O2 utilization curv matched that of the seed bank. Substrate utilization was demonstrated by a decrease in COD and TOC coincident with the rapid O2 uptake. Bacterial growth reached a peak level of over 10^6 organismsa/ml on the third day of testing. Growth decreased thereafter to less than 10^4 organisms/ml at the end of the test.

Reliability : (4) not assignable

19.10.2004 (71)

Type : Anaerobic
Inoculum :

Remark : The anaerobic pseudo first order rate constants are published as follows:

ion	rate constant
NO3(-)	0.53
Fe(3+)	0.17
SO4(2-)	0.1

These are laboratory-derived values.

Reliability : (4) not assignable

19.10.2004 (72)

Remark : It is shown that 80-100 mg/l degrades aerobically in 5 days and anaerobically in 10-25 days.

Reliability : (4) not assignable

19.10.2004 (73)

Remark : Ethanol is biodegraded in aerobic systems using activated sludge, sewage (including filtered and settled), wastewater,and soil inocula. Five day theoretical BOD values range from37-86%. Anaerobic degradation (thermophilic digestion, 54 °C) of ethanol (5 ml of a 5% aqueous ethanol solution) produced approximately 1000 ml gas/g sample using seed whichhad been prepared in a synthetic medium.

Reliability : (4) not assignable
19.10.2004 (35)

Remark : The aerobic half-life of ethanol in aqueous systems has been estimated to be between 6.5 and 26 hours, based upon a river die-away test for one sample of water from one river. The anaerobic half-life has been estimated to be between 26 and 104 hours based upon estimated unacclimated aqueous aerobic biodegradation half-life.
Reliability : (4) not assignable
19.10.2004 (35)

Type : Aerobic
Inoculum : activated sludge
Contact time :
Degradation : 89 (±) % after 14 day(s)
Result :
Control substance : Aniline
Kinetic : %
%

Remark : Concentration of test substance: 100mg/l
Concentration of activated sludge (as the concentration of suspended solid): 30mg/l
Volume of test solution: 300ml
Temperature: 25C
Result : Initiation time before degradation started: 3 days
Reliability : (4) not assignable
Results not available in detail and intermediate times only available in graphical form. No details of test method given although believed to be to an OECD protocol.
19.10.2004 (74)

Deg. product :
Method : other: modelled data
Year :
GLP :
Test substance :

Remark : BIOWIN v4.01 Results - predictions:

Linear Model: Biodegrades Fast
Non-Linear Model: Biodegrades Fast
Ultimate Biodegradation Timeframe: Days-Weeks
Primary Biodegradation Timeframe: Days
MITI Linear Model: Readily Degradable
MITI Non-Linear Model: Readily Degradable
Reliability : (4) not assignable
19.10.2004 (64)

3.6 BOD5, COD OR BOD5/COD RATIO

BOD5
Method :
Year :
Concentration : related to
BOD5 : mg/l

GLP : no data
COD
Method :
Year :
COD : mg/g substance
GLP : no data
RATIO BOD5 / COD
BOD5/COD : ca. .56

Remark : Results from several studies of BODx values as a % of theoretical oxygen demand (ThOD) are presented.
Result : BOD Values for nonadapted inoculum

BOD5 (4 studies): 37, 44, 45, 74% ThOD (Mean 50%)

BOD	%ThoD (range for 3 studies)
10 days	44-74
15 days	71-95
20 days	75-84

BOD5	%ThOD (1 study)
30 days	79
40 days	78
50 days	77

ThOD = 2.10

BOD5 (20 DegC) = 60% ThOD (acclimated)

COD (5 studies): 90,95,95,97,100% ThOD (Mean 95.4%)

BOD5/COD (calculated from above mean values) = 50/95.4 = 0.52
Source : Verschueren, K. (1996) Handbook of environmental data on organic chemicals. 3rd Edition. Van Nostrand Reinhold Company, New York.
Reliability : (4) not assignable
22.06.2004

3.7 BIOACCUMULATION

Elimination :
Method : other
Year :
GLP : no data
Test substance :

Remark : Not expected to bioconcentrate.
Reliability : (4) not assignable
19.10.2004 (5)

BCF : 3.16
Elimination :
Method : other: modelled
Year :
GLP :
Test substance :

Remark : Bcfwin v2.15

Log Kow (estimated) : -0.14
Log Kow (experimental): -0.31
Log Kow used by BCF estimates: -0.31

Equation Used to Make BCF estimate:
Log BCF = 0.50

Correction(s): Value
Correction Factors Not Used for Log Kow < 1

Estimated Log BCF = 0.500

Reliability : (4) not assignable
19.10.2004 (64)

3.8 ADDITIONAL REMARKS

Memo : Air stripping constant

Remark : K = 0.302/day at 2,220 mg/l
Reliability : (4) not assignable
19.10.2004 (15)

Memo : Anaerobic sludge digestion

Result : Inhibited at 1600 mg/l.
Reliability : (4) not assignable
29.09.2003 (15)

Memo : Impact on biodegradation

Remark : 50% Inhibition of NH3 oxidation by Nitrosomonas at 4,100 mg/l.
Reliability : (4) not assignable
29.09.2003 (15)

Memo : Waste water treatment

Remark : Anaerobic lagoon

	influent mg/l	effluent mg/l
13 lb COD/day/1000 cu.ft	80	35
22 lb " " " " " "	270	120
48 lb " " " " " "	270	130

Reliability : (4) not assignable
19.10.2004 (15)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	Static
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= 13000 measured/nominal
Limit test	:	
Analytical monitoring	:	No
Method	:	other
Year	:	1978
GLP	:	no data
Test substance	:	no data

Remark : Biological observations: None described.

Table of cumulative mortality: Not presented.
Lowest concentration causing 100% mortality: Not stated.
Mortality in controls: Not discussed.
Abnormal responses: Not discussed.
Reference substance: None used.
Any observations (e.g. precipitation) that might cause a difference between measured and nominal values: Not discussed.
Oncorhynchus mykiss and Salmo gairdneri are synonyms
The Columbia National Fisheries Research Laboratory conducted aquatic toxicity tests on more than 400 chemicals during 1965-1978; this is a major research area for the lab. The lab. also participated in the development of standard acute toxicity test methodology and only tests meeting acceptable procedures were included in this compilation.

Test organism: Age fingerlings, Length not stated. Weight 0.8 g.
Loading <=0.8 g/l fish/litre.
Pretreatment: Acclimated for 1-3 day.
Dilution water source: Reconstituted deionized water.
Dilution water chemistry: hardness 40-50 mg/l $CaCO_3$; Alkalinity 30-35 mg/l $CaCO_3$; pH 7.2-7.5.
Stock solution preparation: Not described.
Flow-through state: Static.
Vehicle, solvent and concentration: Not applicable
Solubility: Not applicable.
Exposure vessel type: 18.9 l wide-mouthed jars containing 15 l test solution, not aerated.
Illumination: not stated.
Replicates: 10 fish per concentration; no. of replicates not stated.
Water chemistry on test: Not described.
Test temperature range : 12 deg C +/- 1 degC.
Method of calculating mean measured concentration: Only nominal concentrations used.

Statistical method: Litchfield and Wilcoxon (1949).

Reliability : (2) valid with restrictions
Only nominal concentration measurements are available, However, fugacity data suggests low losses would be expected by evaporation. In addition there are no water quality data available and no GLP data.

However, the data is regarded as reliable with restrictions.
Flag : Critical study for SIDS endpoint
17.11.2004 (75)

Type : flow through
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC100 : = 13480 measured/nominal
Limit test :
Analytical monitoring : No
Method : other
Year : 1974
GLP : no data
Test substance : other TS: Reagent grade

Method : Ethanol concentrations ranged up to 30,000 mg/l
Remark : Biological observations: Some fish lost equilibrium.
Table showing cumulative mortality: Not given.
Lowest dose causing 100% mortality: Not stated.
Mortality of controls: Not discussed.
Abnormal response: No abnormal response noted.
Reference substances: None.
Any other observations affecting concentration: None.
These data were collected by the EPA's Environmental research Lab in Duluth, Minnesota, a lab likely to have significant experience in acute toxicity testing of this kind. These data are rated 'probably reliable'.

Test organism: Age Juvenile, 4-6 wk; Length 1-3.1 cm; Weight Not stated.
Loading: 20 fish/jar in 2 l test water.
Pretreatment: Acclimated to flowing water for 48 h.
Dilution water source: Lake Superior water.
Dilution water chemistry: Not stated.
Stock solution preparation: Ethanol weighed in to measured water and whole shaken.
Vehicle, solvent and concentration: Not applicable
Solubility: Not applicable.
Stability: Not measured.
Exposure vessel type: Covered cyclindrical glass battery jars.
Illumination: 50 ft-c cool, white fluorescent light 16 h/day.
Replicates: 10 fish per concentration, 1 replicate per concentration.
Water chemistry on test: Not described. DOC kept to 4 mg/l during test.
Test temperature range : 18-22 deg C.
Method of calculating mean measured concentration: Concentrations not measured.

Statistical method: Standard graphical.
Result : This LC50 value was within 50% of values previously reported. LC50 for shorter periods were as follows:

1 h >18,000 mg/l
24 h >18,000 mg/l
48 h =13,480 mg/l
72 h =13,480 mg/l

Reliability : (2) valid with restrictions
12.11.2004 (76)

Type : Static
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 4 day(s)
Unit : mg/l
LC50 : > 100 measured/nominal
Limit test : Yes
Analytical monitoring : No
Method : other
Year : 1986
GLP : no data
Test substance : other TS: Reagent grade

Method : Juvenile Pimephales promelas fish (4-8 weeks) were exposed to ethanol for 96 hr. EPA method presumed.
Remark : Biological observations: Not discussed. Minnows were considered dead if they were motionless and failed to respond to prodding.
Table showing cumulative mortality: Not given.
Lowest dose causing 100% mortality: 100% mortality not achieved at any dose.
Mortality of controls: Not discussed.
Abnormal response: No abnormal response noted.
Reference substances: None although several other substances tested.
Any other observations: None.
Test organism: Age juvenile, not specified; weight 0.2-0.5g.
Loading: <0.5 wet weight/l.
Pretreatment: Acclimated; food with-held 24 h.
Dilution water source: Activated carbon-filtered, dechlorinated and tempered Lake Ontario water.
Dilution water chemistry: hardness 130 mg/l CaCO3;
Alkalinity 93 mg/l CaCO3, pH 7.4, TOC 1.8 mg/l, TSS 180mg/l; salinity 26 mg/l Cl
Stock solution preparation: Not described. Max concentration tested 100mg/l.
Vehicle, solvent and concentration: Not applicable
Solubility: Not applicable.
Exposure vessel type: Unsealed cubic Pyrex chromatograph dishes.
Illumination: 50 ft-c cool, white fluorescent light 16h/day.
Replicates: 10 fish per concentration, 1 replicate per concentration.
Water chemistry on test: Not described. DOC kept below 40% of starting value.
Test temperature range : 20 deg C +/- 0.1 deg C.
Method of calculating mean measured concentration: Only nominal concentrations used.
Statistical method: Standard graphical.
Result : The 96 hr LC50 was greater than 100 mg/l, the maximum concentration tested.
Reliability : (2) valid with restrictions
17.11.2004 (77)

Type : Static
Species : Salmo gairdneri (Fish, estuary, fresh water)
Exposure period : 1 day(s)
Unit : mg/l
LC50 : > 11200 measured/nominal
Limit test :

Analytical monitoring : No
Method : other:
Year : 1978
GLP : no data
Test substance : other TS

Method : Fish exposed to ethanol at 6 concentrations up to 30,000 mg/l for 24 hr.
Remark : Biological observations:

Table of cumulative mortality: Not presented.
Lowest concentration causing 100% mortality: in static tests, 25,000 mg/l caused 100% mortality in 3 hr.
Mortality in controls: Not discussed.
Abnormal responses: Not discussed.
Reference substance: None used.
Any observations (e.g. precipitation) that might cause a difference between measured and nominal values: None.
Test organism: Age fingerlings, 9.2 cm +/1 1.1 cm, weight 9.5 +/- 3.8 g.
Source Caribou Trout ranch, Soda Springs, Ihaho
Loading 1 fish/litre..
Pretreatment: Acclimated for at least 2 wk.
Dilution water source: Dechlorinated city tap water.
Dilution water chemistry: hardness 90 mg/l $CaCO_3$; Conductivity 190 muS/cm, pH 8.0.
Stock solution preparation: Not described.
Vehicle, solvent and concentration: Not applicable
Solubility: Not applicable.
Exposure vessel type: PET-lined 20 l vessels.
Illumination: 12 h light: 12 h dark cycle.
Replicates: 10 fish per concentration, 1 replicate per concentration.
Water chemistry on test: Not described.
Test temperature range : 10 deg C.
Method of calculating mean measured concentration: Only nominal concentrations used.
Criterion for death: cessation of respiration.

Statistical method: Litchfield (1949 and APHA (1971).
Result : Nominal concentrations achieved: 0.1, 1.0, 10 and 100 mg/l.
LC50 11,200 mg/l (not achieved).
Test substance : Reagent grade ethano from Standard Chemicals.
Reliability : (2) valid with restrictions
This was a screening study for a flow through sub-lethal study. Limited results are reported. Design was static with no measured concentrations, however short length of study limits impact of these omissions. Study rated reliable with restrictions.
17.11.2004 (78)

Type : Other
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : g/l
LC50 : = 14.2
EC50 : = 14.2
Limit test :
Analytical monitoring : Yes
Method : other:USEPA methodology
Year : 1984
GLP : no data
Test substance : other TS

Remark : A 96-hour LC50 or 15.3 g/l and a 96-hour EC50 or 12.9 g/l were recorded in an earlier study under the same condition but with 95% ethanol supplied by the U.S. Industrial Chemical Co. Bacterial growth appeared in the exposure tanks. Affected fish were hypoactive and lost equilibrium prior to death.
fathead minnows were cultureds from brood stock provided by the USEPA Environmental Research Laboratory (Duluth). Adults were maintained in flow-through st 25 degC with a 16 -h light/dark photoperiod. Organisms were were fed adult brine shrimp (Artemia). Fry were fed freshly hatched brine shrimp nauplii three times daily until 24-h before test start.
Fish were not fed during exposure to ethanol. 2 replicates of 25 fish were exposed to each of 5 test concentrations and an untreated control in a flow-through system. The tank volume was 6.3 l and the volume additions was 6.46 vol/day. Test fish were 29-30 day-old at start and had a mean length of 18.2 +/- 2.22 mm and a mean weight of 0.106 +/- 0.036 g. The loading rate was 0.421 g/l. Reconstituted of filtered Lake Superior water was used for control and dilution water. Water had hardness of 45 mg/l as CaCO3 and an alkalinity of 37.0 mg/l as CaCO3.
Nominal (and range of average measured) concentrations (mg/l) tested were 0 (5.4-8.5), 128 (44-48), 214 (77-79), 356 (130-137), 594 (235-263) and 990 (398-420). Test temperatures ranged 23.1-25.5 degC. pH ranged 7.3-7.5 SU and dissolved O2 ranged 6.1-7.4 mg/l. Test concentrations in one replicate were measured daily by GLC.
Mortality and adverse effects were reported at 0, 12, 21, 24, 48, 52, 72 and 96 hr of exposure and the 96-h EC50/LC50 and 95% confidence limits were estimated using the Trimmed Spearman Karber method.
Test substance : Test substance was 95% pure ethanol (Aldrich Chemical Co.).
Reliability : (2) valid with restrictions
12.11.2004 (79)

Type : Static
Species : Leuciscus idus melanotus (Fish, fresh water)
Exposure period : 48 hour(s)
Unit : mg/l
LC0 : = 7110
LC50 : = 8140
LC100 : = 8690
Limit test :
Analytical monitoring : no data
Method : other
Year : 1978
GLP : no data
Test substance : no data

Remark : Method specified in Deutsche Einheitsverfahren zur Wasser-, Abwasser und Schlamm-Untersuchung L15; Fischtest. Study duration not specified in reference, but authors refer to Mann, H. (1975) Vom Wasser 44, 1 - 3 for details of method. This specifies 48 hours.
Reliability : (4) not assignable
11.11.2004 (80)

Type : Static
Species : Alburnus alburnus (Fish, estuary)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 11000
Limit test :
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : other TS

Remark : A narcotic effect (loss of equilibrium) was seen in the fishas an early response, followed by coma and then death.
Test condition : Fish were tested in filtered brackish water (10/tank) at 10 °C in static tanks. At least 6 concentration and one control were tested. Mortality was recorded and the LC50 was determined by probit analysis.
Test substance : The test substance was analytical grade.
Reliability : (4) not assignable
11.11.2004 (81) (82)

Type : Static
Species : Semolitus atromaculatus (Fish, fresh water)
Exposure period : 24 hour(s)
Unit : mg/l
LC100 : = 7000 - 9000 measured/nominal
Limit test : Yes
Analytical monitoring : no data
Method : other
Year : 1952
GLP : no data
Test substance : other TS: Commercial grade

Remark : This study was to evaluated fish toxicity as a means of determining the critical range of wastewater contamination for 51 different chemicals including ethanol.

Critical range is the concentration range (PPM) above which all four fish died in 24 hours and below which all 4 survived over the same period.
Reliability : (4) not assignable
11.11.2004 (83)

Type : Static
Species : Oryzias latipes (Fish, fresh water)
Exposure period : 15 minute(s)
Unit : mg/l
EC50 : = 1000 - 10000
Limit test :
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Concentration assumed; endpoint is for physiology; EC50 value assumed. Other fish in same battery of tests were

Girella punctata, Chasmichthys dolichognathuis and Pagrus major.
Reliability : (4) not assignable
11.11.2004 (84)

Type : Static
Species : Carassius auratus (Fish, fresh water)
Exposure period : 30 minute(s)
Unit : g/l
: = 1
Limit test :
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Results expressed as 1% (v/v or w/w not known). Endpoint is behaviour (temperature selection).
Reliability : (4) not assignable
11.11.2004 (85)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : Static
Species : Ceriodaphnia sp. (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
LC50 : = 5012 measured/nominal
Analytical monitoring : no data
Method : other: ASTM (see ME)
Year : 1987
GLP : no data
Test substance : other TS: Absolute ethanol

Remark : Method was that recommended by the American Society of Testing and Materials (1980) Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. ASTM Standard E729-80. Philadelphia, Pennsylvania.

Test organism: Cerodaphnia dubia: Source not specified. Organisms were mass cultured an acclimated to temperature for at least 10 weeks and maintained in filtered, autoclaved Lake Huron water. Neonates hatched by isolated gravid females gathered by sieving.
Age: Neonates.
Control group: Dilution water controls.
Test conditions: Ethanol not discussed.
Test temperature: 24 deg C.
Exposure vessel: Covered vials, not aerated, triplicates for each concentration.
Dilution water source: See above.
Dilution water chemistry: Hardness 90 mg/l as CaCO3, Alkalinity 70 mg CaCO3, pH 8.8, TOC 5580 mug/l; TDS 140,000 mug/l; Ca/Mg 2.8. Na/K 4.3.
Lighting: 646 lux +/- 85; 16 hr light, 8 hr dark.
Water chemistry on test: Dissolved Oxygen 8.4-10.3 mg/l, pH

8.2-8.4.
Endpoint assessment: Assessed microscopically.
Test design: Ten individuals per beaker, 3 replicates per concentration. Concentrations of ethanol not specified.
Method of calculating mean measured concentration: Not discussed; Geometric mean LC50's calculated.

Statistical method: Thompson moving averages.
Species: Ceriodaphnia dubia

Biological observations:

Number immobilized as compared to number exposed; Not discussed.

Concentration response with 95% confidence limits (LC50)
5012 mg/l (4233-6913 mg/l).
Cumulative immobilization: Not discussed.
Satisfactory control response?: Unknown.
Cumulative immobilization: Not discussed.
Satisfactory control response?: Unknown.

Result : LC50 values ranged from 6325 to 6772 mg/l at 20 degree C and from 3715 to 6076 mg/l at 24 degree C.
Test substance : Test substance was pure (absolute) ethanol (dehydrated U.S.P.).
Reliability : (2) valid with restrictions
These data are regarded as reliable. Fugacity data suggests low losses would be expected by evaporation, however as no measurements made, only rated reliable with restrictions.
Flag : Critical study for SIDS endpoint
11.11.2004 (86)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
LC50 : = 12340 measured/nominal
Analytical monitoring : no data
Method : other: ASTM (see ME)
Year : 1987
GLP : no data
Test substance : other TS: Absolute ethanol

Remark : Biological observations:

Number immobilized as compared to number exposed; Not discussed.

Concentration response with 95% confidence limits (LC50)
1,2340 mg/l (11,065-13,948 mg/l).
Cumulative immobilization: Not discussed.
Satisfactory control response?: Unknown.
Cumulative immobilization: Not discussed.
Satisfactory control response?: Unknown.
LC50 values ranged from 11853 to 13248 mg/l at 20 degree C and from 9268 to 14221 mg/l at 24 degree C.
Method was that recommended by the American Society of Testing and Materials (1980) Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. ASTM Standard E729-80.

Philadelphia, Pennsylvania.

Test organism: Daphnia magna: Source not specified. Stocks maintained in adjusted, autoclaved, aerated Lake Huron water for 3 years before start of study. Neonates hatched by isolated gravid females gathered by sieving.
Age: Neonates.
Control group: Dilution water controls.
Test conditions: Ethanol not discussed.
Test temperature: 20 deg C.
Exposure vessel: Covered beakers, not aerated, triplicates for each concentration.
Dilution water source: See above.
Dilution water chemistry: Hardness 160 mg/l as CaCO3, pH 8.0, TOC 5520 mug/l; TDS 289,550 mug/l; Ca/Mg 5.7. Na/K 4.5.
Lighting: 1916 lux +/- 75; 16 hr light, 8 hr dark.
Water chemistry on test: Dissolved Oxygen 7.6-8.9 mg/l, pH 7.8-8.4.
Endpoint assessment: Assessed microscopically.
Test design: Ten individuals per beaker, 3 replicates per concentration. Concentrations of ethanol not specified.
Method of calculating mean measured concentration: Not discussed; Geometric mean LC50's calculated.

Statistical method: Thompson moving averages.

Test substance : Test substance was pure (absolute) ethanol (dehydrated U.S.P.).

Reliability : (2) valid with restrictions
These data are regarded as reliable. Fugacity data suggests low losses would be expected by evaporation, however as no measurements made, only rated reliable with restrictions.

11.11.2004 (87)

Type : other: not specified
Species : Artemia salina (Crustacea)
Exposure period : 1 day(s)
Unit : mg/l
LC50 : = 1833 measured/nominal
: =
Analytical monitoring : no data
Method : other
Year : 1994
GLP : no data
Test substance : no data

Method : Artemia salina 24 h nauplius larvae were exposed to unspecified nominal concentrations of ethanol for 24 h.

Remark : Biological observations:

Number immobilized as compared to number exposed; Not discussed.

Concentration response with 95% confidence limits (LC50) 1,834 mg/l (1,324-2,538 mg/l).
Cumulative immobilization: Not discussed.
Satisfactory control response?: Unknown.
Cumulative immobilization: Not discussed.
Satisfactory control response?: Unknown.
Test organism: Artemia salina hatched from dry eggs supplied by San Francisco Bay Brand hafter hydration in distilled

water. Cysts were incubated. in synthetic sea water for 24 h at 25 deg C with continuous side illumination and slight aeration.
Age: 24-h-old nauplius larvae.
Control group: Used but not described.
Test conditions: Synthetic seawater was porepared using 35% Synthetica sea salt and distilled deionized seawater.
Test temperature: 25 deg C.
Exposure vessel: Plastic 16 mm petri dishes.
Dilution water source: See above.
Dilution water chemistry: Not described.
Lighting: Incubated in the dark.
Water chemistry on test: Not discussed.
Endpoint assessment: Organisms considered dead if they did not move during 10 sec observation.
Test design: Ten larvae per dish, 3-5 replicates per concentration; experiment repeated 5 times. Concentrations of ethanol not specified.
Method of calculating mean measured concentration: Nominal concentrations only.

Statistical method: Litchfield and Wilcoxon.

Result : Further studies involved older larvae:

48 h LC50 850 mg/l
72 h LC50 695 mg/l

Sensitivity to ethanol was therefore age-related.

Reliability : (2) valid with restrictions

11.11.2004 (88)

Type : Static
Species : other: Paramecium caudatum (ciliate Protozoon)
Exposure period : 4 hour(s)
Unit : mg/l
LC50 : = 5840
Limit Test : No
Analytical monitoring : no data
Method : other
Year : 1989
GLP : no data
Test substance : other TS

Remark : Age of test species: 48hrs
Control group: None mentioned. A number of other solvents tested as well as pesticide compounds.
Test conditions: Stock solutions split-pea medium.
Test temperature range 25 deg C +/- 2 deg.
Exposure vessel: not specified. Test in 100 ml containing 1 ml of culture containing 1,500-2000 stationary phase organisms/ml.
Dilution water source: not specified .
Dilution water chemistry: Not measured.
Lighting: not specified.
Water chemistry on test: Not measured. Medium was Chalkley's isotonic inorganic salt (Patterson, 1982)
Endpoint assessment: lethal concentration at which all animals died in 10 mins; death indicated by lack of swimming and or rupture of cell. Also, 4 hr median lethal concentraton (LC50).
Replicates: 5
Number of concentrations for 4hr LC50 evaluation: 0.1, 0.2, 0.4, 0.8, 1.2, 2

		% v/v (790 to 15,800 mg/l)
Result	:	10 minute LC50=5% v/v (39,000mg/l)
Test substance	:	commercial absolute alcohol
Reliability	:	(2) valid with restrictions Method details not comprehensive. Results available for each concentration but only in graphical form. However, study sufficiently well reported to rate reliable.

17.11.2004 (89)

Type	:	Static
Species	:	other aquatic crustacea: Palaemonetes kadiakensis
Exposure period	:	18 hour(s)
Unit	:	g/l
EC50	:	= 10.1 measured/nominal
Analytical monitoring	:	No
Method	:	
Year	:	1981
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method	:	Organisms exposed to 4 concentrations of ethanol in the range 1% v/v to 2% v/v. Test conducted at 23 degC.
Remark	:	Biological observations: Number immobilized as compared to number exposed; mortality ranged from 0 to 100%. Concentration response with 95% confidence limits (LC50) 1.28% v/v (1.18-1.38). Cumulative immobilization: Not discussed. Satisfactory control response?: Unknown. Test organism: Palaeomonetes kadlakensis caught in a nearby lake. Age: Juveniles. Control group: None mentioned. Test conditions: Stock solutions preparation not discussed. Test temperature range 23 deg C +/- 1 deg. Exposure vessel: 2 litre beakers containing 100 ml test medium. Each dilution tested in duplicate. Dilution water source: Aerated deionized deep well water. Dilution water chemistry: Not measured. Lighting: 1 h of typical fluorescent light illumination, 15.5 h 10% normal illumination then 1.5 h typical illumination. Water chemistry on test: Not measured. Endpoint assessment: Organisms considered dead if they did not respond to light, sound vibration or gentle probing. Test design: Five organisms per beaker, two beakers per concentration, at least 5 concentrations of ethanol. Method of calculating mean measured concentration: Not described. Nominal concentration: Range from 1% v/v to 1.5% v/v according to graph. Measured concentration: Not measured. Statistical method: Probit.
Result	:	LC50 quoted as 1.28% v/v (1.18 to 1.38) which is equivalent to 10.1 (9.3 to 10.9) g/l. Control response not known.
Reliability	:	(2) valid with restrictions

11.11.2004 (90)

Type	:	Static
Species	:	Daphnia pulex (Crustacea)
Exposure period	:	18 hour(s)

Unit	:	g/l
EC50	:	= 12.1 measured/nominal
Analytical monitoring	:	No
Method	:	
Year	:	1981
GLP	:	no data
Test substance	:	other TS

Method : Organisms exposed to 4 concentrations of ethanol in the range 1% v/v to 2% v/v. Test conducted at 23 degC.

Remark : Biological observations:

Number immobilized as compared to number exposed; mortality ranged from 0 to 100%. Concentration response with 95% confidence limits (LC50) 1.53% v/v (1.17-1.80).
Cumulative immobilization: Not discussed.
Satisfactory control response?: Unknown.
Test organism: Daphnia pulex caught from a nearby pond.
Age: less than 24 h old when used.
Control group: None mentioned.
Test conditions: Stock solutions preparation not discussed.
Test temperature range 23 deg C +/- 1 deg.
Exposure vessel: 50 ml culture tubes containing 25 ml test medium. Tubes loosely capped, not aerated. Each dilution tested in duplicate.

Dilution water chemistry: Not measured.
Lighting: 1 h of typical fluorescent light illumination, 15.5 h 10% normal illumination then 1.5 h typical illumination.
Water chemistry on test: Not measured.
Endpoint assessment: Organisms considered dead if they did not move after being swirled under a light.
Test design: Ten organisms per tube, two tubes per concentration, at least 4 concentrations of ethanol.
Method of calculating mean measured concentration: Not described.
Nominal concentration: Range from 1% v/v to 2% v/v according to graph. Measured concentration: Not measured.
Statistical method: Probit.

Result : LC50 reported as 1.53% v/v (1.17 to 1.80) which is equivalent to 12.1 (9.2 to 14.2) g/l. Control response not known.

Test substance : USP grade, 95% ethanol

Reliability : (2) valid with restrictions

11.11.2004 (90)

Type	:	Static
Species	:	other aquatic crustacea: Hyalella azteca
Exposure period	:	18 hour(s)
Unit	:	g/l
EC50	:	= 8.2 measured/nominal
Analytical monitoring	:	No
Method	:	
Year	:	1981
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : Organisms exposed to 4 concentrations of ethanol in the range 1% v/v to 2% v/v. Test conducted at 23 degC.

Remark : Biological observations:

Number immobilized as compared to number exposed; mortality ranged from 20 to 100%. Concentration response with 95% confidence limits (LC50) 1.04% v/v (0.761-1.28).
Cumulative immobilization: Not discussed.
Satisfactory control response?: Unknown.
Test organism: Hyalella azteca caught from a nearby slough and maintained in aquaria with added aerated water and aeration.
Age: Juveniles with 14-16 antenna segments
Control group: None mentioned.
Test conditions: Stock solutions preparation not discussed.
Test temperature range 23 deg C +/- 1 deg.
Exposure vessel: 400 ml beakers containing 100 ml test medium. Beakers covered with aluminium foil. Each dilution tested in duplicate.
Dilution water source: Aerated deionized deep well water.
Dilution water chemistry: Not measured.
Lighting: 1 h of typical fluorescent light illumination, 15.5 h 10% normal illumination then 1.5 h typical illumination.
Water chemistry on test: Not measured.
Endpoint assessment: Organisms considered dead if they did not respond to light, sound vibration or gentle probing.
Test design: Ten organisms per beaker, two beakers per concentration, at least 5 concentrations of ethanol.
Method of calculating mean measured concentration: Not described.
Nominal concentration: Range from 0.8% v/v to 2% v/v according to graph.
Measured concentration: Not measured.
Statistical method: Probit.

Result : LC50 reported as 1.04% v/v (0.761 to 1.28) which is equivalent to 8.2 (6.0 to 10.1) g/l. Control response not known.

Reliability : (2) valid with restrictions

11.11.2004 (90)

Type : Static
Species : Artemia salina (Crustacea)
Exposure period : 1 day(s)
Unit : mg/l
LC50 : = 23874
Analytical monitoring : no data
Method : other: Artoxkit M
Year : 1992
GLP : no data
Test substance : other TS

Method : This test used the Standard Operating Procedures for the ARTOXKIT M test modified as follows:

The procedures of Vanhaecke (1980) and Vanhaeck & Persoone (1981) were followed for the hatching of the cysts and collection of the nauplii. For moulting of instar I to instar II-III larvae the nauplii were transferred after 18-24 h from the start of cyst rehydration to 100 ml Erlenmeyer flasks containing fresh artificial seawater with continuous aeration and illumination for a further 24 hr.

LC50s were calculated with the corresponding 95% confidence limits using the trimmed Spearman Karber method.

Control: Sodium dodecyl sulphate.

An artificial seawater (35 g/l salt) was used:

NaCl 23.9g/l
MgCl2.6H20 10.83g/l
CaCl2 1.15g/l
SrCl2.6H2) 4mg/l
KCl 682mg/l
KBr 9.9mg/l
Na2SO4 9.06mg/l
NaHCO3 200mg/l
NaF 0.3mg/l
H3BO3 2.7mg/l

Result : The EC50 for artemia was 519 mmol/l +/- 29.1 (23.874g/l +/- 1.33).
Test substance : analytical grade from Sigma Chemical Company.
Reliability : (2) valid with restrictions
11.11.2004 (91) (92)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 1 day(s)
Unit : g/l
EC50 : = 10.7 measured/nominal
Analytical monitoring : no data
Method : OECD Guide-line 202
Year :
GLP : no data
Test substance : other TS

Method : 1984 version of OECD method. 3 replicates. Statistics: trimmed Spearman-Karber method. No further data given.
Result : Result quoted as 233mmol/l. No confidence limits given.
Test substance : No specific data. At least 97% pure, bbut possibly >99% or pharmacopia purity.
Reliability : (4) not assignable
11.11.2004 (92)

Type : Static
Species : other: Brachionus calyciflorus
Exposure period : 1 day(s)
Unit : g/l
LC50 : = 29.6 measured/nominal
Analytical monitoring : no data
Method : other: Rotoxkit F
Year :
GLP : no data
Test substance : other TS

Method : This test used the Standard Operating Procedures for the ROTOXKIT F test modified as follows:

The contents of a vial containing B. calyciflorus cysts was emptied into a small disposable polystyrene Petri dish. 5 ml of EPA water was then added after which the whole was covered and incubated at 25C +/-1 in light (19.5uE.m-2) for 18-20hrs. 24 well plates used.

LC50s were calculated with the corresponding 95% confidence limits using the trimmed Spearman Karber method.

Control: Potassium dichromate

EPA water (<2 weeks old and continuously aerated):

KCl 4mg/l
NaHCO3 296g/l
MgSO4 60mg/l
CaSO4.2H2O 60mg/l

Result : Result quoted as 644mmol/l (+/-40.6) which is equivalent to 29.6g/l (+/-1.9).
Test substance : analytical grade from Sigma Chemical Company.
Reliability : (2) valid with restrictions
11.11.2004 (91) (92)

Type : Static
Species : other: Brachionus plicatilis
Exposure period : 1 day(s)
Unit : g/l
LC50 : = 35.4 measured/nominal
Analytical monitoring : no data
Method : other: Rotox M
Year :
GLP : no data
Test substance : other TS

Method : This test used the Standard Operating Procedures for the ROTOXKIT M test modified as follows:

The contents of a vial containing B. plicatilis cystes , suspended in a saline medium of 55g/l, were poured into a simall disposable polystyrene Petri dish. 5ml of dieonised water was added to bring the salinity of the hatching medium to 15g/l. The whole was incubated at 25C+/-1 in light (19.5uE.m-2) for 24-28 hours. 24 well plates used.

LC50s were calculated with the corresponding 95% confidence limits using the trimmed Spearman Karber method.

Control: Cupric sulphate.

An artificial seawater (15 g/l salt) was used:
NaCl 11.32g/l
MgCl2.6H20 1.97g/l
CaCl2 0.54g/l
KCl 0.36mg/l
MgSO4.7H2O 2.39g/l
NaHCO3 70mg/l
H3BO3 10mg/l

Result : Quoted LC50 was 770 mmol/l (+/- 34.5) which is equivalent to 35.4 (+/- 1.6) g/l.
Test substance : analytical grade from Sigma Chemical Company.
Reliability : (2) valid with restrictions
11.11.2004 (91) (92)

Type : Static
Species : other: Streptocephalus proboscideus
Exposure period : 1 day(s)
Unit : g/l
LC50 : = 18.8 measured/nominal
Analytical monitoring : no data
Method : other: Streptox F
Year :
GLP : no data
Test substance : other TS

Method : This test used the Standard Operating Procedures for the STREPTOXKIT F test modified as follows:

Hatching of the cysts was initiated 24hrs before the start of the test, in cylindrico-conical tube containing 100-125ml of EPA water at 25C +/-1 in light (19.5uE.m-2) and aerated. As described by Centeno (1992) hatched larvae (intstar I) were tranferred afte 16-18hrs from the start of the rehydration into Erlenmeyer flasks containing fresh medium and incubated for a further 5-7hrs to moult to the intart II-III stage.

Control: Potassium dichromate

LC50s were calculated with the corresponding 95% confidence limits using the trimmed Spearman Karber method.

EPA water (<2 weeks old and continuously aerated):
KCl 4mg/l
NaHCO3 296g/l
MgSO4 60mg/l
CaSO4.2H2O 60mg/l

Result : Quoted LC50 of 409mmol/l (+/- 12.9) which is equivalent to 18.8g/l (+/- 0.6).
Test substance : analytical grade from Sigma Chemical Company.
Reliability : (2) valid with restrictions
11.11.2004 (91) (92)

Type :
Species : Other
Exposure period : 1 day(s)
Unit : mg/l
IC50 : = 13100 - 20900
Analytical monitoring : no data
Method : other
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : IC50 is for population growth in this freshwater protozoan, Tetrahymena pyriformis (Ciliata).
Result : Three incubation periods were evaluated with the following results:

3 hour 20900 mg/l
6 hour 17700 mg/l
9 hour 13100 mg/l

Reliability : (4) not assignable
21.08.2003 (93)

Type :
Species : Other
Exposure period : 1 day(s)
Unit : mmol/l
EC50 : = 258
LC50 : = 590
Analytical monitoring : no data
Method : other
Year : 1999
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : EC50 values are for development inthe protozoan Spirostomum ambiguum.
Reliability : (4) not assignable
21.08.2003 (94)

Type :
Species : other aquatic crustacea
Exposure period : 1 day(s)
Unit : mg/l
LC50 : = 31700
Analytical monitoring : no data
Method : other
Year : 1994
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : The species used was the Fairy shrimp, Streptocephalus rubricaudatus.
Reliability : (4) not assignable
21.08.2003 (95)

Type : Static
Species : Palaemonetes pugio (Crustacea)
Exposure period : 4 day(s)
Unit : g/l
LC50 : = 12.07 measured/nominal
Analytical monitoring : no data
Method : other
Year : 1997
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Age at start: Adult male and female grass shrimps from local estuaries, Gulf Breeze, Fla, USA.

Acclimation: to 25 Cel and 20 ppt salinity for 2 weeks.

Embryos: Collected for test at embryo cap stage.

Ethanol dosage: increasing concentrations (range not given)

Duration of exposure: 12 days

Mortality and hatching recorded daily.
Result : Mean LC50 at 4 days: 12.07 g/l
Mean LC50 at 12 days: 3.63 g/l
Conclusion : If ethanol is used as a solvent in this developmental toxicity test it should not exceed 1 g/l in the test solution.
Reliability : (4) not assignable
29.09.2003 (96)

Type :
Species : Daphnia sp. (Crustacea)
Exposure period : 1 day(s)
Unit : mg/l
EC50 : = 12300 - 13400
Analytical monitoring : no data

Method : other
Year : 1996
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : The 2 day range was 10100 to 11200 mg/l.
Reliability : (4) not assignable
21.08.2003 (97)

Type :
Species : Daphnia pulex (Crustacea)
Exposure period : 1 day(s)
Unit : mmol/l
EC50 : = 251.07
Analytical monitoring : no data
Method : other
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
21.08.2003 (98)

Type :
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC50 : > 10000
Analytical monitoring : no data
Method : other
Year : 1977
GLP : no data
Test substance : no data

Remark : Although the figure given in the paper is referred to as an LC50, it is better defined as an EC50, as the end-point studied is immobilization.
Twenty-four-hour-old Daphnia exposed to a series of dilutions of ethanol in tap water. Swimming ability measured after 24 hours.
Reliability : (4) not assignable
11.11.2004 (99)

Type :
Species : Daphnia magna (Crustacea)
Exposure period : 2 day(s)
Unit : g/l
LC100 : = 1
Analytical monitoring : no data
Method : other
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Unit of concentration expressed as % (v/v or w/w not known).
Reliability : (4) not assignable
21.08.2003 (100)

Type : Static

Species : Daphnia magna (Crustacea)
Exposure period : 2 day(s)
Unit : g/l
EC50 : > 100
Analytical monitoring : no data
Method : other
Year : 1995
GLP : no data
Test substance : no data

Remark : Result is expressed in ppm,
Reliability : (4) not assignable
21.08.2003 (101)

Type :
Species : Daphnia magna (Crustacea)
Exposure period : 1 day(s)
Unit : mg/l
EC50 : > 10000
Analytical monitoring : no data
Method : other
Year : 1989
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Same value recorded for 2 day exposure.
Reliability : (4) not assignable
21.08.2003 (102)

Type :
Species : Daphnia magna (Crustacea)
Exposure period : 1 day(s)
Unit : mg/l
EC50 : = 2500
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Effect is on physiology; EC50 (2 day) = 2000 mg/l.
Reliability : (4) not assignable
21.08.2003 (103)

Type :
Species : Daphnia magna (Crustacea)
Exposure period : 1 day(s)
Unit : mmol/l
EC50 : = 297.7
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
21.08.2003 (104)

Type :

Species : Artemia sp. (Crustacea)
Exposure period : 2 day(s)
Unit : mg/l
LC50 : = 25500
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
21.08.2003 (105)

Type :
Species : Artemia sp. (Crustacea)
Exposure period : 1 day(s)
Unit : mg/l
LC50 : = 25500 - 27000 measured/nominal
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
21.08.2003 (105)

Type : Static
Species : Artemia salina (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
TLm : > 10000
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Static test carried out in the laboratory at 24.5 C. TLm (concentration causing 50% mortality) determined graphicallyfrom measurements at an unspecified number of concentrations.
Reliability : (4) not assignable
21.08.2003 (106)

Type :
Species : Other
Exposure period : 2 day(s)
Unit : mg/l
EC50 : = 11963
Analytical monitoring : no data
Method : other
Year : 1990
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Endpoint is EC50 for growth in the freshwater protozoan Tetrahymena pyriformis (Ciliata).
Reliability : (4) not assignable

21.08.2003 (107)

Type :
Species : Other
Exposure period : 2 day(s)
Unit : mmol/l
IC50 : = 259.67
Analytical monitoring : no data
Method : other
Year : 1993
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : IC50 is for population growth in this freshwater protozoan. Tetrahymena pyriformis (Ciliata).
Reliability : (4) not assignable
21.08.2003 (108)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Chlorella vulgaris (Algae)
Endpoint : growth rate
Exposure period : 4 day(s)
Unit : mg/l
NOEC : < 500 measured/nominal
LOEC : = 500 measured/nominal
EC50 : = 1000 measured/nominal
Limit test :
Analytical monitoring : No
Method : other
Year : 1996
GLP : no data
Test substance : no data

Method : Growth rate measured as chlorophyll content and biomass accumulation at concentrations of 0.05% (500 mg/l) and higher (range 500 to 10,000 mg/l).
Remark : Cells removed before measurement: cells were not removed.

Biological observations:
+Cell density at each flask/each measuring point: Not given.
+Growth curves: Chlorophyll content plotted over time for each concentration, including control.

Percent biomass/growth rate inhibition per concentration
Observations at 500, 1000, 2000, 5000 and 10,000 mg/l, the growth inhibition was 37%, 54%, 69%, 86% and 95%
Laboratory culture: isolated from lake Geneva in 1980.

Cultivation method: Cultures were grown in Algal Assay Procedure (1971) medium in 500 ml flasks containing 250 ml algal suspension.

Cells were not removed from medium prior to measurement; cell density not given.

Controls consisted of algal suspensions without solvent in each experiment.

Dilution water source not specified.

Growth/test medium: Algal assay Procedure (1971) medium with 15 mg/l NaHCO3 and 12 mg/l K2HPO4.

Exposure vessel type: 20 x 125 mm test tubes containing about 20 ml of suspension and ethanol. 3 Tubes per test concentration were used.

Water chemistry (pH) on test: Not described.

Stock solution preparation: Not described.

Light levels and quality during exposure: 100 microE/m^2-sec except when reduced to 1.5 microE/m^2-sec 20 min before and during measurement of chlorophyll content by fluorescence.

Test design: Ethanol was tested three times at each concentration (0, 0.05, 0.1, 0.3, 0.5 and 1%).

Method of calculating mean measured concentrations: Only nominal concentrations were used.

Result : Growth was inhibited 54% at an ethanol concentration of 1000 mg/l, the value approximating the EC50.

Growth inhibition was 37% at 500 mg/l.

Growth was significantly inhibited (p=0.05) at all concentrations of ethanol.

Test condition : Test temperature 21 +/- 1 deg C with continuous illumination at 100 microE/m^2-sec.

Conclusion : Growth was inhibited 48% at an ethanol concentration of 10,000 mg/l; this approximates the ErC50.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

11.11.2004 (109)

Species : other aquatic plant: Lemna gibba
Endpoint : growth rate
Exposure period : 7 day(s)
Unit : mg/l
NOEC : = 280 measured/nominal
LOEC : > 280 measured/nominal
EC50 : = 4432 measured/nominal
Limit test :
Analytical monitoring : No
Method : EPA OTS 797.1160
Year : 1986
GLP : no data
Test substance : other TS: 100% dehydrated

Method : Lemna gibba (Fat Duckweed) was exposed to alcohol in water at nominal concentrations 0, 1.0, 1.7, 2.8, 4.7, 7.8, 13, 21, 36 etc to 21,000 (21 concentrations) for 7 days. Maintained at 25 degC with 6461 +/- 323 lux continuously; 5382 +/- 89 on test. Grown on Hoagland's with a pH of 4.6 to 5.4. Medium renewed weekly. Acclimation period was 8 weeks.

Remark : Cells removed before measurement: Plant fronds counted visually. Biomass measured by dry weight of plants and

fronds

Biological observations:
+Cell density at each flask/each measuring point: Not applicable.
+Growth curves: Not shown.

Percent biomass/growth rate inhibition per concentration
Observations: Results were not given for each of the 21 occurrences.%
EPA procedures as described by Holst (1986) and Holst and Edwanger (1982).
Laboratory culture: Obtained from Smithsonian Institution.

Cultivation method: Cultures were grown in Hoaglands medium with pH 4.6 to 5.4 medium was renewed weekly.

Acclimation period was 8 weeks.

Plants were not removed from medium prior to measurement; Plant density not given.

Controls consisted of Lemna cultures without ethanol in each experiment.

Dilution water source not specified.

Growth/test medium chemistry: Hoaglands. Water hardness 636 mg/l as CaCO3; Alkalinity 23 mg/l as CaCO3, Conductivity 5000 micromhos/cm, pH 4.5 to 5.1.

Exposure vessel type: 250 ml vessels; Shimadzu closures covered with paraffin. Each concentration replicated 3 times

Water chemistry (pH) on test: pH ranged 4.6 to 5.1..

Stock solution preparation: Not described.

Light levels and quality during exposure: Mean lux 5382 +/- 89 during exposure.

Test design: Ethanol was tested three times at each concentration (1.0 to 21,000 mg/l, plus control). Only nominal concentrations were used.

Method of calculating mean measured concentrations: Only nominal concentrations were used.

Statistical method: EC50 - regression analysis; NOEL: Dunnett's test.

Result : The EC50 (4432 mg/l) was within the 95% confidence interval 845 to 8018 mg/l for plant growth. The EC50 for biomass (dry weight) was 5987 mg/l (1640 to 10,293) mg/l.
Conclusion : Ethanol was the least toxic of 8 compounds tested.
Reliability : (1) valid without restriction
11.11.2004 (110)

Species : other aquatic plant: Lemna minor 6591
Endpoint : growth rate
Exposure period : 7 day(s)

Unit	:	mg/l
NOEC	:	= 778 measured/nominal
LOEC	:	> 778 measured/nominal
EC50	:	= 3690 measured/nominal
Limit test	:	
Analytical monitoring	:	No
Method	:	EPA OTS 797.1160
Year	:	1986
GLP	:	no data
Test substance	:	other TS: 100% dehydrated

Method : Lemna minor (duckweed) was exposed to alcohol in water at nominal concentrations 0, 1.0, 1.7, 2.8, 4.7, 7.8, 13, 21, 36 etc to 21,000 for 7 days. Maintained at 25degC with 6461 +/- 323 lux continuously; 5382 +/- 89 on test. Grown on Hoagland's with a pH of 4.6 to 5.4. Medium renewed weekly. Acclimation period was 8 weeks.

Remark : EPA procedures as described by Holst (1986) and Holst and Edwanger (1982).
Laboratory culture: Obtained from Geobotanischen Institute, Zurich, Switzerland.

Cultivation method: Cultures were grown in revised Hoaglands medium with pH 4.6 to 5.4; medium was renewed weekly.

Acclimation period was 8 weeks.

Plants were not removed from medium prior to measurement; Plant density not given.

Controls consisted of Lemna cultures without ethanol in each experiment.

Dilution water source not specified.

Growth/test medium chemistry: Hoaglands. Water hardness 636 mg/l as CaCO3; Alkalinity 23 mg/l as CaCO3, Conductivity 5000 micromhos/cm, pH 4.5 to 5.1.

Exposure vessel type: 250 ml vessels; Shimadzu closures covered with paraffin. Each concentration replicated 3 times

Water chemistry (pH) on test: pH ranged 4.6 to 5.1..

Stock solution preparation: Not described.

Light levels and quality during exposure: Mean lux 5382 +/- 89 during exposure.

Test design: Ethanol was tested three times at each concentration (1.0 to 21,000 mg/l, plus control). Only nominal concentrations were used.

Method of calculating mean measured concentrations: Only nominal concentrations were used.

Statistical method: EC50 - regression analysis; NOEL: Dunnett's test.
Test highly reliable.

Cells removed before measurement: Unclear. Plant fronds counted visually. Biomass measured by dry weight of plants and fronds

Biological observations:
+Cell density at each flask/each measuring point: Not applicable.
+Growth curves: Not shown.

Percent biomass/growth rate inhibition per concentration
Observations: Results were not given for each of the 21 occurrences.%

Result : The EC50 (4690 mg/l) was within the 95% confidence interval 81 to 167,764 mg/l for plant growth. The EC50 for biomass (dry weight) was 6986 mg/l (3155 to 10,817) mg/l. Of three other clones of Lemna minor, 7120 and 7136 were much more resistant to ethanol with EC50 values of at least 10,000 mg/l and NOEL values of at least 1000 mg/l.
Conclusion : Ethanol was the least toxic of 8 compounds tested.
Reliability : (1) valid without restriction
11.11.2004 (110)

Species : Selenastrum capricornutum (Algae)
Endpoint : growth rate
Exposure period : 4 day(s)
Unit : mg/l
NOEC : < 500 measured/nominal
LOEC : = 500 measured/nominal
EC50 : = 10000 measured/nominal
Limit test :
Analytical monitoring : No
Method : other
Year : 1996
GLP : no data
Test substance : no data

Method : Growth rate measured as chlorophyll content and biomass accumulation at concentrations of 0.05% (500 mg/l) and higher (range 500 to 10,000 mg/l).
Remark : Cells removed before measurement: cells were not removed.

Biological observations:
+Cell density at each flask/each measuring point: Not given.
+Growth curves: Chlorophyll content plotted over time for each concentration, including control.

Percent biomass/growth rate inhibition per concentration
Observations at 500, 1000, 2000, 5000 and 10,000 mg/l, the growth inhibition was 14%, 19%, 26%, 37% and 48%
EPA guidance from 1975 recommends maximum solvent concentration of 0.05% and 0.01% for acute and chronic tests and higher concentrations often occur in practice. Ethanol as solvent in such tests has a significant effect on growth rate of the test alga.
Laboratory culture: Obtained from EPA at Corville, OR.

Cultivation method: Cultures were grown in Algal Assay Procedure (1971) medium in 500 ml flasks containing 250 ml algal suspension.

Cells were not removed from medium prior to measurement; cell density not given.

Controls consisted of algal suspensions without solvent in each experiment.

Dilution water source not specified.

Growth/test medium: Algal Assay Procedure (1971) medium with 15 mg/l NaHCO3 and 12 mg/l K2HPO4.

Exposure vessel type: 20 x 125 mm test tubes containing about 20 ml of suspension and ethanol. 3 Tubes per test concentration were used.

Water chemistry (pH) on test: Not described.

Stock solution preparation: Not described.

Light levels and quality during exposure: 100 microE/m^2-sec except when reduced to 1.5 microE/m^2-sec 20 min before and during measurement of chlorophyll content by fluorescence.

Test design: Ethanol was tested three times at each concentration (0%, 0.05%, 0.1%, 0.2%, 0.5% and 1%).

Method of calculating mean measured concentrations: Only nominal concentrations were used.

Result : Growth was inhibited 48% at an ethanol concentration of 10,000 mg/l, the value approximating the EC50.

Growth inhibition was 14% at 500 mg/l.

Growth was significantly (p=0.05) inhibited at all concentrations of ethanol.

Reliability : (1) valid without restriction

11.11.2004 (109)

Species : Chlamydomonas sp. (Algae)
Endpoint : growth rate
Exposure period : 2 day(s)
Unit : g/l
NOEC : = 7.89 measured/nominal
LOEC : = 19.7 measured/nominal
Limit test :
Analytical monitoring : no data
Method : other
Year : 1980
GLP : no data
Test substance : no data

Method : Ethanol concentrations were 0.5, 1.0, 2.5 and 5.0 %v/v (equivalent to 3.95, 7.89, 19.7 and 39.5g/l respectively) in stocks grown on agar slants at 25 degC with continuous aeration and diurnal light cycle of 12 hr. Before counting, 5% glutaraldehyde added to test systems.

Species: Chlamydomonas eugametos.

Remark : Note whether cell removed prior to measurement: 5% glutaraldehyde was added to test systems. 1 ml samples analyzed by haemocytometer or Coulter counter.

Biological observations: #Cell density: Absolute measurements not given
Growth curves: Not given
%Biomass/growth rate inhibition: No inhibition at ethanol concentrations of 0.5 or 1.0%/ At 2.5% cell number was 57% of control. At 5.0%, growth was completely inhibited
Observations: None described.
Test organism: Bacteria-free Chlamydomonas eugametos culture collection No. 9.
Method of cultivation: Stocks grown on agar slants, liquid cultures made 3-4 days before assay
Liquid cultures grown at 25 degC with continuous aeration and diurnal light cycle of 12 hr.
Controls were used but are not dicussed. Tests for ethanol and other solvents were controls for tests for herbicides dissolved in these solvents.
Test conditions: temperature 25 degC.
Growth/Test medium chemistry: Not described. Grown in nutrient medium.
Dilution water source: Not described.
Exposure vessel type: 150 ml in 250 Erlenmeyer flasks aerated. 1x10^6 cells suspended in 20ml nutrient medium in 50 ml flasks not aerated.
Water chemistry in test (pH):Not described.
Stock solutions preparation: Not described.
Light levels and quality: 12 h diurnal at 200 microEm^2/s PPFD.
Test design: Solvents including ethanol were tersted at 4 concentrations, each concentration was tested at least twice.
Method of calculating mean measured concentrations: Not described.
Statistical test: Duncan's Multiple Range.

Result : Cell number was 57% of control at 2.5% (19.7g/l) and there was complete inhibition at 5% (39.5g/l).
Reliability : (2) valid with restrictions
11.11.2004 (111)

Species : Skeletonema costatum (Algae)
Endpoint :
Exposure period : 5 day(s)
Unit : mg/l
NOEC : = 3240 - 5400
EC50 : = 10943 - 11619
Limit test :
Analytical monitoring : no data
Method : other
Year : 1989
GLP : no data
Test substance : other TS: 100% dehydrated

Method : Growth inhibition in Skeletonema costatum evaluated by cell number count.
Remark : Laboratory culture: Bigelow Lab. for Ocean Sciences, West Boothbay Harbour, Maine, USA.
Cultured in revised ASP12 medium at 20degC with 14 hr light at 4304 lux +/- 161/day. Agitated daily and transferred every 7 days. Acclimated for 4 weeks. Test temperature 19.5 to 20.6 degC.
Controls consisting of Skeletonema in medium without ethanol were used.

Temperature range 19.5-20.6 degC.
Growth/Test medium chemistry: Not described.
Dilution water source: Not described.
Exposure vessel type: 100 ml covered with parafilm; each concentration tested in triplicate.

Stock solutions prepared with double-distilled sterile water.
Light levels and quality: Mean lux 4304 +/- 8.2 with a 14 h light/10 h dark cycle.

Test design: 5 or more concentrations plus control each replicated 3 times.

Method of calculating mean measured concentrations: Only nominal concentratiuons used.

Statistical methods: Not described.
Note whether cells removed prior to measurement: Not stated.
Biological observations:
#Cell density at each flask at each measuring point:
Not given.
#Growth curves: Not given but growth was stimulated before inhibition began.
Percent biomass/growth rate inhibition, observations: Not given.

Result : EC50 for total cell count was 11,619 mg/l (7923 to 15,314) and for total cell volume, 10943 mg/l (7061 to 14,826) mg/l.
Conclusion : The authors state that, using EPA criteria, ethanol can be judged 'practically nontoxic, by this test. Ethanol was used as a carbon source, stimulating growth before inhibition began.
Reliability : (2) valid with restrictions
11.11.2004 (112)

Species : Chlorella pyrenoidosa (Algae)
Endpoint : growth rate
Exposure period : 10 day(s)
Unit : mg/l
EC50 : = 1180
Limit test :
Analytical monitoring : no data
Method : other
Year : 1988
GLP : no data
Test substance : other TS

Remark : Alga: Chlorella pyrenoidosa from National Research Council of Canada.
Culture: Axenic in 250 Erlenmeyer fl;aks containing nitrogen free medium incubated at 25 degC

End point: Growth (biomass) measured by optical density over time.

Concentration of ethanol: 0.4 to 3.0%

Exposure period: 10 to 14 days (precise duration not specified).

EC50 value was the concentration required to cause a 50% reduction in growth.
Result was converted from an EC50 value of 1.18% v/v.
Stratton also studied the effects of ethanol on 5 further species of algae, using the same system but with the concentration of ethanol tested ranging from 0.1 to 8%.
The reported EC50 values were as follows:
Anabaena sp. 6312 mg/l
A. variabilis 10020 mg/l
A. inaequalis 8048 mg/l
A. cylindrica 7653 mg/l
Nostoc sp. 22644 mg/l

Test substance : Test sustance was absolute ethanol.
Reliability : (2) valid with restrictions
11.11.2004 (113) (114)

Species : Cyclotella sp. (Algae)
Endpoint : Biomass
Exposure period : 4 day(s)
Unit : g/l
EC0 : > 2.37 measured/nominal
Limit test :
Analytical monitoring : no data
Method : other
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Species of marine diatom from the intertidal region of the Gulf of Mexico were maintained and tested in Guillard's f/2 medium enriched with artificial sea salt mix.

A 1% (v/v) solution of ethanol was further diluted with test medium at concentrations of 0.2, 0.25 and 0.3 ml/100ml (1.58, 1.97, 2.37g/l respectively). Tests were carried out in test tubes containing 25 ml medium and in triplicate. Each tube was inoculated from exponentially growth cells at an initial density of 4000 cells/ml. The culture was incubated on a shaker for 96 h at 30 degC under cool white light producing 100 muEm-2s irradiation in continuous cycle. Growth was measured spectrophotometrically at 525nm. Statistical analysis was by Student's t-test with significance at $p<0.05$.

Result : There was no significant effect on the growth rate.
Reliability : (4) not assignable
Only a very limited range of concentrations was studied which in most cases produced no effect on growth. No chemical monitoring was carried on the solutions prepared. Results are only reported in summarized, basic, graphical form and only as a percentage of control response. There are insufficient data reported.
11.11.2004 (115)

Species : Navicula sp. (Algae)
Endpoint : Biomass
Exposure period : 4 day(s)
Unit : g/l
EC0 : > 2.37 measured/nominal
Limit test :
Analytical monitoring : no data
Method : other
Year : 1995
GLP : no data

Test substance : as prescribed by 1.1 - 1.4

Method : Species of marine diatom (Navicula saprophila species) from the intertidal region of the Gulf of Mexico were maintained and tested in Guillard's f/2 medium enriched with artificial sea salt mix.

A 1% (v/v) solution of ethanol was further diluted with test medium at concentrations of 0.2, 0.25 and 0.3 ml/100ml (1.58, 1.97, 2.37g/l respectively). Tests were carried out in test tubes containing 25 ml medium and in triplicate. Each tube was inoculated from exponentially growth cells at an initial density of 4000 cells/ml. The culture was incubated on a shaker for 96 h at 30 degC under cool white light producing 100 muEm-2s irradiation in continuous cycle. Growth was measured spectrophotometrically at 525nm. Statistical analysis was by Student's t-test with significance at $p<0.05$.

Result : There was an apparent increase in growth rate but no clear dose response relationship.

Reliability : (4) not assignable
Only a very limited range of concentrations was studied which in most cases produced no effect on growth. No chemical monitoring was carried on the solutions prepared. Results are only reported in summarized, basic, graphical form and only as a percentage of control response. There are insufficient data reported.

11.11.2004 (115)

Species : Nitzschia sp. (Algae)
Endpoint : Biomass
Exposure period : 4 day(s)
Unit : g/l
EC0 : > 2.37 measured/nominal
Limit test :
Analytical monitoring : no data
Method : other
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Species of marine diatom (Nitzschia pussilla species) from the intertidal region of the Gulf of Mexico were maintained and tested in Guillard's f/2 medium enriched with artificial sea salt mix.

A 1% (v/v) solution of ethanol was further diluted with test medium at concentrations of 0.2, 0.25 and 0.3 ml/100ml (1.58, 1.97, 2.37g/l respectively). Tests were carried out in test tubes containing 25 ml medium and in triplicate. Each tube was inoculated from exponentially growth cells at an initial density of 4000 cells/ml. The culture was incubated on a shaker for 96 h at 30 degC under cool white light producing 100 muEm-2s irradiation in continuous cycle. Growth was measured spectrophotometrically at 525nm. Statistical analysis was by Student's t-test with significance at $p<0.05$.

Result : There was no significant effect on the growth rate.

Reliability : (4) not assignable
Only a very limited range of concentrations was studied which in most cases produced no effect on growth. No chemical monitoring was carried on the solutions prepared. Results are only reported in summarized, basic, graphical form and only as a percentage of control response. There are insufficient data reported.

11.11.2004 (115)

Species : Nitzschia sp. (Algae)
Endpoint : Biomass
Exposure period : 4 day(s)
Unit : g/l
EC0 : > 2.37 measured/nominal
Limit test :
Analytical monitoring : no data
Method : other
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Species of marine diatom from the intertidal region of the Gulf of Mexico were maintained and tested in Guillard's f/2 medium enriched with artificial sea salt mix.

A 1% (v/v) solution of ethanol was further diluted with test medium at concentrations of 0.2, 0.25 and 0.3 ml/100ml (1.58, 1.97, 2.37g/l respectively). Tests were carried out in test tubes containing 25 ml medium and in triplicate. Each tube was inoculated from exponentially growth cells at an initial density of 4000 cells/ml. The culture was incubated on a shaker for 96 h at 30 degC under cool white light producing 100 muEm-2s irradiation in continuous cycle. Growth was measured spectrophotometrically at 525nm. Statistical analysis was by Student's t-test with significance at $p<0.05$.

Result : There was an apparent increase in growth rate but no clear dose response relationship.

Reliability : (4) not assignable
Only a very limited range of concentrations was studied which in most cases produced no effect on growth. No chemical monitoring was carried on the solutions prepared. Results are only reported in summarized, basic, graphical form and only as a percentage of control response. There are insufficient data reported.

11.11.2004 (115)

Species : Nitzschia sp. (Algae)
Endpoint : Biomass
Exposure period : 4 day(s)
Unit : g/l
Limit test :
Analytical monitoring : no data
Method : other
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Species of marine diatom (Nitzschia dissipata species) from the intertidal region of the Gulf of Mexico were maintained and tested in Guillard's f/2 medium enriched with artificial sea salt mix.

A 1% (v/v) solution of ethanol was further diluted with test medium at concentrations of 0.2, 0.25 and 0.3 ml/100ml (1.58, 1.97, 2.37g/l respectively). Tests were carried out in test tubes containing 25 ml medium and in triplicate. Each tube was inoculated from exponentially growth cells at an initial density of 4000 cells/ml. The culture was incubated on a shaker for 96 h at 30 degC under cool white light producing 100 muEm-2s irradiation in continuous cycle. Growth was measured spectrophotometrically at 525nm. Statistical analysis was by Student's t-test with significance at $p<0.05$.

Result : There was a dose reponse decrease in growth but with promotion at the lower concentration, the decrease in growth at the higher concentration was only limited. The results is therefore difficult to interpret.
Reliability : (4) not assignable
Only a very limited range of concentrations was studied which in most cases produced no effect on growth. No chemical monitoring was carried on the solutions prepared. Results are only reported in summarized, basic, graphical form and only as a percentage of control response. There are insufficient data reported.
11.11.2004 (115)

Species : other algae: Cylindricotheca sp.
Endpoint : Biomass
Exposure period : 4 day(s)
Unit : g/l
IC50 : = 1.97 measured/nominal
Limit test :
Analytical monitoring : no data
Method : other
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Species of marine diatom from the intertidal region of the Gulf of Mexico were maintained and tested in Guillard's f/2 medium enriched with artificial sea salt mix.

A 1% (v/v) solution of ethanol was further diluted with test medium at concentrations of 0.2, 0.25 and 0.3 ml/100ml (1.58, 1.97, 2.37g/l respectively). Tests were carried out in test tubes containing 25 ml medium and in triplicate. Each tube was inoculated from exponentially growth cells at an initial density of 4000 cells/ml. The culture was incubated on a shaker for 96 h at 30 degC under cool white light producing 100 muEm-2s irradiation in continuous cycle. Growth was measured spectrophotometrically at 525nm. Statistical analysis was by Student's t-test with significance at $p<0.05$.
Result : There was a significant dose response decrease with an apparent IC50 of around 0.25ml/100ml (equivalent to 1.97g/l). However, taking all results from the study into account, the authors describe ethanol to be non-toxic.
Reliability : (4) not assignable
Only a very limited range of concentrations was studied which in most cases produced no effect on growth. No chemical monitoring was carried on the solutions prepared. Results are only reported in summarized, basic, graphical form and only as a percentage of control response. There are insufficient data reported.
11.11.2004 (115)

Species : other algae: Thalassiosira weissflgii sp.
Endpoint : Biomass
Exposure period : 4 day(s)
Unit : g/l
EC0 : > 2.37 measured/nominal
Limit test :
Analytical monitoring : no data
Method : other
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Species of marine diatom from the intertidal region of the Gulf of Mexico were maintained and tested in Guillard's f/2 medium enriched with artificial sea salt mix.

A 1% (v/v) solution of ethanol was further diluted with test medium at concentrations of 0.2, 0.25 and 0.3 ml/100ml (1.58, 1.97, 2.37g/l respectively). Tests were carried out in test tubes containing 25 ml medium and in triplicate. Each tube was inoculated from exponentially growth cells at an initial density of 4000 cells/ml. The culture was incubated on a shaker for 96 h at 30 degC under cool white light producing 100 muEm-2s irradiation in continuous cycle. Growth was measured spectrophotometrically at 525nm. Statistical analysis was by Student's t-test with significance at $p<0.05$.

Result : There was an apparent increase in growth rate but no clear dose response relationship.

Reliability : (4) not assignable
Only a very limited range of concentrations was studied which in most cases produced no effect on growth. No chemical monitoring was carried on the solutions prepared. Results are only reported in summarized, basic, graphical form and only as a percentage of control response. There are insufficient data reported.

11.11.2004 (115)

Species : Scenedesmus subspicatus (Algae)
Endpoint : growth rate
Exposure period :
Unit : mg/l
IC10 : = 400 measured/nominal
Limit test :
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : no data

Reliability : (4) not assignable
11.11.2004 (116)

Species : Scenedesmus subspicatus (Algae)
Endpoint : other: inhibition of protoplast O2 production
Exposure period :
Unit : mg/l
IC10 : = 460 measured/nominal
Limit test :
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : no data

Reliability : (4) not assignable
11.11.2004 (116)

Species : Chlorococcum sp. (Algae)
Endpoint : Biomass
Exposure period : 24 hour(s)
Unit : mg/l
EC10 : > 1000
Limit test :

Analytical monitoring : no data
Method : other
Year :
GLP :
Test substance :

Method : In fresh water; method not specified.
Reliability : (4) not assignable
29.09.2003 (117)

Species : Dunaliella bioculata (Algae)
Endpoint : Biomass
Exposure period : 2 day(s)
Unit : mg/l
EC50 : = 1000 measured/nominal
LC100 : = .05
Limit test :
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Two concentrations of ethanol were tested, 0.1% (1000 mg/l) and 0.05% (500 mg/l).
Result : Growth was reduced 10% at 1000 mg/l. The NOEC and LOEC were not calculated.
Reliability : (4) not assignable
11.11.2004 (118)

Species : Microcystis aeruginosa (Algae, blue, cyanobacteria)
Endpoint : Biomass
Exposure period : 8 day(s)
Unit : mg/l
EC0 : = 1450 measured/nominal
Limit test :
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Difference in growth rate between ethanol-containing cultures and controls measured turbidimetrically. Toxic threshold concentration determined.
Reliability : (4) not assignable
11.11.2004 (119)

Species : Scenedesmus quadricauda (Algae)
Endpoint : growth rate
Exposure period : 7 day(s)
Unit : mg/l
LOEC : = 5000
Limit test :
Analytical monitoring : No
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Ethanol tested in double-distilled water in a cell multiplication inhibition test. Endpoint measured was the toxic threshold.
Reliability : (4) not assignable
11.11.2004 (120)

Species : other algae: see method details for species tested
Endpoint : Biomass
Exposure period : 96 hour(s)
Unit : g/l
Limit test :
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : other TS

Method : Algae obtained from University of Texas algal collection. The following species tested:

Gleocystis ampla
Scenedesmus obliquus
Nannochloris sp.
Tetraselmis sp.
Chlorella ellipsoidea
Chlorococcum sp.

Growth medium prepared according to Bold (1949). pH adjusted to 8 with HCL or NaOH.
Solution concentrate: 1ml/100ml growth medium
Test concentrations: 0.05, 0.1, 0.2ml solution concentrate in 100ml growth medium.
Assays carried out in 25ml medium; triplicate samples and triplicate cultures.
Innoculation: exponentially growing cells at 4x10E3 cells/ml.
Temperature: 30C
Light: cool-white 100uEm-2s in continuous cycle.
Measurement: spectrophotometrically at 525nm on a Fisher electrophotometer.
Statistical method: student's t test at $p<0.05$
Result : All algal species stimulated and all concentrations growed faster than control, some almost doubling in rate. No inhibition seen.
Test substance : Supplied by JT Baker, Phillipsburgh NJ.
Reliability : (3) invalid
Key details reported. No details of growth medium reported in paper but referenced elsewhere. No analytical monitoring or details of water used. Results presented as percentage of control - control value not available separately. Results only available graphically but this is not a major limitation bearing in mind result obtained. However, since there is ambiguity between the way the test concentrations are described in the method details versus the way they are reported in the results section, the study cannot be considered robust since concentrations cannot be determined with confidence.
17.11.2004 (121)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type : Aquatic
Species : Pseudomonas putida (Bacteria)
Exposure period : 16 hour(s)
Unit : mg/l
LOEC : = 6500
Analytical monitoring : no data
Method : other: see remarks
Year : 1980
GLP : no data
Test substance : no data

Remark : Stock solutions of the test compound were prepared under sterile conditions and diluted to test concentrations (by a factor of 2) with bidistilled water.The inoculated 4-parallel dilution series was prepared in 300-ml Erlenmeyer flasks, stoppered with cotton-lined plastic caps. The first flask of each series contained 160 ml of test solution. Subsequent dilutions from this flask were prepared by adding 80 ml of preliminary pollutant dilution and 80 ml bidistilled water. Each flask of the dilution series was inoculated to 100 ml by adding 5 ml of stock solution I, 5 ml of stock solution II, and 10 ml of bacterial cell suspension from the preliminary culture. Blank controls (not containing inoculum) were prepared by adding 5 ml of stock solution I, 5 ml of stock solution II, and 10 ml saline solution (0.50 g NaCl/l sterile, bidistilled water).Stock solution I: 20.0 g D(+)glucose, 4.240 g NaNO3, 2.40 g K2HPO4, 1.20 g KH2PO4, and 30 ml trace elements solution dissolved in 500 ml bidistilled water and sterilized for 30 minutes.Stock solution II: 0.20 g FeSO4o7H20 and 4.00 MgSO4o7H20 dissolved in 1000 ml sterile, bidistilled water. Flasks were cultured at 25 deg. C for 16 hours. The concentration of bacterial cells was measured turbidimetrically and expressed as the extinction of the primary light of monochromatic radiation at 436 nm for a 10 mm layer. Bacterial cell growth inhibition was graphically determined. Both the highest non-toxic test concentration and the lowest toxic test concentration were plotted. A 3% reduction in cell growth was used as the value indicating the onset of inhibitory action.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
11.11.2004 (122)

Type : Aquatic
Species : Photobacterium phosphoreum (Bacteria)
Exposure period : 15 minute(s)
Unit : g/l
EC50 : = 32.1 measured/nominal
Analytical monitoring : no data
Method : other: Microtox
Year : 1994
GLP : no data
Test substance : other TS

Method : Standard MICROTOX assay procedure (Microbiotics 1989). Unclear whether exposure time was 5 minutes or 15 minutes. Four replicates.
Test substance : No specific data. At least 97% pure, bbut possibly >99% or pharmacopia purity.

Reliability : (4) not assignable
11.11.2004 (92)

4.5.1 CHRONIC TOXICITY TO FISH

Species : Pimephales promelas (Fish, fresh water)
Endpoint : other: development, histology and mortality
Exposure period : 42 day(s)
Unit : g/l
EC50 : = 1 measured/nominal
Analytical monitoring : no data
Method : other
Year : 1999
GLP : no data
Test substance : no data

Remark : This study assesses the effects of waterborne exposure of nonylphenol and nonylphenol ethoxylate on the secondary sex characteristics and gonads of the fathead minnow. Ethanol was used as a carrier solvent for the test substances. Two controls were used in the experiment, one pure water and the second ethanol at 0.00001% (v/v or w/w not stated.) This is equivalent to approximately 0.08mg/l (assuming w/w) or 0.1ppm (assuming v/v).Testicular lesions were evaluated according to the severity of relative or absolute Sertoli cell proliferation and the percentage of seminiferous tubules affected.
Result : There was no dose dependent trends in mortality of either males or females exposed to the test substance or controls. Survival rates of males ranged from a low of 50% to 100%, for females from 60% to 100%.
Survival rates

MALES	Expt1	Expt 2
Water control	100%	50%
Ethanol 0.1ppm	100%	50%
FEMALES	Expt1	Expt 2
Water control	100%	100%
Ethanol 0.1ppm	50%	100%

There was no change in the gross appearance of the fatpad of males exposed to ethanol compared to the control. There was no significant difference in the incidence of histological lesions observed. There was as difference observed in one of two experiments in the median size of the tubercules and fatpad. There is no discussion in the paper on the relevance of this difference which was not seen in a second experiment. There was also no dose response trend for these end points for the actual test substances in the study, which varied randomly over a similar range.
Reliability : (3) invalid
This study was carried out using a single, very low concentration of ethanol and did not produce any results of clear significance. It cannot be used to derive a chronic NOEC for ethanol.
22.09.2003 (123)

Species : other: Acipenser transmontanum (White sturgeon)
Endpoint : other: mortality

Exposure period : 24 day(s)
Unit : mg/l
LC0 : = 1000 measured/nominal
Analytical monitoring : no data
Method : other
Year : 1998
GLP : no data
Test substance : no data

Remark : The authors describe this 24 day test "Acute".
Reliability : (3) invalid
The primary goal of this study was to investigate the possibility of using early life stages in both acute and sublethal toxicological testing of forest industry effluents into the Fraser River. The larval stage was more sensitive to toxicants than the fry stage and studies were inconclusive because immature stages did not withstand the additional handling during determination of body mass. The investigators had concerns about the reliability of this testing method and, in any case, ethanol was used only as a carrier solvent control at a concentration of 1 ppm.
04.10.2003 (124)

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species : Ceriodaphnia sp. (Crustacea)
Endpoint : Mortality
Exposure period : 10 day(s)
Unit : mg/l
LC50 : = 1284 - 2638
Analytical monitoring : no data
Method : other
Year : 1991
GLP :
Test substance : as prescribed by 1.1 - 1.4

Method : Follows the basic methodology for the three brood test proposed by Mount and Norberg (Mount, D.J. and Norberg, T.J. (1984) A seven-day life cycle cladoceran toxicity test. Environ. Toxicol. Chem. 3, 425 - 434).Analytical methods used for test substance: no data given.Vehicle used: not required. Statistical methods: For LC50: Probit, moving average and nonlinear interpolation. Calculation of point estimates and other corresponding 95% confidence intervals made using a program written by Stephan (1977, Methods for calculating an LC50, In Mayer FL et al (eds), Aquatic toxicology and hazard assessment, ASTM STP 634: 65-84). Calculation of EC50: statistical package SAS GLM (1987, SAS/STAT guide for personal computers, version 6th ed, SAS institute Inc, Cary, NC) used to generate regression equations. NOELs calculated using Dunnett's t-test.Test organism: Daphnia magna strauss 1820 populations (of British origin) had been maintained in the Dow Chemical Company Laboratory since 1982 without drastic changes in population. Population maintained at 25C for past 3 years and sustained on Ankistrodesmus convulutus (reared in medium based on Provasoli and Pintner (1968, Ecological implications of in vitro nutritional requirements of algal flagellates", Ann NY

Acad. Sci. 56, 839-851.) and Nitzschia frustulum Kutzing cultured in ES-I-Si, a medium developed by Provasoli (1968, "Media and prospects for the cultivation of marine algae", in Watanabe A et al, Cultures and collections of algae, Proceedings of a US-Japan conference, Hakone 1966.) Algal diet axenic.Test conditions: The. testing conditions followed the basic tenets of the original three-brood test proposed by Mount and Norberg (1984) but were revised in that they emphasize the needs of the animals in terms of space and diet. Details of the conditions may be found in in Cowgill and Milazzo (1989). Test vessels were wide mouth clear glass jars graduated in milliliters to contain 150mL. Into each jar was fitted a glass tube, 3.5 cm diameter. which had affixed to one end a nytex screen of 243 µm mesh for C. dubia or 1000 µm mesh for D. magna, These screens were affixed to the glass tubes with silicone glue. After the screens were glued to the glass tubes, three glass beads, 8 mm in diameter, were affixed to the underside equidistant from each other. This was covered with a glass petal dish 5.5 cm in diameter. The jar containing the screened tube was filled with double distilled water and autoclaved for 10 mins at a pressure of 124 kPa. This procedure was repeated three times, renewing the distilled water each time, before the equipment was used for a test. This procedure accomplished the complete removal of all effects of the silicone glue. Only glass vessels were used.

Remark : Species: Ceriodaphnia dubia.
Using the USEPA classification scheme, ethanol would be classified as practically non toxic based on survival.
Based on reproductive parameters, it would be classified as slightly toxic.

Test condition : Analysis of Lake Huron water used in culturing and testing

Al	140	
NH3 total	ND(IO)	
B	40	
Ca	18700	
Cr	8	
Cu	5	
F	80	
Fe	17	
Pb	(5)	ND (5)
Mg	7800	
Mn	(5)	ND (5)
K	1040	
Si	3400	
Na	4800	
S	6000	
Zn	8	
Total dissolved solids	118000	
Total suspended solids	NA	
Total organic carbon	1600	

Test conditions
Test vessel: Capacity 150ml,
Content 100ml
Screen composition: Nytex,
Screen mesh size, 243µm
Light lux: 670 ± 100 lux
Photoperiod, 16 h light, 8 h dark
Temperature 25 ± 2

Dissolved oxygen, 8.0 ± 1.5mg/L
pH: 8.2 ± 0.2
Dilution water
Hardness, as mg CaCO3,/L: 90-110
Alkalinity, as mg CaCQ3/L: 55-75
Habitat: Environmental chamber
Habitat changing frequency: Every other day
Diet: algae (A. convolutes, N. frustulum)
Feeding rate (cells/vessel): A. convolutes 9x10^6, N. frustulum 1.8x10^6
Feeding frequency: daily
Age of organisms, <12h (all from fourth brood.)
Number of control broods: 3
Permitted control loss, 20%
Number of organisms/ vessel: 1
Number of organisms/Concentration: 10
Number of organisms!/control: 20
Test length, days: 7-10
Variables monitored Daily: light, temperature, survival, progeny
Variables monitored every second day: w
ater quality variables in renewed solutions
Variables monitored at Test termination: Survival, total progeny, adult weight
Endpoints: Survival, total progeny, dry adult weight, number of broods, mean brood size, loss of control limited to 20% (LC50/EC50/NOEC)
Test ended when the control animals had produced three broods.
Test concentrations used: not specified.

Reliability : (2) valid with restrictions
This is a well reported study, particularly regarding the methodology of testing. The main weakness is lack of detail on test concentrations used and on the analytical method used to assess test substance concentration.

11.11.2004 (125)

Species : Ceriodaphnia sp. (Crustacea)
Endpoint : reproduction rate
Exposure period : 10 day(s)
Unit : mg/l
NOEC : = 9.6
EC50 : 26 - 38
LC50 : = 1806
Analytical monitoring : no data
Method : other: see free text
Year : 1984
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Follows the basic methodology for the three brood test proposed by Mount and Norberg (Mount, D.J. and Norberg, T.J. (1984) A seven-day life cycle cladoceran toxicity test. Environ. Toxicol. Chem. 3, 425 - 434).Analytical methods used for test substance: no data given.Vehicle used: not required.Statistical methods: For LC50: Probit, moving average and nonlinear interpolation. Calculation of point estimates and other corresponding 95% confidence intervals made using a program written by Stephan (1977, Methods for calculating an LC50, In Mayer FL et al (eds), Aquatic

toxicology and hazard assessment, ASTM STP 634: 65-84). Calculation of EC50: statistical package SAS GLM (1987, SAS/STAT guide for personal computers, version 6th ed, SAS institute Inc, Cary, NC) used to generate regression equations. NOELs calculated using Dunnett's t-test.Test organism: Daphnia magna strauss 1820 populations (of British origin) had been maintained in the Dow Chemical Company Laboratory since 1982 without drastic changes in population. Population maintained at 25C for past 3 years and sustained on Ankistrodesmus convulutus (reared in medium based on Provasoli and Pintner (1968, Ecological implications of in vitro nutritional requirements of algal flagellates", Ann NY Acad. Sci. 56, 839-851.) and Nitzschia frustulum Kutzing cultured in ES-I-Si, a medium developed by Provasoli (1968, "Media and prospects for the cultivation of marine algae", in Watanabe A et al, Cultures and collections of algae, Proceedings of a US-Japan conference, Hakone 1966.) Algal diet axenic.Test conditions: The. testing conditions followed the basic tenets of the original three-brood test proposed by Mount and Norberg (1984) but were revised in that they emphasize the needs of the animals in terms of space and diet. Details of the conditions may be found in in Cowgill and Milazzo (1989). Test vessels were wide mouth clear glass jars graduated in milliliters to contain 150mL. Into each jar was fitted a glass tube, 3.5 cm diameter. which had affixed to one end a nytex screen of 243 µm mesh for C. dubia or 1000 µm mesh for D. magna, These screens were affixed to the glass tubes with silicone glue. After the screens were glued to the glass tubes, three glass beads, 8 mm in diameter, were affixed to the underside equidistant from each other. This was covered with a glass petal dish 5.5 cm in diameter. The jar containing the screened tube was filled with double distilled water and autoclaved for 10 mins at a pressure of 124 kPa. This procedure was repeated three times, renewing the distilled water each time, before the equipment was used for a test. This procedure accomplished the complete removal of all effects of the silicone glue. Only glass vessels were used.

Result : Results based on total progeny
EC50 26mg/l (95% CI 0.5-1443)
NOEL 9.6mg/l
Results based on number of broods
EC50 38mg/l (95% CI 0.6-2554)
NOEL 16mg/l
Results based on mean brood size
EC50 33mg/l (95% CI 0.6-1820)
NOEL 9.6mg/l

Test condition : Analysis of Lake Huron water used in culturing and testing

Al	140
NH3 total	ND(IO)
B	40
Ca	18700
Cr	8
Cu	5
F	80
Fe	17
Pb	ND (5)
Mg	7800
Mn	ND (5)
K	1040

Si	3400
Na	4800
S	6000
Zn	8
Total dissolved solids	118000
Total suspended solids	NA
Total organic carbon	1600

Test conditions
Test vessel: Capacity 150ml, Content 100ml
Screen composition: Nytex, Screen mesh size 243µm
Light lux: 670 ± 100 lux
Photoperiod, 16 h light, 8 h dark
Temperature 25 ± 2
Dissolved oxygen, 8.0 ± 1.5mg/L
pH: 8.2 ± 0.2
Dilution water
Hardness, as mg CaCO3,/L: 90-110
Alkalinity, as mg CaCO3/L: 55-75
Habitat: Environmental chamber
Habitat changing frequency: Every other day
Diet: algae (A. convolutes, N. frustulum)
Feeding rate (cells/vessel): A. convolutes 9x10^6, N. frustulum 1.8x10^6
Feeding frequency: daily
Age of organisms, <12h (all from fourth brood.)
Number of control broods: 3
Permitted control loss, 20%
Number of organisms/ vessel: 1
Number of organisms/Concentration: 10
Number of organisms!/control: 20Test length, days: 7-10.
Variables monitored Daily: light, temperature, survival, progeny
Variables monitored every second day: water quality variables in renewed solutions.
Variables monitored at Test termination: Survival, total progeny, adult weight
Endpoints: Survival, total progeny, dry adult weight, number of broods, mean brood size, loss of control limited to 20% (LC50/EC50/NOEC)
Test ended when the control animals had produced three broods.
Test concentrations used: not specified.

Reliability : (2) valid with restrictions
This is a well reported study, particularly regarding the methodology of testing. The main weakness is lack of detail on test concentrations used and on the analytical method used to assess test substance concentration

11.11.2004 (125)

Species : Daphnia magna (Crustacea)
Endpoint : Mortality
Exposure period : 11 day(s)
Unit : mg/l
NOEC : = 9.6
LC50 : = 454
Analytical monitoring : no data
Method : other: see freetext
Year : 1990
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Follows the basic methodology for the three brood test proposed by Mount and Norberg (Mount, D.J. and Norberg, T.J. (1984) A seven-day life cycle cladoceran toxicity test. Environ. Toxicol. Chem. 3, 425 - 434).Analytical methods used for test substance: no data given.Vehicle used: not required.Statistical methods: For LC50: Probit, moving average and nonlinear interpolation. Calculation of point estimates and other corresponding 95% confidence intervals made using a program written by Stephan (1977, Methods for calculating an LC50, In Mayer FL et al (eds), Aquatic toxicology and hazard assessment, ASTM STP 634: 65-84). Calculation of EC50: statistical package SAS GLM (1987, SAS/STAT guide for personal computers, version 6th ed, SAS institute Inc, Cary, NC) used to generate regression equations. NOELs calculated using Dunnett's t-test.Test organism: Daphnia magna strauss 1820 populations (of British origin) had been maintained in the Dow Chemical Company Laboratory since 1982 without drastic changes in population. Population maintained at 25C for past 3 years and sustained on Ankistrodesmus convulutus (reared in medium based on Provasoli and Pintner (1968, Ecological implications of in vitro nutritional requirements of algal flagellates", Ann NY Acad. Sci. 56, 839-851.) and Nitzschia frustulum Kutzing cultured in ES-I-Si, a medium developed by Provasoli (1968, "Media and prospects for the cultivation of marine algae", in Watanabe A et al, Cultures and collections of algae, Proceedings of a US-Japan conference, Hakone 1966.) Algal diet axenic.Test conditions: The. testing conditions followed the basic tenets of the original three-brood test proposed by Mount and Norberg (1984) but were revised in that they emphasize the needs of the animals in terms of space and diet. Details of the conditions may be found in in Cowgill and Milazzo (1989). Test vessels were wide mouth clear glass jars graduated in milliliters to contain 150mL. Into each jar was fitted a glass tube, 3.5 cm diameter. which had affixed to one end a nytex screen of 243 µm mesh for C. dubia or 1000 µm mesh for D. magna, These screens were affixed to the glass tubes with silicone glue. After the screens were glued to the glass tubes, three glass beads, 8 mm in diameter, were affixed to the underside equidistant from each other. This was covered with a glass petal dish 5.5 cm in diameter. The jar containing the screened tube was filled with double distilled water and autoclaved for 10 mins at a pressure of 124 kPa. This procedure was repeated three times, renewing the distilled water each time, before the equipment was used for a test. This procedure accomplished the complete removal of all effects of the silicone glue. Only glass vessels were used.

Remark : Using the USEPA classification scheme, ethanol would be classified as practically non toxic based on survival. Based on reproductive parameters, it would be classified as slightly toxic.

Result : LC50 (48hr) 9248mg/l (95% CI 7560-12600)
LC50 (9 day) 454mg/l (95% CI 232-814)
NOEL (11 day) 9.6 mg/l

Test condition : Analysis of Lake Huron water used in culturing and testing

Al	105
NH3 total	ND(IO)
B	332

Ca	45050
Cr	ND(5)
Cu	13
F	75
Fe	12
Pb	ND (5)
Mg	7600
Mn	ND (5)
K	2485
Si	4760
Na	5700
S	5585
Zn	15
Total dissolved solids	233500
Total suspended solids	1125
Total organic carbon	1400

Test conditions
Test vessel: Capacity 150ml, Content 100ml
Screen composition: Nytex, Screen mesh size 1000µm
Light lux: 2150 ± 300 lux
Photoperiod, 16 h light, 8 h dark
Temperature 25 ± 2
Dissolved oxygen, 8.0 ± 1.5mg/L
pH: 8.2 ± 0.2
Dilution water Hardness, as mg CaCO3,/L: 160-180
Alkalinity, as mg CaCO3/L: 40-52
Habitat: Environmental chamber
Habitat changing frequency: Every other day
Feeding rate (cells/vessel): A. convolutes 18x10^6, N. frustulum 3.6 x10^6
Feeding frequency: daily
Age of organisms, <12h (all from fourth brood.)
Number of control broods: 3
Permitted control loss, 20%
Number of organisms/ vessel: 1
Number of organisms/Concentration: 10
Number of organisms!/control: 20
Test length, days: 9-11
Variables monitored Daily: light, temperature, survival, progeny
Variables monitored every second day: water quality variables in renewed solutions
Variables monitored at Test termination: Survival, total progeny, adult weightEndpoints: Survival, total progeny, dry adult weight, number of broods, mean brood size, loss of control limited to 20% (LC50/EC50/NOEC)
Test ended when the control animals had produced three broods.
Test concentrations used: not specified.

Reliability : (2) valid with restrictions
This is a well reported study, particularly regarding the methodology of testing. The main weakness is lack of detail on test concentrations used and on the analytical method used to assess test substance concentration.

11.11.2004 (125)

Species : Daphnia magna (Crustacea)
Endpoint : reproduction rate
Exposure period : 11 day(s)
Unit : mg/l

NOEC : = 9.6
EC50 : 14 - 26
LC50 : = 454
Analytical monitoring : no data
Method : other
Year : 1990
GLP : no data
Test substance : other TS: reagent grade

Method : Follows the basic methodology for the three brood test proposed by Mount and Norberg (Mount, D.J. and Norberg, T.J. (1984) A seven-day life cycle cladoceran toxicity test. Environ. Toxicol. Chem. 3, 425 - 434).Analytical methods used for test substance: no data given.Vehicle used: not required.Statistical methods: For LC50: Probit, moving average and nonlinear interpolation. Calculation of point estimates and other corresponding 95% confidence intervals made using a program written by Stephan (1977, Methods for calculating an LC50, In Mayer FL et al (eds), Aquatic toxicology and hazard assessment, ASTM STP 634: 65-84). Calculation of EC50: statistical package SAS GLM (1987, SAS/STAT guide for personal computers, version 6th ed, SAS institute Inc, Cary, NC) used to generate regression equations. NOELs calculated using Dunnett's t-test.Test organism: Daphnia magna strauss 1820 populations (of British origin) had been maintained in the Dow Chemical Company Laboratory since 1982 without drastic changes in population. Population maintained at 25C for past 3 years and sustained on Ankistrodesmus convulutus (reared in medium based on Provasoli and Pintner (1968, Ecological implications of in vitro nutritional requirements of algal flagellates", Ann NY Acad. Sci. 56, 839-851.) and Nitzschia frustulum Kutzing cultured in ES-I-Si, a medium developed by Provasoli (1968, "Media and prospects for the cultivation of marine algae", in Watanabe A et al, Cultures and collections of algae, Proceedings of a US-Japan conference, Hakone 1966.) Algal diet axenic.Test conditions: The. testing conditions followed the basic tenets of the original three-brood test proposed by Mount and Norberg (1984) but were revised in that they emphasize the needs of the animals in terms of space and diet. Details of the conditions may be found in in Cowgill and Milazzo (1989). Test vessels were wide mouth clear glass jars graduated in milliliters to contain 150mL. Into each jar was fitted a glass tube, 3.5 cm diameter. which had affixed to one end a nytex screen of 243 µm mesh for C. dubia or 1000 µm mesh for D. magna, These screens were affixed to the glass tubes with silicone glue. After the screens were glued to the glass tubes, three glass beads, 8 mm in diameter, were affixed to the underside equidistant from each other. This was covered with a glass petal dish 5.5 cm in diameter. The jar containing the screened tube was filled with double distilled water and autoclaved for 10 mins at a pressure of 124 kPa. This procedure was repeated three times, renewing the distilled water each time, before the equipment was used for a test. This procedure accomplished the complete removal of all effects of the silicone glue. Only glass vessels were used.
Remark : Using the USEPA classification scheme, ethanol would be classified as practically non toxic based on survival. Based on reproductive parameters, it would be classified as

slightly toxic.

Result : Results based on total progeny
EC50 14mg/l (95% CI 0.8-274)
NOEL 9.6mg/l
Results based on number of broods
EC50 26mg/l (95% CI 1-640)
NOEL 16mg/l
Results based on mean brood size
EC50 15mg/l (95% CI 0.9-278)
NOEL 9.6mg/l

Test condition : Analysis of Lake Huron water used in culturing and testing

Al	105
NH3 total	ND(IO)
B	332
Ca	45050
Cr	ND(5)
Cu	13
F	75
Fe	12
Pb	ND (5)
Mg	7600
Mn	ND (5)
K	2485
Si	4760
Na	5700
S	5585
Zn	15
Total dissolved solids	233500
Total suspended solids	1125
Total organic carbon	1400

Test conditions
Test vessel: Capacity 150ml, Content 100ml
Screen composition: Nytex, Screen mesh size 1000µm
Light lux: 2150 ± 300 lux
Photoperiod, 16 h light, 8 h dark
Temperature 25 ± 2
Dissolved oxygen, 8.0 ± 1.5mg/L
pH: 8.2 ± 0.2
Dilution water Hardness, as mg CaCO3,/L: 160-180
Alkalinity, as mg CaCO3/L: 40-52
Habitat: Environmental chamber
Habitat changing frequency: Every other day
Feeding rate (cells/vessel): A. convolutes 18x10^6, N. frustulum 3.6 x10^6
Feeding frequency: daily
Age of organisms, <12h (all from fourth brood.)
Number of control broods: 3
Permitted control loss, 20%
Number of organisms/ vessel: 1
Number of organisms/Concentration: 10
Number of organisms!/control: 20
Test length, days: 9-11
Variables monitored Daily: light, temperature, survival, progeny
Variables monitored every second day: water quality variables in renewed solutions
Variables monitored at Test termination: Survival, total progeny, adult weightEndpoints: Survival, total progeny, dry adult weight, number of broods, mean brood size, loss of control limited to 20% (LC50/EC50/NOEC)

Test ended when the control animals had produced three broods.
Test concentrations used: not specified.

Reliability : (2) valid with restrictions
11.11.2004 (125)

Species : Palaemonetes pugio (Crustacea)
Endpoint : other: embryo aute toxicity
Exposure period : 12 day(s)
Unit : g/l
LC50 : = 3 - 4.5
Analytical monitoring : no data
Method : other: see freetext
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Adult male and female grass shrimp were collected by dip net during spring and autumn 1995 from relatively uncontaminated local estuaries near the U. S. Environmental Protection Agency. Gulf Ecology Division. Gulf Breeze, Florida. The grass shrimp were placed in ice chests with water from the collection site, transported to the laboratory . The grass shrimp were acclimated slowly to 251 and 20-ppt salinity over a 4-h period and were maintained communally in glass aquaria with flow-through seawater at 250C and 20-ppt salinity. Adult grass shrintp were held in the aquaria for at least two weeks before use.For each test, gravid female grass shrimp were examined with a dissecting microscope for presence of embryos in the tissue cap stage (2-3 d after oviposition. Female grass shrimp with embryos at the tissue-cap stage were placed under a dissecting microscope and the embryos gently removed from female pleopods and separated from other embryos using forceps and fine-tip probes. Separated embryos were washed three times in filtered 2O-ppt seawater.SEATOX, a 4-d test protocol, was used as a screening test that extended from 2-3 d prior to hatch through the hatching period (Rayburn 1996, Characterisation of grass shrimp embryotoxicity test using the water soluble fraction of no 2 fuel oil. Mar Poll Bull, 32(12) 860-8). Embryos at the tissue cap stage (3 d after Oviposition were collected and placed individually into wells of 24-well plastic tissue culture plates. These were placed into an incubator at 27C and gently rotated at 60rpm. Six days later (9d after oviposition) the embryos were removed and re-examined microscopically. Normal embryos exhibited well developed eyes, a beating heart and visible limbs. Dead or abnormal embryos were discarded and replaced with normal embryos from excess plates kept in the same conditions. Plates of embryos were then randomly selected for a given exposure concentration and the seawater was removed and replaced with 2 ml of test solution. Plates were returned to the 27C incubators and examined daily for mortalities and hatching. The test was terminated after a 4-d exposure, 13d after oviposition. Three tests were performed with 5-6 solvent different concentrations (no further data).Statistical analysis: LC50s with confidence intervals were calculated by Litchfield-Wilcoxon probit analysis.

Remark : The overall control mortality was 7.4% (16/216).

Result : LC50 (4 day) average 12.07g/l (of three replicates, values 12.39, 12.15, 11.6). The mortality curve was extremely sharp and demonstrated a linear concentration response curve.LC50 (12 day) average 3.63g/l (of three replicates, values 4.5, 3,31, 3.00). The range of concentrations over which mortality occured was much broader that with the 4 day test.
Reliability : (2) valid with restrictions
29.09.2003 (126)

Species : Palaemonetes pugio (Crustacea)
Endpoint : other: embryo teratogenesis
Exposure period : 12 day(s)
Unit : g/l
NOEC : > .079
LOEC : = .39
Analytical monitoring : no data
Method : other: see freetext
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Adult male and female grass shrimp were collected by dip net during spring and autumn 1995 from relatively uncontaminated local estuaries near the U. S. Environmental Protection Agency. Gulf Ecology Division. Gulf Breeze, Florida. The grass shrimp were placed in ice chests with water from the collection site, transported to the laboratory . The grass shrimp were acclimated slowly to 251 and 20-ppt salinity over a 4-h period and were maintained communally in glass aquaria with flow-through seawater at 250C and 20-ppt salinity. Adult grass shrimp were held in the aquaria for at least two weeks before use.For each test, gravid female grass shrimp were examined with a dissecting microscope for presence of embryos in the tissue cap stage (2-3 d after oviposition. Female grass shrimp with embryos at the tissue-cap stage were placed under a dissecting microscope and the embryos gently removed from female pleopods and separated from other embryos using forceps and fine-tip probes. Separated embryos were washed three times in filtered 2O-ppt seawater.Three 12-d tests were performed according to Rayburn (Rayburn 1996, Characterisation of grass shrimp embryotoxicity test using the water soluble fraction of no 2 fuel oil. Mar Poll Bull, 32(12) 860-8) using 24-well plastic tissue culture plates for each solvent tested. Each well contained a single embryo and 2 ml of test solution (static). Each treatment dilution or control was conducted within a single 24-well tissue culture plate N = 24). Plates were placed on rotary shakers (60 rpm) in incubators in the dark and kept at 27 ± IC. Plates were removed from the incubator daily and each embryo was examined for abnormalities of the eye, yolk, heart, head, hepatopancreas, and telson. Both the number and type of abnormality were recorded. Survival was determined by structural integrity of the embryo during the first four days of the test, and thereafter by the presence or absence of heartbeat. Mortalities and hatching were recorded daily. Exposures were terminated after 12 d(14--I5 d after oviposition). Three tests were conducted. LC50 values with 95% confidence intervals and coefficients of variation werc

calculated from total mortalities.Treatment concentrations: 0.079, 0.39, 0.79, 1.97, 3.95, 7.9 g/l.Statistical analysis: LC50s with confidence intervals were calculated by Litchfield-Wilcoxon probit analysis.

Remark : The overall; control mortality was 7.4% (16/216).

Result : Developmental abnormalities were not noted at the 0.079g/l concentration but were seen at 0.39 and 0.79g/l and resulted in delayed hatching. Two embryos failed to hatch properly and had swimming difficulties as larvae. Malformations were detected in 21.7% of the embryos exposed to the 3 lowest concentrations and most of the malformed embryos died before the end of the assay. Developmental delay was detected 6 days after oviposition at the three highers concentrations. By 9 days nearly all the embryos exposed to the highest concentration died before the end of the assay and all embryos that died were malformed before the died. Only the 1.97g/l treatments had larvae that survived with malformations.

Reliability : (2) valid with restrictions

11.11.2004 (126)

Species : Palaemonetes pugio (Crustacea)
Endpoint : Mortality
Exposure period : 12 day(s)
Unit : mg/l
LC50 : = 2000 - 9100
Analytical monitoring : no data
Method : other
Year : 1997
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Adult male and female grass shrimps were collected using push nets from a site in Escambia Bay, Pensacola, Florida. Collections were made in the months of Sept. 1995, March 1996, and May 1996. For the Sept 1995 collection, 70 gravid females and 60 males were collected during high tide. Water temperature was 28.3C and salinity was 20 ppt. For the March 1995 collection, 180 non-gravid females and 150 males were collected during low tide. Water temperature was 16.80 C and salinity was 8 ppt. For the final collection in May 1996, 200 gravid females and 200 males were collected during high tide. Water temperature was 28.1C and salinity was 3 ppt. Shrimp were placed into coolers filled with site water, transported to the Gulf Ecology Division Laboratory, Gulf Breeze, FL, and identified as Palaemonetes pugio (Williams 1984). Approximately 60 female and 40 male shrimp from each of three collections were transferred into flow-through aquaria (80 L) and maintained on a flow rate of- 24L per hour, salinity of 19-22 ppt, and temperature of 19-25C for approximately a six-month period. Protective habitats were not furnished in the aquaria. Under these laboratory conditions, the shrimp were able to reproduce and supply adequate numbers of embryos for experiments described here and elsewhere (Little 1968). Shrimp were fed - 2.5 grams of flake food (Tetramin R) daily and twice a week with 25 ml of concentrated brine shrimp (Artemia saline) nauplii.

Experiments were conducted with over a 9 month period using embryos from shrimp that had been maintained in aquaria for different periods of time, from 1-160 days. A single gravid female shrimp with a clutch of embryos 3-d (after oviposition) was selected for testing. Embryos were removed from the female, placed in disposable 24-well flat bottom plastic culture plates and individually exposed to 2 ml of ethanol at five different dilutions (0.05, 0.10, 0.50, 1.0 and 2.0% v/v%) based on 12-d LC5O values from Rayburn and Fisher (1996). Dilutions were made with histological grade 100% EtOH and 20 ppt 0.22 pm filtered natural sea

water. Filtered sea water was also used as control. Embryos were placed on rotators (Model G2, New Brunswick Scientific Co.) in an incubator maintained at 2700±1 for 12 d. Rotators were set at 60 rpm to provide a gentle agitation of the embryos in the test wells. After a 12-d exposure, embryos were examined for mortality. 12-d LC5O values and 95% confidence intervals (Cl) were calculated using the trimmed Spearman-Karber method (Hamilton et al. 1977). Average of mean 1 2-d LC5O values and coefficients of variation (CV) were calculated according to Steel and Torrie (1980).

Five LC50s were determined from the Sept collection over a period from 30-160 days from collection, Four LC50s from the March collection (2-60 days) and two LC50s from the May collection (1-30 days.)

Result : 11 tests performed in total in saltwater solutions containing 0.37% to 1.10% v/v ethanol. Average control mortality 11.7% with a standard error of 3.2%. Three results showed mortalities over 16.7%. One results produced a very low value (order of magnitude lower. High control mortality was observed and it was not possible to calculate an LC50. The average value from the remainder was an LC50 value 0.53% (4.18g/l) SD=0.20% (1.58g/l). Embryos oviposited in the field had greater sensitivity (2-10x) than those ovipositied in the laboratory.

Reliability : (2) valid with restrictions

11.11.2004 (127)

Species : other aquatic crustacea
Endpoint : Mortality
Exposure period : 28 day(s)
Unit : mmol/l
: = 25 - 150
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Behaviour = 25 mmol; Growth = 150 mmol; Mortality = 75-150 mmol.

Reliability : (4) not assignable

05.10.2003 (128)

Species : other aquatic crustacea
Endpoint : Mortality
Exposure period : 56 day(s)
Unit :
Analytical monitoring : no data
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
This otherwise robust study of the acute and chronic toxicity of pentachlorophenol in 95% ethanol referred to a 'solvent control' but the concentration of ethanol was not given, nor was a median lethal concentration determined for the solvent control. Mortality rate in controls was high for Calamoecia lucasi sublethal tests because of the difficulties experienced in their culture.

05.10.2003 (129)

Species	:	other aquatic mollusc: Littorina littorea
Endpoint	:	other: morphological changes (imposex)
Exposure period	:	
Unit	:	
	:	=
Analytical monitoring	:	no data
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The study was primarily on the effects of tributyl tin, Pb and Sn. Ethanol only controls received 50 or 100 ng/l.
Result	:	Ethanol significantly increased length of penis confounding the interpretation of the effects of heavy metal compounds.
Reliability	:	(4) not assignable

22.09.2003 (130)

Species	:	Daphnia magna (Crustacea)
Endpoint	:	Reproduction rate
Exposure period	:	21 day(s)
Unit	:	mg/l
NOEC	:	> 10
	:	= .1
Analytical monitoring	:	no data
Method	:	other
Year	:	1995
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	This study was designed to identify possible physiological and biochemical target sites for the oestrogenic effect of diethylstilbestrol on the freshwater crustacean daphnia magna. Ethanol was used as the solvent for the DES. The controls used in the experiment a water and solvent , the latter at 0.001% ethanol (w/w or v/vnot specified but would approximate to 10mg/l).
Result	:	Exposure to 0.001% ethanol had no adverse effect on survival of the test species.
Reliability	:	(3) invalid This study only used a single ethanol concentration and did not establish a LOEL. It cannot be considered valid to use to asses the chronic toxicity of ethanol to invertebrates.

05.10.2003 (131)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

Species	:	other terrestrial plant: Allium cepa (onion)
Endpoint	:	Growth
Exposure period	:	6 day(s)
Unit	:	mg/l
EC50	:	= 7890 - 15780 measured/nominal
Method	:	other

Year : 1984
GLP : no data
Test substance : no data

Method : Winter-rested onions (Allium cepa), 1.5 to 2.0 cm diameter, were fitted into 10 cm x 1.5 cm (dia) test tubes after skinning and exposure of root primordia. Test liquids were added and the whole incubated at about 20 degC, protected against direct sunlight. The original form of the Allium test had root growth initiated in distilled water and a modified test involved placing onions directly into test solutions.

Root tips were examined microscopically following e.g. Feulgen or orcein staining.

Root lengths were measured and compared with controls (%).

For compounds other than ethanol the test was compared with other mutagen tests.

Result :

Ethanol concentration	Root length (cm)	%Control
2%	2.88	33.8
1%	5.74	67.3
0.1%	7.34	86.1
Control	8.52	100

Density of ethanol = 789 g/l

Reliability : (2) valid with restrictions
This study is primarily an evaluation of this test methodology as a standard short-term test in environmental monitoring. For ethanol, only the root growth inhibition end-point is reported. Given the detail presented in this test development and evaluation this is considered to be valid with restrictions.

12.11.2004 (132)

Species : Lactuca sativa (Dicotyledon)
Endpoint : Emergence
Exposure period : 3 day(s)
Unit : mg/l
EC50 : = 5382 measured/nominal
Method : other
Year : 1977
GLP : no data
Test substance : no data

Method : Lettuce fruits (Lactuca sativa cv. Great Lakes) were germinated at 30 degC in the absence or presence of concentrations of a wide variety of aliphatic organic compounds including ethanol. The inhibitory activity of the compounds was expressed as the millimolar concentration of compound producing 50% inhibition at 30 deg C.

Result : Effect of Ethanol on Lactuca sativa Reproduction

ENDPOINT: 72 h EC50 of = 117 mM (equivalent 5382 mg/l)
Measurement: Germination.

Reliability : (2) valid with restrictions
This seems to be a well conducted study. There is some concern regarding the specific germination temperatures of different lettuce cultivars which may affect reproducibility. This study is considered to be valid with restrictions; see also Meyer, H. 1971.

12.11.2004 (133)

Species : other terrestrial plant: Lactuca sativa (Lettuce) and Pisum sativum (Pea)
Endpoint : Emergence
Exposure period : 2 day(s)
Unit :
Method : other
Year : 1971
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Peas (Pisum sativum cv. Alaska) and lettuce seeds (Lactuca sativa cv. Grand Rapids) were treated for 24 and 44 hours with absolute ethanol or water. Lettuce was germinated in light and peas in the dark.

Result : Percent germination

	24 h	44 h
Lettuce		
Treatment:		
None	65	65
Abs Ethanol	20	16
Peas		
Treatment:		
None	80	80
Abs Ethanol		

Reliability : (2) valid with restrictions
Although this study was instituted to investigate solvents as carriers of substances into seeds it shows that ethanol inhibits germination in lettuce but not in peas. This is confirmatory to the findings of other studies. This study, although deficient in detail, is regarded valid with restrictions; see also Reynolds T., 1977.

12.11.2004 (134)

Species : other terrestrial plant: Zea mays mays (Corn)
Endpoint : other: coleoptile and root size and respiration
Exposure period : 1 day(s)
Unit : mg/l
inhibition respiration : = 3000 - 4000 measured/nominal
Method : other
Year : 1958
GLP : no data
Test substance : no data

Method : Maize (Purdue hybrid) tissues were established in Warburg reaction vessels for 9 h before total growth and respiration were determined. Seedlings were raised in the dark at 30 degC in tap water and sections 6 mm long were cut 2-3 mm from the tips of coleoptiles. Primary leaves were removed. CO2 was determined by absorption onto KOH soaked filter paper.

All studies were carried out at 25 degC. Tips were grown with and/or without ethanol (0, 0.05%, 0.1%, 0.2%, 0.3% and ranging up to 1.5% vol)

and indole-3-acetic acid.

Respiratory Quotient (RQ) was also determined.

Result : The threshold concentration for the inhibitory effect on growth and respiration was approx. 0.1% and the degree of inhibition was increased markedly through 0.2 to 0.3% ethanol.

The growth of coleoptiles was more sensitive to ethanol than that of the roots. Oxygen uptake was less sensitive to added ethanol in coleoptiles. Respiratory quotients were depressed by addition of ethanol. The inhibitive effect of ethanol was 0.4%.

Reliability : (2) valid with restrictions
This report gives experimental detail and clear evidence of an effect of ethanol. Although old, the study can be regarded to be valid with restrictions.

12.11.2004 (135)

Species : other terrestrial plant: Solanum tuberosum (Potato)
Endpoint : other: tuber respiration
Exposure period : 1 day(s)
Unit :
Method : other
Year : 1935
GLP : no data
Test substance : no data

Method : Irish Cobbler potatoes were exposed to concentrations of a number of chemical species and rates of respiration were determined and expressed and total CO_2 per 100 g in 48 h.

Result : Respiration expressed as total carbon dioxide per 100 g in 48 h:

Controls: 76.9
Ethanol 0.063 mmol/l: 50.3 (2.9 mg/l)
Ethanol 0.25 mmol/l: 60.9
Ethanol 1 mmol/l: 164.7 (46 mg/l)
Ethanol 2 mmol/l: 105.2
Ethanol 8 mmol/l: 44.1 (368 mg/l)

Reliability : (2) valid with restrictions
This old study is well controlled and yields a respiration rate end point in which low and high dosages of ethanol are shown to inhibit respiration, with a significant increase in respiration at 1 mmol/l. There are data from a later study by Rychter (1979) supporting this observation together with corresponding changes in respiratory enzyme activities. This study is therefore as regarded valid with restrictions.

12.11.2004 (136)

Species : Avena sativa (Monocotyledon)
Endpoint : Growth
Exposure period : 7 day(s)
Unit :
Method : other
Year : 1958
GLP : no data
Test substance : no data

Method : 15 Replicates per treatment were studied in an experiment in which oat plants were pretreated in darkness with or without 5% carbon dioxide, with or without ethanol (0.2%, 0.3%) for 3 days from the time of planting.

Result	:	The growth of both mesocotyls and coleoptiles was depressed by each factor during pretreatment. Between 3 and 7 days in darkness, both treatments promoted mesocotyl by increments of 33.6 and 38.8 mm. Both carbon dioxide and ethanol are therefore seen to prolong the meristematic phase.
Reliability	:	(2) valid with restrictions Ethanol is seen to stimulate growth in oat seedlings in this study with comprehensive controls. The observed effect may be considered a positive finding and the endpoint to be of uncertain toxicological significance.

12.11.2004 (137)

Species	:	other terrestrial plant: Solanum tuberosum (Potato)
Endpoint	:	other: Tuber respiration
Exposure period	:	2 day(s)
Unit	:	?g/l
respiratory inhibition	:	> 1578 measured/nominal
Method	:	other
Year	:	1979
GLP	:	no data
Test substance	:	no data

Method	:	Potato (cv. Norchip) were preconditioned for 2 wk following harvest. 6 Whole tubers weighing ca 100 g were placed in 2L kjars and ventilated with different gas mixtures at a rate of 400 ml/min containing ethanol, acetaldehyde or acetic acid vapours. Respiration and respiratory intermediates were evaluated.
Result	:	An increase in the concentration of ethanol from 500 to 5000 mul/l led to a progressive increase in respiration. Higher concentrations (20,000 mul/l) reduced respiratory upsurge. Ethanol induced a decline in the level of 2-phosphoglyceric acid and phosphoenolpyruvate while leading to the accumulation of tricarboxylic acid cycle intermediates including isocitrate and alpha-ketoglutarate. This was similar to but independent of the action of ethylene.
Reliability	:	(2) valid with restrictions This study is more recent than Miller (1935) and gives similar results together with supporting respiratory enzyme values. Although the endpoint is of uncertain toxicological significance the study is regarded as valid with restrictions because of the complementary findings in the earlier Miller study.

12.11.2004 (138)

Species	:	other terrestrial plant: Helianthus tuberosus (Girasole)
Endpoint	:	other: cell enzyme activity and pigmentation
Exposure period	:	3 day(s)
Unit	:	
Method	:	other
Year	:	1979
GLP	:	no data
Test substance	:	no data

Method	:	Slices (1 mm thick) of Jerusalem artichoke were incubated in a dark room at room temperature in 2 L Erlenmeyer flasks with 1.5 ml distilled water or distllled water containing the test compound. Solvents were avoided as these affected induction patterns. The incubation medium was vigorously bubbled with a stream of filtered, hydrated air. Microsomal fractions were prepared and tri-cinnamic acid 4-

hydroxylase activity determined. Cytochrome P-450 and microsomal proteins were estimated. These were shown to reach maximum activity 24 h after slicing.

Ethanol, butanol, isopropanol, methanol, phenobarbital, Mn and Fe were included in medium for up to 72 hr.

Remark : This study shows that cytochrome P450 is inducible in plant cells by ethanol.

Result : Effect of Ethanol on Helianthus tuberosus Enzyme(s)

Concentration / Dose 300.00mM
ENDPOINT:
hydroxylase activity 72h 15% +/- 9%

P450 content 72h 85 +/- 25 pmol/mg protein

Concentration / Dose 0.00mM (WATER)
ENDPOINT:
hydroxylase activity 72h 13% +/- 1%

P450 content 72h 35 +/- 2 pmol/mg protein

Reliability : (2) valid with restrictions
The endpoint is of uncertain toxicological significance but may have interpretative value.

12.11.2004 (139)

Species : other terrestrial plant: Saccharum officinarum (Sugarcane)
Endpoint : other: root abundance
Exposure period : 28 day(s)
Unit :
Method : other
Year : 1940
GLP : no data
Test substance : no data

Method : Sugar cane sets were immersed in ethyl alcohol solutions of strength 1, 2, 4, 6, 8 and 10% and root primordia and production was monitored.

Result : A very marked increase in root production was noted, especially at marginal temperatures

Reliability : (2) valid with restrictions
Ethanol stimulated root production in sugar cane sets which is considered a positive response. The endpoint is of uncertain toxicological significance.

12.11.2004 (140)

Species : other terrestrial plant: Solanum tuberosum (Potato)
Endpoint : other: shoot size and abundance (15 days); tuber enzymes (5 days)
Exposure period : 15 day(s)
Unit :
Method : other
Year : 1931
GLP : no data
Test substance : no data

Method : Irish Cobler potatoes were dipped in water or ethanol solutions (20, 40 and 80 ml/l) for 24 hours and then evaluated after 5 days for peroxidase and catalase activities in their juice.

After 15 days the number of sprouts on 12 pieces were

counted and their total length recorded.

Other compounds evaluated included acetaldehyde, ethylene chlorohydrin, potassium thiocyanate and thiourea.

Result :

Conc. ml/l	Peroxidase	Catalase	Sprouts	Total length
0 (water)	2.18	10.0	2	0.2
20 ml/l	1.94	11.9	7	1
40 ml/l	2.36	14.5	12	3
80 ml/l	2.13	17.5	12	7

Reliability : (2) valid with restrictions
Ethanol stimulated potato tubers into growth from dormancy, an effect that may be regarded either a positive or negative effect. There were control groups in this old but apparently well conducted study and the dosage is a single immersion. However, the endpoint is of uncertain toxicological significance.

12.11.2004 (141)

Species : other terrestrial plant: Daucus carota (Wild carrot)
Endpoint : Growth
Exposure period : 28 day(s)
Unit : mg/l
EC50 : = 395 measured/nominal
Method : other
Year : 1978
GLP : no data
Test substance : no data

Result : Cultures of carrot cells exposed to 2% ethanol showed arrested growth and soybean cell growth could be decreased by about 50% by 0.05% ethanol. Concentrations of 2% could be reduced to nontoxic levels after 20-25 days by volatilization.

(Density of ethanol = 789 g/l; 0.05% is approximately 395 mg/l).

Reliability : (3) invalid
This study evaluated the possible effects of compounds used as herbicide solvents on plant cell growth and ultrastructure. The results show ethanol to have cytotoxic activity in carrot and soybean cells in vitro, with wide variation in cytotoxic dose between the two species. The endpoint is of uncertain toxicological significance and relevance. Overall, this study is therefore regarded as invalid.

12.11.2004 (142)

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

Type : filter paper
Species : Eisenia fetida (Worm (Annelida), soil dwelling)
Endpoint : Mortality
Exposure period : 48 hour(s)
Unit : other: microgram/cm^2 filter paper
LC50 : = 100 - 1000 calculated
Method : other
Year : 1984
GLP : no data
Test substance : no data

Method : Eisenia foetida earthworms were obtained from Bert's Bait Farm, Irvine KY. They were housed in Nalgene boxes filled with moist peat moss and rabbit manure and stored at 18 degC. Cornmeal was added as an additional food source and calcium carbonate was added to prevent pH falling below 5.5. Mature worms with developed clitellum, weighing 370 to 450 mg were selected for exposure to test substances.

Worms were exposed individually to deposits of test subtances on filter paper for 48 hr, in the dark, and mortality was recorded.

Glass shell vials (22 mm x 85 mm) were lined with Whatman No. 1 filter paper strips (9.5 x 6.8 cm = 65cm2) and placed in cardboard scintillation vial trays. Chemical concentrations were expressed in microg/cm^2.

For volatile solvents, microlitre quantities were added to pre-moistened vials when high concentrations were used (as for ethanol)

After inserting earthworms, the vials were capped and stored in the dark in a horizontal position for 48 hr.

Death was ascertained by gentle prodding of the anterior end. A minimum 100 earthworms were used to determine each LC50 in a replicated tests involving a geometric series of 5 to 7 exposure concentrations.

LC50 was determined by the Litchfield-Wilcoxon log dose-effect probit transformation method.

Remark : This study could be regarded as an 'inhalation' exposure study. It is possible to calculate the vapour loading in the test vessels from their dimensions and the dose of ethanol used. This equates to 200-2000mg/litre.

Test substance : All chemicals tested in this study were of either technical or analytical grade.

Conclusion : Regarded as moderately toxic (LC50 <1000 mg/square cm) in this test

Reliability : (2) valid with restrictions
This experimentally robust study involving 90 chemical species demonstrated the unpredictability of chemical toxicity to different animal species. The 'dosage', presented interms of concentration in a given area of substrate, is difficult to interpret.

12.11.2004 (143)

Type : other: aquatic
Species : Lumbricus sp. (Worm (Annelida), soil dwelling)
Endpoint : Mortality
Exposure period : 96 hour(s)
Unit : other: mg/l
LC50 : > 100 measured/nominal
Method : other
Year : 1986
GLP : no data
Test substance : no data

Method : Methodology is proposed for simultaneous multispecies testing by static immersion of test organisms in 20 l of test solution.

Result : Asellus intermedius (pillbug), Daphnia magna (water flea), Dugesia tigrina (flatworm), Gammarus fasciatus (Sideswimmer), Helisoma trivolvis (snail), Lumbricus variegatus (segmented worm) and Pimephales promelas (fathead

minnow) all gave a 96 h LC50 of >100 mg/l.

Reliability : (3) invalid

10.08.2003 (144)

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

In Vitro/in vivo : In vivo
Type : Metabolism
Species : Human
Number of animals
Males : 12
Females : 12
Doses
Males : 150 mg/cu m; 750 mg/cu m and 1500 mg/cu m; and exceeding MAK
Females : 150 mg/cu m; 750 mg/cu m and 1500 mg/cu m and exceeding MAK
Vehicle : other: air
Route of administration : inhalation
Exposure time : 4 hour(s)
Product type guidance :
Decision on results on acute tox. Tests :
Adverse effects on prolonged exposure : no significant effects
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product :
Method : Other
Year : 1994
GLP : no data
Test substance : no data

Method : 12 Male and 12 female healthy volunteers carried out a day of training and then 3 experiments at intervals of about 4 days between each. Inhalation exposures to ethanol were at 80 ppm (150 mg/m^3), (smell threshold); 400 ppm (750 mg/m^3) and 800 ppm (1500 mg/m^3) for 4 hours. In a second series 8 males and 8 females were exposed to concentrations above the MAK upper limit, 1.e. 1000 ppm (1900 mg/m^3) and at hourly changing levels to a maximum 3610 mg/m^3. Blood alcohol levels were measured and subjects were evaluated by the Swedish Performance Evaluation System.

Statistical tests included analysis of variance, F-test and correlation coefficients.

Remark : The highest dose was below the current MAK value of 1900 mg/cu m (= 1000 ppm) and it is concluded that the maximum blood alcohol level will remain below 0.001% both in men and in women. Regression analysis of the data shows that the blood ethanol concentration (BEC) can be modelled using the following equation:

[BEC] = [exposure (ppm)] × 0.0029 (with a 7% error for 95% confidence).

Result : Blood alcohol levels were between 0.00023 and 0.0021 mg/ml in the first series and 0.00066 and 0.0056 mg/ml in the second (Units of concentration not clear in results). There was good correlation between inhalation exposure concentrations and resultant blood ethanol concentrations.

In both experiments there were no significant exposure-related effects in the psychological performance variables in both men and women. In the second experiments where concentrations varied about the MAK there were no significant effects at and below the MAK but at concentrations above the MAK, exposure was troublesome.

Source : CEFIC Ethyl Alcohol Group
Reliability : (2) valid with restrictions
This appears to be a well-run study evaluating a broad range of air

concentrations in relation to the MAK value. It is not clear from the original paper and its translation whether the blood alcohol levels expressed as 'promille' are mg/l or parts per million, former most likely, although this does not materially affect the conclusions.

Flag : Critical study for SIDS endpoint

24.06.2004 (145)

In Vitro/in vivo : In vivo
Type : Absorption
Species : Human
Number of animals
Males : 11
Females :
Doses
Males :
Females :
Vehicle : other: inspired air
Route of administration : other: oral drink in orange juice followed by prolonged inhalation
Exposure time : 1 hour(s)
Product type guidance :
Decision on results on acute tox. Tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product :

Method : Expired and inspired air and blood was collected and analysed for ethanol following ingestion of 0.7 g/kg bodyweight of ethanol followed by prolonged (more than 1 hr) inhalation exposure to ethanol in air (14 mg/l).

All experiments were performed on 11 normal, adult, male human subjects. Concentrations of ethanol in atmospheric air were determined by quadrupole mass spectrometer. Air was sampled into 20 ml syringes which were then injected with 4 ml of freshly boiled hot water and shaken for 8 minutes to transfer ethanol from air to water. Whole blood aliquots were precipitated with 0.33 M perchloric acid (1:8). Supernatants were taken for enymatoc analysis or gas chromatographic analyses.

Result : About 55% of ethanol in air was absorbed by adult volunteers and fractional absorption was not affected by variations in tidal volume (0.7 to 2.1 litres), nor by the presence of systemic blood alcohol levels up to 50 times higher than that of inspired air. Absorption fractions were about 0.55 and the concentration in end expiratory air did not fall below some 30% of the of the inspired air.

Source : CEFIC Ethyl Alcohol Group

Conclusion : Inspired ethanol is a significant contributor to elevations of blood alcohol concentration.

Reliability : (2) valid with restrictions
This well-designed study with intention for use in forensic studies gave consistent respiratory uptake values and fractional uptake values across all 11 volunteers and values close to previously published data. There was robust support for the conclusion that inspired ethanol can be considered as a source of elevation of blood alcohol concentration.

Flag : Critical study for SIDS endpoint

24.06.2004 (146)

In Vitro/in vivo : In vivo
Type : Excretion
Species : Human
Number of animals
Males : 976
Females : 114
Doses
Males :
Females :
Vehicle :
Route of administration : other: drinking
Exposure time :
Product type guidance :
Decision on results on acute tox. Tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product :
Method : Other
Year : 1996
GLP : no data
Test substance : other TS: alcoholic beverage

Method : 150 Volunteers (20-60 year-old) received neat whisky (40% v/v) or an ethanol cocktail (15-20% v/v) at a dose of 0.35, 0.51, 0.68, 0.85 or 1.05 g ethanol/kg bodyweight. To evaluate possible interference by gastric acid secretion, a further 12 volunteers also received cimetidine, ranitidine or omeprazole to suppress acid secretion. Additionally, 16 male and 4 female chronic acoholics with blood alcohol levels greater that 250 mg/dl were recruited. Two consecutive blood samples were taken from each of 188 suspected drunk drivers and for subjects with falling blood alcohol concentration (176), the frequency distribution of apparenmt rates of disappearance were plotted.
Result : The mean blood alcohol levels for driving under the influence (DUI) suspects was 1.88 +/- 0.748 mg/ml in males and 1.86 +/- 0.702 for females. The overall mean rate of alcohol elimination from DUI suspects was 0.191 +/- 0.049 mg/ml/hr with 95% limits spanning from 0.09 to 0.29 mg/ml/h. The slowest rate of ethanol disappearance was in a healthy male who ingested 0.68 g ethanol per kg bodyweight after an 8 hr fast; the beta-slope was 9 mg/dL/h. The fastest rate of disappearance was observed in a male chronic alcoholic during detoxification; the beta-slope was 36 mg/dL/h. This 4-fold observed difference should be considered when the pharmacokinetics of ethanol become an issue in drinking and driving trials, for example, when retrograde estimations are attempted.
Source : CEFIC Ethyl Alcohol Group
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
24.06.2004 (147)

In Vitro/in vivo : In vivo
Type : Metabolism
Species : Human

Number of animals
Males :
Females :
Doses
Males :
Females :
Vehicle :
Method : Other
Year : 1987
GLP : no data
Test substance :

Remark : The oxidation of ethanol in mammals, in partucular humans and the regulation of the rate of ethanol oxidation by enzymes, is reviewed.
Result : Ethanol is metabolized by conversion to acetaldehyde which can then be further oxidized to acetate which is then converted to CoA ester before entering the general pathways for fat oxidation. Ethanol is oxidized by either cytoplasmic dehydrogenase (ADH) or microsomal enzymes (the microsomal ethanol oxidation system, MEOS) to acetaldehyde. Genetic and environmental factors that can alter the rates of ethanol oxidation are discussed.
Source : CEFIC Ethyl Alcohol Group
Conclusion : During the oxidation of ethanol at blood levels of about 10 mM, acetaldehyde is present at about below 2 microM which indicates that the removal of acetaldehyde is via an enzyme of very low Km.

Alcohol dehydrogenase is responsible for the oxidation of approximately 90% of ingested ethanol.
Reliability : (4) not assignable
24.06.2004 (148)

In Vitro/in vivo : In vivo
Type : Metabolism
Species : Human
Number of animals
Males :
Females :
Doses
Males :
Females :
Vehicle :

Remark : In the major pathway for alcohol metabolism, ethanol is metabolised to acetaldehyde mainly by alcohol dehydrogenase (ADH) which is then further metabolised by aldehyde dehydrogenase (ALDH) to acetic acid. ADH and ALDH can exist in multiple isoenzymes and ADH polymorphism leads to individual differences in alcohol sensitivity and metabolism. Deficiency of the ALDH2 isozyme (ALDH2*2) can lead to flushing syndrome due to deficient metabolism of the aldehyde intermediate species. Such a genetic deficiency is only found prevalently amongst people of Mongoloid origin (including Japanese and Chinese.)
Reliability : (4) not assignable
Review article.
12.11.2004 (149)

In Vitro/in vivo : In vivo
Type : Metabolism
Species : Human

Number of animals
Males : 3
Females :
Doses
Males : 0.1, 0.4g/kg 16% solution for oral, 7% solution for i.v.
Females :
Vehicle : Water
Route of administration : other: intravenous and peroral
Exposure time : 6 hour(s)
Product type guidance :
Decision on results on acute tox. Tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product :
Method :
Year :
GLP : no data
Test substance :

Method : Subject ages 29, 33, 34. One carried the low Km isozyme of aldehyde dehydrogenase (ALDH) and received both low and high doses of ethanol. Peroral administration was at the rate of 0.1g/kg per 2.5mins and i.v. at 0.1g/kg per 10 mins. Dosing was either after 12hrs fasting or 15 minutes after a light meal (100g bread, boiled egg, 200ml milk.)

Blood samples were collected from the mean cubital vein and deproteinized by the PCA method (Okada 1982, Eriksson 1982). Laboratory temperature 24-26C. Ethanol and acetaldehyde determined by gas chromatography.

Result : i.v. concentrations - fasted

ALDH type	Dose	Peak EtOH	Peak Acetald.
normal	0.4	20mM	9uM
deficient	0.4	16mM	25uM
normal	0.1	5.5mM	<2uM
deficient	0.1	3.5mM	14uM

p.o. concentrations - fasted

ALDH type	Dose	Peak EtOH	Peak Acetald.
normal	0.4	3.5mM	8uM
deficient	0.4	11mM	21uM
normal	0.1	3.5mM	<2uM
deficient	0.1	2.5mM	14uM

0.1g/kg dose - after meal

ALDH type	Route	Peak EtOH	Peak Acetald.
normal	p.o	1.5mM	<2uM
deficient	p.o	1.0mM	28uM
normal	i.v	5.5mM	<2uM

deficient i.v 3.0mM 21uM

Conclusion : Peak blood ethanol concentrations lower in peroral than i.v. dosing. Whilst there were differences in peak concentrations there was no marked difference in elimination rate between the normal and deficient ALDH types (both 0.12-0.13mg/ml/hr)

Reliability : (2) valid with restrictions
No analytical information provided. Some data only available in graphical form. Otherwise reasonably well reported.

12.11.2004 (150)

In Vitro/in vivo : In vivo
Type : Metabolism
Species : Human
Number of animals
Males : 30
Females :
Doses
Males : 0.4g/kg
Females :
Vehicle :
Route of administration : oral unspecified
Exposure time :
Product type guidance :
Decision on results on acute tox. Tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product :

Method : Volunteers split equally between those with normal aldehyde dehydrogenase (ALDH) and those with the low Km isozyme of ALDH, as determined from their hair roots by an isoelectric technique (Harada 1978). Mean ages and body weights were not significantly different between the groups. Blood samples were collected from the mean cubital vein at 0.5, 1, 2, 3 and 4 hrs after start of ethanol intake and deproteinized by the PCA method (Okada 1982, Eriksson 1982). Laboratory temperature 24-26C. Ethanol and acetaldehyde determined by gas chromatography.

Widmark's beta60 value in each subject was calculated from the slope of the elimination curve by linear least squares regression of the psuedo liner portion. The C0 value was calculated from the the regression line y intercept. the r value was derived from the dose divided by C0. Ethanol elimination rate ER was calculated from beta60 and r. Computer calculations were used to derive the Michaelis Menton parameters.

Result : Widmark model

	normal ALDH	Deficient ALDH	
beta 60	0.16 ±0.033	0.14 ±0.017	mg/ml/hr
C0	0.61 ±0.095	0.56 ±0.053	mg/ml
r	0.67 ±0.11	0.72 ±0.067	l/kg
ER	104.91±10.55	96.88±11.73	mg/kg/hr

Michaelis Menton model

normal ALDH	Deficient ALDH

Vmax 0.18 ±0.032 0.15 ±0.026 mg/ml/hr
Km 0.047 ±0.053 0.037 ±0.039 mg/ml
k12 0.087 ±0.064 0.060 ±0.037
k12 0.093 ±0.071 0.073 ±0.049
Vd 0.61 ±0.91 0.66 ±0.068 l/kg

Conclusion : According to the data presented, there is a slight but not a significant difference in the ethanol elimination rates between those with normal ALDH and those with deficient ALDH.

Reliability : (2) valid with restrictions
There are some apparent discrepancies between the conclusions in the text and the data. Insufficient information is available to check the statistics provided. Little analytical information is presented. Assuming the data to be correct, the study is considered reasonably well reported and reliable.

12.11.2004 (150)

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : = 9.8 - 11.6 ml/kg bw
Species : Mouse
Strain : NMRI
Sex : male/female
Number of animals : 5
Vehicle : physiol. saline
Doses : 3 doses lying between LD16 and LD84
Method : other
Year : 1976
GLP : no data
Test substance : other TS: Analytical grade

Remark : Method: Ethanol administered to SPF NMRI mice (5 per sex per group) by gavage, diluted as necessary with physiological saline (0.9% NaCl). Volume administered was 20 ml/kg, or where necessary, 30 ml/kg. Quantities of solvent were varied so that at least three mortality values between 16 and 84% were obtained.

All deaths occurred within 24 h. No signs or necropsy findings described.

LD50 calculated by probit analysis and is for both sexes combined.
Time of death: All occurred within 24 hr. Individual times not given.
Description, severity, time of onset and duration of clinical signs at each dose level: Not described.
Necropsy findings: Not done.
Potential target organs: Not discussed.
Sex comparison: Not given; LD50 is for both sexes combined. Values cited are 95% confidence limits.

Result : Values cited are 95% confidence limits. Average LD50=10.5ml/kg, which is equivalent to 8300mg/kg.

LD50's for i.v. and i.p. routes also determined:-

i.v. 2.8
i.p. 4.0

Test condition : Age of animals: not given. Animals (5 of each sex) were housed in polycarbonate cages in air-conditioned rooms at a temperature of 22 deg. C and relative humidity of 55%.
Food (Ssniff Standard diet R from Intermast GmbH, Bockum-Hovel) and water were available ad lib.
Doses: Not stated. At least 3 doses between LD16 and LD84 were used.

Doses per time period: One.
Volume administered or concentration: 20 ml/kg total volume.
Post dose observation period: 7 days.
Exposure duration: Not applicable.

Test substance : Test substance was analytical grade.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
12.11.2004 (151)

Type : LD50
Value : = 15010 mg/kg bw
Species : Rat
Strain :
Sex : Female
Number of animals : 8
Vehicle : other: gavaged after 5% gum acacia
Doses : 16,17,18,19,20,21 ml/kg
Method : other
Year : 1992
GLP : no data
Test substance : other TS

Remark : 19 ml/kg converts to 15.01 g/kg bw.
Method used female rats only, 8 per dose and 7 dose levels.

Ethanol gavage was preceeded by gum acacia gavage, intended to reduce local irritation in stomach.

Post dose observation period was 24 h.

Potential target organs, male-female comparison, necropsy findings not reported.
Time of death: Individual times not given.
Description, severity, time of onset and duration of clinical signs at each dose level: Inebriation to gait disturbance, dose-related decrease inresponse to painful stimuli, respiratory depression and coma.
Necropsy findings: Diffuse congestion of the gastric mucosa without gross haemorrhage or ulceration.
Potential target organs: Not discussed.
Sex comparison: Not applicable.

Result : Clinical observations ranged from inebriation to gait disturbance and dose-related decrease in response to painful stimuli, repiratory depression and coma. Deaths were due to cardiorespiratory failure.

Test condition : Age of animals: Adults, 180 g. Animals were housed at a temperature of 22-26 degC with 12 hr light-12 hr dark cycle.
Food and water were available ad lib.
Doses: 16, 17, 18, 20, 21 and 22 ml/kg.
Doses per time period: One.
Volume administered or concentration: See above.
Post dose observation period: 24 hrs.
Exposure duration: Not applicable.

Test substance : Test substance was 99.8% ethanol and 0.1% methanol.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
12.11.2004 (152)

Type : LD50
Value : = 7000 - 11000 mg/kg bw

Species : Rat
Strain : Wistar
Sex : Male
Number of animals : 10
Vehicle : no data
Doses : six to 8 dose levels
Method : other
Year : 1970
GLP : no data
Test substance : no data

Method : Rats were about 100 days old in one experiment, 10-12 months old in another. Six to eight dose levels with a dose interval of 1.1 used. Ethanol given as a 40% w/v solution,
Remark : Results range of values for old rats to young rats.

Time of death: All deaths occurred within 24 hr. Individual times not given.
Description, severity, time of onset and duration of clinical signs at each dose level: Not described.
Necropsy findings: Not conducted.
Potential target organs: Cause of death was respiratory failure.
Sex comparison: Not applicable.
Test condition : Age of animals: About 100 days or 10-12 mth. Food and water were available ad lib.
Doses: 6-8 dose levels, not described.
Doses per time period: One.
Volume administered or concentration: As a 40% w/v solution.
Post dose observation period: 24 hrs.
Exposure duration: Not applicable.
Conclusion : Old rats were considerably more sensitive than young rats.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
12.11.2004 (153)

Type : LD50
Value : = 14.6 ml/kg bw
Species : Rat
Strain : Sprague-Dawley
Sex : Male
Number of animals : 6
Vehicle : other: none
Doses :
Method : other
Year : 1971
GLP : no data
Test substance : other TS: analytical grade

Method : Age at start of treatment: Older rats (300-470 g). Dosing by straight needle in undiluted form in non-fasted animals. LD50 determined by method of Litchfield and Wilcoxon.
Result : 95% confidence limits of result: 12800-16700mg/kg.
Reliability : (4) not assignable
Very little method descrption was given.
12.11.2004 (154)

Type : LD50
Value : = 7800 mg/kg bw
Species : Rat
Strain : Sprague-Dawley

Sex : male/female
Number of animals :
Vehicle : other: none
Doses :
Method : other
Year : 1971
GLP : no data
Test substance : other TS: analytical grade

Method : Age at start of treatment: 14 day old rats (16-50 g). Dosing by straight needle in undiluted form in non-fasted animals. LD50 determined by method of Litchfield and Wilcoxon.
Number of animals: 6-12
Result : 95% confidence limits of result: 6300-9700mg/kg.
Reliability : (4) not assignable
Very little detail provided of test method
12.11.2004 (155)

Type : LD50
Value : = 11500 mg/kg bw
Species : Rat
Strain : Sprague-Dawley
Sex : Male
Number of animals : 6
Vehicle : other: none
Doses :
Method : other
Year : 1971
GLP : no data
Test substance : no data

Method : Age at start of treatment: young adult rats (80-160 g). Dosing by straight needle in undiluted form in non-fasted animals. LD50 determined by method of Litchfield and Wilcoxon.
Result : 95% confidence limits of result: 18800-2700mg/kg.
Reliability : (4) not assignable
Very little detail given of method used.
12.11.2004 (155)

Type : LD50
Value : 11170 - 16710 mg/kg bw
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Method:
Reliability : (4) not assignable
12.11.2004 (156)

Type : LD50
Value : = 7060 mg/kg bw
Species : Rat
Strain :
Sex :

Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP : no data
Test substance : no data

Reliability : (4) not assignable
12.11.2004 (157)

Type : LD50
Value : ca. 11850 mg/kg bw
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP : No
Test substance : other TS

Remark : BASF Test.
LD50 value was between 11850 and 12640mg/kg.
test substance was ethanol at 90%,70% 50% and 30%.
Reliability : (4) not assignable
12.11.2004 (158)

Type : LD50
Value : = 9500 mg/kg bw
Species : Mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP : no data
Test substance : other TS

Test substance : Test substance was 95% ethanol.
Reliability : (4) not assignable
12.11.2004 (159)

Type : LD50
Value : = 3450 mg/kg bw
Species : Mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Method not specified.

Reliability : (4) not assignable
12.11.2004 (160)

Type : LD50
Value : > 790 mg/kg bw
Species : Mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year : 1972
GLP : No
Test substance : no data

Remark : Converted from 10ml/kg.
Reliability : (4) not assignable
12.11.2004 (161)

Type : LD50
Value : 5060 - 7850 mg/kg bw
Species : Rabbit
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Value = 6300 mg/kg body weight
Reliability : (4) not assignable
12.11.2004 (162)

Type : LDLo
Value : = 7000 mg/kg bw
Species : Rabbit
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Method not specified.
Reliability : (4) not assignable
12.11.2004 (163)

Type : Other
Value : = 9900 mg/kg bw
Species : Rabbit
Strain :
Sex :
Number of animals :
Vehicle :

Doses :
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Although the value was reported as an LD50 value, in a later publication (Munch J.C. 1972. Ind. med. Surg. 41, 31) it is said to be the minimum lethal dose.
Reliability : (4) not assignable
12.11.2004 (164)

Type : LDLo
Value : = 6000 mg/kg bw
Species : Cat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year : 1936
GLP : no data
Test substance : no data

Remark : Method not specified.
Reliability : (4) not assignable
12.11.2004 (165)

Type : Other
Value : 5500 - 6500 mg/kg bw
Species : Dog
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year : 1875
GLP : no data
Test substance : no data

Remark : Time of death reported to be "12 to 14 hours". Value is reported as the lethal dose.
Reliability : (4) not assignable
12.11.2004 (166)

Type : Other
Value :
Species : Dog
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method :
Year :
GLP : no data
Test substance : other TS

Remark : 30% aqueous ethanol.

Dogs were dosed by gavage with 4ml/kg (ca. 3160mg/kg) or 8mls/kg (ca. 6320mg/kg) of 33% aqueous ethanol solution. (4ml/kg dosed within 1 hour, 8ml/kg dosed within 2 hours). Liver function was tested by use of Bromosulphothalein (BSP) tests. The 8ml/kg ethanol dosed group gave an mean increased BSP retention time of ca. 10% that of the 4ml/kg group.

Reliability : (4) not assignable

12.11.2004 (167)

Type : LD50
Value : = 5560 mg/kg bw
Species : guinea pig
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year :
GLP : no data
Test substance : no data

Reliability : (4) not assignable

12.11.2004 (168)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC50
Value : > 60000 ppm
Species : Mouse
Strain : CD-1
Sex : male/female
Number of animals : 6
Vehicle :
Doses : 40,000, 50,000 and 60,000 ppm
Exposure time : 60 minute(s)
Method : other
Year : 1985
GLP : no data
Test substance : other TS: 95% USP

Remark : The sexes of the animals were not specified and the numbers given are estimates as 12 animals per exposure concentration were used.

Time of death: Not applicable, no deaths.
Description, severity, time of onset and duration of clinical signs at each dose level: Slight to moderate ataxia occurred and recovery time was more than 4 hours at all exposure levels.
Necropsy findings: Not applicable
Potential target organs: Not applicable.
Sex comparison: Not applicable

Result : No LC50 was determined as no deaths occurred at any of the exposure concentrations.

Slight to moderate ataxia was observed and recovery from this exceeded 4 hours at all exposure levels.

		Necropsy and target organ study not applicable.
Test condition	:	Age of animals: Not stated but weighed 25-30 g. Animals were caged with wood-chip bedding in a room at a temperature of 22-24 deg. C and 121 hr light/12 hr dark cycle. Doses: 40,000, 50,000 and 60,000 ppm for different exposure duration. Doses per time period: One exposure pereiod per exposure level. Volume administered or concentration: Not applicable. Post dose observation period: 72 days. Exposure duration: 60, 30 and 10 minutes.
Reliability	:	(2) valid with restrictions The study is reasonably well reported but there are the following deviations from an ideal protocol. Exposure period only 60 minutes. Species mouse rather than preferred rat. Observations reported for only 3 days rather than 14. Volume of chamber 29 litres (above 20 litres) No detailed observations of effects. No pathology No detailed reporting of findings down to individual animal.
Flag	:	Critical study for SIDS endpoint
12.11.2004		(169)

Type	:	LCLo
Value	:	> 29.43 mg/l
Species	:	Rat
Strain	:	Other
Sex	:	no data
Number of animals	:	12
Vehicle	:	
Doses	:	saturated air
Exposure time	:	7 hour(s)
Method	:	other
Year	:	1981
GLP	:	No
Test substance	:	no data
Remark	:	12 rats were exposed to a saturated vapour concentration of the test substance at a temperature of 20 degrees C. There were no deaths. The mean atmospheric concentration of test substance was 29.43mg/l. BASF Test
Reliability	:	(4) not assignable
12.11.2004		(170)

Type	:	Other
Value	:	
Species	:	Mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Exposure time	:	
Method	:	other
Year	:	1982
GLP	:	no data
Test substance	:	no data
Remark	:	13,300 ppm for 1.33 hours caused ataxia. 23,940 ppm for 1.25 hours caused narcosis. 29,300 ppm for 7 hours caused narcosis and deaths.

31,900 ppm for 0.33 hours caused ataxia.
Reliability : (4) not assignable
18.11.2004 (171)

Type : Other
Value :
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Exposure time : 7 hour(s)
Method : other
Year : 1982
GLP : No
Test substance : other TS

Remark : 12 rats were exposed to a saturated atmosphere of the test substance at 20 degrees C for seven hours. No deaths resulted.
50% ethanol in water
BASF test.
Reliability : (4) not assignable
12.11.2004 (172)

Type : LC50
Value : = 5.9 mg/l
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Exposure time : 6 hour(s)
Method : other
Year : 1980
GLP : No
Test substance : no data

Remark : BASF test
Reliability : (4) not assignable
12.11.2004 (173)

Type : LC50
Value : = 124.7 mg/l
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Exposure time : 4 hour(s)
Method : other
Year : 1980
GLP : No
Test substance : no data

Remark : BASF test
Reliability : (4) not assignable
12.11.2004 (174)

Type : LC0
Value : = 16000 ppm
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Exposure time : 8 hour(s)
Method : other
Year :
GLP : no data
Test substance : no data

Source : Patty's Toxicology of INdiustrial Chemicals
Reliability : (4) not assignable
29.09.2003 (175)

Type : Other
Value :
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Exposure time : 8 hour(s)
Method : other
Year :
GLP : no data
Test substance : no data

Remark : No details of method. Some deaths occurred at 16000 ppm andat 32000 ppm.
Reliability : (4) not assignable
12.11.2004 (176)

Type : Other
Value :
Species : Rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Exposure time :
Method : other
Year : 1918
GLP : No
Test substance : no data

Remark : Duration of exposure varied from 0.5 to 21.75 hours. No effects at 3260 ppm for 6 hours but drowsiness by 8 hours. Incoordination at 5660 ppm for 1.75 hours and light narcosis at 6400 ppm for 12 hours. At 12,400 to 12,700 ppm there was deep narcosis by 8.5 hours and deaths by 21.75 hours. Deep narcosis and death occurred at 44,000 ppm by 6.5 hours.
Reliability : (4) not assignable
12.11.2004 (177)

Type : Other
Value :
Species : guinea pig
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Exposure time :
Method : other
Year :
GLP : No
Test substance : no data

Remark : Duration of exposure varied from 3.75 to 24 hours.
No overt effects at 6400 ppm for 8 hours or 9080 ppm for 5.25 hours. Light narcosis and incoordination at 12,850 to 13,300 ppm for 8.75 to 24 hours. No effects at 19,260 ppm for 3.75 hours, but 20,000 ppm for 6.5 hours caused incoordination, and 21,900 ppm for 9.8 hours caused deep narcosis and death.
Reliability : (4) not assignable
12.11.2004 (177)

Type : LC50
Value : = 39 mg/l
Species : Mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Exposure time : 4 hour(s)
Method : other
Year :
GLP : no data
Test substance : no data

Reliability : (4) not assignable
12.11.2004 (178)

Type : Other
Value :
Species : guinea pig
Strain : Hartley
Sex : Male
Number of animals :
Vehicle : other: 0.9% saline
Doses : concentration 31, 62,5, 125, 250mM
Exposure time :
Method : other
Year : 1994
GLP : no data
Test substance : no data

Method : Animals with tracheas cannulated with polyethylene tubes, artificially ventilated (tidal vol 10ml/kg). Changes to resistance to inflation measured by pressure required to overinflate by 2x tidal volume for 2 breaths. Animals subjected to 15mins saline aerosol, followed by 15second bursts of ethanol containing aerosol, with a 5 minute gap before next burst of

ethanol aerosol, with increasing concentrations used. 46.4% of aerosol measured as deposited in lungs by radiolabel technique.

Remark : Study designed to assess if ethanol in aerosol form causes bronchoconstriction.
Result : Ethanol did not cause bronchorestriction.
Reliability : (4) not assignable
18.11.2004 (179)

Type : other: volunteer study
Value :
Species : Human
Strain :
Sex : male/female
Number of animals : 6
Vehicle : other: saline
Doses : 0, 25% in aerosol form
Exposure time : 30 minute(s)

Method : Volunteers: Healthy; 2 atopic, 5 non-atopic; 4 women, 2 men; 5 non-smokers, 1 regular smoker; age 28-45yrs.
Inhalation via the mouth of an aerosol, particle size 0.5-4.0um. 5 day interval between exposures.
Lung function assessed by recording partial and maximum expiratory flow volume at time zero and repeatedly during the 4 hrs after exposure (1 sec forced expiratory volume and flow rate at 40% of the forced vital capacity.)
Mean of three repeats used.
Statistical analysis: student's t test.
Ethanol concentrations were measured using Draeger tubes in inhaled (measured in breathing tube) and expired air (5 & 30 mins after exposure.)
Result : Subjects reported coughing at start of exposure and 3 reported chest tightness at end. None reported signs of intoxication normally associated with ethanol ingestion. No symptoms were experienced with the saline control.
Ethanol decreased the maximum expiratory flow rates for the whole of the 4 hour period after exposure (8-37% statistically significant reduction for the first 90 minutes after exposure.) There was no significant effect on the one second forced expiratory volume.
The ethanol concentration in inspired air was 0.18-0.2% (1800-2000ppm) and in exhaled air for 30 minutes post exposure 0.06-0.1% (600-1000ppm)
Reliability : (4) not assignable
18.11.2004 (180)

Type : Other
Value :
Species : Mouse
Strain : C57BL
Sex : Female
Number of animals :
Vehicle :
Doses : single group exposed to 25-38mg/l
Exposure time : 24 hour(s)
Method : other
Year : 1986
GLP : no data
Test substance :

Method : Animals 12-24 weeks old.
Caged mice placed in perspex inhalation chambers.
Feed: CRM pellets (K&K Greefe), freely available during exposure.
Aged matched control mice used
Blood obtained in heparinised syringes (cardiac puncture) then mixed with

EDTA. Hb, RBC and WBC determined by Coulter counter. PVC measured using microhaematocrit tubes. Platelet count determined (after 100x dilution in formal citrate) using a Neubauer counting chamber. Reticulocytes counted on unfixed smears of supervitally -stained blood. Blood films stained by the May-Grunwald-Giemsa method and differential leucocyte counts performed on 500 consecutive nucleated cells. Femoral marrow expekked into heparinised Hank's solution, dispersed into a single cell suspension and used for determination of marrow cellularity or deoxyuridine suppression values.
Quantification of granulocyte-macrophage progenitor cells: Femoral marrow expelled into MEM alpha medium. Samples dispersed into single cell medium, washed twice, assays of CFU-GM performed on each marrow cell suspension in triplicate. Details of procedure given in reference.

Result : Many mice showed locomotor depression and ataxia. Blood ethanol levels were in the range 150-560mg/dl. Ethanol exposed mice developed leucopenia, neutropenia, lymphopenia, monocytopenia, thrombocytopenia but not anaemia or macrocytosis. There was no effect on deoxyuridine suppression values or number of granulocyte-macrophage progenitor cells. There was a slight reduction in the number of megakaryocytes.

Reliability : (4) not assignable

18.11.2004 (181)

5.1.3 ACUTE DERMAL TOXICITY

Type : LDLo
Value : = 20000 mg/kg bw
Species : Rabbit
Strain : no data
Sex : no data
Number of animals : 4
Vehicle :
Doses : no data
Method : other
Year : 1968
GLP : no data
Test substance : no data

Remark : Dosage translated from 200 Proof. Reported that dose used killed 1 out of four animals.

Reliability : (4) not assignable
No details of method reported therefore not possible to assess compliant with relevant testing protocol. No reference source quoted for reported data.

12.11.2004 (182)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type : LC50
Value : = 9450 - 9710 mg/kg bw
Species : Mouse
Strain : other: HS
Sex : male/female
Number of animals : 10
Vehicle : physiol. Saline
Doses : 6, 8 and 10 g/kg in 20% w/v solution
Route of admin. : i.p.
Exposure time : 24 hour(s)
Method :

Year : 1995
GLP : no data
Test substance : no data

Remark : Time of death: All deaths occurred within 30 min. Individual data were not given.
Description, severity, time of onset and duration of clinical signs at each dose level: Not described.
Necropsy findings, included doses affected, severity and number of animals affected: Not done.
Potential target organs: Not discussed.
Sex comparison: LD50 males; 9.71 g/kg. LD50 females; 9.45 g/kg.
Result : The LD50 vales were 838 to 1127 (9.71 g/kg) in males and 8.45 to 1049 g/kg (9.45 g/kg) in females.
Test condition : Age of animals: 25-30 days. Animals were housed in Plexiglass cages with aspen shavings in a climate-controlled room with 12 hr light and 12 hr dark cycle. Food and water were available ad lib.
Doses: 6, 8 and 10 g/kg.
Doses per time period: One.
Volume administered or concentration: 10 ml/kg total volume as a 20% w/v solution.
Post dose observation period 24 hr.
Exposure duration: Not applicable.
Reliability : (2) valid with restrictions
12.11.2004 (183)

Type : LD50
Value : = 9000 mg/kg bw
Species : Mouse
Strain : Swiss Webster
Sex : Male
Number of animals : 8
Vehicle : Water
Doses : 6 ranging 5000 to 11000 mg/kg bw
Route of admin. : i.p.
Exposure time :
Method : Other
Year : 1979
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : The LD50 was calculated using the Lichfield-Wilcoxon method.
Time to death, clinical signs, necropsy findings and potential target organs not reported.

Time of death: Not reported.
Description, severity, time of onset and duration of clinical signs at each dose level: Not reported.
Necropsy findings, included doses affected, severity and number of animals affected: Not reported.
Potential target organs: Not discussed.
Sex comparison: Not applicable.
Result : The LD50 in male mice was 9.2 g/kg bodyweight with a 95% confidence interval of 8.9 to 9.4 mg/kg.
Test condition : Age of animals: Not stated but weighed 25-30 g. Animals were housed in plastic cages in a climate-controlled room with 12 hr light and 12 hr dark cycle. Food and water were available ad lib.
Doses: Not stated but at least 6 doses between 5.0 and 11.0

g/kg.
Doses per time period: One.
Volume administered or concentration: 0.2 to 0.25 ml using a 20% w/v solution.
Post dose observation period 7 days.
Exposure duration: Not applicable.

Reliability : (2) valid with restrictions
12.11.2004 (184)

Type : LD50
Value : = 5100 - 6710 mg/kg bw
Species : Rat
Strain : Wistar
Sex : Male
Number of animals : 10
Vehicle :
Doses : Six to eight dose levels
Route of admin. : i.p.
Exposure time :
Method : Other
Year : 1970
GLP : no data
Test substance : no data

Method : Rats were about 100 days old in one experiment, 10-12 months old in another.
Remark : Results are values for old rats to young rats.

Time of death: All within 24 hr, individual times not reported.
Description, severity, time of onset and duration of clinical signs at each dose level: Not reported.
Necropsy findings, included doses affected, severity and number of animals affected: Not conducted.
Potential target organs: Cause of death was respiratory failure.
Sex comparison: Not applicable.

Source : EU Existing Chemicals Programme HEDSET
Test condition : Age of animals: About 100 days or 10-12 mth. Food and water were available ad lib.
Doses: Not stated but 6 to 8 doses with interval 1.05.
Doses per time period: One.
Volume administered or concentration: A 15% w/v solution.
Post dose observation period 24 hrs.
Exposure duration: Not applicable.

Reliability : (2) valid with restrictions
12.11.2004 (153)

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 4 hour(s)
Number of animals : 6
Vehicle :
PDII :
Result : not irritating
Classification : not irritating
Method : OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"

Year : 1981
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Ethanol was applied to six shaved New Zealand white albino rabbits for 4 hours under exposure chamber of 6 cm2.
Draize scoring criteria.
Mean score for erythema was 1.0 after 1 and 24 hours.
Scores for erythema and oedema were 0.0 at all other time points.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
17.11.2004 (185)

Species : Rabbit
Concentration : 95 %
Exposure :
Exposure time :
Number of animals : 4
Vehicle : Water
PDII :
Result : slightly irritating
Classification : not irritating
Method : other
Year : 1971
GLP : no data
Test substance : other TS

Remark : Classification according to Directive 67/548/eec is not possible from the data presented in this paper.
Method was a modified Draize test employing groups of 4 rabbits and 24-hour covered application.
The average score was 0.5 out of a possible 8 (scores of 0.62, 0.62 and 0.25 were recorded for 3 repetitions).
Test substance : Test compound was 95% ethanol.
Reliability : (2) valid with restrictions
12.11.2004 (186)

Species : Rabbit
Concentration :
Exposure :
Exposure time :
Number of animals :
Vehicle :
PDII :
Result : not irritating
Classification :
Method : other
Year : 1979
GLP : No
Test substance : as prescribed by 1.1 - 1.4

Remark : After Fed Reg vol 38 No 187 27-05-1973 1500.41
Reliability : (4) not assignable
12.11.2004 (187)

Species : Rabbit
Concentration :
Exposure :
Exposure time :
Number of animals :

Vehicle :
PDII :
Result : not irritating
Classification :
Method : Draize Test
Year : 1978
GLP : No
Test substance : other TS

Remark : ethanol 96%
Reliability : (4) not assignable
12.11.2004 (188)

Species : Human
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 4 hour(s)
Number of animals : 31
Vehicle :
PDII :
Result : not irritating
Classification : not irritating
Method : other
Year : 2004
GLP : no data
Test substance : no data

Method : Application of 0.2ml ethanol on a 25mm plain Hill Top chamber containing a Webril pad to the skin of human volunteers for 4 hours. Full details given in York (1996)) and Basketter (1997). Treatment sites assessed for irritation on a four point scale at 24, 48 and 72hrs after pad removal. Any weakly positive reaction (mild erythema or dryness across most of contact site) considered a positive reaction. Interpretation of results in terms of EU classification done by statistical comparison with a concurrent positive control (20% sodium dodecyl sulphate).
Result : One out of 31 subjects produced a positive result. Positive control produced a reaction in 15 out of 31 subjects Ethanol therefore considered non-irritant.
Reliability : (4) not assignable
17.11.2004 (189)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : 100 other: microlitre
Exposure time :
Comment :
Number of animals :
Vehicle :
Result : moderately irritating
Classification : Irritating
Method : OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year : 1987
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Method: six New Zealand white rabbits, application of 100 microlitre into the lower conjunctival sac. Draize scoring

		criteria.
Result	:	Average scores

	24hr	48hr	72hr
Conjunctivitis	2.50	2.61	2.06
Chemosis	1.67	1.17	0.83
Iritis	0.50	0.33	0.00
Corneal Opacity	1.00	1.50	1.00

Test substance : Test substance was neat ethanol.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
12.11.2004 (190)

Species : Rabbit
Concentration : 100 % active substance
Dose :
Exposure time :
Comment :
Number of animals : 3
Vehicle : None
Result : moderately irritating
Classification : not irritating
Method : OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year : 1998
GLP : Yes
Test substance : other TS: 100% ethanol

Method : The method was fundamentally OECD Guideline 405 with instillation of 0.1 ml, observation for 7 days and standard grading scales for lesions. However, a Modified Maximum Average Score (MMAS) was derived by averaging the individual animal weighted scores at each time of observation and then selecting the highest (maximum) of these averages. This is a preferred result for this end point as it is a recent study carried out to a recognized protocol that is reported in detail.
Result : Average scores

	Day 1	Day 2	Day 3
Corneal opacity	1.33	1.33	0.66
Iritis	0.33	0.66	0.33
Conjunctival redness	2.66	2.00	1.66
Chemosis	1.66	1.66	0.66

Individual animal observations reported. Full reversal of all symptoms in animals within 14 days. Most persistent effect conjunctival redness, still present, grade 1, at 7 days (last observation time before 14 day observation.)
Test substance : Concentration undiluted/100%
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
12.11.2004 (191)

Species : other: human, rabbit
Concentration :
Dose :
Exposure time :
Comment : other: review of published work
Number of animals :
Vehicle :
Result :
Classification :

Method :
Year : 1986
GLP :
Test substance : other TS

Remark : Routes include direct contact with and without anesthesia, vapour exposure, injection into orbit, acute and chronic alcohol intoxication and fetal alcohol syndrome.
Test substance : spirits, toiltery solutions; various concentrations alcohol
Conclusion : 1. Splashes of alcoholic spirit (20-50% alcohol causes stinging discomfort and reflex closure with no lastic effects.
2. On rabbit cornea, 50% alcohol causes mild reaction graded 20 on a scale of 100.
3. repeated applicatuon of 7 drops of 40 to 80% alcohol caused loss of corneal epithelium and endothelium followed by haemorrhage into conjunctiva, inflitration and vascularization of corneal stroma.
4. Shaving lotions etc may contain up to 90% alcohol; severe reactions with slow recovery may occur, possibly due to other components.
5. High vapour concentrations may cause stinging and watering of eyes above 0.25%.

By other routes - ocular effects are not irritation.
Reliability : (4) not assignable
Flag : Critical study for SIDS endpoint
12.11.2004 (192)

Species : Rabbit
Concentration :
Dose :
Exposure time :
Comment :
Number of animals :
Vehicle :
Result : not irritating
Classification : not irritating
Method : OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year : 1983
GLP : No
Test substance : other TS

Remark : Pure ethanol
Reliability : (4) not assignable
12.11.2004 (193)

Species : Rabbit
Concentration :
Dose :
Exposure time :
Comment :
Number of animals :
Vehicle :
Result : not irritating
Classification :
Method : other
Year : 1979
GLP : No
Test substance : as prescribed by 1.1 - 1.4

Remark : After Fed Reg vol 38 no. 187 27-09-1973
Reliability : (4) not assignable
12.11.2004 (194)

Species : Rabbit
Concentration :
Dose :
Exposure time :
Comment :
Number of animals :
Vehicle :
Result : moderately irritating
Classification :
Method : Draize Test
Year : 1978
GLP : No
Test substance : other TS

Remark : 96% ethanol.
Reliability : (4) not assignable
12.11.2004 (195)

Species : Rabbit
Concentration :
Dose :
Exposure time :
Comment :
Number of animals :
Vehicle :
Result : highly irritating
Classification :
Method : other
Year : 1982
GLP : no data
Test substance : other TS

Remark : Classification of the results according to Directive 67/548/EEC was not possible based on the data presented in the paper.
Method apparently complied with the main requirements of the OECD protocol (1979). Groups of six rabbits each had 100 microulitre test substance instilled into the lower conjunctival sac of one eye. In one group the eyes were not rinsed out, while intwo further groups, rinsing was performed after 4 and 30 seconds respectively. Scoring was carried out based on AFNOR recommendations (Association Francaise de Normalisation, 1982).
When classified according to AFNOR recommendations, ethanol was severely irritating if not rinsed out of the eye, and very irritating if rinsed out of the eye after 4 or 30 seconds. Reclassification of the results based only on readings taken (without rinsing) at the observation times specified in the OECD protocol, gave rise to a classification of "severely irritant".
Test substance : Test substance was ethanol 90 %?.
Reliability : (3) invalid
12.11.2004 (196)

5.3 SENSITIZATION

Type : Mouse ear swelling test
Species : Mouse
Number of animals : 23
Vehicle :
Result : not sensitizing
Classification : not sensitizing
Method : other
Year : 1988
GLP : no data
Test substance : no data

Remark : Age at start of treatment: 6-8 weeks
Acclimation: 7 days

On day 0, mice (9 males and 10 females) were injected s.c. with 0.05 ml of the test substance in complete Freund's adjuvant (scapular region) and the test substance was also applied topically to the abdomen (amount not specified). On days 3, 5, 7, 10, 12 and 14 they received topical applications to the shaved abdomen, and a second scapular s.c. injection of 0.05 ml in CFA was given on day 7. On day 26, the thickness of the left ear was measured using a mobile disk caliper with an accuracy of 0.01 mm immediately prior to application of the test substance to both sides of the ear. Left ear thickness was measured again on days 27 and 28 (i.e. 24 and 48 hours after challenge). No increase in ear thickness following challenge application of ethanol.

Result : Measurement of 94 untreated mice showed an ear thickness of 0.214 mm with a typical variation of 0.002 mm. There was no statistically significant increase in ear thickness following challenge application of ethanol. Average ear thickness before: 21.66 +- 1.85 mm.
Average thickness after: 21.69 +/- 1.91 (Swelling 0.1%).
Known moderate and strong sensitizers applied as controls produced significant swelling in this study.
Test substance : Test substance was 95% ethanol.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
12.11.2004 (197)

Type : Guinea pig maximization test
Species : guinea pig
Number of animals : 10
Vehicle :
Result : not sensitizing
Classification : not sensitizing
Method : other
Year : 1984
GLP : Yes
Test substance : other TS

Remark : Effective concentrations of ethanol used in the induction phase were:

Subst. 1; intradermal: 25%
Subst. 1; topical: 37.5%
Subst. 2 intradermal and topical: 37.5%

Effective concentratiuons of ethanol used in the challenge phase:

Subst. 1 1st and 2nd challenges: 25%, 47.5%
Subst. 2 1st Challenge: 37.5%, 60% and 71.25%
Subst. 2 2nd Challenge: 22.5%, 60% and 71.25%
Method: Test procedure was based on that of Magnusson and Kligman (1969) J. Invest. Derm. , 52, 269.

Test animals were femnale Dunkin-Hartley albino guinea pigs; 10 test and 10 controls.

Result :

No skin reactions were evoked at challenge with the polyalkalene glycol in 75% ethanol in either test or control group animals.

Although this study was not primarily carried out to assess ethanol, it can be reliably concluded that ethanol did not show any signs of sensitizing propert

Reliability : (2) valid with restrictions
There is no detailed information provided on method used other than reference to a second source document. No positive control was used.

Flag : Critical study for SIDS endpoint

12.11.2004 (198)

5.4 REPEATED DOSE TOXICITY

Type : Sub-chronic
Species : Rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 90 days
Frequency of treatm. : Daily
Post exposure period :
Doses : 1,2,3,4,5%,10%w/w ethanol in liquid diet
Control group :
NOAEL : = 2 %
LOAEL : = 3 %
Method : other
Year : 1986
GLP : no data
Test substance : other TS: pure

Method : Age at study start: 43 days
No. of animals per sex per dose : 10
Ethanol supplied in nutritionally balanced liquid diet.
Controls received diet without ethanol.
Parameters recorded: Bodyweights weekly, food consumption daily.
Blood aspartate aminotransferase and alanine aminotransferase levels determined at termination.
Liver and kidneys were examined macroscopically and microscopically at necropsy and the spleen was weighed.

No statistical tests for significance were used.

Remark : 2% dose calculated to be equivalent to 2400mg/kg/day

Result : Bodyweight: All groups gained weight though final weights decreased with

dose.
Food/water consumption: Consumption in the 10% group was reduced relative to controls (182 ml diet/kg-d versus 195 ml diet/kg-d).
Clinical signs: No adverse signs were observed
Ophthalmology, haematology: Not examined.
Clinical biochemistry: Serum liver enzymes were not affected by treatment and kidney findings were minimal.
Mortality and time to death: No deaths occurred.
Fross pathology: Liver yellowing, dosage-related.
Histopathology: Hepatic centrilobular steatosis increased in severity with dose as did the frequency and severity of Mallory bodies (hyaline) and acidophilic degeneration and
necrosis. Most liver findings were absent or mild at 2% w/w ethanol but became more significant at 3% and higher dose.

Reticulo-endothelial cell proliferation was slight at 1 and 2%. A few kidney casts were noted in animals from the 1-3% dose groups and there were a few calcifications in the 3-5% groups. Slight tubular fatty change occurred in all groups.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
12.11.2004 (199)

Type : Sub-chronic
Species : Mouse
Sex : Male
Strain : B6C3F1
Route of admin. : drinking water
Exposure period : 90 days
Frequency of treatm. : 7 days/week ad libitum
Post exposure period :
Doses : 5% w/v in deionized water
Control group : yes, concurrent vehicle
NOAEL : < 5 %
LOAEL : = 5 %
Method : other: NTP 13-wk toxicity protocol
Year : 1996
GLP : Yes
Test substance : as prescribed by 1.1 - 1.4

Method : Age at study start: 43-46 days
Number of animals per sex per group: 10
Ethanol was diluted in deionized water.
No satellite animals were included.
Parameters observed were bodyweights, water consumption and clinical observations weekly. Sperm motility was assessed at termination.
Complete necropsies were performed.
Statistical tests were t-tests and F-tests.

Remark : The 5% dose was calculated as equivalent to 7300-9400 mg/kg body weight over the various urethane dose groups, based on average body weight and drinking water consumption.

Result : LOAEL dose was much greater than 5% w/v for observed body and organ weight increases and decreased sperm count.

No premature deaths occurred.

Bodyweight-relative liver weight was increased and there were increases in absolute heart, liver, kidney and lung weight

Minimal nephropathy occurred in 30% of treated animals and in 10% controls. Sperm count in the cauda epididymis was decreased.

Source : U.S. Environment Protection Agency High Production Volume, Chemical Right to Know Program.
Reliability : (2) valid with restrictions
Single dose used did not allow a NOAEL to be determined so therefore only reliable with restrictions.
12.11.2004 (200)

Type : Sub-chronic
Species : Mouse
Sex : Female
Strain : B6C3F1
Route of admin. : drinking water
Exposure period : 90 days
Frequency of treatm. : 7 days/week ad libitum
Post exposure period :
Doses : 5% w/v in deionized water
Control group : yes, concurrent vehicle
NOAEL : = 5 %
LOAEL : > 5 %
Method : other: NTP 13-wk toxicity protocol
Year : 1996
GLP : Yes
Test substance : as prescribed by 1.1 - 1.4

Method : Age at study start: 43-46 days
Number of animals per sex per group: 10
Ethanol was diluted in deionized water.
Satellite animals were not included.
Parameters observed were bodyweights, water consumption and clinical observations weekly. Vaginal cytology was assessed before termination.
Complete necropsies were performed.
Statistical tests were t-tests and F-tests.
Oestrus cycle length was determined.
Remark : Bodyweight: Unaffected by treatment
Food/water consumption: water consumption lowered in ethanol group.
Clinical signs: None noted
Ophthalmological, haematological and blood chemistry findings: Not examined.
Mortality and time of death: No premature deaths occurred.
Gross pathology: Time spent in dioestrus and pro-oestrus was increased.
Organ weight changes: Ethanol treatment did not affect organ weights.
Histopathology: Non-neoplastic lesions did not significantly differ from controls

The 5% dose was calculated as equivalent to 17000-24000 mg/kg body weight over the various urethane dose groups, based on average body weight and drinking water consumption.
Result : NOAEL effects were body and organ weights and oestrous cycle length.

The only treatment-related change in female mice was the time spent in dioestrus and pro-oestrus but it was unclear whether this was significant. Cycle length was unchanged.
Reliability : (1) valid without restriction
Highly reliable. Single dose but sufficient to determine a NOAEL.
12.11.2004 (201)

Type : Sub-chronic
Species : Rat
Sex : Female
Strain : Fischer 344

Route of admin.	:	drinking water
Exposure period	:	90 days
Frequency of treatm.	:	7 days/week ad libitum
Post exposure period	:	
Doses	:	5% w/v in deionized water
Control group	:	yes, concurrent vehicle
NOAEL	:	< 5 %
LOAEL	:	= 5 %
Method	:	other: NTP 13-wk toxicity protocol
Year	:	1996
GLP	:	Yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Age at study start: 43-46 days
Number of animals per sex per group: 10
Ethanol was diluted in deionized water.
Satellite animals were included for haematological and clinical chemistry examination at 3 and 23 days.
Parameters observed were bodyweights, water consumption and clinical observatons weekly. Haematology, blood chemistry and vaginal cytology was assessed before study termination.
Complete necropsies were performed.
Statistical tests were t-tests and F-tests.

Remark : The 5% dose was calculated as equivalent to 4800-5600 mg/kg body weight over the various urethane dose groups, based on average body weight and drinking water consumption.

Result : Body and organ weights were unaffected by treatment while alanine aminotransferase was decreased and serum bile acids were increased at week 13. NOAEL and LOAEL were not achieved at this dosage.

No clinical signs, ophthalmological, haematological, or organ weight changes were observed.

No premature deaths occurred.

Minimal nephropathy occurred in 40% test animals and in 0% of controls. No liver lesions were found in controls but hepatodiaphragmatic nodules were observed in ethanol-exposed animals.

Reliability : (2) valid with restrictions
Single dose used did not allow a NOAEL to be determined so therefore only reliable with restrictions.

12.11.2004 (200)

Type	:	Sub-chronic
Species	:	Rat
Sex	:	Male
Strain	:	Fischer 344
Route of admin.	:	drinking water
Exposure period	:	90 days
Frequency of treatm.	:	7 days/week ad libitum
Post exposure period	:	
Doses	:	5% w/v in deionized water
Control group	:	yes, concurrent vehicle
NOAEL	:	> 5 %
Method	:	other: NTP 13-week toxicity protocol
Year	:	1996
GLP	:	Yes
Test substance	:	as prescribed by 1.1 - 1.4

Method : Age at study start: 43-46 days
Number of animals per sex per group: 10

Ethanol was diluted in deionized water.
Satellite animals were included for haematological and clinical chemistry examination at 3 and 23 days.
Parameters observed were bodyweights, water consumption and clinical observations weekly. Haematology, blood chemistry and sperm motility was assessed at termination.
Complete necropsies were performed.
Statistical tests were t-tests and F-tests.

Remark : The 5% dose was calculated as equivalent to 2800-4100 mg/kg body weight over the various urethane dose groups, based on average body weight and drinking water consumption.

Result : There was a 20% decrease in thymus weight relative to controls. Reticulocyte count was increased and serum bile acid concentration increased. Some other blood biochemical parameters differed inconsistently from control values at day 3 or 23. Reproductive tissues and sperm counts were not affected by treatment.

Reliability : (1) valid without restriction
Highly reliable. Single dose but sufficient to determine a NOAEL.

12.11.2004 (201)

Type : Sub-chronic
Species : Rat
Sex : no data
Strain : no data
Route of admin. : oral feed
Exposure period : Up to 36 weeks
Frequency of treatm. : Continuous
Post exposure period : None
Doses : 2 ml ethanol/rat/day given to 6 rats
Control group : yes, concurrent no treatment
Method : other
Year :
GLP : No
Test substance : other TS

Remark : Body weight gain decreased in treated rats. Haematological effects evident at all time points ($p < 0.05$) included reductions in erythrocytes, haematocrit and haemoglobin concentration, while erythrocyte sedimentation rate, MCV and MCH were increased. A significant fall in total white bloodcells was also seen in treated rats ($p < 0.001$) at all time points. Lymphocytes were reduced while neutrophils were increased. Monocytes were increased at 10 and 14 weeks only.
Six rats given alcohol equivalent to approximately 8 g/kg body weight/day (based on body weight mid-way through the study). Six control rats given isocaloric amount of sucrose. Body weights monitored. Haematological parameters measured after 10, 14, 18 and 22 weeks.

Reliability : (4) not assignable

12.11.2004 (202)

Type : Sub-chronic
Species : Rat
Sex : male/female
Strain : Other
Route of admin. : Inhalation
Exposure period : 90 days
Frequency of treatm. : Continuous
Post exposure period : no data
Doses : 86 mg/m3, 15 rats exposed

Control group : Yes
Method : other
Year : 1970
GLP : no data
Test substance : other TS

Remark : Both Long-Evans and Sprague-Dawley rats were used in the study.
In this study, 15 male and female Princeton-derived guinea-pigs, 3 male New Zealand albino rabbits, 3 male squirrel monkeys and 2 male beagle dogs were exposed at the same time as the rats. No deaths, signs of toxicity or histopathological effects were reported in these species either.
One control group (123 rats) used for experiments with 5 chemicals. Blood samples taken before and after exposure to determine haemoglobin concentration, haematocrit and total
leukocyte counts. Sections of heart, lung, liver, kidney and spleen retained for histopathological examination from about half of the rats. Limited number of biochemical and histochemical determinations carried out.
Result : None of the treated rats died and no signs of toxicity were evident. Histopathological examination revealed non-specific circulatory and inflammatory changes that were not considered to be chemically induced.
Test substance : Test substance was absolute ethanol (USP grade).
Reliability : (3) invalid
Insufficient detail reported. Single low dose limits value of study.
12.11.2004 (203)

Type : Sub-chronic
Species : Rat
Sex : Male
Strain : Wistar
Route of admin. : drinking water
Exposure period : 8 month
Frequency of treatm. : Continuous
Post exposure period : None
Doses : ca. 7.7 g/kg body weight/day given to 6 rats.
Control group : Yes
Method : other
Year :
GLP : no data
Test substance : other TS

Remark : Ethanol was given as a 10% solution in drinking water for 8 months after which a range of haematological parameters was studied. Control rats received pure drinking water.
Result : Body weight gain was unaffected in the treated rats. Following ethanol exposure the osmotic fragility of the erythrocytes was increased. There were no statistically significant effects on haematocrit, haemoglobin concentration, erythrocyte count, reticulocyte count, MCV, MCH or MCHC.
Reliability : (4) not assignable
12.11.2004 (204)

Type : Chronic
Species : Other

Sex : male/female
Strain : Other
Route of admin. : Other
Exposure period : up to 5 years
Frequency of treatm. : Continuous
Post exposure period : None
Doses : Ethanol added to diet and drinking water of a group of 4 baboons in increments to reach 25 g/kg body weight/day after 1 year.
Control group : Yes
Method : other
Year :
GLP : no data
Test substance : other TS

Remark : Ethanol was administered in a semi-liquid diet and also in the drinking water to a treated group of 3 male and 1 femaleanimal and a control group of 1 male and 1 female animals. The baboons were weighed and given blood tests regularly. Liver biopsies were performed every 3 months for 2 years, then every 6 months. The treated baboons were studied for 9, 18, 48 and 60 months.
Species: Baboon
Strain: Papio
Result : The ethanol-containing diet had no effect on body weight gain. Moderate fatty change was seen in the livers of the animals treated for 18 and 48 months, while the livers of those treated for 9 and 60 months were normal. No cirrhosis was evident at post-mortem.
Test substance : Test substance was absolute alcohol.
Reliability : (4) not assignable
12.11.2004 (205)

Type : Chronic
Species : Other
Sex : no data
Strain : Other
Route of admin. : oral feed
Exposure period : up to 22 months
Frequency of treatm. : Continuous
Post exposure period : None
Doses : Ethanol added to diet of a group of 9 baboons to provide 50% of their total calorific intake as alcohol.
Control group : Yes
Method : other
Year :
GLP : no data
Test substance : no data

Remark : A figure of 80 mg/kg body weight day is given in the paper, which may relate to the total volume of liquid diet consumedor to the mean intake of ethanol (which would equate with 63g ethanol/kg body weight/day).
An additional group of 6 treated baboons and their pair-fed controls had been given a solid diet with either ethanol or carbohydrates in the drinking water for periods of from 17 to 34 months. They were then changed to the liquid diet foran average of 17 months. When the ethanol was given in a solid diet, no lesions more severe than fatty liver were seen, while with the liquid diet, one baboon developed hepatitis (after 29 months on the solid and 19 months on theliquid diet), two developed incomplete cirrhosis (after

30 months on the solid and 15 months on the liquid diet) and one developed complete cirrhosis (after 34 months on the solid and 19 months on the liquid diet).
Ethanol was administered in a liquid diet to 9 treated baboons and 9 pair-fed controls which were given isocaloric carbohydrate. Liver biopsies were performed at regular intervals. The baboons were exposed for from 8 to 22 months,the average exposure being 15 months.
Species: Baboon
Strain: Papio hamadryas or olive and yellow

Result : The ethanol-containing diet reduced body weight gain, and inebriation was observed. Fatty liver developed in all treated baboons, and the liver triglyceride content increased progressively. Mild inflammation, cellular degeneration and and some fibrosis were noted in the liver, and ultrastructural changes were seen in the mitochondria and endoplasmic reticulum. Three baboons fed ethanol for 9 months, and one treated for 12 months developed alcoholic hepatitis. When two of these animals were biopsied at 20 months, cirrhosis was found.
Serum cholesterol and gutamic-oxaloacetic transaminase activity were increased in the treated animals, while haematocrit and haemoglobin values tended to be lower.

Reliability : (4) not assignable

12.11.2004 (206)

Type : Sub-chronic
Species : Rat
Sex : Male
Strain : Wistar
Route of admin. : Other
Exposure period : Up to 85 days
Frequency of treatm. : Continuous
Post exposure period : None
Doses : 80 ml ethanol/kg body weight/day given to 16 rats
Control group : Yes
Method : other
Year : 1985
GLP : no data
Test substance : no data

Remark : Body weight gain decreased in treated rats. Haematological effects evident at all time points ($p < 0.05$) included reductions in erythrocytes, haematocrit and haemoglobin concentration, while erythrocyte sedimentation rate, MCV andMCH were increased. A significant fall in total white bloodcells was also seen in treated rats ($p < 0.001$) at all time points. Lymphocytes were reduced while neutrophils were increased. Monocytes were increased at 10 and 14 weeks only.
Treated rats were fitted with two gastrostomy canulae. A low-fat liquid diet was infused through one, and ethanol solution through the other. Controls were given the diet and glucose solution (which was isocaloric with the administered ethanol) through one canula. An initial dose of 8 g ethanol/kg body weight/day was gradually increased to12 g ethanol/kg body weight/day over 85 days. Blood sampleswere taken at 2, 4, 6 and 12 weeks. The rats were killed at15 days (n=2), 30 days (n=7), 45 days (n=2) and 85 days (n=5). Their livers were removed and subjected to microscopy and lipid analysis.

Result : Body weight gain was unaffected. Fatty degeneration of the liver was seen in all exposed rats. The degree of severity varied, but correlated with the duration of intoxication, and with blood alcohol levels (p <0.001). Mild, focal mononuclear cell infiltration and necrosis of hepatocytes was mainly found in rats with severe fatty degeneration. Liver triglycerides were elevated (p <0.001). No similar effects were seen in the controls.
Reliability : (3) invalid
Study inadequately reported with regard to dosing regime.
12.11.2004 (207)

Type : Sub-acute
Species : Rat
Sex : Male
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 4 week
Frequency of treatm. : Continuous
Post exposure period : None
Doses : Ethanol given in the diet at a concentration of 6% v/v to 30 rats.
Control group : Yes
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Cell proliferation (crypt cell production rate) was examinedin the gastrointestinal tract (oesophagus, stomach, duodenum, ileum, proximal colon and rectum) of 30 ethanol-treated rats and their pair-fed controls given an isocaloric liquid diet containing 36% total calories as either ethanol or carbohydrate. Blood samples were taken at the time of death.
Result : The crypt cell production rate was increased 2.1 fold in the rectal mucosa of treated rats (p <0.005) but not in the other gut tissues examined. The proliferative compartment of the crypt was also expanded towards the colonic lumen in ethanol-treated rats (p <0.001). Serum gastrin concentrations were significantly increased (p <0.01). All tissues of the gastrointestinal tract were normal by light microscopy.
Reliability : (4) not assignable
17.11.2004 (208)

Type : Sub-acute
Species : guinea pig
Sex : no data
Strain : no data
Route of admin. : Inhalation
Exposure period : 10.5 weeks
Frequency of treatm. : Every day for first 2 weeks; thereafter 4 hours/day, 6 days/week.
Post exposure period : no data
Doses : 3000 ppm
Control group : yes, concurrent no treatment
Method : other
Year :
GLP : No
Test substance : other TS

Remark : 3000 ppm is equivalent to 5643 mg/m3.
Guinea-pigs were exposed to ethanol vapour on 64 occasions.

Precise number exposed not specified but at least 3. Body weights recorded weekly, blood counts and urinalysis carriedout every 2 weeks.

Result : No untoward effects other than slight transient albuminuria in one animal.
Test substance : Test substance used was "denaturing formular 2b", containing0.5% benzene.
Reliability : (4) not assignable
12.11.2004 (209)

Type : Sub-acute
Species : Rat
Sex : Male
Strain : Sprague-Dawley
Route of admin. : drinking water
Exposure period : 12 week
Frequency of treatm. : Continuous
Post exposure period : None
Doses : 3.26 M (equivalent to 10.2 g/kg body weight/day by the end of the study) given to 12 rats.
Control group : yes, concurrent vehicle
Method : other
Year :
GLP : no data
Test substance : no data

Remark : No overt signs of toxicity. Body weight gain was reduced inthe treated rats. No effects on liver function (as measuredby GOT, GPT and serum albumin) or haematological indices. One treated rat had increased serum creatinine but there were no microscopic effects on the kidneys. Organ weights were unaffected and there was no gross pathology at autopsy.Microscopic examination of the liver revealed fatty degeneration in 10/12 treated rats, an effect not seen in the controls.
Two groups of 12 rats given either pure water or drinking water containing ethanol. Rats weighed weekly. Blood samples collected at the end of study for determination of haemoglobin, leukocytes, differential white cell count, serum creatinine, GPT, GOT and serum protein. Liver, kidneys, heart and spleen weighed and samples of liver, pancreas, kidney and heart taken for microscopic examination.
Reliability : (4) not assignable
12.11.2004 (210)

Type : Chronic
Species : Monkey
Sex : male/female
Strain : Other
Route of admin. : oral feed
Exposure period : up to 48 months
Frequency of treatm. : Continuous
Post exposure period : None
Doses : Ethanol given as 40% of total ingested calories to a group of 4 monkeys.
Control group : Yes
Method : other
Year : 1983
GLP : no data
Test substance : no data

Remark : Eight monkeys were given a nutritionally adequate liquid diet which provided 50% of their total calorific intake as ethanol. Four control animals were given isocaloric amountsof carbohydrate in the same diet. Liver biopsies were taken at 3, 12 and 24 months, and at 40 or 48 months when the monkeys were killed. Detailed description of methods not provided.
Strain: Macaca radiata
Result : There were no effects on body weight gain or on relative liver weights. Control liver biopsies were normal. Fatty infiltration was seen in biopsies from the treated monkeys at all time points, but no necrosis, inflammation, fibrosis or effects on hepatic collagen were found.
Reliability : (3) invalid
12.11.2004 (211)

Type : Sub-chronic
Species : Monkey
Sex : Male
Strain : Other
Route of admin. : Other
Exposure period : 3 month
Frequency of treatm. : twice daily
Post exposure period : None
Doses : Ethanol comprised 40% of the ingested calories in a group of 14 animals.
Control group : Yes
Method : other
Year :
GLP : no data
Test substance : no data

Remark : A dose level in g/kg body weight can not be derived from theinformation presented in the report of this study.
Fourteen rhesus monkeys were given a nutritionally adequate liquid diet containing 40% of the total calories as alcohol,twice a day by gavage for 3 months. A control group of 12 monkeys received the same diet, with the ethanol replaced bycarbohydrate. At the end of the study, a complete necropsy was performed, and an unspecified range of tissues was examined microscopically.
Result : The treatment had no effect on body weight gain. Marked accumulation of triglycerides, cholesterol and phospholipids occurred in the serum and liver. Although generalized fatty change was evident in the liver, cirrhosis did not develop. The relative heart weight was increased and microscopic effects on the heart were observed (fatty change in the myocardium, focal myocytolysis, atrophy of muscle bundles and early fibrosis). Triglyceride and cholesterol ester levels were increased in the heart. ECGs were normal.
There were no effects on haematological parameters or gross or microscopic effects on the pancreas, kidneys, spleen or lungs.
Reliability : (4) not assignable
12.11.2004 (212)

Type : Sub-acute
Species : Rat
Sex : Male
Strain : Sprague-Dawley
Route of admin. : Gavage

Exposure period : Up to 10 weeks
Frequency of treatm. : Daily
Post exposure period : None
Doses : 5 g/kg body weight/day given to groups of 4 or 6 rats.
Control group : Yes
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Groups of 4 rats were treated with ethanol daily for 0, 1, 2, 5 or 10 weeks, and then given a final dose of 5 g ethanol/kg body weight 3 hours prior to being killed. The livers were removed and the mitochondria and microsomes wereexamined for evidence of diene conjugation (a method for detecting lipid peroxidation).

Further groups of six rats were treated daily for 0, 1, 2, 3, 5 or 7 weeks prior to being killed. The livers were thenremoved and enzyme activities determined.

Control rats were given isocaloric sucrose in both cases.

Result : Mitochondrial lipid peroxidation was increased in 2 of 4 rats at week 0 (i.e. after an acute dose of ethanol but withno pre-treatment), 3 of 4 rats after 1 week of ethanol treatment, and in all treated rats from week 2 onwards. Microsomal peroxidation was not seen at week 0, but was evident in treated rats from 2 weeks onwards.
The activities of hepatic glutathione peroxidase and glutathione reductase were increased by ca. 45 and 14% respectively at all time points.

Reliability : (4) not assignable
12.11.2004 (213)

Type : Sub-acute
Species : Rat
Sex : Male
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 14 days
Frequency of treatm. : Continuous
Post exposure period :
Doses : 10mg/l for 3 days then 25mg/l
Control group : yes, concurrent no treatment
Method : other
Year : 1988
GLP : no data
Test substance : other TS

Remark : Study designed to assess the effects of ethanol on immune and hematopoietic systems. Full method details provided in reference.

Result : Ethanol blood levels measured at 169+/-14mg%. No weight changes were observed.
A decrease in cellularity was found in the spleen, thymus and bone marrow. Red and white blood cell counts and haemoglobin concentration were not affected. Ethanol treatment did alter the relative proportions of lymphocytes and polymorphonuclear leukocytes in the peripheral blood. In the bone marrow, granulocyte macrophage progenitor cells were not affected but there was a decline in erythroid progenitor cells. The proliferation ability of splenic lymphocytes when stimulated by mitogens was unaffected.

Test substance : 95% ethanol.
Reliability : (4) not assignable
18.11.2004 (214)

Type : Sub-acute
Species : Rat
Sex : Male
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 3, 6, 9, 26 day groups
Frequency of treatm. : Continuous
Post exposure period :
Doses : 20mg/l
Control group : Other
Method : other
Year : 1979
GLP : no data
Test substance : no data

Method : Full details provided in reference.
Additional set of animals treated sub-cutaneously daily with pyrazole and a control set treated with saline.
Ethanol levels assayed twice daily and in triplicate.
Blood ethanol levels measured in duplicate by gas chromatography - details of method provided in reference.
Also measured:
- plasma retention of sodium sulfobromopthalein
- Plasma activity of glutamic pyruvic transaminase and - glutamic oxalacetic transaminase
- Liver triglycerides
- Phagocytic function
- Liver and spleen histopathology.
Statistical analysis by student's t test.

Remark : Whilst a well reported study, the results are of limited value in assessing the toxic properties of ethanol relevant to its use as a chemical substance.

Result : Ethanol exposure produced a small but noticeable retardation in bodyweight gain.
Initial exposure produced lethargy, ataxia and intoxication but animals adapted and appeared normal at the end of the study.
Blood ethanol levels in the ethanol-saline group peaked on day 9 at 126+/-40mg/100ml and declined by day 26. In the ethanol-pyrazole group they peaked at 219mg/100ml (+/- 35). No blood ethanol was measured in either the air-saline or air-pyrazole controls..
Liver triglycerides were raised (doubled) for the ethanol groups at the earlier time points but were the same as the controls by day 26. Plasma triglycerides showed no consistent pattern.
Plasma glutamic pyruvic transaminase levels were raised by 20% in the ethanol-saline group compared to the control.
Liver samples from the ethanol-saline group exhibited mild vacuolisation for the early time periods, but this was not seen at 26 days.
No other parameters were significantly effected between the ethanol-saline and air-saline groups.

Reliability : (2) valid with restrictions
17.11.2004 (215)

Type : Sub-acute
Species : Rat
Sex : Male
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 35 days

Frequency of treatm. : Continuous
Post exposure period :
Doses : see method details
Control group : yes, concurrent no treatment
Method : other
Year : 1990
GLP : no data
Test substance : no data

Method : Detailed method provided in reference.
Ethanol vapour concentration adjusted to maintain a blood ethanol level of 200-300mg/dl (sufficient to produce signs of intoxication and ataxia levels 2-3 as defined by Majchrowijz (1975).
Rats sacrificed within 1 hour of exposure, lungs and livers removed, weighed and snap frozen in liquid nitrogen.
Blood ethanol levels determined (method provided in reference).
Liver and lung glutathione and malonaldehyde levels measured (method details provided.) Enzyme assays also carried out and vitamin E levels measured.
Result : Ethanol exposed rats showed retarded weight gain. Lung and liver weights not affected but a moderate decrease in hepatic total protein and a small decrease in pulmonary soluble protein observed with ethanol exposure.
Ethanol did not affect levels of glutathione of vitamin E in the lung but levels were significantly diminished in the liver. However, this change disappeared if levels expressed per gram of protein. Ethanol had no effect on malondialdehyde levels in either tissue..
Of the enzymes, catalase and superoxide dismutase levels were significantly increased in the lung by ethanol exposure. Other enzyme levels (glutathione peroxidase and reductase) were not affected. There was no effect on anti-oxidant enzyme levels in the liver.
Conclusion : Ethanol exposure does not produce a significant degree in oxidative stress in rat lung.
Reliability : (4) not assignable
17.11.2004 (216)

Type : Sub-acute
Species : Rat
Sex : Male
Strain : Other
Route of admin. : oral feed
Exposure period : 3 to 4 weeks (not further defined)
Frequency of treatm. : Continuous
Post exposure period : no data
Doses : ethanol consumed by groups of 12 rats equivalent to 12.1 to 16.9 g/kg body weight/day
Control group : Yes
Method : other
Year :
GLP : no data
Test substance : no data

Remark : In the first experiment, rats received 36% of their total calories as either ethanol or isocaloric dextrin maltose.
In the second experiment, they received 36% of their calorific intake as ethanol or isocaloric fat. The ethanol was administered in a liquid diet at 5 g/100 ml diet. At the end of the study, the small intestine was removed for examination.
Strain: CD
Result : Reduced body weight gain was seen in the ethanol-treated rats regardless of whether ethanol was substituted for

carbohydrate or fat (p <0.01). There was no effect on smallintestine weight. The intestinal villi were shorter and contained fewer cells in the treated rats (p <0.001), but showed no haemorrhagic erosions. Effects on the crypts indicative of cellular proliferation were seen (increased epithelial cell count and mitotic index, increased thymidine kinase activity and higher incorporation of tritiated thymidine into intestinal DNA).

Reliability : (4) not assignable
12.11.2004 (217)

Type : Sub-acute
Species : Mouse
Sex : Female
Strain : C57BL
Route of admin. : Inhalation
Exposure period : 20-43 days
Frequency of treatm. : Continously
Post exposure period :
Doses : 10-25mg/l
Control group : yes, concurrent no treatment
Method : other
Year : 1986
GLP : no data
Test substance :

Method : Animals 12-24 weeks old.
Caged mice placed in perspex inhalation chambers.
Feed: CRM pellets (K&K Greefe), freely available during exposure.
Aged matched control mice used
Blood obtained in heparinised syringes (cardiac puncture) then mixed with EDTA. Hb, RBC and WBC determined by Coulter counter. PVC measured using microhaematocrit tubes. Platelet count determined (after 100x dilution in formal citrate) using a Neubauer counting chamber.
Reticulocytes counted on unfixed smears of supervitally -stained blood.
Blood films stained by the May-Grunwald-Giemsa method and differential leucocyte counts performed on 500 consecutive nucleated cells.
Femoral marrow expekked into heparinised Hank's solution, dispersed into a single cell suspension and used for determination of marrow cellularity or deoxyuridine suppression values.
Quantification of granulocyte-macrophage progenitor cells: Femoral marrow expelled into MEM alpha medium. Samples dispersed into single cell medium, washed twice, assays of CFU-GM performed on each marrow cell suspension in triplicate. Details of procedure given in reference.

Result : Mice exposed to ethanol developed thrombocytopenia only and none of the more extensive effects seen following short exposure to higher concentrations. There was no effect on bone marrow. Effects are only believed to occur following exposures >22-24mg/l

Reliability : (4) not assignable
18.11.2004 (181)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : TA97, 98, 100, 104 and 1535
Test concentration : 1, 3, 10, 33, 100, 333, 1,000, 3,333, 10,000 microgram/plate
Cycotoxic concentr. : Not determined
Metabolic activation : with and without
Result : Negative
Method : Other

Year : 1992
GLP : no data
Test substance : other TS: 91% pure

Method : No. of replicates: 5 + complete repeat of experiment.
Frequency of dosing: Once, including pre-incubation.
Positive and negative controls: Positive controls were included.
No. of metaphases analyzed: Not applicable.
Solvent used: Not applicable.
Follow-up: Not applicable.
Criteria for evaluating results: Combination of magnitude of increase in number of his+ revertants and shape of dose-response curve.
Positive controls (-S9): sodium azide (for TA1535, TA100), 9-aminoacridine (TA97), 4-nitro-o-phenylenediamine (TA98), methylmethane sulphonate (TA104).
Positive control (+S9): 2-aminoanthracene.
Solvent control: water.
Remark : A preincubation assay. This test is considered to be highly reliable in view of inclusion in NTP mutagenicity testing program, conducted in 5 strains over a wide range of concentrations, with and without two metabilic induction systems in two concentrations.
Result : Negative.

Test-specific confounding factors: None.
Dose-effected related observations: Ethanol at any dose did not produce a 2-fold increase in his+ revertants in the absence or presence of rat or hamster liver extracts.
Mitotic index: Not applicable.
Conclusion : Ethanol failed to induce reversions in any S. typhimurium tester strain with or without metabolic activation over a wide range of doses up to 10 mg/plate.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
12.11.2004 (218)

Type : Ames test
System of testing : Salmonella typhimurium/microsome test
Test concentration : 100 microlitre
Cycotoxic concentr. : Not determined
Metabolic activation : with and without
Result : Negative
Method : Other
Year : 1982
GLP : no data
Test substance : no data

Method : Test design: Salmonella/microsomal assays were carried out by making post-mitochondrial preparations from livers of male Sprague-Dawley rats induced with Aroclor 1254. Reversion of all strains by 5 microgram/plate of the promutagen 2-aminoanthracene was included in each assay system.

Ethanol was one of 25 chemicals examined by spot testing with 5 microgram with and without S9 mix. Compounds positive in the spot test were then subject to plate incorporation testing (not necessary for ethanol). Ethanol was actually being used as an inert solvent.

Positive and negative controls: Both negative controls and positive (2-

aminoanthracene, 4-nitro-o-phenylene diamine [frameshift mutagen], 9-aminoacridine [frameshift mutagen]and sodium azide [base-pair substitutions]) were used.

The Salmonella histidine auxotrophs hisTA98, hisTA100, hisTA1535, hisTA1537 and hisTA1538 were used.

Result : No evidence of mutagencity was observed for ethanol with and without S9 mix. 9 Of 25 chemicals demonstrated potential mutagenicity in the spot test but only one of these, DEA laureth sulphate, gave a positive test in the plate incorporation test.

Reliability : (2) valid with restrictions
Spot testing with confirmatory incorporation testing with 4 positive controls indicates a valid study. However, there is no mention of Good Laboratory Practice.

12.11.2004 (219)

Type : Ames test
System of testing : Salmonella/microsome
Test concentration : 10, 100, 500, 1000 microgram/plate
Cycotoxic concentr. :
Metabolic activation : With
Result : Negative
Method : Other
Year : 1975
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Ethanol was one of 300 chemicals tested in the standard Salmonella/microsome Ames test using human or rat liver S9 mix. The test method is given in detail in Ames, McCann and Yamasaki (1975) Mutat Res. and is reviewed in McCann & Ames (1975) Ann N.Y. Acad Sci.

Salmonella typhimurium strains TA1535, TA1537, TA100 and TA98 were used.

Remark : It is noted that there was a high correlation between carcinogenicity and mutagenicity (90%; 156 carcinogens in 174 mutagens) whereas few noncarcinogens showed any degree of mutagenicity.

Result : There were <70 revertents per 10,000.

Reliability : (1) valid without restriction
The methodology of this test is now accepted and repeatedly used as a standard in vitro test for mutagenicity. It is considered robust for detecting environmental carcinogens. This study is regarded as valid without restrictions.

12.11.2004 (220)

Type : Ames test
System of testing : Salmonella typhimurium LT2 strains
Test concentration : 100 microlitre
Cycotoxic concentr. : not presented
Metabolic activation : with and without
Result : Negative
Method : Other
Year : 1983
GLP : no data
Test substance : no data

Method : Different concentrations of 9delta-tetrahydrocannabinol and positive mutagen controls were added in 0.1 ml proportions together with 0.1 ml of a 16 hr nutrient broth culture of the bacterial test strain or 0.1 ml of the culture and 0.5 ml of S-9 mix. These were then poured onto minimal glucose agar plates to form an even layer across the agar.

Duplicate plates were made for each strain and plates were incubated for 48 hr at 37 degC. Colonies were counted on a Quebec colony counter. Background lawn and unreverted bacteria were evaluated by microscopy. Appropriate control combinations and growth study plates were prepared.

Remark : Salmonella typhimurium strains TA1538, TA1537, TA1535, TA100 and TA98.
This study incorporated ethanol as a negative control in an evaluation of the mutagenicity of delta9-tetrahydrocannabinol and other mutagens. Absolute ethanol was used as the solvent for 9-aminoacridine, one of the positive mutagen controls.

Result : No evidence of mutagencity was observed in the absence or presence of S9 mix.

Reliability : (2) valid with restrictions
The study gave the results expected for positive controls and ethanol evaluated as a solvent to a positive control gave negative results with and without S-9 mix. However, there is no mention of Good Laboratory Practice.

12.11.2004 (221)

Type : other: Ames reversion test and DNA repair test in E. coli
System of testing : Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538 and E.coli strains
Test concentration : Tested to "toxicity limit" (not defined).
Cycotoxic concentr. :
Metabolic activation : with and without
Result : Ambiguous
Method : Other
Year : 1984
GLP : no data
Test substance : other TS

Method : Ethanol tested in revised plate incorporation test as described in Maron, D.M. & Ames, B.N. (1983) Revised methods for the Salmonella mutagenicity test, Mutation Res.113;173-215.

The Ames reversion test was conducted with his- S. typhimurium strains TA1535, TA1537, TA1538, TA98, TA100 and, in part, TA97.

S9 Mix contained 10% liver S9 fractions from Sprague-Dawley rats pre-treated with Aroclor 1254.

Mutagenic potency was expressed by dividing the number of revertants in excess of controls by the corresponding amount of ethanol in nmoles.

The genotoxic activity of Escherichia coli was assessed using strains WP2 (repair proficient), WP67 and CM871.

A ratio of more than 2 between the MIC's in repair proficient (rep+) and -deficient (rep-) strains was considered to be sufficient.

Result : All strains of Salmonella typhimurium showed no reversion in the presence of ethanol with potency (revertants/nmole of <0.00006.

In the DNA repair test there was equivocal activity in the 2 hr preincubation assay in the presence of S9, otherwise, ethanol was inactive in the absence of S9 and in the spot test.

Test substance : Reagent grade

Reliability : (2) valid with restrictions
Consistency of results in the two tests for 71% of the substances tested together with an overall predictive accuracy of 64.5% for the reversion test and 72.4% for the DNA-repair test in 75 compounds classified for their

carcinogenic activity, demonstrated the validity of this study on comparability of methods. TRhis study is considered to be valid with restrictions.
12.11.2004 (222)

Type : Ames test
System of testing : Salmonella typhimurium strains TA97 and TA102
Test concentration :
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method :
Year : 1984
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Plate incorporation test (as described in Maron and Ames - 1983. Muta Res 113, 173.) S9 mix contained 10% liver S9 from SD rats pretreated with Aroclor 1254.

Mutagenic potential expressed by dividing number of revertants in excess of controls by corresponding amount of compound in nmoles.
Result : Ethanol was negative for mutagenic activity in the Ames reversion test using strain TA97 but showed a reproducible increase in revertants over controls in TA102 but this was less than two-fold increase which is not normally considered to be biologically significant in the Ames test. It was however repeatable.

The authors tentatively classified it as an uncertain or questionable mutagen. However, considering the response against the high dose used (160mg/plate) suggests that this is an excessively conservative conclusion and that the balance of evidence points to a negative result.
Test substance : Source from Carlo Erba
Reliability : (2) valid with restrictions
12.11.2004 (223)

Type : Ames test
System of testing :
Test concentration :
Cycotoxic concentr. :
Metabolic activation : with and without
Result :
Method :
Year : 1997
GLP : yes
Test substance :

Remark : Ethanol has been reported as being compatible with the Salmonella/microsome test at 200ul/plate in the plate incorporation assay and up to 100 ul/plate in the pre-incubation assay (Maron et al., 1981). At Safepharm Laboratories, ethanol has been used as one of the validated vehicle controls for more than 10 years. The typical dose volume used is 100ul/plate, which is equivalent to 79mg/plate, or approximately 16 times the normal maximum recommended dose level of 5mg/plate used in regulatory mutagenicity tests. In 1998 it was used as the vehicle for 18 test materials, which was approximately 5% of the total number of studies completed in that year. The mean, minimum and maximum frequencies of revertant colonies for the ethanol vehicle control plates were all comparable to the 1998 vehicle control history profile for all vehicle controls used at Safepharm Laboratories in 1998.
Reliability : (2) valid with restrictions

29.06.2004 (20)

Type : DNA damage and repair assay
System of testing : 343/636 (genotype uvrB+/recA+/lac-) and DNA repair deficient 343/591 (uvrB-/recA-/lac+)
Test concentration : Up to 1720 mmol/l
Cycotoxic concentr. : >1720 mmol/l
Metabolic activation : with and without
Result : negative
Method : other
Year : 1992
GLP : no data
Test substance : other TS

Method : Differential DNA repair test as described by Mohn, G.R. et al. (1984) Methodologies for the direct and animal mediated determination of various genetic effects in derivatives of strain 343/113 of E. coli K-12, in: B.J. Kilbey et al. (Eds.) Handbook of Mutagenicity Test Procedures, 2nd edn., Elsevier, Amsterdam, pp. 189 - 215.

For each concentration of test compound 100 ul of test compound or the solvent, 100 ul of bacterial mix and 500 ul S9 mix (where used) were made up to 1 ml with buffered saline. The mixture was incubated at 37 degC in the dark before seeding NR agar plates.

The relative survival of DNA repair deficient and proficient bacteria were calculated.

Solvent: not specified, but since ethanol was a solvent used for other compounds and a high concentration was used, it is likely no solvent was used.
Controls: The positive control was 4-nitroquinoline-N-oxide without S9 mix. No positive control was used for the S9 mix as this had been previously validated.
Statistical methods: confidence interval determined according to the variance of each strain, determined from an experiment with 100 untreated samples. A reduction in number of colonies by 2 standard deviations taken as significant.
Remark : This study was a screening test of 61 compounds, giving a mixture of positive and negative results.
Result : In both the absence and presence of S9 mix, the high dose of 1720 mmole/l ethanol gave a negative result in DNA repair deficient strain of E. coli.
Test substance : Test substance was of the highest purity obtainable from commercial sources.
Reliability : (2) valid with restrictions
Although conducted to a standard published method this paper does not present method detail in full. There was an overall concordance of 80% between this and the results from Ames tests on the 51 chemicals studied . The study is therefore considered to be valid with restrictions.
12.11.2004 (224)

Type : Bacterial forward mutation assay
System of testing : Escherichia coli RK+ (replicative killing competent strain CHY832)
Test concentration : 11 to 23% v/v
Cycotoxic concentr. : 17% v/v
Metabolic activation : without
Result : positive
Method : other
Year : 1985
GLP : no data

Test substance : other TS: see TS

Method : Test design: The test strain carries a lethal gene (RK+) that is repressed below 39 degC and derepressed above this temperature. After treatment with ethanol at 30 degC cells were plated and cultured at 42 degC to detect RK- mutants.

No. of replicates: 3 per concentration.
Frequency of dosing: exposure to ethanol for 10 min before plating.
Positive and negative controls: Negative controls were used.
No. of metaphases analyzed: Not relevant.
Solvent: with and without DMSO.
Evaluation criteria: Positive result when mutation index twice that of control.

Remark : The authors suggest that there is a threshold concentration below which ethanol is not genotoxic. This concentration appears to be the upper limit for cellular tolerance to ethanol.

Whilst positive, the massive concentration at which this result was seen can be extrapolated to conclude that the result would be negative at more conventional test concentrations.

Result : The 5 ethanol preparations showed similar dose-response curves for induction of RK- mutants with thresholds of 18-19% v/v. Addition of dimethylsulfoxide lowered the thresholds by around 5% to 13-15%.

Test-specific confounding factors: None.
Dose-effect related observations: All ethanol preparations induced RK- mutants with mutation indices of 2 or more. Steep dose-reponse curves showed threshold at 18-19% v/v.

Frequency of reversions etc: All preparations gave mutation indices of up to 50 at the highest dose tested.

Mitotic idex: Not relevant.

Test substance : Synthetic anhydrous 100%, synthetic 95%, 95% grain alcohol, 96.6% grain alcohol and dehydrated absolute 100% grain alcohol.

Conclusion : The positive result could be due to trace contaminants in ethanol, a bacterial metabolite, direct mutagenic effect of ethanol and indirect effect of ethanol.

Reliability : (2) valid with restrictions
12.11.2004 (225)

Type : Bacterial reverse mutation assay
System of testing : Escherichia coli
Test concentration : 140 or 180 mg/ml
Cycotoxic concentr. :
Metabolic activation : with and without
Result : positive
Method : other
Year : 1984
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : The escherichia coli selector strain CHY832 deleted for bio-uvr-chlA was used with and without S9 activation to examine the mutagenic potential of 48 environmental chemicals including ethanol. The study was run parallel with the McCann and Ames Mutatest.

Result : In the Mutatest, ethanol was negative for mutagenicity at 10000 microg/ml with S9. In the RK test, ethanol was positive for mutagenicity at 180000 microg/ml without S9.

Reliability : (4) not assignable
12.11.2004 (226)

Type : Chromosomal aberration test
System of testing : Human peripheral lymphocyte
Test concentration : 1% v/v
Cycotoxic concentr. : Not recorded
Metabolic activation : without
Result : negative
Method : other
Year : 1985
GLP : no data
Test substance : other TS: analytical grade

Method : No of replicates: 2 (as solvent control to two other substances).
Duration of treatment: 24 hours.
Number of metaphases analyzed: 100 or 200.
In vitro actication, chromosomal aberrations in blood cultures (without and with S9 mix delivered via an improvised dialysis bag); sister chromatid exchange and C-mitotic effects and polyploidies in blood cultures were studied.
Remark : All compounds produced C-mitoses, polyploidies and micronuclei, the latter interpreted as resulting from errors in the anaphase distribution of chromosomes by spindle disturbances rather than from structural chromosome aberration.
Result : Ethanol produced 3 aberrations in 100 metaphases in one study and 4 aberrations in 200 metaphases in another study.
Reliability : (2) valid with restrictions
This study appears to be well conducted with appropriate controls. The study is regarded as valid with restrictions.
Flag : Critical study for SIDS endpoint
12.11.2004 (227)

Type : Chromosomal aberration test
System of testing :
Test concentration :
Cycotoxic concentr. :
Metabolic activation : with and without
Result :
Method :
Year : 1997
GLP : yes
Test substance :

Remark : During the 1990's, ethanol was used as a vehicle in a sufficient number of studies at Safepharm Laboratories to demonstrate that it is not clastogenic to either human lymphocytes or to Chinese hamster lung cells (CHL). As in gene mutation assays, the dose volume of 1% (100ul/10ml) exceeds the maximum recommended dose levels suggested by the OECD test guideline. In the data for 1997, in both cell types, the mean values for ethanol controls were slightly higher than the overall control means but the maximum values were similar in all cases.
Reliability : (2) valid with restrictions
30.06.2004 (20)

Type : Chromosomal aberration test
System of testing : Chinese hamster ovary cell
Test concentration : 5%
Cycotoxic concentr. : >5%
Metabolic activation : no data
Result : negative

Method : other
Year : 1989
GLP : no data
Test substance : other TS

Method : This study examined the potentiating effect of ethanol on other clastogens in Chinese hamster ovary cells in vitro.

Plating rate: 3x10^5 cells per petri dish.
Number of replicates: not given.
Frequency of dosing: single dose for 3 hrs.
Positive controls: Methyl methanesulphonate, bleomycin, mitomycin.
Number of metaphases analysed: 100-200 per treatment.
Information cited on aberrant metaphases, chromatid breaks and exchanges, chromosome types (break or ring/dicentric).
Solvent: double distilled water.
Statistical methods: chi-square analysis to assess if effects between two treatments give statistically significant differences.

Result : Treatment with ethanol alone (5% for 3 hours) had no clastogenic activity as demonstrated in lack of induction of chromosome breaks and chromatid exchanges. Tabulated data is reported for 0 and 4% ethanol. Ethanol was found to potentiate the clastogenicity of known clastogens (those used as positive controls) with a clear dose response relationship.
Test substance : Absolute ethanol (ex Merck)
Reliability : (2) valid with restrictions
Key data that would be required to assess compliance with the OECD protocol are not reported in this study. However, it does appear to be otherwise reliable.

12.11.2004 (228)

Type : Cytogenetic assay
System of testing : human lymphoblastoid cells
Test concentration : 1% and 2%
Cycotoxic concentr. : Not stated
Metabolic activation : no data
Result : negative
Method : other
Year : 1992
GLP : no data
Test substance : no data

Method : This study involved ethanol (1% or 2%) alone as a control in an interaction study to evaluate the effect of ethanol on lobeline sulfate and bleomycin. Two cell lines evaluated, one derived from a female with multiple primary malignancies and the second from a patient with cutaneous melanoma.

Number of replicates: 3
Frequency of dosing: no data
Controls: negative and positive (bleomycin)
Number of metaphases analysed: 100.
Solvent: no data, presumed water.
Statistical method: student t test. Results quoted as number of chromatid breaks per cell, with comparison made with cultures with no treatment.

Result : Ethanol (1%) alone showed breakage rates not significantly different from controls.
Reliability : (4) not assignable
Study reasonably well reported. Study not designed primarily to assess clastogenicity of ethanol and some details, required to assess compliance with OECD protocol, are not reported. Appears to be reliable with restrictions.

12.11.2004 (229)

Type	:	Cytogenetic assay
System of testing	:	mouse embryo
Test concentration	:	22, 65, 220 and 650 mM plus control
Cycotoxic concentr.	:	
Metabolic activation	:	no data
Result	:	negative
Method	:	other
Year	:	1991
GLP	:	no data
Test substance	:	no data

Method : This study investigated whether acetaldehyde, the primary metabolite of ethanol, is responsible for evoking the observed embryotoxicity, embryolethality, chromosome-breaking activity and induction of sister chromatid exchange in mouse embryos in vitro.
4-Methylpyrazole was used to inhibit alcohol dehydrogenase.
It is shown that mouse oocytes as well as morulae and blastocysts are able to xidise ethanol in the presence of NAD+

Result : Embryotoxicity in pre-implantation embryos was due to acetaldehyde.

Reliability : (4) not assignable

12.11.2004 (230)

Type	:	Cytogenetic assay
System of testing	:	human lymphoid cells
Test concentration	:	2%, 4%, 6%, 8% and 10%
Cycotoxic concentr.	:	8% and 10%
Metabolic activation	:	no data
Result	:	positive
Method	:	other
Year	:	1991
GLP	:	no data
Test substance	:	no data

Method : This study was part of a cocarcinogen evaluation involving cigarette smoke condensates in vitro. Concentrations of ethanol were included as controls.

Result : Ethanol alone showed no demonstrable clastogenic activity as measured by the frequency of chromatid breaks per cell. At relatively high doses (below cytotoxic doses of 8% and 10%)
ethanol inhibited DNA and chromosome repair systems. At 4% there was pronounced uncoiling of chromatids and at 6% the uncoiling was difficult to identify mitotic figures.

Reliability : (4) not assignable

12.11.2004 (231)

Type	:	Cytogenetic assay
System of testing	:	Human lymphocytes
Test concentration	:	0.8 and 1% ethanol (equivalent to 6.31 and 7.89 mg/ml respectively)
Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	negative
Method	:	other
Year	:	1984
GLP	:	no data
Test substance	:	no data

Remark : Peripheral lymphocytes were exposed to ethanol for 24 hours and 100 metaphases were analyzed per treatment.

Result : There was no increase in either chromatid breaks or isochromatic lesions at 0.8 or 1% ethanol.
Reliability : (4) not assignable
12.11.2004 (232)

Type : Cytogenetic assay
System of testing : Human lymphocyte and Chinese Hamster Ovary cells
Test concentration : 0.5 to 10 mg/ml
Cycotoxic concentr. :
Metabolic activation : with and without
Result : ambiguous
Method : other
Year :
GLP : no data
Test substance : other TS

Method : Human lymphocytes and Chinese hamster ovary cells were grown for 12 to 48 hors in the presence of 0.5 to 10 mg/ml in the absence or presence of S9 liver homogenate.
Remark : This paper is only an abstract.
Result : CHO cells in SP metabolised ethanol to acetaldehyde. Acetaldehyde produced a dose-dependent increase in chromosome damage below 5 mg/ml in the same study.
Reliability : (4) not assignable
12.11.2004 (233)

Type : Cytogenetic assay
System of testing : Chinese hamster ovary cells
Test concentration : 160 mmol/l (equivalent to 7.37 mg/ml)
Cycotoxic concentr. :
Metabolic activation : without
Result : negative
Method : other
Year : 1987
GLP : no data
Test substance : no data

Remark : Cells were incubated with ethanol for 30 minutes at 30°C. 100 cells were scored for chromosome aberrations. Controls contained 3% DMSO.
Result : No increase in chromosome aberrations was seen in the absence of a metabolic activation system.

The S2 fraction from Zea mays induced chromosome aberrations (gaps, breaks and exchanges) when tested on the cells in the absence of ethanol. In the presence of ethanol and S2 fraction, the aberration rate was increased.
Reliability : (4) not assignable
12.11.2004 (234)

Type : Chromosomal aberration test
System of testing : human lymphocytes
Test concentration : 25, 150 and 500 mg/100 ml
Cycotoxic concentr. :
Metabolic activation : without
Result : negative
Method : other
Year : 1973
GLP : no data
Test substance : no data

Remark : Blood obtained fron 6 men and 4 women and serum was cultured in the

		presence of 25 mg, 150 mg or 500 mg/100 ml ethanol for 3 days. Cells from each culture were examined for chromosome gaps, breakages, re-arrangements and aneuploidy. Blastic transformation was studied by Thomas' method.
Result	:	Ethanol had no effect on chromosomes in vitro.
Reliability	:	(4) not assignable
12.11.2004		(235)

Type	:	Cytogenetic assay
System of testing	:	Human lymphocytes
Test concentration	:	1.16, 2.32, 3.48 mg/ml
Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	positive
Method	:	other
Year	:	1977
GLP	:	no data
Test substance	:	no data
Remark	:	A significant dose-related increase ($p < 0.05$) in chromosome aberrations (particularly chromatid and chromosome gaps and breaks) was seen at all dose levels. Cells from 5 donors incubated with ethanol for 50 hours. 100 metaphases/donor screened for chromosome aberrations.
Reliability	:	(4) not assignable
12.11.2004		(236)

Type	:	Mouse lymphoma assay
System of testing	:	Mouse lymphoma L5178Y cells, TK +/-
Test concentration	:	0.092, 0.184, 0.369, 0.553, 0.738 mol/l without activation; 0.414, 0.465 and 0.517 mol/l with activation
Cycotoxic concentr.	:	Maximum concentration with metabolic activation caused <10% fall in growth
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: Clive et al. (1979)
Year	:	1988
GLP	:	no data
Test substance	:	no data
Method	:	Test design: mouse lymphoma cell TK +/- forward mutation assay with and without metabolic activation. No. of replicates: 3 per dose level but 6 for negative control. Frequency of dosing: One 4 h exposure. Positive and negative controls: Negative (no ethanol) only. Number of metaphases analyzed: Not relevant. Solvent/vehicle: Not discussed. Follow up: Not relevant. Criteria for evaluating results: 2-fold or greater increase in mutation frequency at 10% or greater total growth cf. controls. Statistical test 2-tailed Student's t-test.
Remark	:	Results are supported by those of Amacher, D., et al. (1980) Mutat. Res. 72:447-474. Test specific confounding factors: None. Dose-effect related observations: No clear-cut dose-effect related observations were seen. Frequency of reversions etc.: Without activation, mutation index values from lowest to highest dose were 1.3, 1.1, 1.2,

1.1 and 1.6. With metabolic activation these values were 1.1, 1.3 and 1.8.

Mitotic index: Not strictly applicable. Total growth cf. controls were 88, 84, 53, 34 and 17% from lowest to highest concentrations in the absence of activation. With activation, total growth was 43, 24 and 6% from lowest to highest concentration.

Result : Only at the maximum concentration, with metabolic activation was total growth <10% control.

Without activation, the lowest and highest concentrations of ethanol produced statistically significant increases in mutation frequency.

See Remarks.

Conclusion : Ethanol is judged not to have significant mutagenic activity in this system.

Reliability : (2) valid with restrictions

12.11.2004 (237)

Type : Mouse lymphoma assay
System of testing : Mouse lymphoma L5178Y cells, TK +/-
Test concentration : Up to 7.79 x 10-1 M (equivalent to ca. 35.9 mg/ml)
Cycotoxic concentr. : More than 2%
Metabolic activation : without
Result : negative
Method : other
Year : 1980
GLP : no data
Test substance : other TS

Method : Cells determined free of mycoplasma before use. Stock cells treated weekly with THMG mixture to reduce spontaneous mutant levels.
No. of replicates: 3 Plates each and two controls
Frequency of dosing: Once.
Positive and negative controls: Positive controls were included. 10 Noncarcinogens and 13 putative animal carcinogens were tested.
Solvent used: none.
Criteria for evaluating results: Gene mutation at the thymidine kinase (TK) locus in trifluorothymidine-resistant L5178Y mouse lymphoma cells.

Cytotoxicity test: 6x10E5 cells/ml suspension, 5 log range of concentrations used as range finder. Estimated ID50 used as median dose for main study. Protocol: 3 hours treatment followed by cell washing. Cell counts at 24 and 48 hours.

Mutagenicity test protocol: As cytotoxicity test then split with cells resuspended in soft agar cloning medium, with or without trifluorothymidine.

Remark : Mouse lymphoma thymidine kinase assay, as described in Amacher, D.E. et al. (1979) Point mutations at the thymidenekinase locus in L5178Y mouse lymphoma cells. I. Application to genetic toxicology testing. Mutation Res., 64, 391 - 406.

Result :

Concentration	Cell survival	Mutants/10E4 survivors
0	100%	0.73
0.173	91%	0.69
0.26	82%	0.77

0.346	81%	0.81
0.433	75%	0.74
0.52	63%	0.92
6.06	52%	0.68
6.93	36%	0.60
7.79	3%	0.72

No increase in mutants at clearly cytoxic concentrations.

Test substance : Purity not specified
Reliability : (2) valid with restrictions
12.11.2004 (238)

Type : Mammalian cell gene mutation assay
System of testing : S49 mouse lymphoma cells
Test concentration : 1%
Cycotoxic concentr. :
Metabolic activation : with
Result : negative
Method : other
Year : 1983
GLP : no data
Test substance : no data

Method : This study evaluated ethanol as the solvent control in a study of the induction of dexamethasone, 6-thioguanine and ouabain resistance.

Materials:
Dulbecco's modified Eagle's medium with 4.5g glucose/l, heat inactivated horse serum and fetal calf serum, Bacto Agar, ICR 191 and ethanol ex Sigma. S49.1 ML cells from P Coffino, San Francisco, originally isolated by Horibata and Harris (1970).

Cells grown in stationary medium without antibiotics at 37C in a humidified CO2 incubator. Freshly cloned cultures frozen at -80C. Stock cultures frequently discarded and repaced with thawed frozen ones to prevent build up of mutants. Cells stained with trypan blue for counting.

Mutagenic treatment and selection:
4 hour treatment. Cells (6x10E6/dose) centrifuged and re-suspended in medium containing S9. Maximum solvent concentration 1%. Positive control used.

Criteria for positive result: survival >=40%, factor by which frequency elevated compared to control >3, frequency of 6-TG mutants elevated against controls.

Result : The dexamethasone resistance marker was induced at the highest frequency and was expressed within 3 days after mutagenesis. Ethanol had no effect on the mutagenesis of
this marker.

Surviving fraction: 100%
Mutant frequency: 0
Mean and standard deviation of control: 104 +/- 9
Elevation of mutant freq. compared to control: 0

Reliability : (2) valid with restrictions
12.11.2004 (239)

Type : Micronucleus test in vitro
System of testing : Male Chinese hamster lung fibroblast (V79) cells
Test concentration : 50 microlitre/ml
Cycotoxic concentr. :

Metabolic activation : without
Result : negative
Method : other
Year : 1984
GLP : no data
Test substance : other TS

Method : This study evaluated the micronucleus assay by comparison with sister chromatid exchange results for known mutagens/carcinogens. Ethanol, methanol, butanol and propanol were also examined.

Micronucleus induction was studied in vitro in cells treated with ethanol (50 microliter/ml) for 1 hour.
Result : No significant induction of micronuclei was evoked by ethanol in V79 Chinese hamsetr cells.
Test substance : Absolute ethanol.
Reliability : (4) not assignable
12.11.2004 (240)

Type : Sister chromatid exchange assay
System of testing : Chinese hamster ovary cells
Test concentration :
Cycotoxic concentr. :
Metabolic activation :
Result : negative
Method :
Year : 1977
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Methanol, ethanol (0.1% w/v), propanol, butanol and acetaldehyde (0.0005% and 0.001% w/v) were evaluated for effect on SCE in CHO cells in vitro.
Result : SCE in ethanol treated cells occurred at 4.83 SCE/mitosis versus 4.52 in controls.

In acetaldehyde treated cells, there were 13.56 SCE/mitosis at the lowest concentration and 28.25 SCE/mitosis at the highest concentration versus 4.69 SCE/mitosis in controls.
Conclusion : It is acetaldehyde rather than ethanol responsible for an increase in SCE in CHO cells.
Reliability : (4) not assignable
12.11.2004 (241)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Micronucleus assay
Species : rat
Sex : male
Strain : other: BD6
Route of admin. : drinking water
Exposure period : 10-30 days
Doses : 5% or 10%
Result : negative
Method : other
Year : 1993
GLP : no data
Test substance : other TS

Method : No of rats: 51 in 3 separate experiments.
Weight: 180-200 g
Diet and drinking water: standard rodent dioet; water ad libitum alone or with added ethanol.
Exposure period: 10-30 days.

Investigations: At end of exposure animals were killed and pulmonary alveolar macrophages and bone marrow erythroblasts were harvested. Both cytotoxic and cytogenetic effects were examined.
Remark : Assuming that rat drinking water consumption is 100ml/kg/day, 5% ethanol in drinking water would be equivalent to 5000mg/kg, well above the normal upper limit stated in OECD 474)
Result : No effect on micronucleus incidence was observed.

Polynucleated PAM were enhanced.

10% dose was cytotoxic to bone marrow
Test substance : 5% or 10% in drinking water as part of a co- clastogenicity study with tobacco smoke.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
12.11.2004 (242)

Type : Micronucleus assay
Species : rat
Sex : male
Strain : Wistar
Route of admin. : drinking water
Exposure period : 3 or 6 week
Doses : 10% or 20% ethanol in the drinking water given to one to four rats per dose and exposure period
Result :
Method : other
Year : 1980
GLP : no data
Test substance : other TS

Remark : Age of animals at start: Adult.
No. of animals per dose: 1, 2 or 4.
Dosage: 10% or 20% v/v
Vehicle Control: Tap water.
Duration of test: 3 or 6 weeks.
Frequency of treatment: Daily
Sampling: Hepatocytes, bone-marrow cells and blood lymphocytes for micronuclei, micronuclei in polychromatic erythrocytes and for sister chromatid exchanges and chromosomal aberrations.
Group sizes in this study small.
Result : Negative. Drinking ethanol did not affect the incidence of micronuclei in bone marrow cells or hepatocytes at either ofthe two dose levels.

Also, drinking ethanol did not affect the incidence of chromosome aberrations in bone marrow cells or cultured lymphocytes at either of the two dose levels. Frequencies of sister chromatid exchanges in blood lymphocytes are significantly enhanced in rats exposed to either dose and at the higher dose, ethanol increased the frequency of micronuclei and chromosomal aberration in polychromatic erythrocytes.
Reliability : (2) valid with restrictions
12.11.2004 (243)

Type : Micronucleus assay
Species : mouse

Sex : male
Strain : Swiss
Route of admin. : drinking water
Exposure period : 27 days
Doses : up to ca. 65 g/kg body weight/day
Result : negative
Method : other
Year : 1977
GLP : no data
Test substance : other TS: see TS

Method : Age at study start: 72-75 days.
No. of animals per dose: 3 in negative control, 5 in ethanol groups and 6 in positive control.
Vehicle: Water.
Duration of test: 27 days.
Frequency of treatment: Ethanol ad libitum; for positive control, ethyl methanesulfonate by injection 30 and 6 h before sacrifice.
sampling times: Sacrificed on 27th day and 4 slides of stained bone marrow taken from each mouse.
Controls: see above.
Parameters observed: Bodyweight
Organs/tissues at necropsy: Bone marrow smears only.
Criteria for evaluating results: An average of 4000 polychromatic erythrocytes and corresponding normochromic cells were counted for each animal. The % of cells with micronuclei and groups means were calculated.
Criteria for selecting MTD: Not discussed. 2 animals receiving 40% over the last 2 wk died.

Remark : Age of animals at start: 72-75 days
No. of animals per dose: 3 or 5
Dosage: Two groups of mice. Group 1 given 10% alcohol in the drinking water for 6 days, then 20% for 7 days followed by 30% for 14 days. Group 2 given 10% for 6 days, 30% for 7 days, then 40% for 14 days. Control group was untreated.
Duration of test: Total 26 days.
Controls: Ethyl methyl sulfonate opr dimethylsulfoxide.
Investigations: Bone marrow preparations were made and examined for polychromatic and normochromatic erythrocytes.
This investigation suffers from the limitation of a relatively short period of alcohol ingestion. However, these data were considered sufficiently reliable by US EPAS for inclusion in the GeneTox Program report and for this reason have been assigned a reliability score of 2.
Time weighted average concentrations of ethanol were 23% and 33%.
Actual intakes were not determined.

Result : 40% level in drinking water is equivalent to an intake of approximately 65 g/kg body weight/day.

The P/N ratio was not affected by ethanol but was significantly increased in the positive
(ethylmethylsulfonate) control. The incidence of micronuclei was significantly increased in the positive control group but not by ethanol.
Mortality at each dose level: 2 aniumals receiving 40% ethanol died possibly of dehydration. 2 positive control animals and 0 negative control animals died.
Mutations etc observed: The %PCE's with micronuclei in negative control, low dose, high dose and positive control groups were 0.37, 0.26, 0.24 and 0.88 respectively.
Clinical signs: Not discussed.
Body weight changes: Not affected by treatment.
Food/water consumption: Not discussed.

Test substance : Test substance: "distilled ethanol".

Reliability : (2) valid with restrictions
12.11.2004 (244)

Type : Cytogenetic assay
Species : rat
Sex : male
Strain : other: CD
Route of admin. : oral feed
Exposure period : 6 weeks
Doses : 36% of dietary energy
Result : positive
Method : other
Year : 1981
GLP : no data
Test substance : other TS

Method : Age of animals at start: Weanling fed until 130-150 g weight.
No. of animals per dose: 16 males per pair-fed group
Rat strains: CD
Dosage: 12 to 16 g/kg bodyweight/day representing 36% of total energy intake.
Duration of test: 6 weeks
Frequency of treatment: Fed ad libitum in pair fed groups with group fed diet without alcohol.
Controls: Untreated only.

Examinations: Blood samples examined for frequency of micronuclei, polychmomasia, orthochromasia.
Blood ethanol concentrations were measured at 9am by tail tip excision.
Statistics: student t test.

Remark : Dose of 6g/kg given by gavage the day before sacrifice produced no change in bone marrow cell population, mitotic index or percentage of cells with micronuclei.

In the authors' opinion, the decreased proportion of nucleated cells most likely reflects hypoplasia, conceivably from cytogenetic damage of stem cells.

Result : Blood ethanol concentrations 149mg/100ml +/- 20mg.

Despite pair feeding equal consumption of calories, ethanol fed animals had significantly lower bodyweight.

Ethanol treatment significantly ($P<0.001$) decreased the number of nucleated cells per rat relative to pair-fed controls (4929 ± 774 versus 7996 ± 708) and significantly ($P<0.05$) increased the percentage of nucleated cells undergoing mitosis to 2.43 ± 0.47 from 1.48 ± 0.38.

Ethanol significantly ($P<0.001$) increased the number of erythrocytes per rat from 4789 ± 525 to 7595 ± 390 with significant increases in both polychromatic and orthochromatic components. The number of erythrocytes with micronuclei, per rat, was increased significantly ($P<0.01$) from 35 ± 7 to 71 ± 12 of which both polychromatic and orthochromatic components were equally affected. This was also reflected in the percentage of erythrocytes with micronuclei although significant only with respect to polychromatics.

Overall, the percentage of erythrocytes with micronuclei increased from 0.74 ± 0.13 ti 0.94 ± 0.16 ($p<0.03$) with the PCEs increaseing from 0.95 ± 0.13 to 1.30 ± 0.20 ($p<0.05$) and the OCEs increasing from 0.67 ± 0.12 to 0.84 ± 0.16 (not significant.)

Reliability : (2) valid with restrictions

12.11.2004 (245)

Type : Micronucleus assay
Species : mouse
Sex : male/female
Strain : other
Route of admin. : i.p.
Exposure period : 2 day
Doses : 0.3 ml of 20, 40 or 60% ethanol injected into groups of 4 males and 4 females
Result :
Method : other
Year : 1977
GLP : no data
Test substance : no data

Remark : Age at start of treatment: 10 weeks.
Strain: Parkes.
Dosages: Equivalent to 0.62, 1.24 and 1.86 g/kg body weight.
Route of administration: i.p.
Period of treatment: 2 consecutive days.
Controls: no treatment.
Examinations: Femur bone marrow was collected 6 hours after the last dose and erythrocytes were examined for micronuclei and chromatin masses.
Result : Positive. Statistically significant increases ($p < 0.05$) in the occurrence of micronuclei were seen at 0.62 and 1.24 g/kg body weight. At 1.86 g/kg body weight, the occurrence was increased, but statistical significance was not attained. No sex difference was evident.
Reliability : (3) invalid
The data show several inconsistencies that indicate that they may not be valid. The numbers of observed micronuclei are presented in both tabular and graphical form but the data appear to be inconsistent between the two forms of presentation. Also, the tabulated data report a control group mean micronucleus frequency of 4.63% in NCEs and 5.6% in PCEs. These values are 20-50 times the typical frequency seen in laboratory mice used in standard regulatory tests. This difference indicates that either the mice used in this study were grossly abnormal in this respect or that there may have been a problem with the technical quality of the slides or the slide scoring method used. The data also indicate similar frequencies of micronuclei in NCEs and PCEs. This cannot have occurred as a result of exposure to ethanol because the kinetics of the development of NCEs from PCEs does not allow for the observation of equivalent numbers of micronucleated erythrocytes of the two types at the single time-point of 30 hours that was used. Because of the apparent errors in this report, and the deviations from internationally recognized procedures, it is considered that a reliable conclusion cannot be drawn from this data.
12.11.2004 (246)

Type : Cytogenetic assay
Species : Chinese hamster
Sex : male/female
Strain :
Route of admin. : oral feed
Exposure period : 9 weeks
Doses : 10% v/v
Result : negative
Method : other
Year : 1979
GLP : no data

Test substance : no data

Method : Age at start of study:10-20 weeks.
Dosages: Ethanol (10%) in liquid feed (11 animals); salt water control (36 animals), cyclophosphamide (6 animals), ethanol + cyclophosphamide (8 animals); patulin (6 animals); ethanol + patulin (7 animals); aflatoxin B (10 animals) and ethanol + aflatoxin B (7 animals).

Investigation: Bone marrow examined after 9 weeks for chromatid breaks, isochromatid breaks, chromatid translations and mitosis with multiple aberrations.
Result : Ethanol alone had no effect on bone marrow chromosomes in either sex.
Reliability : (2) valid with restrictions
12.11.2004 (247)

Type : Cytogenetic assay
Species : hamster
Sex : male/female
Strain : other
Route of admin. : drinking water
Exposure period : 12 week
Doses : 10% in the drinking water during week 1, 15% in weeks 2 - 3, 20% in weeks 4 - 12.
Result : negative
Method : other
Year : 1981
GLP : no data
Test substance : other TS: Absolute, extra pure

Remark : Age at study start: 10-20 weeks.
No. animals/dose: Ethanol treated 8 females, 9 males.
Controls 9 females, 7 males.
Vehicle: Water.
Duration of test: 12 weeks.
Frequency of treatment: Drinking water ad libitum. Controls received plain water.
Diet: (Altromin 7024)
Dosage: 10% v/v in the first week; 15% during the second and third week and 20% from the 4th to the 12th week. Fluid intake approximately 5.2 ml/hamster/day. Maximum intake therefore up to 26 g/kg body weight/day in males and 33 g/kg body weight/day in females.

Investigations: Some animals in each group were exposed to cigarette smoke during the last 4 weeks. Bone marrow was examined for chromosomal aberrations.
Ethanol also failed to induce chromosome aberrations in bone marrow cells in a similar study by the same group in which hamsters were given 10% v/v ethanol in the diet for 9 weeks (Korte, A. et al. (1979). The influence of ethanol treatment on cytogenetic effects in bone marrow cells of Chinese hamsters by cyclophosphamide, aflatoxin B1 and patulin. Toxicology 12, 53-61.
Result : 2.3% aberrant metaphases detected in controls, 3.7 % in treated animals. No sex difference evident. Mitotic index significantly elevated in smoke treated group (P <0.001) but not in ethanol controls.
Reliability : (2) valid with restrictions
12.11.2004 (248)

Type : Cytogenetic assay
Species : hamster
Sex : male/female

Strain : other
Route of admin. : drinking water
Exposure period : 46 week
Doses : 10 % v/v ethanol in the drinking water given to 5 females and 2 males
Result : negative
Method : other
Year : 1981
GLP : no data
Test substance : other TS

Method : Age at study start: 15 mth. Animals housed individually.
No. animals/dose: Controls 3 males, 2 females. Ethanol 2 males, 5 females.
Vehicle: Water.
Doses were given as 10% v/v (180 g/kg/day).
Duration of test: 46 wk.
Frequency of treatment: Drinking water ad libitum. Controls received plain water.
Sampling: Blood taken in the 47th week. Two samples per animal analyzed.
Clinical observations: None.
Organs examined at necropsy: None.
Criteria for examining results: Chromosomal aberrations in lymphocytes included chromatid breaks, isochromatid breaks and chromatid translocations. An aberrant metaphase cell contained at least one aberration.
Criteria for selecting MTD: None.
Statistics: Chi square test.
Remark : Group size in this study was small and ingested dose was uncertain. Hamsters are reported to have ingested about 1.4 ml/g body weight/week (157g/kg body weight/week). This figure may have been erroneous as the hamsters appear to have consumed ca. 45 ml fluid/week, corresponding to an approximate intake of 17 g ethanol/kg body weight/day.
Result : The rate of aberrant metaphases was higher in the ethanol-treated group than in the control group (10.8 versus 7.7%) but the difference was not statistically significant ($p > 0.25$).

Mortality at each dose level: None.
Clinical signs: None described.
Body weight changes: Did not change significantly.
Food/water consumption changes: Animals consuming ethanol ate 30% less food than controls.
Test substance : Test substance was ethanol absolute, extra pure, Merck.
Reliability : (2) valid with restrictions
12.11.2004 (249)

Type : Dominant lethal assay
Species : mouse
Sex : male
Strain : other
Route of admin. : gavage
Exposure period : 5 day
Doses : 10 or 40% ethanol in water (dose volume 2 ml/kg) to groups of 15 mice
Result : ambiguous
Method : other
Year : 1982
GLP : no data
Test substance : no data

Remark : Age at start of treatment: 10-12 weeks

Strain: CFPL or Alderly Park.
Treatment: Mice dosed with ethanol in distilled water on 5 consecutive days.
Dosages: equivalent to 0.25 of the MTD (10% ethanol) and the MTD (40% ethanol). Actual doses administered were 0.16 and 0.63 g/kg body weight/day by oral gavage.
Controls: treated with distilled water only.
Mating: Immediately after completion of dose schedule, each male was caged sequentially with 2 undosed females each week for 8 consecutive weeks. All females were killed and examined 18 days after first being caged with males.
Implantation sites and dead implants/female were recorded.
Replicates: In 3 different laboratories.

Result : No effect on pregnancy rate. Occasional positive results with regard to preimplantation loss during weeks 7 and 8 (reduction in the number of implants/male). It was suggested that in most cases this was due to a lower number of implants in the corresponding control groups. There were also occasional increases in the number of postimplantation deaths/male although the majority of the post implantation results were not significant.

Conclusion : Ethanol is unlikely to be a dominant lethal mutagen, at least up to the maximum tolerated dose.

Reliability : (1) valid without restriction
This is a highly reliable study that was well reported and compliant with OECD protocols

12.11.2004 (250)

Type : Dominant lethal assay
Species : mouse
Sex : male/female
Strain : Swiss
Route of admin. : other: i.p. (acute) and drinking water (chronic)
Exposure period : 3 days (acute) and 11 weeks (chronic)
Doses : 1.26 g/kg/day and 1.04 g/mouse/day
Result : positive
Method : other
Year : 1994
GLP : no data
Test substance : no data

Method : Age at study start: 12-16 weeks (25-30 g).
Strains: Inbred Swiss, C57Bl6 and CBA
No. animals/dose: Controls 3 males, 2 females. Ethanol 2 males, 5 females.
Dosage: 0.1 ml 40% alcohol i.p. (acute study). 5% in drinking water increased by 5% every week to 40% and then at 40% for 4 weeks.
Equivalent dose 0.13 g/mouse/day at 5%; 1.04 g/mouse/day at 40%.
Period of treatment: 3 days (acute), 11 weeks (chronic).
Vehicle: Water.
Replicates: 2 or 3
Mating: 4-day schedule post last treatment.
Investigations: Uterine contents, deciduomas, post-implantation losses.

Remark : This study was designed to reproduce the results of Badr using i.p. injection rather than intubation, but it was unable to do. The authors concluded that ethanol did not have a significant dominant lethal effect but caused some pre-implantation loss, which might be due to an effect on the fertilization capacity of sperm.

Result : In Swiss mice, the mutagenic index based on both pre- and post-implantation lethality was consistently positive.

There was a marked reduction (34% and 30%) in the number of pregnant females at the first two mating times in the treated group and a significant

decrease in total and live implants in the second mating. There was no increase in dead implants from the first two matings and only a small increase at the third mating (P<0.05).

Reliability : (2) valid with restrictions
12.11.2004 (251)

Type : Dominant lethal assay
Species : mouse
Sex : male/female
Strain : C3H
Route of admin. : oral feed
Exposure period : 4 weeks in males, then mated with untreated females
Doses : 0%, 20% and 30% of ethanol derived calories
Result : negative
Method : other
Year : 1982
GLP : no data
Test substance : other TS: 95%

Method : Age at start of treatment: 10 weeks.
Strain: C3H/HE mice.
Feeding: ad libitum.
Environment: temperture and humidity controlled constant with diurnal daylight/dark rhythm.
Dosage: 0%, 20% or 30% of isocaloric diet made up of ethanol-derived calories.
Replicates: pair-fed regimen.
Sampling: Weekly blood for blood alcohol concentration.
Investigations; Implantation sites, dead, resorptions and live foetuses counted.
Statistical analysis: Undernutrition and gender factors considered.

Result : No differences were found between the litters of alcohol-treated males and controls in terms of number of implantation sites, prenatal mortality, foetal weight, sex ratio or frequency of soft tissue malformations.

Conclusion : Paternal alcohol consumption does not grossly alter foetal growth and development in C3H mice.

Reliability : (2) valid with restrictions
12.11.2004 (252)

Type : Dominant lethal assay
Species : mouse
Sex : male/female
Strain : other: CF1
Route of admin. : oral feed
Exposure period : 5 weeks
Doses : 5% v/v liquid diet (28% ethanol-derived calories)
Result : positive
Method : other
Year : 1991
GLP : no data
Test substance : other TS: 95% USP

Remark : Age of animals at start: 8-9 weeks; 30.1 g
No. of animals per dose: 10 per group
Dosage: 5% v/v in liquid diet representing 28% of total energy intake.
Duration of test: 5 weeks
Frequency of treatment: Fed ad libitum in pair fed groups with group fed diet with alcohol replaced by sucrose.
Mating: 3 Females were housed with each male and examined daily for presence of vaginal plug to a maximum 6 days.
Examinations: Tail blood samples examined for haematocrit.

Females were housed until day 14 when ovaries and uteri were scored for dominant lethal mutations. A mutation index (MI) was calculated: MI/100 = (no of corpora lutea + dead foetuses - total foetuses)/no of corpora lutea. This study was part of a co-mutagencity study involving delta9-tetrahydrocannabinol and Trenimon.

Result : Ethanol caused minimal impairment of fertility at this dosage, but increased the frequency of dominant lethal mutations.

Reliability : (2) valid with restrictions

12.11.2004 (253)

Type : Dominant lethal assay
Species : rat
Sex : male
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 5 week
Doses : 6% ethanol in the diet for 1 week, then 10% given to 6 rats
Result : positive
Method : other
Year : 1976
GLP : no data
Test substance : other TS

Method : Animals: weight at start of treatment: 318 g (males); 259 g (females). No of animals: 12 males; 25 females.
Environment: Individually housed, 22 degC, RH 45% +/- 10 with diurnal light cycle (nocturnal 20:00 to 8:00hrs.)
Dosage: Six treated rats were given a 6% v/v ethanol-containing liquid diet (providing 35% of calories.) This was increased to 10% v/v after 7 days exposure (58% of dietary calories.
Controls: Six controls given an isocaloric amount of sucrose.
Diet 'Metrecal' (chocolate or vanilla.)
Duration of treatment: 15 days (males). females on lab chow and water.
Investigations: After treatment each male was placed in a cage with 2 females every night. No food or water was provided during this time. The experiment was continued for 5 weeks, with the males still receiving alcohol during the day. The males were killed on day 36. Blood samples were collected.
Pregnancies were terminated on day 20 of gestation and litter size and foetal mortality was assessed.

Result : Treated males showed signs of intoxication and considerable weight gain compared to controls. Treated animals were much less succesful at mating; the numbers of successful matings were 6/12 in the treated group and 13/13 in the controls. The number of offspring/litter was greater in the controls ($p < 0.01$). A higher incidence of early resorption was seen in the treated group ($p < 0.01$).

From graphical data presented in the reference, it is possible to estimate ethanol consumption as being in the range 7.2 to 14.4 g/kg/day.

Test substance : Test substance was 95% v/v ethanol.

Reliability : (2) valid with restrictions
Only six pregnancies examined in treatment group and males also chronically treated with ethanol such that the quality of the study is reduced.

12.11.2004 (254)

Type : Dominant lethal assay
Species : rat
Sex : male
Strain : Long-Evans
Route of admin. : drinking water

Exposure period	:	60 day
Doses	:	20% v/v ethanol solution given to 10 rats
Result	:	positive
Method	:	other
Year	:	1982
GLP	:	no data
Test substance	:	other TS

Method : Age at Study start: Not stated. Animals 200-300 g and were acclimated for 2 wk before mating.
No of animals/group: 10.
Dosage: Level of alcohol in the drinking water equivalent to a daily dose of 15.7 g/kg body weight.
Vehicle: Distilled water.
Duration of Test: Males treated for 60 days then mated with 3 females over three weeks.
Frequency of treatment: Ad libitum for 60 days.
Sampling: Testicular tissue examined after the third mating.
Uterine contents examined on gestation day 20.
Controls: Untreated males.
Clinical observations: male bodyweights before and after 60 day exposure and at sacrifice.
Histopathology: Testicular tissue.
Criteria for evaluation: Dominant lethal index calculated as 100%x(1-litter size in treated group/litter size in control group).
Criteria for selection of MTD: Not discussed.

Remark : Diluted to 20% v/v in distilled water.

Result : Both the number of resorptions and the percentage of litters with resorptions were increased. The index of dominant lethal mutations declined from 16.4 to 7.8 over three successive matings.

Absolute and bodyweight relative testicular weights were decreased by ethanol treatment (20%) and seminiferous tubule diameters were decreased together with an increase in the number containing cellular debris.

Mortality at each dose: None.
Mutations etc. Not relevant.
Clinical signs: No adverse signs were observed.
Body weights: Male bodyweights were unaffected by ethanol treatment.
Food/water consumption: Not presented.

Test substance : Test substance was USP alcohol, 200 proof.

Reliability : (2) valid with restrictions

12.11.2004 (255)

Type	:	Dominant lethal assay
Species	:	rat
Sex	:	male
Strain	:	Wistar
Route of admin.	:	drinking water
Exposure period	:	up to 35 days
Doses	:	up to 30% alcohol in the drinking water given to an unspecified number of rats
Result	:	negative
Method	:	other
Year	:	1980
GLP	:	no data
Test substance	:	no data

Remark : Age at start of treatment: 6-7 weeks.

Treatment: Three groups of rats (numbers unspecified) were treated as follows:
group I, 30% ethanol for 4 days; group II, 15% ethanol for 5 days, then 20% for 30 days; group III, 15% for 5 days, 20% for a further 5 days, 25% for 10 days and then 30% ethanol for the final 15 days. One control group was untreated, while a positive control group was exposed to x-rays (200 R) prior to mating. After treatment, each male was paired with 2-3 females per week for 8 consecutive weeks. The females were killed 10 - 11 days after removal from the males and examined for live and dead implantations.
Investigations: Dead implantations, reduction of live implantations and total implantations were enumerated.

Result : There were no significant differences in the numbers of dead, live and total implantations at the pre-or postimplantation levels in the control (untreated) or ethanolic groups. In the positive control group there was a high incidence of dead implants and a reduction in the number of live implants. The pregnancy rate was lower in group II, but this was not thought to be treatment-related, as the effect was not seen at 30%.

Reliability : (2) valid with restrictions

12.11.2004 (256)

Type : Dominant lethal assay
Species : mouse
Sex : female
Strain : no data
Route of admin. : oral unspecified
Exposure period : single dose
Doses : 5 ml/kg body weight given to 31 mice
Result : negative
Method : other
Year : 1975
GLP : no data
Test substance : no data

Remark : Females in pro-oestrus given a single dose of ethanol and mated on same day (2 females to 1 male). Pregnant females then dissected. Control group of 33 mice.
Route of administration: presumably gavage.

Result : Ethanol produced no dominant lethal effects at a daily dosage of 5 ml/kg p.o.

Reliability : (4) not assignable

12.11.2004 (257)

Type : Dominant lethal assay
Species : mouse
Sex : female
Strain : C3H
Route of admin. : gavage
Exposure period : single dose 1, 1.5 or 2 hr after mating
Doses : 1 ml of 12.5% ethanol or distilled water
Result :
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Age at study start:10-12 weeks.
Strain: (C3H x C58BL)F1
No. animals/dose: 47 ethanol treated vs. 43 controls mated at 1 hr; 24 ethanol treated vs. 24 controls mated at 1.5 hr; Three further experiments involved mating 1 hr (29 vs.

25 controls) or 2 hours (118 vs. 110) after ethanol treatment, with uterine analysis.
Vehicle: Distilled water.
Duration of test: 17 days
Investigations: Number of implantations per pregnant female, number of living embryos per pregnant female and % dead implants.
Sampling: Eggs taken and examined microscopically; first-cleavage embryos taken for chomosome analysis.
Clinical observations: None.
Organs examined at necropsy: None.

Result : Treatment with ethanol at 1 or 1.5 hr after mating did not affect the number of implantations per pregnant female, the number of living embryos per pregnant female and the % dead implants. However, the pooled data (118 ethanol-treated vs. 110 controls) for rats treated at 2 hr post mating showed a significant increase in late (post 11 days) deaths (P = 0.002).

Ethanol treatment was associated with a higher number of abnormal cells (P=0.039), ie trisomy possibly the result of clastogenicity or lagging of chromosomes in M-II.

Reliability : (4) not assignable
12.11.2004 (258)

Type : Dominant lethal assay
Species : mouse
Sex : male
Strain : CBA
Route of admin. : gavage
Exposure period : 3 day
Doses : 0.1 ml of 40 or 60% ethanol (1.24 and 1.88 g/g bodyweight)
Result : positive
Method : other
Year : 1975
GLP : no data
Test substance : no data

Remark : Age of animals at start: About 7-10 weeks.
No. of animals per dose: 13 at lowest dose; 6 at the highest dose.
Mouse strains: CBA/Fa Cam and CBA/Jackson
Dosage: 0.1 ml 40% ethanol solution (1.24 g/g bodyweight) daily by oral gavage, both strains; and 0.1 ml 60% (1.88 g/g) in CBA/J
Vehicle: Distilled water.
Duration of test: Mated with untreaed females about every 4 days for 7 weeks.
Frequency of treatment: Gavaged once daily on 3 consecutive days.
Controls: Untreated only.
Replicates: 2 in CBA/Jackson mice
Mating: Each mouse was mated to 2 females and to further females after 3 days,and then new females 7 more times at 4-day intervals.
Sampling: Pregnant females were sacrificed 13-15 days after conception.
Pregnant CBA/Fa females were allowed to produce their first litters, and the size and sex ratios were recorded. Young were examined for abnormalities.
Pregnant CBA/Jackson females were killed and dissected 13 - 15 days after conception. Dead and live implants were recorded.
Doses of ethanol administered equivalent to 1.24 and 1.88 g/kg body weight.

Identical results are reported in another paper (Badr, F.M. et al. (1977) Adv. exp. Med. Biol. 85A, 25 - 46), but the number of mice exposed to 40% ethanol is given as 25 and there are said to be 20 controls.

Result : Dead implants increased and live implants decreased significantly (P<0.01) compared to controls. The dominant lethal mutation index increased to a maximum of 46% in low
dose ltters and 67% in high dose litters.

It was evident that late spermatids were most affected by ethanol treatment.

Reliability : (3) invalid
No examination of the uterine contents was undertaken yet this was taken as evidence of post-implantation loss due to dominant lethal mutations. The method that was used to calculate the dominant lethal index is only appropriate for very potent mutagens. The study did not take into account the fact that genetic effects can result in a reduction in mating frequency, fertilization frequency or implantation frequency. This study cannot be considered reliable.

12.11.2004 (259)

5.7 CARCINOGENICITY

Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : drinking water
Exposure period : 179 weeks
Frequency of treatm. : ad libitum
Post exposure period : none
Doses : 0, 10% in drinking water
Result :
Control group : yes, concurrent vehicle
Method : other
Year : 1986
GLP : yes
Test substance : other TS

Method : Drinking water: tap water. Replace daily after measuring consumed volume.
Animals: Treatment started at 39 weeks old (breeders), 7 days before mating or from embryo life (offspring) and continued until death. 100 animals per dose level.
Animals housed in groups of 5, markrolon cages, stainless steel wire top, white wood shavings for bedding.
Temperature: 23+/-2C, relative humidity 50-60%
Animals weighed weekly for 13 weeks, then biweekly to 104 weeks then every 8 weeks. Status and behaviour recorded 3x daily and clinical examination every 2 weeks.
Tissue necropsy: Skin and subcutaneous tissue, brain, pituitary gland, Zymbal glands, parotid glands, submaxilliary glands, Harderian glands, cranium (oral/nasal cavities, ear ducts), 5 head sections, tongue, thyroid, parathyroid, pharynx, larynx, thymus, mediastinal lymph nodes, trachea, lung, mainstem bronchi, heart, diaphragm, liver, spleen, pancreas, kidneys, adrenal glands, esophagus, stomach (fore/glandular), intestine (4 levels), bladder, prostate, gonads, interscapular fat pad, subcutaneous and mesenteric lymph nodes and any other organs with visible lesions. All slides examined by the same group of pathologists
Statistical analysis: chi-squared test.

Result : Intake of food and water was reduced but there was no significant difference in bodyweight or survival, except females in the 104-152 week age range showed higher mortality. No effects were visible from gross inspection.

Increased tumour incidences reported.

total malignant tumours (M+F)
Total malignant mammary tumours (F)
Head and neck carcinomas, especially of oral cavity, lips and tongue (M+F)
Squamous cell carcinoma of forestomach (M+F)
Interstitial adenomas of the testes (M)
Sertoli cell tumours (ovary) (F)
Adenocarcinoma of uterus
Pheochromoblastoma (M+F)
Osteosarcoma of the head and other sites (M+F)

Test substance : ethanol >99.8% supplied by Carlo Erba, Milan. Material replaced every 3 months.

Reliability : (4) not assignable
No detail other than histopathology reported. Animals were allowed to live out their natural lives. No mortality or historical incidence data available. Statistical analysis and basis of conclusions unclear. Study is severly limited by the single high dose used with no intermediate concentrations.

18.11.2004 (260)

Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : drinking water
Exposure period : 2 years
Frequency of treatm. : ad libitum daily
Post exposure period :
Doses : 3300 mg/day (males) 80 and 5000 mg/day (females) approx.
Result : ambiguous
Control group : yes, concurrent vehicle
Method : EPA OPPTS 870.4300
Year : 2002
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : Groups of 48 male and 48 female mice were exposed to 2.5% or 5% ethanol in drinking water ad libitum for 2 years. This study was designed and conducted to determine the long-term toxicity and carcinogenicity of urethane in ethanol with control groups consuming drinking water alone or containing ethanol as specified above.

Draft report issued 2002.

Result : Ethanol caused a marginal exposure-related increase in survival in males but had no effect on the survival of females. There was evidence of an ethanol-induced reduction in water consumption that was more marked in males than in females.

There was equivocal evidence of carcinogenic activity of ethanol in MALE based on increased incidences of hepatocellular neoplasms.

There was no evidence of carcinogenic activity of ethanol in FEMALE mice exposed to either concentration of ethanol.

Overall, the findings were insufficient to establish a definitive effect of ethanol on the carcinogenicity of urethane in this strain of mouse.

Reliability : (2) valid with restrictions
Well reported and reliable study from a reputable toxicology laboratory. However, only a single and very high dose used limit ultimate value, hence

only reliable with restrictions.
12.11.2004 (261)

Species :
Sex :
Strain :
Route of admin. :
Exposure period :
Frequency of treatm. :
Post exposure period :
Doses :
Result :
Control group :
Method :
Year : 1999
GLP :
Test substance :

Remark : Ethanol or the metabolites may be important factors for the human carcinogenic effect of alcoholic drinks. This was concluded as a result of the observation that all kinds of alcoholic drinks, if taken in sufficient quantity, can lead to an increased incidence of certain types of tumour (Blot 1992; IARC 1988). The mechanisms of ethanol-induced carcinogenisis have not been clearly settled. Carcinogenisis of the mouth, throat, larynx and possibly also gullet, as a result of the local effects of the ethanol, on the one hand, must be distinguished from the carcinogenic effects in the liver and breast, which arise from the systemic availability of ethanol or its metabolites, on the other. A local mechanism, among others, has been postulated because the frequency of cancer in the mouth and throat was increased in persons who used, but as a rule did not swallow, mouth washes with an ethanol content of at least 25% (Blot 1992). Mechanistically, because of its physical-chemical properties, ethanol could change the barrier function of the cell membrane thereby making the penetration of carcinogenic substances into the cell easier. This hypothesis is supported by the synergistic effect of ethanol and tobacco smoke, with its many carcinogenic constituents, in relation to a carcinogenic effect in the region of the mouth and throat. In addition, the influence of ethanol on the expression of enzymes that metabolise particular foreign substances, from which an increased activation of the carcinogens might result, could be responsible (Blot 1992; IARC 1988). An increase in the incidence of tumours induced by various carcinogens when ethanol is administered simultaneously has been demonstrated in several animal experiments. For example, ethanol increases the carcinogenic effect of certain nitrosomines in the upper gastrointestinal and respiratory tracts or the vinyl-chloride-induced hepatocarcinogenisis (Anderson et al. 1995; Blot 1992; IARC 1988; Mufti et al. 1997; Seitz et al. 1992).

To date, it has not been convincingly shown that ethanol acts as a complete liver carcinogen. It is possible that the formation of liver cirrhosis, which is as a precancerous lesion independent of the initiating factors, plays a causal role. The induction of cytochrome-P450-2E1 and the resulting increased formation of reactive oxygen species, and also the increased lipid peroxidation, has been assigned an important role in alcohol-induced liver damage (Brooks 1997). A modulating effect of ethanol on hepatocellular carcinogenisis by other initiating factors, such as infection with hepatitis B or C viruses, has also been discussed. Further, the induction of various enzymes which metabolise foreign substances and the changing of the membrane properties of the endoplasmic reticulum and the cell by ethanol could alter the toxicokinetics and bio-availability of particular carcinogens or pre-carcinogens and thus promote the formation of hepatocellular or extrahepatic carcinomas (Anderson et al. 1995; Farber

1996; Seitz et al. 1992).

The increased rate of breast cancer which has been linked to the consumption of alcoholic drinks, may possibly result from the demonstrated influence of ethanol upon the hormone system. However, the in-vitro animal investigations available at present offer no conclusive mechanistic explanation for the epidemiological findings (Blot 1992; Longnecker 1995; Singletary 1997). In how far the weak genotoxic properties of ethanol or acetaldehyde and the reactive oxygen species formed during ethanol metabolism are involved in the carcinogenisis has not been clarified. With alcoholics and persons with a genetically determined reduced ALDH activity, at least, increased acetaldehyde concentrations have been found in the peripheral venous blood (Eriksson and Fukunaga 1993). Indications as to the critical role of acetaldehyde in the aetiology of tumours in the upper digestive tract caused by ethanol have been observed in Japanese with the inactive ALDH2 genotype which leads to increased acetaldehyde concentrations after consumption of ethanol: 40 alcohol-dependent and 29 non-dependent patients with squamous cell carcinoma of the oesophagus and groups of 55 and 28 control persons respectively were examined with respect to their ALDH2 genotype. In the case of the alcoholics, and also the non-alcoholics with an ALDH*2~ allelomorph which codes for an inactive ALDH2, a clearly increased risk of gullet cancer was found. The OR was 7.6 95% -CI; 2.8 - 20.7) for alcoholics and 12.1(95%-CI; 3.4-42.8) for non-alcoholics. The alcoholics consumed ca. 120 g ethanol per day, the non-alcoholics ca. 55g. Perturbing factors such as smoking and diet could not be taken into account because of the small size of the groups (Yokoyama et al. 1996a). Supporting this, a relationship between the frequency of primary tumours in the oesophagus and the ALDI-12 genotype was observed in 33 Japanese male alcoholics with squamous cell carcinoma of the oesophagus. Of 17 patients with the genotype for the inactive ALDH2, 13 had multiple primary tumours as opposed to only S from 16 with the active ALDH2. The difference was statistically significant (p '~ 0.01). The age and drinking and smoking habits of the patients with single and multiple carcinomas were not a distinguishing feature. The prevalence of further tumours in the upper regions of the respiratory passages and digestive tract was also higher; 29.4% for the patients with inactive ALDH2 as
compared with 6.3% of the patients with active ALDH2. Since the cells of the oesophagus epithelium show no ALDH2
activity, the authors assume that the observed differences between the patients with active and those with inactive ALDH2 are the result of an increased systemic availability of acetaldehyde in the latter group (Yokoyama et al. I996).
Investigations of a possible carcinogenic effect due to occupational exposure to ethanol inhalation are not available to date.

It can be proved that the consumption of alcoholic drinks leads to an increase of the incidence of tumours in various locations. The corresponding epidemiological investigations have been documented in detail by the IARC. In summary, many retrospective and some prospective cohort studies with alcoholics, or brewery workers with a high permitted consumption of alcohol during working hours, showed a clear connection between the consumption of alcoholic drinks and the appearance of tumours of the mouth, throat, larynx, oesophagus and liver. Many case-control studies have confirmed the connections and given indications as to the dose-effect relationships that are, however, subject to large uncertainties. According to these studies, the relative risks of tumours of the mouth, throat or larynx are, in part, already significantly increased by the daily consumption of ca. 10 g of ethanol. Mostly, the effects were independent of the preferred type of alcoholic drink (IARC 1988). In the meantime, a connection between the taking of alcoholic drinks and an

increased risk of breast cancer may also be assumed. Similarly, there are indications of a relationship between the consumption of alcoholic drinks and the increased incidence of colorectal tumours (Blot 1992; Longnecker 1995; Singletary 1997).
There are no investigations of the carcinogenic effect of ethanol under an inhalative condition relevant to the work-place.

The available animal experiments with chronic oral dosing with alcohol or alcoholic drinks are described in detail elsewhere (IARC 1988). For mouse, rat and hamster there is in the majority no indication concerning treatment-related increased incidence of tumours. But, hardly any of the investigations met the requirements of a valid carcinogenicity study because, for example, of the absence of suitable control groups, because the number of animals was too small or because of an inadequate histopathalogical assessment (LARC 1988).

Studies which essentially fulfill the required criteria are discussed in more detail in the following. In an
investigation on the influence of ethanol on vinylchloride-induced hepatocarcinogenisis, a control group of 80 male Sprague-Dawley rats received drinking water with 5% ethanol (v/v) for 30 months. If an average drinking water consumption of 15 ml/day and a body weight of 300 g is assumed, then from this a mean dosage of 2 g ethanol/kg BW per day can be estimated. A further control group of the same size received pure water. Thus, there was no compensation of the increased calorie intake which may be assumed for the ethanol group. The survival rate after 18 months was comparable in the two groups; 73% (ethanol treated) and 70%. Up until the end of the study, there were 8 hepatocellular carcinomas and 29 hyperplastic nodules in the ethanol group and 1 corresponding carcinoma and 10 nodules in the untreated group. 57 cases of tumours of the endocrine system were observed in the ethanol-treated group; in the control group only 8. The incidences were 26/79 compared with 8/80 for tumours of the hypophysis, 14/79 and 0/80 for tumours of the adrenal gland, 14/79 and 0/80 for tumours of the pancreas (not further specified) and 3/79 and 0/80 for testicular tumours. In total, 91 tumours were diagnosed in the alcohol group, 44% of which were classified as malign. In the control group there were 16 tumours, of these 5 were malign (Radike et al. 1981).

Sprague-Dawley rats (50 animals per dose and sex) received a semi-synthetic liquid feed with 1 or 3% ethanol for 2 years. A dosage of ca. 1 and 3 g ethanol/kg BW per day can be estimated from the food consumption. In place of ethanol, the control groups received in their diet an equi-calorific quantity of glucose. From week 104 until the end of the experiment (week 120) all animals received a normal liquid diet. The survival rates in the ethanol-treated groups were not reduced in comparison with the corresponding control values. The body weights in the higher dosage group were significantly reduced in comparison with the corresponding control values; for the males from the 13th week and for the females from the 69th week. The maximum body-weight reduction was ca. 15%. The weights of the kidneys and livers of the ethanol-treated animals did not deviate significantly from the corresponding control values. In the histological assessment, various nonneoplastic findings arose significantly more often in the ethanol-treated group than in the corresponding control group. In the male animals of both dosage groups these were cystic and focal degeneration of hepatocytes and chronic inflammation in the pancreas. In addition, the higher dosage group showed increased fibrosis in the bile duct, and the lower dosage group hyperplasia in the pancreas, C-cell hyperplasia of the thyroid gland and demyelination of the peripheral nerves. In the female animals of both dosage groups focal hyperplasia in the adrenal cortex was noticeable; in the higher dosage group only, C-cell

hyperplasia of the thyroid gland, inflammation of the clitoral gland, pigmentation of the mandibular lymph nodes and also demyelination of the peripheral nerves. The analysis of the neoplastic findings gave no indication of a significant ethanol-induced change in the tumour spectrum or tumour frequency. In the female animals of the lower dosage group, fibroma, fibroadenoma and adenoma of the mammary gland were statistically significantly increased. In contrast, tumours of the islet cells in the pancreas were reduced. In the higher dosage group, the number of (not more exactly specified) neoplasia of the hypophysis was increased, the number of adenoma of the adrenal cortex reduced. Overall, the total number of tumours in the higher dosage group was significantly reduced. The authors concluded from their results that ethanol itself has no carcinogenic effect (Holmberg and Ekstrom 1995).

The influence of a life-long ethanol consumption on life-expectancy was investigated on male CS7BL-mice. At first the animals received 3.5% ethanol (v/v) in their drinking water from the 14th to the 19th week of life. After that they were divided into three groups which received 3.5, 7.5 or 12% ethanol in their drinking water until the end of their lives. Each group comprised 100 animals which were caged separately. Two control groups, also with 100 animals each, were maintained. In one group each animal was individually caged while in the other the animals were held 5 to a cage. From the consumption of liquid the authors calculated mean ethanol doses for the three exposed groups as 2.8, 7.3 and 11.1 g/kg BW per day. The blood-ethanol concentrations were measured for the first time in the 13th week of the experiment and additionally at three further points in time 3 months apart and at different times of the day. The values averaged over all measurements were, with rising dose, 66, 142 and 268 mg/kg blood (approximately equal to mg/l blood). The development of body weight in the 5 groups was not different. The average life in the middle dosage group was statistically significantly raised with respect to that of the control group with individual caging. In the other groups there was no difference from the control. On the natural death of the animals, or after killing in extremis, a detailed histological examination was carried out. Of the animals caged individually, between 72 and 89 animals per group were examined; of those caged in groups only 55 on account of cannibalism. There were no significant differences between the frequency, type and severity of the non-neoplastic liver damage observed in the 5 groups. No damage attributable to the treatment with ethanol could be detected in the other organs (brain, lungs, heart, pancreas, spleen, kidneys, small intestine, testes) which were also examined histologically. The type and frequency of the neoplasia in the individual groups gave no indication of a significant, ethanol-related change in the tumour spectrum or incidence (Schmidt et al. 1987). Thus, overall, the results of those animal experiments carried out with a convincing methodology are contradictory. The incidences of tumours in male Sprague-Dawley rats following chronic consumption of ethanol in their drinking water were raised, but not, however, in the case of male and female animals of the same strain to which ethanol was administered in a liquid feed. A drinking water study with mice also gave negative results. Since the MTD was not reached in all three studies, the strength of their evidence in the assessment of the carcinogenic potential of ethanol is in any event limited.

Reliability : (2) valid with restrictions
Whilst this is a review it is from a reputable body (German MAK commission).

12.11.2004 (262)

Species : Mouse
Sex :
Strain : Other
Route of admin. : drinking water

Exposure period : Life (up to 160 weeks)
Frequency of treatm. : Continuous
Post exposure period : None
Doses : 100 males/group given 3.5 to 15% solutions for 5 weeks followed by 3.5, 7.5 or 12% v/v ethanol in water for life.
Result :
Control group : Yes
Method : other
Year : 1987
GLP : no data
Test substance : no data

Result : Treatment had no adverse effects on survival. There were increased incidences of liver sarcomas (probably lymphomas) at 7.5% (13/87) and 12% (10/72) compared with 4/79 in controls and 6/77 at 3.5% ethanol. No increase in liver carcinomas was found.
Reliability : (3) invalid
Study design inadequate: not all mice were necropsied, as many were autolysed, histopathology was inadequately reported and no female mice were used.
12.11.2004 (263)

Species : Rat
Sex : male/female
Strain : Long-Evans
Route of admin. : Other
Exposure period : Days 11 to 21 of pregnancy inclusive
Frequency of treatm. : Daily in two divided doses
Post exposure period : All rats exposed in utero followed until natural death (up to 2 years 6 months).
Doses : 7 g/kg body weight/day to 20 dams in two divided doses
Result :
Control group : Other
Method : other
Year : 1987
GLP : no data
Test substance : no data

Method : One male and one female pup from each of 20 ethanol-treated litters were selected for lifetime study at age 6 months.

Route of administration: rat litters exposed in utero. Dams gavaged with ethanol on days 11 to 21 of gestation. Litters were transferred to untreated surrogate mothers within 24 hours of birth.

Control groups consisted of litters from pair-fed (isocaloric sucrose solution) an ad lib fed (lab. feed) mothers.
Result : Minimal detail reported in this study which was part of an investigation into the effect of in utero ethanol exposure on longevity. Six unspecified tumours were found in 6/20 males and 6/20 females in each group except female pair-fed controls which had 8/20 animals with tumours. The authors concluded that there was no significant difference in tumourincidence. Alcohol-exposed females had significantly shorter lifespans (about 2 weeks) than pair fed and ad lib. fed controls ($p < 0.02$). A similar but less marked effect on longevity was seen in males.
Reliability : (3) invalid
Small group sizes (n=20) and absence of tumour histopathology make this study inadequate.
12.11.2004 (264)

Species : Mouse

Sex	:	Male
Strain	:	C3H
Route of admin.	:	Other
Exposure period	:	mothers treated either during pregnancy or for 1 week beginning when the pups were 1 week old
Frequency of treatm.	:	Continuous
Post exposure period	:	15 months
Doses	:	0.5 or 5% in the drinking water
Result	:	
Control group	:	Other
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data

Remark	:	Method: moths given ethanol in their drinking water either during pregnancy or when pups 1 week old. Control group not clearly described. Route of exposure: transplacental and neonatal.
Result	:	In offspring treated during embryogenesis, there were liver tumours (diagnosed grossly and described as hepatomas) in 3/25 exposed to 0.5% and 1/10 exposed to 5%. In offspring treated via the milk, liver tumours developed in 5/31 exposed to 0.5% and 5/45 exposed to 5%. The incidence in pooled control males was 27/62, significantly higher (p <0.005) than in the (pooled) exposedgroups.
Reliability	:	(3) invalid Study design inadequate: short treatment duration, females not examined, tumours not examined histopathologically. No information on initial group sizes.

12.11.2004 (265)

Species	:	Hamster
Sex	:	Male
Strain	:	other: outbred Syrian golden
Route of admin.	:	oral feed
Exposure period	:	29 weeks in total over a 33 week period
Frequency of treatm.	:	Daily
Post exposure period	:	up to a further 19 months
Doses	:	19 males given a liquid diet containing 6% ethanol.
Result	:	
Control group	:	Yes
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data

Remark	:	Nineteen males were maintained on a liquid diet containing 6% ethanol (w/v) from age 9 to 29 weeks, then left untreatedfor 4 weeks, then treated with 6% ethanol in the diet for a further 9 weeks. It is unclear how the control group of 21 males was treated.
Reliability	:	(3) invalid Study design inadequate: small group sizes, no females used, short exposure duration.

12.11.2004 (266)

Species	:	Rat
Sex	:	Male
Strain	:	Fischer 344
Route of admin.	:	oral feed
Exposure period	:	26 weeks

Frequency of treatm. :
Post exposure period : 63 weeks
Doses : 26 rats given 6% ethanol in liquid diet
Result :
Control group : yes, concurrent vehicle
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Method: Groups of 26 nine-week old rats were given a liquid diet containing 0 or 6% ethanol for 26 weeks and then fed a normal laboratory diet for a further 63 weeks prior to sacrifice.
Result : No statistically significant difference in tumour incidence was seen.
Reliability : (3) invalid
Study design inadequate: short exposure period, no females used, group sizes too small. A dietary level of 6% ethanol corresponded to 35% total calories.
12.11.2004 (267)

Species : Rat
Sex : Male
Strain : other: Holtzman
Route of admin. : oral feed
Exposure period : up to 370 days
Frequency of treatm. : Continuous
Post exposure period : no data
Doses : 15 males given 5 g/kg bw/day
Result :
Control group : yes, concurrent vehicle
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Method: Treated rats given a diet containing alcohol equivalent to 35% of total calories. Control rats pair fed.
Result : No liver tumours seen in either group.
Reliability : (3) invalid
Study design inadequate: no female rats used, small numbers of animals used, study duration too short, pathological examination limited to the liver.
12.11.2004 (268)

Species : Mouse
Sex : male/female
Strain : C57BL
Route of admin. : Gavage
Exposure period : 50 weeks
Frequency of treatm. : twice per week
Post exposure period : no data
Doses : 0.2 ml of 40% ethanol in water to 36 males and 32 females
Result :
Control group : No
Method : other
Year :
GLP : no data
Test substance : no data

Result : No treatment-related tumours.
Reliability : (3) invalid
Study quality inadequate: limited dose of ethanol administered, short

duration and no control group.
12.11.2004 (269)

Species : Rat
Sex : male/female
Strain : other: BDVI
Route of admin. : Gavage
Exposure period : 78 weeks
Frequency of treatm. : twice per week
Post exposure period : unclear - see remarks
Doses : 25 rats per sex given unspecified amounts of 40% ethanol.
Result :
Control group : yes, concurrent no treatment
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Method: Observation period unclear, but survivors killed when 120 weeks old.
Result : No obvious effects on survival. No treatment-related tumours.
Reliability : (3) invalid
Study design inadequate: small group sizes, unspecified doses, short exposure period.
12.11.2004 (270)

Species : Rat
Sex :
Strain : Sprague-Dawley
Route of admin. : Gavage
Exposure period : Life
Frequency of treatm. : Daily
Post exposure period : None
Doses : 40 rats/group given 0.5 ml/day of 30 or 50% ethanol
Result :
Control group : Yes
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Method: 10 control rats were treated with saline.
Result : Average lifespans were 500 and 396 days for the low and highdose groups. No lifespans given for the controls. No oesophageal, stomach or hepatic tumours were seen in any group.
Reliability : (3) invalid
Study inadequately reported. Limited pathological examination carried out.
12.11.2004 (271)

Species : Hamster
Sex : Male
Strain : other: outbred Syrian golden
Route of admin. : drinking water
Exposure period : 29 weeks
Frequency of treatm. : Continuous
Post exposure period : 47 weeks
Doses : groups of 27 males given 7.4 or 18.5% aqueous ethanol
Result :
Control group : yes, concurrent vehicle
Method : other
Year :

GLP : no data
Test substance : no data

Remark : Method: control group of 27 males given tap water.
Result : No statistically significant differences in tumour incidence were observed.
Reliability : (3) invalid
Study design inadequate: short treatment duration, no females used, small group sizes.
12.11.2004 (272)

Species : Hamster
Sex :
Strain : Other
Route of admin. : drinking water
Exposure period : up to 46 weeks
Frequency of treatm. : Continuous
Post exposure period : None
Doses : 20 hamsters of each sex given 5% (w/v) aqueous ethanol
Result :
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : no data

Result : No tumours of pancreas, common duct or gall-bladder observed.
Reliability : (3) invalid
Study design inadequate: no tissues examined other than pancreas, common duct and gall bladder. Group sizes small, exposure period short, no controls.
12.11.2004 (273)

Species : Hamster
Sex : male/female
Strain : Other
Route of admin. : drinking water
Exposure period : up to 807 days
Frequency of treatm. : 5 days/week
Post exposure period : None
Doses : 7 males and 3 females given 19.5% aqueous ethanol
Result :
Control group : No
Method : other
Year :
GLP : no data
Test substance : no data

Result : No tumours reported.
Reliability : (3) invalid
Study inadequately reported. Group sizes were small and no controls were used.
12.11.2004 (274) (275)

Species : Mouse
Sex : Female
Strain : other: C3H/St
Route of admin. : drinking water
Exposure period : 80 weeks
Frequency of treatm. : Continuous
Post exposure period : no data
Doses : 12% in water to 15 females

Result :
Control group : yes, concurrent vehicle
Method : other
Year :
GLP : no data
Test substance : no data

Result : Mammary tumours developed in 8/11 treated and 22/27 control mice. Though the incidences did not differ, median time to tumour appearance was significantly (p <0.001) shorter in the treated group (8 months versus 14.2 months of age).
Reliability : (3) invalid
Study design inadequate: small group sizes, no males used, no histopathological examination of tumours.
12.11.2004 (276)

Species : Rat
Sex : Male
Strain : Wistar
Route of admin. : drinking water
Exposure period : up to 40 weeks
Frequency of treatm. : Continuous
Post exposure period : None
Doses : 10 rats given 10% aqueous ethanol
Result :
Control group : yes, concurrent vehicle
Method : other
Year :
GLP : no data
Test substance : no data

Result : No tumours observed in either group.
Reliability : (3) invalid
Study inadequate to assess carcinogenicity: group sizes too small, only male rats used, duration of exposure short.
12.11.2004 (277)

Species : Rat
Sex : Male
Strain : Sprague-Dawley
Route of admin. : drinking water
Exposure period : up to 30 months
Frequency of treatm. : Continuous
Post exposure period : no data
Doses : 80 males given 5% v/v in water
Result :
Control group : yes, concurrent vehicle
Method : other
Year :
GLP : no data
Test substance : no data

Result : At 18 months survival was about 70% in both groups. Hepatocellular carcinomas were found in 1/80 controls and 8/79 treated rats (p = 0.016). Hyperplastic nodules occurred in the liver of 10 controls and 29 treated rats. In the treated group, 57/59 had endocrine tumours compared with 8/80 controls. Of these, the treated group showed 26 pituitary (P= 0.00004), 14 adrenal, 14 pancreatic and three testicular tumours. All eight control group tumours were pituitary tumours.
Reliability : (3) invalid
Isocaloric and isonutrient diets not used, no females were included in the

study. Some histopathology inadequately described.
12.11.2004 (278)

Species : Rat
Sex : male/female
Strain : Wistar
Route of admin. : drinking water
Exposure period : up to 23 months
Frequency of treatm. : each day or on alternate days
Post exposure period : no data
Doses : 15% aqueous ethanol daily or 55% aqueous ethanol on alternate days, each to groups of 20-25 rats of each sex.
Result :
Control group : yes, concurrent vehicle
Method : other
Year :
GLP : no data
Test substance : no data

Result : Survivors were killed at 23 months. No excess of tumours ofany kind was found in either treated group.
Reliability : (3) invalid
Study design inadequate due to small group sizes. Ethanol intakes at the high concentration were lower.
12.11.2004 (279)

Species : Rat
Sex :
Strain : Sprague-Dawley
Route of admin. : drinking water
Exposure period : Life
Frequency of treatm. : 5 days/week
Post exposure period : None
Doses : 25% ethanol in water to 48 rats
Result :
Control group : yes, concurrent no treatment
Method : other
Year :
GLP : no data
Test substance : no data

Remark : Method: Control group consisted of 48 rats.
Result : No adverse effects on survival. No statistically significant increase in tumour incidence reported.
Reliability : (3) invalid
Data inadequately reported.
12.11.2004 (280)

Species : Mouse
Sex : male/female
Strain : other: C57BL and CF1
Route of admin. : Dermal
Exposure period : up to 802 (CF1) or 830 days (C57BL)
Frequency of treatm. : three times/week
Post exposure period :
Doses : unspecified amounts of 50% aqueous ethanol applied to 31 female and 33 male C57BL mice and to 57 male CF1 mice.
Result :
Control group : No
Method : other
Year :

GLP : no data
Test substance : no data

Remark : Method: no data.
Result : Only one skin papilloma was seen in a C57BL mouse.
Reliability : (3) invalid
Study inadequately designed and reported.
12.11.2004 (281)

Remark : In addition to studies in which ethanol alone was investigated, a number of carcinogencity studies have been carried out where ethanol has been co-administered with other compounds (IARC, 1988). Such studies have generally involved the use of ethanol as a vehicle for another test material of interest or have been carried out where mechanistic or other reasons have required the deliberate study of the co-exposure of ethanol with another test material e.g. the use of ethanol as an experimental hepatotoxin in carcinogenicity studies of vinyl chloride. These studies are generally inadequate in one or more aspects: short duration, small group sizes, inadequate reporting, inconsistent reporting and inadequate pathological examination. They are consequently inadequate for determining the effects of ethanol.
Reliability : (4) not assignable
12.11.2004 (282)

5.8.1 TOXICITY TO FERTILITY

Type : Two generation study
Species : Mouse
Sex : male/female
Strain : CD-1
Route of admin. : drinking water
Exposure period : 105 weeks
Frequency of treatm. : ad libitum
Premating exposure period
Male : Parental 7 days; F1 74 days
Female : Parental 7 days; F1 74 days
Duration of test :
No. of generation studies : 2
Doses : 5, 10 and 15% v/v in water
Control group : yes, concurrent no treatment
NOAEL parental : = 15 %
NOAEL F1 offspring : = 10 %
NOAEL F2 offspring : < 15 %
Result : No observed effect on fertility
Method : other: NTP protocol
Year : 1985
GLP : no data
Test substance : other TS: 92%

Method : Age at onset: P animals 6 weeks at receipt, 11 weeks at first exposure.
No. of animals per sex per group: 20 also 20 F1 animals at the high dose mated at 74 days old.
Ethanol administered in deionized, filtered water.
P generation dosed for 7 days pre-mating and then for 98 days. F1 animals continued on dosing until mating.

Animals mated in cohabiting pairs; litters were proof of pregnancy.

Litters were not standardized.
Clinical signs, oestrous length etc. were not evaluated.
Epididymal and vas sperm were evaluated for concentration, motility and morphology in F1 males only.
High dose F1 animals had liver, kidney/adrenal and male sex organs weighed at termination.
F2 data were for litter sizes etc. Only.

Result : Parental/F1 data: Ethanol treatment had no effect on bodyweights and on the proportion of breeding pairs producing at least 1 litter during the continuous breeding phase or the number of litters per pair. Fertility indices were 97, 100, 100 and 94% in the controls and 5%, 10%, 15% ethanol groups respectively.

Offspring data: F1 offspring of the 15% ethanol pairs had fewer live pups per litter. their F2 offspring weighed less as pups than control pups, males, females or both sexes. Fertility indices in F1 matings were 85% and 85% in the controls and 15% ethanol groups respectively. Other reproductive performance indices e.g. gestation index, changes in lactation and changes in oestrous cycles were not studied

Effects on sperm and male reproductive organs: In the F1, 15% ethanol group there was a significantly decreased %motile sperm but no changes in sperm concentration, %abnormal sperm or %tailless sperm. There was a significant decrease in testis, epididymis and seminal vesicle weight

Gestation index, changes in lactation and changes in oestrus cycles were not studied.

Haematological, clinical biochemical, gross pathological and histopathological changes were not studied.

Mortality in P animals is reported but not discussed. F1 males from the 15% group at adulthood had decreased bodyweight and and decreased weight of testis and epididymides and seminal vesicles. In F2 females, relative liver and kidney/adrenal weights were increased.

Offspring toxicity:
Litter size and weights: Not given.
Sex ratios: Not influenced by treatment
Viability index: Not reported. Litters born to P at 15% ethanol had reduced number of live pups per litter.
Post natal survival until weaning: Not reported.
Postanatal growth: Pups born in final F1 generation of animals exposed to 15% ethanol pre- and post-natally weighed less than controls at birth and days 21 and 74.
Vaginal opening or preputial separation: Not studied.
Anogenital distance: Not measured.
Organ weights: Described above.
Gross pathology: Not examined.

Conclusion : Overall, ethanol in drinking water at concentrations up to 15% (equivalent to 20.7 g/kg/day) had no demonstrable effect on fertility in this two-generation study.

Reliability : (1) valid without restriction
Well reported study but not to a standard protocol. Very high doses used and no NOAEL identified for all endpoints.

Flag : Critical study for SIDS endpoint

12.11.2004 (283)

Type : One generation study
Species : Mouse
Sex : Male

Strain : Swiss Webster
Route of admin. : oral feed
Exposure period : 49 days
Frequency of treatm. : ad libitum
Premating exposure period
Male : Sequential matings through 7 weeks of exposure
Female : None
Duration of test : 7 weeks
No. of generation studies : 1
Doses : 10% and 25% of ethanol-derived calories
Control group : Yes
NOAEL parental : = 10 %
NOAEL F1 offspring : = 25 %
Result : Fertility not affected
Method : other
Year : 1989
GLP : no data
Test substance : no data

Method : Number/age of animals: 20 males per group, 75 days old at start of treatment.
Vehicle etc: Ethanol providing 10 or 20% of calories in a nutritionally balanced liquid diet. Two control groups consisting diets compensated or not for ethanol calories.
Dosing schedules: Males were given ethanol or control treatments for 7 weeks prior to mating with untreated females.
Mating procedure: 2 females per male for 4 hours; vaginal plugs were treated as evidence of pregnancy. Females were allowed to give birth and offspring were counted, weighed, culled and re-weighed at 21 days. Litters were standardized by culling at birth to 8 per dam.

Parameters assessed: Vital and functional observations were maintained on P and F1 generations.

Sperm quality, anogenital distance and organs at necropsy were not evaluated.

Remark : Parental data
Bodyweight: Paternal bodyweights were less at 25% ethanol-derived calories than at 10 or 0%. Offspring bodyweights were not affeceted by treatment.
Food/water consumption: High-dose males consumed less diet. (NB pair-fed controls).
Clinical signs: None reported.
Fertility index: At least 80% for each ethanol concentration at each time point. Feretility was at least as great as in pair-fed controls.
Precoital interval: Not measured.
Duration of gestation: To term.
Gestation index: Not given.
Changes in lactation, oestrus cycles, sperm, haematology, clinical chemistry, gross pathology, no. of implantations, no.of corpora lutea, ovarian primordial follicle count, organ weight change and histopathology: Not studied.
Mortality: Not reported.

Offspring toxicity:
No dose-related observations were made.
Litter sizes and weights: Not affecetd by paternal exposure.
Sex and sex ratios: Not affected by paternal, exposure.
Viability index: Not measured.
Post natal survival: No mortality reported.

Effects on offspring: Not studied.
Postnatal growth: Not affected by day 21.
Vaginal opening: Not studied.
Anogenital distance, organ weights and gross pathology: Not studied.

Result : No toxic responses were noted in treated males other than decreased bodyweight gain at 25% ethanol-derived calories in diet. Fertility over 7 weeks of treatment was not affected.

No adverse effects on offspring were noted as a function of either level of paternal ethanol treatment or duration of treatment.

Fertility was at least as great as in pair-fed or standard controls.

Reliability : (2) valid with restrictions
Well reported study but not to a standard protocol. Very high doses used.

12.11.2004 (284)

Type : One generation study
Species : Rat
Sex : Female
Strain : other: Holtzmann
Route of admin. : oral feed
Exposure period : 8 or 16 weeks before mating
Frequency of treatm. : ad libitum daily
Premating exposure period
Male : no treatment
Female : 3 or 6 weeks
Duration of test :
No. of generation studies :
Doses : 5% in liquid feed
Control group : Yes
NOAEL parental : < 5 %
NOAEL F1 offspring : <= 5 %
Result : Fertility not affected although oestrous cycle length was prolonged and irregular
Method : other
Year : 1982
GLP : no data
Test substance : no data

Method : No. and age of animals: 10 per group, age 20 days. No F2 generation.
Ethanol was supplied in a liquid diet for 16 weeks prior to mating or for 8 weeks followed by 8 weeks on standard diet. Dosing ended after mating.
Two Control groups were used, one receiving standard diet the other pair-fed with 5% ethanol in diet.
Mating procedure was by 1:1 cohabitation with a fertile male for 14 hr. proof of pregnancy was a sperm-positive vaginal smear. Study ended with delivery of F1 pups.
Oestrous cycle length and pattern was recorded.
Growth performance in F1 pups was followed.
Statistical test was one-way ANOVA.

Remark : Parental data:
Effect of duration of exposure, not dose, was assessed.
Administration of 5% for 16 weeks, not 8 weeks, increased oestrus cycle length and irregularity. Age to vaginal patency was increased by both regimen. No abnormalities of
ovaries or uteri were found.
Bodyweight: Maternal bodyweights were measured but not reported.
Offspring bodyweights were not affected by treatment.
Food/water consumption: Not reported but must have been recorded.
Clinical signs: None reported.

Fertility index: At least 80% for each ethanol concentration at each time point. Fertility was at least as great as in pair-fed controls.
Precoital interval: Not measured.
Duration of gestation: Not reported.
Gestation index: All females delivered live litters.
Changes in lactation, oestrus cycles, sperm, haematology, clinical chemistry, gross pathology, no. of implantations, no.of corpora lutea, ovarian primordial follicle count, and organ weight change: Not studied.
Histopathology: All ovaries and uteri examined were normal.
Mortality: None reported.

Offspring toxicity:
No dose-related observations were made.
Litter sizes and weights: Not affecetd by maternal exposure.
Sex and sex ratios: Not given.
Viability index: Not measured.
Post natal survival: No mortality reported.
Effects on offspring: Not studied.
Postnatal growth: Not studied.
Vaginal opening: Average age of vaginal patency was 72-77 days in both groups and significantly older than in control groups (41-58 days).
Anogenital distance, organ weights and gross pathology: Not studied.

Result : No adverse effect on fertility, litter size or neonatal bodyweight was detected. Irregular cycles and longer oestrous cycles were noted in rats fed for 16 weeks but not after 8 weeks with 8 weeks recovery period.

Average age of vaginal patency was 72-77 days in both groups of ethanol-treated rats versus 41-58 days in controls.

Reliability : (2) valid with restrictions
Well reported study but not to a standard protocol. Very high doses used and no NOAEL identified.

12.11.2004 (285)

Type : One generation study
Species : Rat
Sex : Female
Strain : other: Holtzmann
Route of admin. : oral feed
Exposure period : 55 days
Frequency of treatm. : ad libitum daily
Premating exposure period
Male : None
Female : 50-55 days
Duration of test :
No. of generation studies :
Doses : 2.5% and 5% in feed, estimated 8-12 g/kg/day and 12-14 g/kg/day
Control group : no data specified
NOAEL parental : = 2 %
Result : Ovarian function was suppressed at the high dose
Method : other
Year : 1982
GLP : no data
Test substance : no data

Method : No. and age of animals: 8-11 per group aged 20 days at start.
Vehicle etc: Ethanol was supplied in a liquid diet.
Dosing schedule; Pair-fed controls were used at each dose for P animals with ethanol in diet ad lib for 50-55 days.
No matings attempted so no F1 and F2 animals.
Litter standardization: Not applicable.

Animals were weighed weekly and examined daily for vaginal patency. Once patent, vaginal lavages were made daily.
Oestrous cycle length and pattern: - length but not pattern determined.
Sperm examination: Not applicable.
F1 and F2 observations: Not applicable.

Remark : This study was to assess ovarian function as a possible factor in fertility studies following ethanol exposure. It is an adjunct to a study by the same authors demonstrating absence of effect on fertility but evidence of disturbed ovarian function.

Parental and offspring data are not applicable in this study.

Result : Ovarian function was supressed in rats that achieved blood alcohol levels of 250 mg/100 ml.

Animals receiving 5% ethanol(but not 2.5%) in liquid diet had longer time to vaginal patency, failed to begin oestrus cycles, showed decreased bodyweight gain, had ovaries containing only a single generation of corporea lutea, had infantile vaginal and uterine epithelium and decreased uterine and ovarian weight.

Reliability : (2) valid with restrictions
Well reported study but not to a standard protocol. Very high doses used.

12.11.2004 (286)

Type : One generation study
Species : Rat
Sex : Female
Strain : Wistar
Route of admin. : oral feed
Exposure period : 49 days (animal age 28-77 days)
Frequency of treatm. : Daily
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 5% in feed, estimated 12,000 to 14,000 mg/kg/day
Control group : other: See method details
Method : other
Year :
GLP : no data
Test substance : no data

Method : Number of animals: 100, ex Charles River Breeding Labs, individually caged.
Ethanol fed animals received a liquid diet with ethanol accounting for 35% of total calories. Pair fed isocaloric controls (using dextrimaltose as alternative to ethanol.)
Controls: ad libitum intact and ad libitum oophorectomized (Feed: Lab Blox F4 ex Best Feeds, Oakdale, Pa)
Gross and microscopic anatomy: Animals sacrificed by exsanguination, 6-7mls of blood kept for analysis. Liver, ovaries, uterus and fallopian tubes removed and weighed, preserved in Bouin's solution. Histological sections stained with hematoxylin and eosin. Liver assessed for degree of fat, necrosis and inflammation. Ovaries assessed for corpus lutea, corpus hemorrhagica, and numbers/development of Graafian follicles.
Uterus/fallopian tubes assessed for thickness of endometrium, type of and secretory activity of the epithelial lining cells and thickness of the muscle wall of each organ. Cervix/vagina examined for thickness of epithelial cell lining and signs of estrogen stimulation.
Plasma steroids and gonadotropins. All assessed by radioimmunoassays

with following levels of detection:
Progesterone 10pg, Estradiol 0.1pg, estrone 0.2pg, corticosterone 20pg, gonadotropin 4ng/ml. All measurements in duplicate.
Liver: Enzyme function assessed by measuring serum alkaline phosphatase, gamma glutamyl transpeptidase, glutamic pyruvic and glutamic oxalacetic transaminase activities.
Blood ethanol: measured using blood samples obtained 1 day before sacrifice between 9-11am and before feeding.
Statistical analysis: Student t test. "Probable significance" at p<0.05 and "significance" at p<0.01

Remark : The reported blood ethanol level was relatively low (110±9mg/dL) but the timing of the sample (taken 09.00 - 11.00hours) was probably inappropriate to detect the peak likely at the usual time of feeding during the previous evening.

Result : Growth (body weight at sacrifice): ethanol fed: 138 +/-5.3g; isocaloric controls: 161.6+/-3.8g. Significant difference (p<0.01).
Anatomy: Livers of alcohol fed animals significantly larger than controls (p<0.01. Treated animals 10.6g+/-0.5, isocaloric controls 6.4g+/-0.3, ad libitum controls 8.3g+/-0.2). Ethanol treated animal livers markedly more fatty in appearance.
Ovaries: Marked weight loss in treated animals (30.6+/-2.2mg versus 75.5+/-3.9mg for isocaloric controls and 91.4mg+/-0.2mg for ad libitum controls). Weight loss significant even when corrected for body weight. Reduction in ovarian mass due to absence of developing follicles, corpus lutea and corpus hemorraghagica.
Histology: Differences in appearance of uterus, cervix and vagina between treated and untreated animals.
Uterus/fallopian tube: Marked weight loss in treated animals (39.0+/-4.1mg versus 180.5+/-18.7mg for isocaloric controls and 306mg+/-15.5mg for ad libitum controls). Weight loss significant even when corrected for body weight.

Plasma steroid and gonadotropin levels (n=25):
Plasma estradiol reduction seen in treated animals (ethanol fed: 27.5+/-1.2pg/ml; isocaloric controls: 33.3+/-1.5pg/ml; ad libitum intact controls: 48.0+/-1.4pg/ml; p<0.01). However no statistically significant reduction relative to oophorectomized control (29.8+/-1.6pg/ml).
Plasma progesterone reduction seen (ethanol fed: 23.3+/-4.3pg/ml; isocaloric controls: 54.3+/-7.3pg/ml; ad libitum intact controls: 41.7+/-6.7pg/ml; p<0.01). However no statistically significant reduction relative to oophorectomized control (18.0+/-0.6pg/ml).
Plasma estrone increase seen (ethanol fed: 156.0+/-26.7pg/ml; isocaloric controls: 114.9+/-13.9pg/ml; ad libitum intact controls: 80.5+/-6.3pg/ml; p<0.01); oophorectomized control (48.0+/-5.2pg/ml).
Plasma corticosterone levels increased (ethanol fed: 74.0+/-9.0ug/dl; ad libitum controls: 48.0+/-6.0ug/dl; p<0.05). However no statistically significant reduction relative to pair fed controls (78.0+/-9.0ug/dl).
Plasma lutenizing hormone levels increased (ethanol fed: 68.7+/-5.7ng/ml; ad libitum controls: 43.5+/-7.0ng/ml; p<0.01). However no statistically significant reduction relative to pair fed controls (79.4.0+/-6.8ng/ml). All significantly less (p<0.01) than oophorectomized ad libitum control.
Plasma follicle stimulating hormone levels not statistically different but all significantly less (p<0.01) than oophorectomized ad libitum control.

Biochemical liver function:
Serum glutamic oxalo-acetic-acid-transaminase and serum glutamic pyruvic transaminase levels increased in treated animals (2.5x control levels). Alkaline phosphatase 50% greater in treated animals (all p<0.01).
Gamma glutamyl transpeptidase activity not detected in any controls but reproducibly measured (2.3+/-IU/ml) in ethanol fed animals.

Blood ethanol levels: 110+/-9.0mg/l. Not detected in any controls.

Reliability : (2) valid with restrictions
Well reported study but not to a standard protocol. Very high doses used and no LOAEL identified.

12.11.2004 (287)

Type : Fertility
Species : Rat
Sex : Male
Strain : Sprague-Dawley
Route of admin. : Gavage
Exposure period : 9 weeks
Frequency of treatm. : twice per day
Premating exposure period
Male : 9 weeks
Female :
Duration of test :
No. of generation studies :
Doses : 0, 2, 3 g/kg
Control group : other: concurrent vehicle and concurrent no treatment
Method : other
Year :
GLP : no data
Test substance : other TS

Method : Animals: Supplied by Charles River, 2 Months old at acquisition; acclimated for 2 weeks.
Environment: 22 +/- 1 degree C; relative humidity 45 +/- 5%; 12 hr:12 hr light:dark cycle.
Feed: Laboratory feed and water ad libitum.
Treatment: After acclimation, groups of 20 male rats were intubated at 0900 and 1600 hrs with 3g/kg ethanol (15% w/v in distilled water), 2g/kg ethanol or vehicle (distilled water) only. Groups receiving 2 g/kg or 0 g/kg were paired fed with those receiving 3 g/kg and a fourth group received no gavage treatment. This continued for 9 weeks before breeding with 70 to 90-day-old females.
Controls: Distilled water by gavage (Group 3) or no gavage treatment (Group 4). Offspring of these groups were compared to evaluate the potential for handling stress.
Evaluation of pups: After birth, pups were examined and weighed and litters were culled to 10 per female. Culled pups were subjected to brain and adrenal gland weight measurements. Offspring were weighed at 7, 14 and 21 days. At 7 days old, three males and 3 females from each of 6 litters were culled and their brain and adrenal gland weights determined. At 21 days, this was repeated with inclusion of more organ weights. Blood alcohol levels were determined after breeding was determined at 1,2,3 & 5 hours after dosing.
Statistical analysis: ANOVA and Duncan's Multiple Range tests on parametric data; Chi-square on non-parametric data.

Remark : Paternal alcohol exposure did not influence litter size, average birth weight per pup or postnatal bodyweights in offspring. A study of runts suggested an influence of ethanol on individual sperm rather than on entire sperm production. The small but significant effect on male:female ratio (53 to 45%) was unexpected and is without explanation. An apparent effect on adrenal gland weight at birth is difficult to interpret, as this did not persist through offspring growth and development. An effect on spleen and heart weight indicates that paternal alcohol exposure may produce gross changes in offspring as well as functional changes.

Result : 6 Males in the top dose group and 1 ad lib control rate died due to illness prior to breeding. Peak blood alcohol level was 338 +/- 15 mg% in the top

dose group and 132 +/- 5 mg% in the lower dose group. There were no adverse effects on male reproductive performance and female fecundity was no affected. Litter sizes and birth weights were not affected. Litter sizes and birth weights were not affected by paternal ethanol intake at either dose. Ethanol treatment in fathers had no effect on offspring growth rate.

There was a significantly higher number of female runts (bodyweight <5.5 g) in the groups sired by rats exposed to ethanol. There was also a significantly higher number of male runts in the groups sired by rats exposed to ethanol but only in comparison to the intubated control; the difference with the non-intubated control was not significant.

The % of males in litters sired by ethanol treated rats was significantly lower than the % sired by vehicle-treated fathers (p<0.04) although the difference with the non-intubated control was not significant. Ethanol treatment at both levels resulted in a significant increase in absolute adrenal gland weight but not in brain/bodyweight ratios. Organ weights (both absolute and bodyweight relative) were unaffected at 7 days but significant reductions of spleen and heart weight were noted at 21 days at the 3g/kg dose level (NOEL=2g/kg).

Conclusion : There was no effect on fertility in a group of 20 male rats given 3 g or 2 g/kg ethanol by oral intubation daily for nine weeks, achieving BAL's of 338±15 and 132±5mg/dL, respectively. Although fertility was unaffected, this study did reveal higher incidences of runted pups in the resulting offspring especially at the highest exposure level (3g/kg).

Reliability : (2) valid with restrictions
Study well enough reported for a valid with restrictions rating

12.11.2004 (288)

Type : Fertility
Species : Mouse
Sex : Male
Strain : C57BL
Route of admin. : oral feed
Exposure period : 35 or 70 days
Frequency of treatm. : Daily
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 5 or 6% in feed calculated to yield 12,000 to 14,000 mg/kg/day
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Animals: supplied by Jackson Laboratories (Bar Harbor, ME).
Age: 60 days.
Environment: 22 ± 1 degree C; relative humidity 45 ± 5%; 14 hr:10- hr light:dark cycle. Animals housed individually.
Feed: Laboratory feed and water ad libitum.
Treatment: Vitamin supplemented (3g/l) chocolate flavoured Carnation Slender containing sucrose in amounts isocaloric to either 5% (v/v) or 6% (v/v) ethanol for 3 days after which on half of the animals in each group received diets containing 5% or 6% ethanol. All mice initially treated for 3 days on contro followed by 70 days treatement for the 5% ethanol group and 35 days for the 6% ethanol group. 48 Hr after cessation of treatment,

mice were anaestheticsed then hemicastrated and the right testis and associated structures weight recorded. Spermatozoal function (number, motility, forward progression, in vitro fertilisation of mouse oocytes) and blood alcohol levels (BAL) were determined from 25ul aliquots of tail blood (head space sampling technique used). After castration, the incision was closed and the mice allowed free access to lab chow and water (no treatment) for a further 70 days. All animals then sacrificed.
Group sizes consisted 5 to 12 individuals (treatment and control groups respectively).
Controls: Vehicle (Carnation Slender containing sucrose as above).
Statistical analysis: Wilcoxon tests; parametric data analysed following appropriate transformations and Newman-Keuls multiple range test.

Remark : Hemicastration was used to evaluate the reversibility of ethanol's effects on male reproductive function. Hemi-castrated pair fed controls were used to minimize the effect of hemicastration on the data (as it is known to produce compensatory effects on the remaining testis).
Except for germ cell desquamation, all effects seen at the 5% ethanol diet were reversible. The authors speculated that Sertoli cells rather than Leydig cells are involved in reproductive failure of abstinent alcoholics.

Result : BAC peaked at 166± 38mg% (1660mg/l, n=15) for the 5% dose and 260± 35mg% (2600mg/l) for the 6% dose, both at day 34/35.
After treatment with either 5% ethanol or 6% ethanol, testicle weight decreased by 24% and 28%, but the effect was reversible and there was no significant difference between the control and recovery animals (10 weeks no treatment, P>0.1).
Seminal vesicle/prostate weights decreased by 20% for those on the 6% diet but the effect was reversible and there was no significant difference between the control and recovery animals (10 weeks no treatment, P>0.1).
There were significant increased frequencies of germ cell desquamation (480% in the 5% treatment group, 400% in the 6% treatment group) and of inactive seminiferous tubules (186% in the 5% treatment group, 567% in the 6% treatment group) Improvement in both parameters was noted in the contralateral organs after 10 weeks alcohol abstinence but all remained significantly elevated except for the % inactive tubules which returned to control group levels in the 5% treatment group. (Note: Germ cell desquamation in 5% treatment recovery group - 95% confidence limit range: 1.2-3.2% lumina showing desquamation versus control level of 0.3-1.0%.)
Quality of spermatogenesis was significantly poorer in testes from both treatment groups compared to their respective controls. After 19 weeks abstinence, some pathology persisted in animals that had been exposed to ethanol although the differences were not significant.
Caudal epididymal sperm content was not significantly affected by treatment with the 5% ethanol diet but was 6% lower in the animals receiving the 6% diet (p<0.01). This difference disappeared following 10 weeks without treatment.
Sperm motility was not significantly affected by treatment with the 5% ethanol diet in the animals receiving the 6% diet molility was reduced by 85%. This difference disappeared following 10 weeks without treatment.
Forward progression was reduced in both treatment groups (apparently more so in the lower treatment group). The difference disappeared in the recovery group that had been receiving 5% ethanol but persisted in the 6% group.
In vitro fertilization of mouse oocytes by epididymal spermatozoa was reduced by 20% in the 5% treatment group and 63% in the 5% treatment group but these differences disappeared in both treatment groups following 10 weeks abstinence.

Source : IARC monographs on the evaluation of carcinogenic risk to humans. Vol 44, Alcohol Drinking. IARC, Lyon, France, 1988.

Conclusion : No NOAEL established (<5% ethanol diet, <166mg% BAC). However, for persistent effects the NOAEL would appear to be close to 5% ethanol diet.

Reliability : (2) valid with restrictions
Not a standard study protocol but appears to be well conducted and reported. Limited and very high doses does not allow a NOAEL to be established.

12.11.2004 (437)

Type : Fertility
Species : Rat
Sex : Male
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 3 to 4 weeks
Frequency of treatm. :
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 22, 23, 25, 27 mg/l (approx 11500, 12000, 13000, 14000ppm)
Control group : yes, concurrent no treatment
NOAEL parental : = 23 mg/l
LOEL parental : = 25 mg/l
Method : other
Year : 1983
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Animals: adults, 330-350 g; acclimated for 3-5 days. Animals housed together in airtight chambers.
Controls: Housed in similar chambers to exposed animals without ethanol vapours present.
Environment: Not described.
Feed: Laboratory feed and water ad libitum during exposure. Feed supplemented with vegetables and peanut bars.
Treatment: During acclimation, all treated rats were exposed to 22 mg/l ethanol vapour in air. After acclimation, groups of 10-12 rats were exposed to ethanol at 23, 25 or 37 mg/l for up to 3 to 4 weeks. Five exposure were run as separate experiments a concurrent controls. After treatment, animals were immediately weighed then sacrificed and trunk blood collected over ice, the plasma separated and frozen for later analysis. Sex organs (testes, seminal vesicles, prostate) were removed and weighed. In one experiment, animals were injected with saline or gonadotropin releasing hormone (GnRH) and sacrificed 10 minutes later.
Blood samples were withdrawn periodically from a tail vein for blood alcohol concentration (BAC) determination.
Plasma testosterone and lutenising hormone (LH) were recorded.
Statistical analysis: Dunnett's and Duncan's tests after analysis of variance.
Remark : This data supports the premise that fertitily effects observed following high doses of ethanol may well be confounded by malnutrition stress.
Result : BACs of 94-127 mg/100ml resulted in smaller increase in bodyweight gain than observed in control animals but was not associated with significant changes in plasma testosterone levels or weights of sex organs. The maximum BAC observed was 187mg/100ml. A BAC of 180 mg/100 ml was associated with an inhibition of testosterone secretion only in animals that had failed to grow but this was not seen in a second experiment with a similar BAC.
In the experiment where GnRH was injected, a BAC of 163mg/100ml was associated with a marked weight loss and reduction of plasma testosterone levels. Basal plasma LH levels were comparable in the control and ethanol treated rats and intravenous administration of GnRH produced comparable

elevations in LH level in the controls and ethanol treated rats.

NOAEL (male fertility) = 127mg/100ml BAC
LOAEL (male fertility) = 163mg/l00ml BAC

Conclusion : Authors' conclusion is that data indicates that in growing animals testosterone secretion appears to be directly related to changes in body weight but not the degree of alcohol exposure. Adequate function of the hypothalamic-pituitary-testicular axis provided normal growth is maintained. Alcohol does not appear to lower pituitary gland sensitivity to GnRH.

Reliability : (2) valid with restrictions
Not a standard protocol but reasonably well reported and a no effect level established by a relevant route of exposure.

12.11.2004 (289)

Type : One generation study
Species : Rat
Sex : Male
Strain : Sprague-Dawley
Route of admin. : Gavage
Exposure period :
Frequency of treatm. : Daily
Premating exposure period
Male : 3 and 9 weeks
Female : not treated
Duration of test :
No. of generation studies : 1
Doses : 2.5 and 5.0g/kg
Control group : yes, concurrent vehicle
Method : other
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Animals: Supplied by Charles River, Portage, MI but test animals bred in laboratory.
Age: 70 days.
Number of animals: 18-26 per dose group.
Environment: 22 ± 1 degree C; relative humidity 45 ± 5%; 12 hr:12 hr light:dark cycle.
Feed: Laboratory feed and water ad libitum.
Vehicle: distilled water.
Controls: Vehicle (distilled water without ethanol) and non-intubated controls to evaluate the effects of intubation stress. Animals in the low and control treated groups were pair-fed to the high dose group animals.
Treatment: Males were bred in once after 3 weeks exposure and twice after 9 weeks of exposure to 75-90-day-old females for 2 weeks or until sperm plugs were observed. Males were intubated throughout the mating period. Females with plugs were separated and housed individually. At 20-days gestation, females from the 3 week exposure breeding and one from the 9 week exposure breeding were killed and their foetuses counted, sexed and weighed. The second female from the 9 week breeding was allowed to deliver its litter, which was similarly assessed.
Blood alcohol levels (BALs) were determined in males after 15 weeks exposure. Animals were sacrificed 1 hour after intubation (previously determined to co-incide with peak BAL) and trunk blood measured. BAL was assessed by reduction of NAD by alcohol dehydrogenase using a Beckman automated analyser.
Statistical analysis: Chi-square, ANOVA and Duncan Multiple Range Tests were used.

Remark : The 3 week exposure period was intended to assess the effect on

postmeotic spermatids and spermatogonia whilst the 9 week exposure peroid was intended to assess the effect on germ cells throughout their maturation prior to as well as meiosis.

Result : Male fertility and litter size was not affected by treatment. Fecundity was not reduced in individual breedings but was significantly reduced at the 5g/kg dose level if the breeding periods were combined. There was no differences between the two control groups.
At the first breeding (3 weeks) the 2.5g/kg and concurrent control males weighed significantly more than ad lib controls. After 9 weeks, only the concurrent control animals weighed significantly more than the ad lib controls.
There did not appear to be any treatment related effects on resorptions and litter size.
The mean BACs for the two ethanol dose groups was 248±14mg% (5g/kg) and 155±9mg% (2.5g/kg).
There was a treatment related increase in the number of male foetuses in the 3 week breeding at 5g/kg, but the effect was not repeated at the 9 week breedings.
At the 3 and 9 week breedings there was a significant dose related increase in fetal weights. There was also a significant increase in placental weights, but only at the 9 week breedings.
There were no treatement related effects on newborns sired by alcohol treated males.

Conclusion : There was no significant effect on male fertility. Female fecundity was reduced at the high dose level when all breedings were combined but litter size was not decreased nor were resorptions significantly increased. The main finding was an increase in fetal weights of offspring sired by alcohol treated males.

Reliability : (2) valid with restrictions
Not a standard protocol but reasonably well reported. Dose levels very high and no clear no effect level established.

12.11.2004 (290)

Type : One generation study
Species : rat
Sex : male
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 5 weeks
Frequency of treatm. : continuous
Premating exposure period
Male : 15 days
Female : no treatment
Duration of test : 36 days
No. of generation studies :
Doses : 6 and 10% in diet; calculated to be much greater than 12,000 mg/kg
Control group : no data specified
NOAEL parental : < 6 - 10 %
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Animals: weight at start of treatment: 318 g (males); 259 g (females). No of animals: 12 males; 25 females.
Environment: Individually housed, 22 degC, RH 45% +/- 10 with diurnal light cycle (nocturnal 20:00 to 8:00hrs.)
Dosage: Six treated rats were given a 6% v/v ethanol-containing liquid diet (providing 35% of calories.) This was increased to 10% v/v after 7 days exposure (58% of dietary calories.

Controls: Six controls given an isocaloric amount of sucrose.
Diet 'Metrecal' (chocolate or vanilla.)
Duration of treatment: 15 days (males). females on lab chow and water.
Investigations: After treatment each male was placed in a cage with 2 females every night. No food or water was provided during this time. The experiment was continued for 5 weeks, with the males still receiving alcohol during the day. The males were killed on day 36. Blood samples were collected.
Pregnancies were terminated on day 20 of gestation and litter size and foetal mortality was assessed.

Result : Treated males showed signs of intoxication and considerable weight gain compared to controls. Treated animals were much less succesful at mating; the numbers of successful matings were 6/12 in the treated group and 13/13 in the controls. The number of offspring/litter was greater in the controls ($p < 0.01$). A higher incidence of early resorption was seen in the treated group ($p < 0.01$). Adverse effects on the testes, seminal vesicles, ductules and in serum testosterone levels were noted.

From graphical data presented in the reference, it is possible to estimate ethanol consumption as being in the range 7.2 to 14.4 g/kg/day.

Test substance : Test substance was 95% v/v ethanol.
Reliability : (2) valid with restrictions
Only six pregnancies examined in treatment group and males also chronically treated with ethanol such that the quality of the study is reduced. General toxicity such as ataxia, lethargy and weight loss during the experiment may confound the results.

12.11.2004 (254)

Type : Fertility
Species : Rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 7 hours
Frequency of treatm. : Daily
Premating exposure period
Male : 6 weeks
Female : None
Duration of test : see method details
No. of generation studies :
Doses : 0, 10000, 16000ppm
Control group : Yes
other: NOAEL paternal : > 16000 ppm
Result : Negative
Method : other
Year : 1985
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Exposure was conducted in 0.5m3 chambers with dynamic air flow (one air change per minute.)

Animals: 18/group. Starting weights 400-500g.
Temperature: 73 +/- 3F. Humidity: average 40-50%.
Exposure period: 2 day non exposure period before mating. Mating period 5 days. Mating confirmed by presence of sperm plugs under cages or vaginal smears. Females housed individually.
Analytical monitoring: Yes (IR analyser - exposures found to be within 11% of nominal). Independently cross-checked with charcoal adsorption tubes.
Parameters measured: weights (daily), food and water intake.

Result : No effect on weight gain, feed or water intake. No effect on fertility or litter sizes.
Previous studies quoted as showing exposures to 10000 and 16000ppm ethanol typically give rise to blood ethanol concentrations of 30 and 500mg/l ethanol. Authors calculate that for rats exposures in excess of 11000ppm are required to begin accumulating ethanol in the blood and that ethanol is no more toxic by the inhalation route than by other routes.
Reliability : (2) valid with restrictions
Well reported study but not to a standard protocol but no pathology on males. Study incomplete as a comprehensive assessment of effects on male fertility. Route of exposure highly relevant.
12.11.2004 (291) (292) (293)

Type : Fertility
Species : Rat
Sex : Female
Strain : CD-1
Route of admin. : s.c.
Exposure period : Once
Frequency of treatm. :
Premating exposure period
Male :
Female :
Duration of test : Once
No. of generation studies :
Doses : 7900 mg/kg bodyweight
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Ovulatory surges of LH and therefore ovulation were blocked by ethanol.
Reliability : (4) not assignable
12.11.2004 (294)

Type : Fertility
Species : Rat
Sex : Male
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 1 week
Frequency of treatm. : 6 hours/day
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 0, 1000ppm
Control group :
Method :
Year : 1985
GLP : no data
Test substance : other TS

Method : Full method details provided in White (1983).
Equilibrium concentrations achieved after 30 minutes.
Temperature 20-22C, relative humidity 40-60%
Controls: air only.

No food or water during treatment.
Statistical methods: student's t test, significant $p<0.05$
Animals sacrificed immediately after exposure or after an 18hr rest period.
Measurement of hormones was by radioimmunoassay.

Remark : Study designed to assess the effect on male reproductive function by quantifying serum concentrations of testosterone and luteinizing hormone.
Result : No significant effect on luteinising hormone or corticosterone. A significant depression of testosterone occurred after the first exposure, but the level returned to normal after 18hrs recovery and was absent at the end of the study.
Test substance : 99% ethanol, supplied by Merck.
Conclusion : The relevance of this transient effect was not considered significant with respect to the ability of the testes to produce testosterone.
Reliability : (2) valid with restrictions
Very restricted range of parameters assessed and exposure short, but within these restrictions, results appear reliable.
17.11.2004 (295)

Type : Fertility
Species : Monkey
Sex : Female
Strain : Macaca Fascicularis
Route of admin. : i.v.
Exposure period : 3-6.5 months
Frequency of treatm. :
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 2900-4400 mg/kg bodyweight
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Amennorrhea, atrophy of the uterus, decreased ovarian mass and significant lowering of LH levels were observed.
Reliability : (4) not assignable
12.11.2004 (296)

Type : One generation study
Species : Rat
Sex : male/female
Strain : Wistar
Route of admin. : oral feed
Exposure period : gestation day 12 to ten days postpartum
Frequency of treatm. :
Premating exposure period
Male :
Female :
Duration of test : in utero and as neonates
No. of generation studies :
Doses : 36% of calorie intake; estimated to be greater than 12,000 mg/kg/day
Control group : no data specified
Method : other
Year :
GLP : no data

Test substance : as prescribed by 1.1 - 1.4

Remark : The authors concluded that there was less phenotypic masculinization at birth in the treated offspring.
Result : In male progeny there was decreased anogenital distance; the weights of the testes and seminal vesicles/prostate were decreased 55 and 110 days postpartum; serum testosterone and luteinizing hormone levels were decreased on day 55 but not on day 110; and sexual motivation and performance were reduced.
Reliability : (4) not assignable
12.11.2004 (297)

Type : One generation study
Species : Rat
Sex : Female
Strain : Wistar
Route of admin. : oral feed
Exposure period : while in utero and as neonates
Frequency of treatm. :
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 36% of calorie intake
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Male offspring gonadal growth and sexual performance was adversely affected.
Reliability : (4) not assignable
12.11.2004 (298)

Type : One generation study
Species : Rat
Sex : Female
Strain : Wistar
Route of admin. : drinking water
Exposure period : Before mating, through gestation and lactation
Frequency of treatm. :
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 12% in drinking water
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : No effect on reproductive performance was noted.
Reliability : (4) not assignable
12.11.2004 (299)

Type : One generation study
Species : Rat
Sex : Male
Strain : Sprague-Dawley
Route of admin. : i.p.
Exposure period : Once
Frequency of treatm. : Once
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 2500 mg/kg bodyweight
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : There was a significant decrease in the level of LH and testosterone with marked attenuation of testicular steroidogenesis.
Reliability : (4) not assignable
12.11.2004 (300)

Type : One generation study
Species : Rat
Sex : Female
Strain : Wistar
Route of admin. : i.p.
Exposure period :
Frequency of treatm. :
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses :
Control group :
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : Information taken from secondary source - no dosage information given.
Result : Ethanol increased prolactin secretion.
Reliability : (4) not assignable
12.11.2004 (301)

Type : One generation study
Species : Rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : Other
Exposure period :
Frequency of treatm. :
Premating exposure period
Male :
Female :

Duration of test :
No. of generation studies :
Doses : 11600 mg/kg bodyweight
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Patterns of LH secretion in both male and female offspring, as adults, indicated an effect on the central mechanisms controling secretion of pituitary LH.
Reliability : (4) not assignable
12.11.2004 (302)

Type : One generation study
Species : Mouse
Sex : Female
Strain : C57BL
Route of admin. : drinking water
Exposure period : before mating, through gestation and lactation
Frequency of treatm. : Daily
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies :
Doses : 10% in drinking water
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : No significant effect on reproductive capacity.
Reliability : (4) not assignable
12.11.2004 (303)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : 17 hours per day
Frequency of treatm. : Daily
Duration of test : Days 1-19 of gestation
Doses : 10,000, 16,000 or 20,000 ppm
Control group : no data specified
NOAEL maternal tox. : = 16000 ppm
NOAEL teratogen. : > 20000 ppm
LOAEL Maternal Toxicity : = 20000 ppm
LOAEL Teratogenicity : >= 20000 ppm
Method : other
Year :
GLP : no data

Test substance : as prescribed by 1.1 - 1.4

Remark : Age at study start - not stated - 176-200 g.

Number of animals per dose per sex: not explicitly stated but approximately 16.
Doses are calculated to be equivalent to 17, 29 and 28 g/kg bodyweight.
Vehicle used - not applicable

Mating conditions: Virgin females were housed with males and vaginal smears were taken.

Foetuses were examined externally and internally for malformations; implants and resorptions were recorded as was litter weight.

No maternal organs were examined and fetuses were examined for external and visceral malformations.
Maternal LOAEL effect was narcosis and lowered food consumption.
Development LOAEL effect - none seen
Development NOAEL effect - visceral or skeletal malformations or variants.
Statistical tests were t-tests or chi-square tests; p=<0.05 regarded significant.
This was considered in the IARC Monograph 1988.

Result : No mortality occurred.

Maternal data are not given for the following:

Number aborting
Number of corporea lutea
(Duration of pregnancy not relevant)
Bodyweights
Haematology and blood chemistry findings
Gross pathology in dams
Organ weight changes
Histopathology incidence and severity.

Maternal data are given for the following:

The number of pregnant per dose level were 15/15, 15/16 and 14/16 in the low, medium and high dosage groups.
The numbers of resorptions were not affected by ethanol inhalation.
Number of implantations were 14-16/litter in all ethanol-treated groups.
Number of corpora lutea were 14-16/litter
Food consumption was lowered in the high-dose group.
Clinical signs: the highest dose induced complete narcosis (described as severe toxicity); lower doses did not cause narcosis but caused hyperactivity after exposure.
Maternal weight gains were not affected by treatment.

Blood alcohol levels ranged 0.02 to 0.04 mg/ml at 10000ppm, 0.43 to 0.53 mg/ml at 16000ppm and 1.48 to 1.93 mg/ml at 20000ppm. Measurements were made on non-pregnant rats and represent the ranges of the average values measured at days 1, 10 and 19.

Foetal data are not given for the following:

Litter size (but are deduced to average 6.0 to 7.1 foetuses/litter across the groups).
Number viable
Postnatal growth (not applicable)
Postnatal survival (not applicable)

Foetal data are given for the following:

Litter weights were not significantly affected by ethanol treatments.
Sex ratio did not differ significantly from controls.
Grossly visible abnormalities are given in detail but the frequency of each did not differ significantly between groups. More litters contained abnormal foetuses in the 20,000 ppm group compared to controls.

Conclusion : No definite evidence of malformations due to ethanol exposure were seen although the incidence of abnormal changes at the highest concentration was of borderline significance.

Reliability : (2) valid with restrictions
Well reported study which established a NOAEL

Flag : Critical study for SIDS endpoint

12.11.2004 (293)

Species : Rat
Sex : male/female
Strain : Wistar
Route of admin. : Gavage
Exposure period : throughout gestation and gestation + lactation
Frequency of treatm. : Daily
Duration of test :
Doses : 1 g/kg/day (12.5% v/v in distilled water)
Control group : yes, concurrent vehicle
Method : other
Year : 1998
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Age at study start 3 mth (females 180 g)

Number of animals: not explicitly stated for parental stock but 252 offspring subjected to further treatments and tests.

Treatment: Ethanol was administered as a 12.5% v/v solution in distilled water by gavage to yield a dosage of 1 g/kg/day to dams throughout gestation alone or through gestation and lactation.

Controls: Sucrose was added to distilled water vehicle to provide isocaloric control.

Mating conditions: Females were housed singly with male for 4 days.

Offspring were appropriately cross-fostered and divided into those that had not experienced exposure to ethanol, those that had experienced ethanol in utero and those that had experienced etanol exposure in utero and throughout lactation.

Dams were weighed during gestation. Offspring were weighed on days 3, 10, 20, 30, 45 and 63.

Offspring were subjected to assessments in Two-Way Active Avoidance (Shuttle-Box) Tests at 9 and 12 weeks and at 5 months.

Blood alcohol levels were determined on gestation day 14 and post natal day 14.

Statistical analysis was by ANOVA and Chi-squared analysis.

Remark : This study is included as a neurotoxicity study in Chapter 5.9.

Result : Mortality was significantly increased (32% versus 7% in controls) in offspring exposed to ethanol during pregnancy with continued postnatal exposure having no significant further effect.

Offspring cross-fostered to dams that had been exposed to ethanol only during pregnancy showed even greater mortality (59%). Growth in offspring was delayed during the first 9
weeks. Learning was impaired in rats of both gender at 9 weeks relative to controls. This remained evident in males, but not females, at 5 months.

No visible malformations observed.

In offspring treated both pre- and post-natally with ethanol, 60% were poor learners compared with 33% in sucrose controls.

Blood ethanol levels were 35.0 +/- 5.8mg/100ml.

Conclusion : Ethanol at a dose of 1 g/kg/day administered to dam rats during gestation and lactation produce growth and behavioural changes in the offspring.

Reliability : (2) valid with restrictions
Single dose used and no NOAEL established. High levels of mortality also a concern; high levels of cannibalism (possibly due to aggressive behaviour linked to alcohol withdrawal) offered as a possible explanation by authors.

12.11.2004 (304)

Species : Mouse
Sex : Female
Strain : C57BL
Route of admin. : oral feed
Exposure period :
Frequency of treatm. : ad lib
Duration of test :
Doses : 17%, 25% and 30% ethanol-derived calories
Control group : Yes
NOAEL maternal tox. : = 17 %
NOAEL teratogen. : = 17 %
LOAEL Maternal Toxicity : = 25 %
LOAEL Teratogenicity : = 25 %
Method : other
Year : 1979
GLP : no data
Test substance : no data

Remark : Age at study start 4-5 months
Dosage: NOAEL and LOAEL doses given as % ethanol-derived calories.
NOAEL (teratogenicity) effect is malformed foetuses and litter weight.
Doses are calculated to be equivalent to 17, 29 and 28 g/kg bodyweight
Ethanol or sucrose was added to provide calories.
Number of animals per dose per sex: not explicitly stated but approximately 16.
Mating conditions: Females were housed singly with proven studs until vaginal plugs were found.
Dams were weighed on days 0, 4, 10 and 18 (at sacrifice).
Foetuses were examined externally and internally for malformations; implants and resorptions were recorded as was litter weight.
No maternal organs were examined and foetuses were examined for external and visceral malformations.
Statistical tests were t-tests or chi-square tests; p=<0.05 regarded sign

Result : No mortality occurred.

Maternal data are not given for the following:

Number pregnant per dose level
Number aborting
Pre- and post-implantation losses
Number of corporea lutea
(Duration of pregnancy not relevant)
Haematology and blood chemistry findings
Gross pathology in dams
Organ weight changes
Histopathology incidence and severity.

Maternal data are given for the following:

The numbers of resorptions were one per litter at the two lower doses and 2/litter at the higher dose.
Number of implantations were 7.3/litter in all ethanol-treated groups.
Maternal weight gains were not affected by treatment.
Rates of diet consumption were 12.02 ml/d, 12.86 ml/d and 10.31 ml/d in the 3 ethanol dosed groups.
Clinical signs included slight tremulousness at the high-dose group when ethanol-containing diet was removed.

Blood alcohol levels ranged 3 mg% to 384mg% across the 3 treatment groups.

Foetal data are not given for the following:

Litter size
Number viable
Sex ratio
Postnatal growth
Postnatal survival

Foetal data are given for the following:

Litter weights were not significantly affected by ethanol treatments.
Grossly visible abnormalities affected limb, eye, brain, heart, uro-genital tract and abdomen. Litter weight was not affected by ethanol-containing diets but malformations were significantly increased by maternal diets containing 25% or more of ethanol-derived calories.

Grossly visible abnormalities, external, soft tissue and skeletal abnormalities affected the limb, eye, brain, heart, urogenital tract and abdomen of foetuses.

Conclusion : The teratogenicity of ethanol is demonstrated.
Reliability : (2) valid with restrictions
Well reported study which established a NOAEL.

12.11.2004 (305)

Species : mouse
Sex : male
Strain : Swiss Webster
Route of admin. : oral feed
Exposure period : 28 days
Frequency of treatm. : ad libitum
Duration of test : 28 days
Doses : 6.3% ethanol in liquid diet (32% ethanol-derived calories)
Control group : yes
NOAEL maternal tox. : < 32 %

NOAEL teratogen.	:	< 32 %
LOAEL Maternal Toxicity	:	= 32 %
LOAEL Teratogenicity	:	= 32 %
Method	:	other
Year	:	1981
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : Age at study start: 190 days.

Number of animals per group is not stated.

Ethanol was added to diet and control diet contained an isocaloric amount of sucrose.

Bodyweights were measured every two days. Blood samples were taken for blood alcohol levels.

Mating: 48 h after ethanol or sucrose diets were removed males were mated with nulliparous females by 5-days cohabitation in the same cage. If no vaginal plugs were found, new females were offered. Mating lasted until 11 days after the last ethanol treatment.

Only pregnancy rate and resorptions were recorded for dams. Corpora lutea were counted although data are not presented. Males were not examined.

Foetal examination included crown-rump length, viability, sex ratio, litter size and weight and grossly visible visceral and skeletal abnormalities.

Remark : These are PATERNAL NOAEL and LOAEL, not maternal. Fertilizaton rate was decreased 1/9 among matings 3-5 days after treatment.

Result : Crown rump length was reduced in the one litter produced by mating 3-5 days after paternal ethanol treatment.
Nine females became pregnant per dose level. There were no abortions although pregnancy rates were reduced. Resorption rates varied 0-27% across mating intervals but were unaffecetd by ethanol treatment.
Implantations, implantation losses and numbers of corpora lutea were not reported.
Paternal bodyweights were unaffected by treatment.
Clinical signs, haematological and blood biochemical parameters were not reported.
Blood alcohol levels reached 296 +/- 19 mg%.
Gross pathology, organ weight changes and histopathological incidences were not studied.

Foetal changes:

Litter size and weights, percentage of live foetuses and sex ratios were unaffecetd by ethanol treatment.
Only 2 abnormalities (undescended testes and body asymmetry) occurred in 95 pups sired by treated males.
Skeletons were not examined.

Conclusion : The role of paternal alcohol intake on anomalies seen in foetal alcohol syndrome was not conclusively established.

Reliability : (2) valid with restrictions
Well reported study but only a single high dose used, which did not allow a no effect level to be established.

12.11.2004 (306)

Species : Mouse
Sex : Female
Strain : CBA
Route of admin. : oral feed
Exposure period : -31 to 17 gestation
Frequency of treatm. : ad libitum
Duration of test :
Doses : 15, 20, 25 and 30% ethanol-derived calories
Control group : Yes
NOAEL maternal tox. : < 15 %
NOAEL teratogen. : < 15 %
LOAEL Maternal Toxicity : = 15 %
LOAEL Teratogenicity : = 15 %
Method : other
Year : 1977
GLP : no data
Test substance : no data

Remark : No early deaths were reported,
No. pregnant per dose level: 8-10
All implants were resobed at the highest concentration of ethanol in diet.
Resorption rates were 2% and 0% in controls and 57%, 72% and 100% in respective treatment groups.
Numbers of implantations were 4.8 and 5.6 in controls and 4.0, 5.5, 5.2 and 0 in the treatment groups.
Pre- and post-implantation losses were not specified.
No. of corpora lutea were not counted.
Clinical signs were not discussed although dams were described as 'alcoholic'.

Blood alcohol levels before mating werer 0 and 0 mg/dl in controls and 73, 121, 174 and 315 mg/dl in treatment groups.

Liver weight relative to body weight measured in 3 females per group before mating were not affected by treatment and there were no histopathological effects seen in liver tissue.

Result : Foetal data:

Litter size is not given. Foetal weights were depressed by treatment with means of 0.97 and 0.95 g in controls and 0.64, 0.33 and 0.51 g in the 3 lowest ethanol dose groups.

Defects included skeletal abnormalities at 100% incidence in all 3 ethanol treated groups. These effects were primarily of the occipital bone but also affected the sternum and ribs.

Visceral abnormalites affected 0% of foetuses in either control group and 36%, 100% and 100% of foetuses in the 3 ethanol treated groups. Dilated brain ventricles were the most frequent anomaly but open eyelids, exencephaly, gastroschisis and heart defects also occurred in the higher dose groups.

Test condition : Age at study start: 60-100 days.

Number of animals per group: at least 8 per group..

Ethanol was added to diet and control diet contained an isocaloric amount of sucrose. Females received ethanol in diet for 10 days before dose graduated to next higher

concentration until there were 10 females in each diet group. Depending on dose group, animals received ethanol for 30 to 80 days before mating.

Bodyweights were measured every two days. Blood samples were taken for blood alcohol levels.

Mating: 48 h after ethanol or sucrose diets were removed males were mated with nulliparous females by 5-days cohabitation in the same cage. If no vaginal plugs were found, new females were offered. Mating lasted until 11 days after the last ethanol treatment.

Only pregnancy rate and resorptions were recorded for dams. Corpora lutea were counted although data are not presented. Males were not examined.

Foetal examination included crown-rump length, viability, sex ratio, litter size and weight and grossly visible visceral and skeletal abnormalitie

Reliability : (2) valid with restrictions
Not a standard study protocol but appears to be well conducted and reported. Limited and very high dose does not allow a NOAEL to be established.

12.11.2004 (307)

Species : Mouse
Sex : Female
Strain : C3H
Route of admin. : oral feed
Exposure period : -31 to day 17 gestation
Frequency of treatm. : Ad libitum
Duration of test :
Doses : 20, 25, 30 and 35% ethanol-derived calories
Control group : Yes
NOAEL maternal tox. : = 20 %
NOAEL teratogen. : < 20 %
LOAEL Maternal Toxicity : = 25 %
LOAEL Teratogenicity : = 20 %
Method : other
Year : 1977
GLP : no data
Test substance : no data

Remark : Maternal data:
No early deaths were reported.
No. pregnant per dose level: 8-10
All implants were resorbed at the highest concentration of ethanol in diet.
Resorption rates were 7% and 0% in controls and 0% 30%, 72% and 100% in respective treatment groups. Early and late resorptions were not distinguished.
Numbers of implantations were 11 and 7.3 in controls and 6.8, 6.5, 6.1 and 0 in the treatment groups. Pre- and post-implantation losses were not specified.
No. of corpora lutea were not counted.
Clinical signs were not discussed. Animals were descriubed as 'alcoholic'.
Bodyweight: Not given.
Food/water consumption: Calorific intake was similar across all groups.
Haematological findings: Not measured.
Blood alcohol levels were 103, 160, 289 and 398 mg/dl in the treatment

groups.
Clinical biochemistry and gross pathology: Not examined.
Organ weight changes: Liver weight relative to bodyweight was not affeceted by treatment.
Histopathology: No liver pathology was detected.

Foetal data:

Litter size and weights: Not given. Foetal weights in controls were depressed (1.14 and 1.27 g in controls and 0.77, 0.50 and 0.58 g in the ethanol dosed groups).
Number viable and sex ratio: Not reported.
Postnatal growth and postnatal survival: Not applicable.
Foetuses showed high rates of skeletal and visceral anomalies at all doses yielding foetuses.

Blood alcohol levels before mating werer 0 and 0 mg/dl in controls and 73, 121, 174 and 315 mg/dl in treatment groups.

Liver weight relative to body weight measured in 3 females per group before mating were not affected by treatment and there were no histopathological effects seen in liver tissue.
These data and test conditions were taken from Source document which did not present the author and reference.

Result : Foetal data:

Litter size is not given. Foetal weights were depressed by treatment with means of 0.97 and 0.95 g in controls and 0.64, 0.33 and 0.51 g in the 3 lowest ethanol dose groups.

Defects included skeletal abnormalities at 100% incidence in all 3 ethanol treated groups. These effects were primarily of the occipital bone but also affected the sternum and ribs.

Visceral abnormalites affected 0% of foetuses in either control group and 36%, 100% and 100% of foetuses in the 3 ethanol treated groups. Dilated brain ventricles were the most frequent anomaly but open eyelids, exencephaly, gastroschisis and heart defects also occurred in the higher dose groups.

Test condition : Age at study start: 60-100 days.

Number of animals per group: at least 8 per group.

Ethanol was added to diet and control diet contained an isocaloric amount of sucrose. Females received ethanol in diet for 10 days before dose graduated to next higher concentration until there were 10 females in each diet group. Depending on dose group, animals received ethanol for 30 to 80 days before mating.

Bodyweights were measured every two days. Blood samples were taken for blood alcohol levels.

Mating: mated in pairs during 1.5 hr periods. Copulation plugs indicative of mating.

Parameters assessed: Blood ethanol levels and relative liver weights before mating and fetal weights and abnormalities.

Organs examined at necropsy: Adult livers. Foetuses were examined for

abnormalities of the skeleton and internal organs.

Foetal examination included crown-rump length, viability, sex ratio, litter size and weight and grossly visible visceral and skeletal abnormalities.

Reliability : (2) valid with restrictions
Well reported study but not to a standard protocol. Very high doses used and no NOAEL identified.

12.11.2004 (308)

Species : Mouse
Sex : Female
Strain : CD-1
Route of admin. : Gavage
Exposure period : 8-14 gestation
Frequency of treatm. : once per day
Duration of test :
Doses : 2,200, 3,600, 5,000 and 7800 mg/kg bw
Control group : Yes
NOAEL maternal tox. : = 2200 mg/kg bw
NOAEL teratogen. : >= 6400 mg/kg bw
LOAEL Maternal Toxicity : = 3600 mg/kg bw
LOAEL Embryotoxicity : > 6400 mg/kg bw
Method : other
Year : 1987
GLP : no data
Test substance : other TS: 200 proof

Method : Age at start: 8-10 wks
Number of animals per dose pewr sex: 6 confirmed pregnant/group.
Ethanol was administered by gavage in distilled water in 10 ml bolus doses.
Clinical observations performed were physical examination and weight recording 6 times through pregnancy. Viability checked twice daily.
Females were paired 1:1 with males and vaginal plugs were indicative of pregnancy.
Parameters assessed were maternal bodyweights, numbers of implantation sites, resorptions, live and dead foetuses, external abnormalities.

No organs were examined at necropsy.

Statistical tests were Bartlett's for homogeneity of variance, one-way ANOVA, Dunnett's, Duncan, Kruskal-Wallis, Dunn's and nested ANOVA.

Remark : Maternal data:

Mortality and day of detah: No control animals died.
Mortality rates in treated groups were 0/6, 1/6, 4/6, 5/6 and 6/6. Day of death not reported.
Number pregnant per dose level: 6
Number aborting: Not reported. Possibly 2 litters were aborted at 5000 mg/kg. A surviving dam at 6400 mg/kg delivered a litter.
Number of resorptions: Not distinguised. Resorptions per litter did not differ from control below 5000 mg/kg.
Mean implantations ranged from 10.5 in controls to 13.83 but no significant difference noted.
Pre- and post-implantation loss: Not reported.
No. of corpora lutea: Not measured.
Duration of pregnancy: dams killed on gestation day 18.
Bodyeight: Not affected by treatment.
Food consumption: not reported.
Clinical signs: Timing and duration not reported. At 3600 mg/kg and above

dams were lethargic with staggering gait and or laboured breathing.
Haematological findings: Not measured.
Clinical biochemistry: Not measured.
Gross pathology: Not reported.
Organ weight changes: Not measured.
Histopathology: Not reported.

Foetal data:

Litter size and weights: Not reported. Group means were not significantly different from controls.
Number viable: Mean number of dead foetuses per litter did not vary significantly with dose and ranged 0 to 0.5. Number of live foetuses per litter differed significantly from controls at 5000 mg/kg.
Sex ratio: Not reported.
Postnatal growth: Not applicable.
Postnatal survival: Not applicable.
Grossly viable abnormalities etc: No externally malformed foetuses were found in treated groups. Other types of abnormality were not sought.

Result : No maternal mortality occurred at 2200 mg/kg but 1/6 dams died at 3600 mg/kg rising to 6/6 at 7700 mg/kg. At doses of at least 3600 mg/kg, dams were lethargic and showed
staggered gait and/or laboured breathing.

At 5000 mg/kg, resorption of litter were increased and live foetuses/litters were decreased. This was not apparent in the one litter at 6400 mg/kg. No other fetal effects were seen.

Foetal data:

Group mean litter weights ranged from 1.33 g (controls) to 0.99 g and did not vary with statistical significance. Mean number of dead foetuses per litter was not dose related and ranged from 0 to 0.5.

No externally visible malformations were found in foetuses from treated animals.

Conclusion : No dose-related adverse effects on foetuses were observed at doses close to those causing acute maternal toxicity.

Reliability : (2) valid with restrictions
Well reported study but not to a standard protocol.

12.11.2004 (309)

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : drinking water
Exposure period : Days 6-15 gestation
Frequency of treatm. : Daily
Duration of test : ad-libitum
Doses : 15% ethanol in water
Control group : yes, concurrent vehicle
NOAEL teratogen. : > 15 %
Result : not developmentally toxic
Method : other
Year : 1978
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Animal details: supplied by Spartan Research Animals, Haslett, Michigan; weight 250-270 g
Acclimation: 2-3 weeks at 72 degF and 45% RH;light cycle 12 hr dark, 12

hr dark.
Dosing: Liquid provided via a ball tipped waterer to minimise evaporation.
Food: Purina lab chow.
Day 0 taken when sperm observed in a vaginal smear.
Blood alcohol determinations in non pregnant animals (using an alcohol dehydrogenase method): 10 am, 2 pm, 10 pm, 2 am and 6 am on day 4 of treatment.
Sacrifice: day 21
Observations:
-Weights (dams daily and offspring)
- Live, dead and resorbed fetuses.
- Crown-rump length
- Number of each sex
- External examination and check for cleft palate.
- One third of fetuses examined histopathologically for soft tissue damage; heads preserved in Bouins solution and examined for soft tissue damage but not skeletal alterations.
- All fetuses preserved in alcohol and subsequently processed for skeletal alterations.
Statistical evaluation: Litter used as experimental unit. Wilcoxon test as modified by Hasseman and Hoel to evaluate incidence of fetal alterations and resorptions. Analysis of variance used for maternal and fetal bodyweights. Level of significance $p<0.05$.

Result : Mean consumption of food and liquid by rats given ethanol was significantly less that that of control rats during the experimental period. As a result, mean gain in body weight of the exposed rats was also significantly less between days 6 and 16 of gestation. Ethanol ingestion did not affect fetal survival adversely, but mean fetal body weight was significantly less than that of the control litters.

No malformed fetuses were found in the experimental litters. No external or soft tissue alterations were observed among the fetuses of the control or experimental litters. Skeletal malformations were not detected in the experimental group but skeletal variants consisting of unfused bones of the skull and cervical vertebra with missing centra occurred in the ethanol litters at an incidence significantly greater than in the control litters.

Reduced fetal body weight (5.41g+/-0.25; control 5.70+/-0.35)
Non fused sternebrae (100 in 18 litters; control 45 in 8)
Vertebrae-missing centra (117 in 18 litters; control 15 in 8)
Wavy ribs (13 in 5 litters; control 3 in 3)
Note: total skeletal fetuses examined 223 in 29 litters

Overall there were no definite teratogenic abnormalities.

Peak blood alcohol levels: 40 mg/100ml blood at 6am.

Test substance : Reagent grade ethyl alcohol 190 proof from US Industrial Chemicals Co.
Reliability : (2) valid with restrictions
Well reported study but only single dose used.

12.11.2004 (310)

Species : Mouse
Sex : Female
Strain : CD-1
Route of admin. : drinking water
Exposure period : days 6-15 of gestation
Frequency of treatm. : Daily
Duration of test : ad libitum
Doses : 15% ethanol in drinking water
Control group : yes, concurrent vehicle
NOAEL teratogen. : > 15 %

Result : no developmental toxicity observed.
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Animals: Virgin mice from Carworth Animals, Portage, Michigan, weight 22-25 g.
Acclimation: 2-3 weeks at 72 degF and 45% RH;light cycle 12 hr dark, 12 hr dark.
Dosing method: Liquid provided via a ball tipped waterer to minimise evaporation.
Food: Purina lab chow.
Day 0 taken when vaginal plug observed in mice.
Blood alcohol determinations in non pregnant animals (using an alcohol dehydrogenase method): 10 am, 2 pm, 10 pm, 2 am and 6 am on day 4 of treatment.
Sacrifice: day 18
Observations:
-Weights (dams daily and offspring)
- Live, dead and resorbed fetuses.
- Crown-rump length
- Number of each sex
- External examination and check for cleft palate.
- One third of fetuses examined histopathologically for soft tissue damage; heads preserved in Bouins solution and examined for soft tissue damage but not skeletal alterations.
- All fetuses preserved in alcohol and subsequently processed for skeletal alterations.
Statistical evaluation: Litter used as experimental unit. Wilcoxon test as modified by Hasseman and Hoel to evaluate incidence of fetal alterations and resorptions. Analysis of variance used for maternal and fetal bodyweights. Level of significance $p<0.05$.

Result : Mice receiving ethanol consumed significantly less food and liquid than control mice. Consumption returned to control levels within two days after removal of the ethanol. Maternal body weight gain reflected the decreased consumption of food and liquid. The number of live fetuses/litter was not significantly affected but fetal weight and length were significantly decreased upon comparison to control values. Other than one fetus with cleft palate and two fetuses from different litters that had exencephaly with open eye, no external or soft tissue alterations were noted among the offspring of dams given ethanol. The incidence of exencephaly, open eye, and cleft palate did not differ significantly from control values. Skeletal malformations were not detected but the incidence of several minor skeletal variants e.g. delayed ossification of the centra of cervical vertebra, non-fused sternebrae and delayed ossification of sternebrae (less than 90% ossified), was significantly increased among the litters of mice ingesting ethanol.

Significant effects in mice:
reduced fetal body weight (0.95g+/-0.12; control 1.11+/-0.11)
Reduced crown rump length (22.2+/-1.0mm; control 23.5+/-1.2mm)
Non fused sternebrae (52 in 18 litters; control 26 in 12)
Delayed ossification (59 in 17 litters; control 7 in 4)
Note: total skeletal fetuses examined 239 in 21 litters

Overall there were no significant teratogenic abnormalities.

Peak blood alcohol levels were 204 mg/100ml at 2am.

Test substance : Reagent grade ethyl alcohol 190 proof from US Industrial Chemicals Co.
Reliability : (2) valid with restrictions

Well reported study but only single dose level used.
12.11.2004 (311)

Species	:	Rabbit
Sex	:	Female
Strain	:	New Zealand white
Route of admin.	:	drinking water
Exposure period	:	Days 6-18 of gestation
Frequency of treatm.	:	Daily
Duration of test	:	ad libitum
Doses	:	15% ethanol in drinking water
Control group	:	yes, concurrent vehicle
NOAEL teratogen.	:	> 15 %
Result	:	not developmentally toxic
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	other TS

Method : Animals: supplied by Langshaws Rabbitry, Augusta, Michigan. Initial animal weights not specified.
Acclimation: 2-3 weeks at 72 degF and 45% RH;light cycle 12 hr dark, 12 hr dark.
Dosing: Liquid provided via a ball tipped waterer to minimise evaporation.
Food: Purina lab chow.
Day 0 taken when mating observed.
Blood alcohol determinations in non pregnant animals (using an alcohol dehydrogenase method): 10 am, 2 pm, 10 pm, 2 am and 6 am on day 4 of treatment.
Sacrifice: day 29
Observations:
-Weights (dams daily and offspring)
- Live, dead and resorbed fetuses.
- Crown-rump length
- Number of each sex
- External examination and check for cleft palate.
- One third of fetuses examined histopathologically for soft tissue damage; heads preserved in Bouins solution and examined for soft tissue damage but not skeletal alterations.
- All fetuses preserved in alcohol and subsequently processed for skeletal alterations.
Statistical evaluation: Litter used as experimental unit. Wilcoxon test as modified by Hasseman and Hoel to evaluate incidence of fetal alterations and resorptions. Analysis of variance used for maternal and fetal bodyweights. Level of significance $p<0.05$.

Result : Liquid consumption of animals receiving ethanol was significantly less than that of controls as was mean body weight (latter difference as statistically significant on days 12 and 18 of gestation.)
The incidence of resorptions among litters of rabbits given ethanol was approximately twice that observed in the control litters; this increase was due primarily to the complete resorption of two litters in the ethanol group. Fetal body measurements and the number of malformed fetuses were comparable between the control and experimental litters. No alterations were observed at an incidence that was significantly increased in the ethanol group compared to the control group. Minor vascular alterations observed have all been found to occur spontaneously in control groups of this strain of rabbit.

Overall there were no teratogenic abnormalities

Peak blood alcohol level was 24mg/100ml at 6am.

Test substance	:	Reagent grade ethyl alcohol 190 proof from US Industrial Chemicals Co.
Reliability	:	(2) valid with restrictions A well reported study but only a single dose used.

02.06.2004 (311)

Species	:	Rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	7 hours
Frequency of treatm.	:	Daily
Duration of test	:	see method details
Doses	:	0, 10000, 16000ppm
Control group	:	Yes
NOAEL maternal tox.	:	> 16000 ppm
other: NOAEL behavioural teratogenicity	:	> 16000 ppm
other: NOAEL fertility	:	> 16000 ppm
Result	:	Negative
Method	:	other: see method details
Year	:	1985
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : Behavioural testing procedures:
Behavioral testing was from days 10 - 90. Female and male pups were selected randomly. For each test, one female and one male were used from each litter. Testers were not aware of the treatment groups to which subjects belonged. Tests used:
1. Rotorod, 9cm in diameter and 10 cm long, and the surface was rough with sand. Rotation speed increased until the animals had five unsuccessful trials.
2. The open field was 1 m in diameter, with an enclosure wall 0.5 m high. Animals were tested for 3 min.
3. Optical digital animal activity monitor. The animal test area was a 40x40x20 cm Plexiglas cage which had 30 photodiodes per side. Activity scores were summed over the 3 days of testing at each age.
4. Running wheel activity over a 24 hr period, separated into day and night activity scores.
5. Two shuttle boxes in sound-attenuated chambers, with 4 cm center partitions. Metal grid floors to which electrical shocks could be applied. 5 sec warning stimulus.
hr/day until they no longer responded sufficiently to receive reinforcement.

Exposure was conducted in 0.5m3 chambers with dynamic air flow (one air change per minute.)

Animals: 18/group. Starting weights 400-500g.
Temperature: 73 +/- 3F. Humidity: average 40-50%.
Exposure period: Males: 6 weeks; 2 day non exposure period before mating. Females: GD 1-20. Mating period 5 days. 7 hours per day.
Mating confirmed by presence of sperm plugs under cages or vaginal smears. Females housed individually.
Analytical monitoring: Yes (IR analyser - exposures found to be within 11% of nominal). Independently cross-checked with charcoal adsorption tubes.
Parameters measured: weights (daily), food and water intake as measures of maternal toxicity.
Parturition: All litters culled to 4 pups of each sex and fostered to untreated dams which had delivered within past 2 days. On PND10 pups individually identified by ear punch and randomly assigned to behavioural study

groups.

Result : No effect on weight gain, feed or water intake. No effect on fertility or litter sizes.
Behavioural testing showed no difference from controls in any of the behavioural tests.
Previous studies quoted as showing exposures to 10000 and 16000ppm ethanol typically give rise to blood ethanol concentrations of 30 and 500mg/l ethanol. Authors calculate that for rats exposures in excess of 11000ppm are required to begin accumulating ethanol in the blood and that ethanol is no more toxic by the inhalation route than by other routes.

Reliability : (2) valid with restrictions
Well reported study but not to a standard protocol. Route of exposure highly relevant.

12.11.2004 (291) (292)

Species : Rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : inhalation
Exposure period : 7 hours
Frequency of treatm. : Daily
Duration of test : see method details
Doses : 0, 10000, 16000pm
Control group : Yes
Method : other: see method details
Year : 1988
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Behavioural testing procedures:
Behavioral testing was from days 10 - 90. Female and male pups were selected randomly. For each test, one female and one male were used from each litter. Testers were not aware of the treatment groups to which subjects belonged. Tests used:
1. Rotorod, 9cm in diameter and 10 cm long, and the surface was rough with sand. Rotation speed increased until the animals had five unsuccessful trials.
2. The open field was 1 m in diameter, with an enclosure wall 0.5 m high. Animals were tested for 3 min.
3. Optical digital animal activity monitor. The animal test area was a 40x40x20 cm Plexiglas cage which had 30 photodiodes per side. Activity scores were summed over the 3 days of testing at each age.
4. Running wheel activity over a 24 hr period, separated into day and night activity scores.
5. Two shuttle boxes in sound-attenuated chambers, with 4 cm center partitions. Metal grid floors to which electrical shocks could be applied. 5 sec warning stimulus.
hr/day until they no longer responded sufficiently to receive reinforcement.
Exposure was conducted in 0.5m3 chambers with dynamic air flow (one air change per minute.) Dosing method described in detail.

Animals: Starting weights: females, 15 per group, 176-200g; males, 18 per group >390g.
Temperature: 24+/-2C. Humidity: ~40%. 12hr light/dark cycle.
Exposure period: Males: 6 weeks; 2 day non exposure period before mating. Females: GD 1-20. Mating (1:1 male:female) period 5 days.
Mating confirmed by presence of sperm plugs under cages or vaginal smears. Females housed individually. Stock males used as sires for controls.
Analytical monitoring: Yes (IR analyser - exposures found to be +/-200ppm of nominal). Independently cross-checked with charcoal adsorption tubes

analysed by gas chromatography.
Parameters measured weekly: weights food and water intake as measures of maternal toxicity.
Parturition: All litters weighed within 16 hrs. Litters less than 3 pups per sex discarded. Offspring weighed on PND 7 and checked for abnormalities
Neurochemical analysis: One female and one male (untested) from 5 litters sacrificed PND 21 for analysis of concentrations of protein and the neurotransmitters acetylcholine. dopamine, norepinephrine, 5-hydroxytryptamine, substance P. beta-endorphin, and Met-enkephalin. Pup brains were separated into the four general brain regions of cerebrum, cerebellum, brainstem meduila-pons), and midbrain and frozen until assayed by sonic homogenization in 8 ml of 0. 1 N HCl.

Statistical Analyses: Behavioral data were analyzed using multivariate analysis of variance or an m-ranking procedure. Repeated measures analyses were conducted where appropriate to $p<0.05$. Neurochemical data were analyzed using Analysis of Variance followed by Duncan's Multiple Range post-hoc tests where a significance was found.

Remark : Authors concluded that industrial inhalation exposure to ethanol may not be expected to produce alarming blood ethanol levels and that inhalation exposures to ethanol which produce narcosis in maternal rats are not teratogenic.

Result : Males: weight gain retarded during 1st week but normal thereafter.
Females: no effect on weight gain. Feed intake retarded during 1st week but normal thereafter at 16000ppm.
No effects on litter size, still births, length of pregnancy, offspring survival.
No effect observed in behavioural study tests.
No effect on dopamine, substance P, beta-endorphin and acetylcholine levels.
Significant effects on norepinephrine, 5-hydroxytryptamine but magnitude and direction of changes not correlated with dose. Level of Met-enkephalin affected at lower but not higher dose.

Reliability : (2) valid with restrictions
Well reported study but not to a standard protocol. Route of exposure highly relevant.

12.11.2004 (291)

Species : Monkey
Sex :
Strain : Macaca Fascicularis
Route of admin. : other: in utero expusure
Exposure period : throughout gestation
Frequency of treatm. : Daily
Duration of test :
Doses :
Control group :
Method : other
Year : 1999
GLP : no data
Test substance : no data

Remark : There was some correlation with cognitive performance and this study is further considered in Neurotoxicity.

Result : Standardised craniofacial cephalograms of 18 macaques exposed weekly to ethanol or sucrose solution in utero were measured at ages 1, 6, 12 and 24 mths showed that there may be a critical period for induction of alcohol-induced craniofacial alterations. These were most prominent at 6 mths and diminished thereafter as underlying changes in
skeletal structure caused disappearance of the thin upper lip and smooth philtrum characteristic of feat alcohol syndrome.

Reliability : (4) not assignable

12.11.2004 (312)

Species : Human
Sex :
Strain :
Route of admin. : oral feed
Exposure period :
Frequency of treatm. :
Duration of test :
Doses :
Control group :
Method : other:literature review
Year : 1999
GLP :
Test substance :

Remark : The large and growing evidence of the effects of parental alcohol consumption on offspring was assessed. Study of clinical cases support the conclusion that chronic heavy maternal drinking consistent with alcohol dependence or alcoholism is an aetiological factor in foetal alcohol syndrome. The effects of this can extend beyond puberty with cognitive and neurobehavioural problems continuing and with the most deleterious of outcomes. Animal studies have demonstrated a dosage relationship in the absence of the confounding factors prevalent in humans (e.g. poor maternal health and tobacco smoke) and that the effects of alcohol are most pronounced on the brain. Individual differences in maternal metabolism and genetic factors may explain why the infants of some problem-drinking mothers are more affected than others but there is also some evidence of a threshold of drinking below which adverse effects cannot be detected. This may be around 30 to 40 g per day (4-5 UK drinks or 2.5 to 3.5 US drinks per day). This level is well above the levels defined as moderate drinking for non-pregnant women.
This is a literature review presented as a book chapter.
Reliability : (4) not assignable
12.11.2004 (313)

Species : Rat
Sex : Female
Strain :
Route of admin. : drinking water
Exposure period : 4 weeks and throughout gestation
Frequency of treatm. :
Duration of test :
Doses : 20% alcohol before mating and 30% through gestation; 3500 mg/kg/day throughout treatment.
Control group :
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Offspring of female Sprague-Dawley rats that were administered ethanol in water as the drinking fluid at a concentration of 20% v/v for four weeks before mating and at a concentration of 30% v/v during gestation were physically and developmentally retarded and failed to catch up with control offspring during the first four weeks postpartum.
Reliability : (4) not assignable
12.11.2004 (314)

Species : Rat
Sex : Female

Strain : Sprague-Dawley
Route of admin. : drinking water
Exposure period : 4 weeks before mating and on days 1 to 20 of gestation.
Frequency of treatm. :
Duration of test :
Doses : 20-30% in water; estimated 4000 mg/kg/day.
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : The offspring of Sprague-Dawley rats given 20% ethanol in the drinking-water four weeks before mating and 30% ethanol in drinking-water until gestation day 20 had retarded skeletal development and decreased body weight but no gross malformation.
Reliability : (4) not assignable
12.11.2004 (315)

Species : Rat
Sex : Female
Strain : Long-Evans
Route of admin. : drinking water
Exposure period : 10 or 90 days before gestation and during gestation
Frequency of treatm. :
Duration of test :
Doses : 9220 mg/kg/day during 10 day pre-exposure; 14500 mg/kg/day in 90 day preexposure and 11,300 mg/kg/day during gestation
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : This is an important study in that it confirms that 5% v/v in feed delivers a dosage of 12,000 to 14,000 mg/kg.
Result : A decrease in foetal bodyweight was noted in each dosage regimen. There were no gross abnormalities or skeletal defects noted.
Reliability : (4) not assignable
12.11.2004 (316)

Species : Rat
Sex :
Strain : Long-Evans
Route of admin. : drinking water
Exposure period : GD 7 to delivery
Frequency of treatm. :
Duration of test :
Doses : 10%
Control group :

Remark : Study was designed to assess ethanol effects on sexual differentiation.
Result : Gestation was prolonged and offspring of each sex showed decreased anogenital distances at birth. Pups nursed by ethanol-drinking mothers had a significantly earlier preputial separation, but there was no effect on adult masculine sex behaviour, plasma testosterone or weights of accessory sex glands.
Reliability : (4) not assignable
12.11.2004 (317)

Species : Mouse

Sex	:	Female
Strain	:	other: not specified
Route of admin.	:	drinking water
Exposure period	:	from 11 weeks before mating and throughout gestation
Frequency of treatm.	:	
Duration of test	:	
Doses	:	10-20% in drinking water; Estimated 3000 to 5000 mg/kg/day
Control group	:	no data specified
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Retardation of muscle growth was seen in offspring killed at 12 weeks of age of inbred mice given 10-20% ethanol in the drinking-water for 11 weeks before mating and 30% ethanol after breeding until delivery. Prenatally, there was suppression of hyperplasia of muscle fibres during myogenesis; postnatally, there was suppression of normal hypertrophy of individual muscle fibres.
Reliability	:	(4) not assignable

12.11.2004 (318)

Species	:	Mouse
Sex	:	Female
Strain	:	C57BL
Route of admin.	:	drinking water
Exposure period	:	Before mating, throughout gestation and lactation
Frequency of treatm.	:	
Duration of test	:	as above
Doses	:	10% (v/v)
Control group	:	no data specified
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Fetotoxicity and structural teratology: When female C57Bl/Crgl mice were given 10% ethanol (v/v) in water as the drinking fluid before mating, throughout gestation and lactation, no significant effect on pup development or behaviour was seen.
Reliability	:	(4) not assignable

12.11.2004 (303)

Species	:	Dog
Sex	:	Female
Strain	:	Beagle
Route of admin.	:	Gavage
Exposure period	:	
Frequency of treatm.	:	twice daily
Duration of test	:	throughout gestation
Doses	:	1800 mg/kg/day
Control group	:	no data specified
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Dogs were administered 1.8 g/kg bw ethanol as a 25% solution by gavage twice daily and were given either a normal-proteino r low-protein diet throughout gestation.
Result	:	Ethanol consumption and low dietary protein intake, independently of each

other, significantly decreased maternal weight gain as well as the weight of the neonates

Reliability : (4) not assignable
12.11.2004 (319)

Species : Dog
Sex :
Strain :
Route of admin. : Gavage
Exposure period : throughout gestation
Frequency of treatm. :
Duration of test :
Doses : 3 or 3.6g/kg bodyweight
Control group :

Result : There were no gross or histological abnormality, a slight decrease in the number of offspring per litter and in pup weight, and an increase in the number of still births. Blood ethanol concentrations were 1.3-1.75 g/l,
Reliability : (4) not assignable
12.11.2004 (320)

Species : Monkey
Sex : Female
Strain : Macaca Fascicularis
Route of admin. : Gavage
Exposure period : Starting before day 10 or on day 40 of gestation
Frequency of treatm. : once per week
Duration of test : throughout gestation as above
Doses : 0.3 - 4.1 g/kg bw
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Spontaneous abortion frequency increased at peak plasma ethanol concentrations above 2 g/l. Developmental alterations were observed consistently in offspring of monkeys with blood levels greater than 1.5 g/l when treatment was initiated at the start of gestation; infants exposed only after gestation day 40 were less consistently abnormal despite higher maternal blood ethanol levels (5.5g/l). There were developmental alterations and an increase in spontaneous abortions at peak plasma ethanol levels above 200 mg/100 ml. Developmental alterations in offspring were consistent at blood alcohol levels in excess of 150 mg/100 ml.
Reliability : (4) not assignable
12.11.2004 (321) (322)

Species : Rat
Sex : male/female
Strain :
Route of admin. : oral feed
Exposure period : from 6 weeks before mating
Frequency of treatm. :
Duration of test :
Doses : Estimated much greater than 12,000 mg/kg/day
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Male Sprague-Dawley rats maintained for six weeks on a liquid diet containing 10% ethanol were paired with untreated females.
Result : There was body weight loss and central nervous system impairment, and only half of the treated animals had successful matings, compared to all of the controls. There was a decrease in litter size and an increase in prenatal mortality among the litters.
Reliability : (4) not assignable
12.11.2004 (323)

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : Days 1-21 of gestation
Frequency of treatm. :
Duration of test :
Doses : 35% of calorie intake; estimated 12150 mg/kg/day
Control group :
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : When Sprague-Dawley rats were given ethanol as 35% of total calories in a liquid diet on gestation days 1-21, offspring had abnormal distribution of nerve fibres in the temporal regions of the hippocampus, which persisted to maturity.
Reliability : (4) not assignable
12.11.2004 (324)

Species : Rat
Sex : Female
Strain : Wistar
Route of admin. : oral feed
Exposure period : Days 6-19 of gestation
Frequency of treatm. :
Duration of test :
Doses : 5% in diet; average dose 11,200 mg/kg (range 12,000-14,000)
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Gestation length, litter survival or birth weight. Pup weight was not affected by treatment from birth to 28 days. Offspring motor activity was significantly increased at 16 days of age.
Reliability : (4) not assignable
12.11.2004 (325)

Species : Rat
Sex : Female
Strain : Long-Evans
Route of admin. : oral feed
Exposure period : Days 6-20 gestation
Frequency of treatm. :
Duration of test :
Doses : 35% of calorie intake, estimated 12,150 mg/kg/day
Control group : no data specified
Method : other

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

Result : Offspring showed lower maximal suckling pressure as well as suckling pattern changes.
Reliability : (4) not assignable
12.11.2004 (326)

Species : Rat
Sex : Female
Strain : Long-Evans
Route of admin. : oral feed
Exposure period : Days 6-20 of gestation
Frequency of treatm. :
Duration of test :
Doses : 35% of calorie intake. Estimated 12,150 mg/kg/day.
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Males showed feminization; females showed masculinization indicating prenatal hormonal disruption.
Reliability : (4) not assignable
12.11.2004 (327)

Species : Rat
Sex : Female
Strain : Wistar
Route of admin. : oral feed
Exposure period : From day 12 of gestation to 10 days post partum
Frequency of treatm. :
Duration of test :
Doses : Estimated 12,150 mg/kg/day; 36% of calorie intake.
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Offspring showed decreased ano-genital distance; decreased male reproductive organ weights 55 and 110 days post partum; lowered sex hormones (LH and testosterone), sexual motivation and performance; lowered phenotypic masculinization.
Reliability : (4) not assignable
12.11.2004 (328)

Species : Rat
Sex : Female
Strain :
Route of admin. : oral feed
Exposure period : 16 weeks
Frequency of treatm. :
Duration of test : 16 weeks
Doses : 5% ethanol in liquid feed
Control group : no data specified
Method : other
Year :
GLP : no data

Test substance : as prescribed by 1.1 - 1.4

Result : Mating of female Holtzman rats fed a liquid diet containing 5% ethanol for 16 weeks with untreated males resulted in no adverse effect on litter size or neonatal body weight.
Reliability : (4) not assignable
12.11.2004 (285)

Species : Rat
Sex : Female
Strain : Wistar
Route of admin. : oral feed
Exposure period : Before mating, throughout gestation and lactation
Frequency of treatm. :
Duration of test : as above
Doses : 12% ethanol in sucrose solution (20-25% calorie intake)
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : When female Wistar rats were given 20-25% of the calories consumed as 12% ethanol in a sucrose solution as the drinking fluid before mating and throughout gestation and lactation, there was no effect on development of offspring
There was no effect on development or offspring.
Reliability : (4) not assignable
12.11.2004 (329)

Species : Rat
Sex : Female
Strain :
Route of admin. : oral feed
Exposure period : 1 year
Frequency of treatm. : Daily
Duration of test :
Doses : Blood alcohol level of 22.8 mmol/l
Control group : yes, concurrent no treatment
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : To study the severity and degree of in utero alcohol effects in relation to the rate of maternal alcohol damage, multiparous 1-year alcohol-fed rats were used, with an appropriate pair-fed control group. During pregnancy, alcoholic dams showed relatively high acetaldehyde levels (41 +/- 19 mumol/l) and blood alcohol levels of 22.8 +/- 14 mmol/l.
Remark : The stage of maternal alcohol illness, as indicated mainly by the extent of liver damage, may play an important role in the frequency and severity of in utero alcohol effects in the rat.
Result : Dams showed marked histological alterations in liver as well as high serum aspartate-aminotransferase, alanine- aminotransferase, alkaline phosphatase, glutamate dehydrogenase, and gamma-glutamyltransferase activities. The increase in serum enzyme levels did not correlate with an increase in hepatic enzyme levels since only glutamate dehydrogenase was enhanced in liver after 1 year of alcohol intake. In addition, except for an increase in low Km aldehyde dehydrogenase activity, there were no changes in liver alcohol metabolizing enzymes in chronic alcohol vs. pair-fed females.

Alcoholic rats showed a high incidence of damage in their progeny (resorptions, immature fetuses, decrease in fetal weight, etc.), and rats with the highest serum levels of theabove enzymes (especially glutamate dehydrogenase and gamma-glutamyl transferase) had severely affected progeny. Rats with minimal histological liver damage, in contrast, did not show resorptions.

Reliability : (4) not assignable
12.11.2004 (330)

Species : Rat
Sex :
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : GD 7 to parturition
Frequency of treatm. :
Duration of test :
Doses : liquid diet containing ethanol as 35% of calories
Control group :

Result : Absence of sexual dimorphism (saccharin preference and maze learning) was seen among offspring, suggesting disrupted perinatal androgen status.
Reliability : (4) not assignable
12.11.2004 (331)

Species : Rat
Sex :
Strain : Long-Evans
Route of admin. : oral feed
Exposure period : GD 6-20
Frequency of treatm. :
Duration of test :
Doses : Liquid diet containing 35% ethanol derived calories
Control group :

Result : Offspring males showed feminized behaviour and females showed masculinized behaviour, suggesting disruption of the hormonal environment prenatally.
Reliability : (4) not assignable
12.11.2004 (332)

Species : Rat
Sex :
Strain : Long-Evans
Route of admin. : oral feed
Exposure period : GD 6-20
Frequency of treatm. :
Duration of test :
Doses : liquid diet containing 35% ethanol derived calories
Control group :

Result : There was evidence of behavioural deficits, which persisted until adulthood. Female offspring showed a variety of deficits in maternal behaviour when adult, which may have been related to prenatal hormonal alterations.
Reliability : (4) not assignable
12.11.2004 (333)

Species : Mouse
Sex : Female
Strain : C3H

Route of admin. : oral feed
Exposure period : Days 0-17 of gestation
Frequency of treatm. :
Duration of test : Days 0-17 gestation
Doses : 4.1% w/v in liquid diet
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Groups of C3H mice were given a liquid diet or a fortified liquid diet, each either alone or with 4.1% w/v ethanol, from days 0-17 of pregnancy; a further group was given an amount of liquid diet equal to that consumed by the group given liquid diet plus ethanol.
Result : Ethanol consumption inhibited foetal growth and development but did not affect litter size irrespective of the diet used.
Reliability : (4) not assignable
12.11.2004 (334)

Species : Mouse
Sex : Female
Strain : other: RAP
Route of admin. : i.v.
Exposure period :
Frequency of treatm. : once on day 3 or 4 of gestation
Duration of test :
Doses : not specified
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Preimplantation effects in a study on the effects of preimplantation exposure: mice were given ethanol intravenously on days 3 and 4 of pregnancy and offspring were examined on day 19 of pregnancy.
Result : On day 19 of gestation the mean foetal and placental weightswere significantly lowered but there was no effect on skeletal development.
Reliability : (4) not assignable
12.11.2004 (335)

Species : Monkey
Sex : Female
Strain : other: rhesus and cynomolgus
Route of admin. : i.v.
Exposure period : 1-2 minutes
Frequency of treatm. :
Duration of test : Gestation days 120-147
Doses : 3g/kg bodyweight
Control group :

Result : In monkeys given 3g/kg bw ethanol intravenously over 1-2 min on gestation days 120-147, transient but marked collapse of umbilical vasculature was observed within 15 min. This resulted in severe hypoxia and acidosis in the fetus, but recovery occurred during the succeeding hour.
Reliability : (4) not assignable
12.11.2004 (336)

Species : Rat
Sex :

Strain : Long-Evans
Route of admin. : oral unspecified
Exposure period : GD 6 – 20
Frequency of treatm. :
Duration of test :
Doses : liquid diets containing ethanol (35% of total calories)
Control group :

Result : Offspring exerted a lower maximal suckling pressure,spent less time suckling during test sessions and displayed an altered suckling pattern.
Reliability : (4) not assignable
12.11.2004 (337)

Species : Rat
Sex : Female
Strain : Long-Evans
Route of admin. : oral unspecified
Exposure period : throughout gestation
Frequency of treatm. :
Duration of test : throughout gestation
Doses : 1 or 2 g/kg bodyweight given daily
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Litter size, litter weight and mean pup weight were lowered but there were no gross malformations or behavioural teratogenic effects.
Reliability : (4) not assignable
12.11.2004 (338)

Species : Rat
Sex : Female
Strain : Long-Evans
Route of admin. : oral unspecified
Exposure period : Days 5-19 of gestation
Frequency of treatm. :
Duration of test : as above
Doses : 6000 mg/kg achieving blood alcohol level of 260 mg/100 ml
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Long-Evans rats administered 6 g/kg bw ethanol orally on gestation days 5-19 had blood ethanol concentrations of over 2.6 g/l. Fetuses had decreased body weight, increased body water and sodium content and decreased lipid-free solid content.
Reliability : (4) not assignable
12.11.2004 (339)

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : oral unspecified
Exposure period : Days 1-21 of gestation
Frequency of treatm. :
Duration of test : as above
Doses : 36% of calorie intake achieving blood alcohol levels of 150-200 mg/100 ml.

Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Sprague-Dawley rats were provided with 18, 25 and 32% protein-derived calories and 36% ethanol-derived calories in a liquid diet on gestation days 1-21. The maternal ethanol blood levels were 1.5-2 g/l. Ethanol caused a significant decrease in fetal body weight and brain weight
but an increase in relative brain weight, irrespective of the protein content of the diet.
Reliability : (4) not assignable
12.11.2004 (340)

Species : Rat
Sex :
Strain : Sprague-Dawley
Route of admin. : oral unspecified
Exposure period : GD 16 until postnatal day 14 or from birth until postnatal day 14
Frequency of treatm. :
Duration of test :
Doses : 36% of total calories in a liquid diet
Control group :

Result : The sexually dimorphic nucleus in the preoptic area of the brain of adult male offspring was significantly decreased in volume.
Reliability : (4) not assignable
12.11.2004 (341)

Species : Rat
Sex :
Strain : Sprague-Dawley
Route of admin. : oral unspecified
Exposure period : GD 7-15
Frequency of treatm. : twice daily
Duration of test : 3 day period
Doses : 4g/kg
Control group :

Result : An increased incidence of resorptions and marginal effect on fetal body weight but no teratogenic effect were observed.
Reliability : (4) not assignable
12.11.2004 (342)

Species : Rat
Sex :
Strain : Sprague-Dawley
Route of admin. : oral unspecified
Exposure period :
Frequency of treatm. : Daily
Duration of test : GD 1-15 or GD 1-20
Doses : 5 or 5g/kg bodyweight
Control group :

Result : In contrast to studies in which gross malformations were not observed, polydactyly and polysyndactyly were reported in the offspring of rats given 5 g/kg bw (but not in those given 6 g/kg bw) per day ethanol. Maximal blood ethanol concentrations of 2.5-3.25 g/l were reported with the two doses
Reliability : (4) not assignable

12.11.2004 (343)

Species : Rat
Sex :
Strain : Long-Evans
Route of admin. : oral unspecified
Exposure period :
Frequency of treatm. :
Duration of test :
Doses : Prenatal treatment with 35% ethanol-derived calories in a liquid diet.
Control group :

Result : Treatment shortened the umbilical cord.
Reliability : (4) not assignable
12.11.2004 (344)

Species : Rat
Sex :
Strain : Long-Evans
Route of admin. : oral unspecified
Exposure period : GD 6-20
Frequency of treatm. :
Duration of test :
Doses : liquid diet containing ethanol as 35% of total calories
Control group :

Remark : The most frequently reported behavioural teratogenic effect is alteration in motor activity.
Result : Increased motor activity of offspring was reported.
Reliability : (4) not assignable
12.11.2004 (345)

Species : Mouse
Sex : Female
Strain : C3H
Route of admin. : oral unspecified
Exposure period :
Frequency of treatm. :
Duration of test :
Doses : 1 x 1 ml of 12.5% alcohol; Estimate 2500 mg/kg.
Control group :
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Remark : The authors proposed that the effect was due to a specific action on the fertilized ovum at the time of second meiotic division, causing aneuploidy, but the numbers of embryos available for examination in this study were inadequate to confirm this hypothesis
Result : Treatment of (C3H x C57Bl)F1 female mice with a single dose of 1 ml of 12.5% ethanol by gavage 2 h after a 30-min mating period produced an increase in late (after day 11) fetal deaths. The same treatment given 1 hr after mating did not produce this effect.
Reliability : (4) not assignable
12.11.2004 (346)

Species : Mouse
Sex : Male
Strain : C3H
Route of admin. : oral unspecified

Exposure period : 4 weeks before mating
Frequency of treatm. : Daily
Duration of test :
Doses : 20-30% of total calorie intake
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Male-mediated developmental effects Male C3H mice were fed ethanol (20 or 30% of total calories) in a liquid diet and, after four weeks of treatment, were mated to untreated females.
Result : The resulting litters showed nochange in the number of implants, prenatal mortality, fetal weight, sex ratio or soft-tissue malformations.
Reliability : (4) not assignable
12.11.2004 (347)

Species : Monkey
Sex :
Strain : other: Cynomolgus
Route of admin. : oral unspecified
Exposure period : GD 20-150
Frequency of treatm. : Daily
Duration of test :
Doses : 5g/kg bw
Control group :

Result : An increase in pregnancy wastage (abortions and still births) was observed but no structural malformation or facial change.
Reliability : (4) not assignable
12.11.2004 (348)

Species : other: ferret
Sex :
Strain :
Route of admin. : oral unspecified
Exposure period : GD 15-35
Frequency of treatm. :
Duration of test : 90 days prior to mating and during gestation or during gestation only
Doses : 1.5g/kg bodyweight as a 25% solution
Control group :

Result : There was a significant increase in the number of fetuses and litters with malformations but no effect on fetal weight or resorptions. The peak blood ethanol concentration was 2 g/l.
Reliability : (4) not assignable
12.11.2004 (349)

Species : Rat
Sex :
Strain : Long-Evans
Route of admin. : other: intragastric
Exposure period : GD 7-15
Frequency of treatm. :
Duration of test :
Doses :
Control group :

Method : Administration of ethanol in combination with an unspecified extract of marijuana containing 9-tetrahydrocanabinol.

Result : Treatment produced a significant decrease in maternal weight gain and an increased incidence of resorptions. The incidence of resorptions was increased with marijuana alone, but the increase was more than additive with the combination of marijuana and ethanol.
Reliability : (4) not assignable
12.11.2004 (350)

Species : Mouse
Sex :
Strain : Swiss Webster
Route of admin. : s.c.
Exposure period : GD 1-15
Frequency of treatm. :
Duration of test :
Doses :
Control group :

Method : Administration of ethanol in combination with an unspecified extract of marijuana containing 9-tetrahydrocanabinol.
Result : Treatment produced a significant decrease in maternal weight gain and an increased incidence of resorptions. The incidence of resorptions was increased with marijuana alone, but the increase was more than additive with the combination of marijuana and ethanol.
Reliability : (4) not assignable
12.11.2004 (350)

Species : Rat
Sex :
Strain : other: hooded
Route of admin. :
Exposure period :
Frequency of treatm. :
Duration of test :
Doses :
Control group :

Result : In hooded rats given a liquid diet containing 37% ethanol-derived calories from day 6 of gestation to time of birth (gestation day 23 for ethanol-exposed rats; day 22 forcontrols), delayed and extended period of cortical neuron generation, reduced number of neurons and altered distribution of neurons were seen.
Reliability : (4) not assignable
12.11.2004 (351)

Species : Rat
Sex :
Strain : other: albino
Route of admin. :
Exposure period :
Frequency of treatm. :
Duration of test :
Doses :
Control group :

Result : Ethanol in combination with lithium carbonate had a synergistic effect on the induction of fetal abnormalities.
Reliability : (4) not assignable
12.11.2004 (352)

Species : Rat
Sex : Female

Strain : Long-Evans
Route of admin. :
Exposure period : 60 days
Frequency of treatm. :
Duration of test :
Doses : 20 ml of 20% alcohol; estimated 4000 mg/kg/day
Control group :
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : An increase in congenital malformations was noted.
Reliability : (4) not assignable
12.11.2004 (353)

Species : Rat
Sex : male/female
Strain :
Route of admin. :
Exposure period : 40-45 days before mating and 5 days post fertilization
Frequency of treatm. :
Duration of test :
Doses : 20% or 24% ethanol crudely calculated to 1100 mg/kg/day.
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Method : Preimplantation effects were studied by the examination of uterine contents of albino rats following consumption of plum brandy (reported as 24% ethanol) or cognac (reported as20% ethanol) for 40-45 days before mating and during pregnancy until the rats were killed on day 5.
Result : Development was retarded, and there was an increased number of pathological morulae and blastocysts.
Reliability : (4) not assignable
12.11.2004 (354)

Species : Rat
Sex : Female
Strain : Long-Evans
Route of admin. :
Exposure period : Days 6-15 of gestation
Frequency of treatm. :
Duration of test :
Doses : 4000 mg/kg
Control group :

Result : In Long-Evans rats given 4 ml/kg bw ethanol as a single oral dose between days 6 and 15 of gestation, a variety of gross malformations was reported in 72-100% offspring compared to 12% of controls.
Reliability : (4) not assignable
12.11.2004 (355)

Species : Rat
Sex :
Strain : Sprague-Dawley
Route of admin. :
Exposure period :
Frequency of treatm. :

Duration of test :
Doses : 15 or 25% ethanol-derived calories
Control group :

Method : Animals were given 15 or 25% ethanol-derived calories in liquid diets 20 days before mating, throughout mating and until gestation day 19; additional groups were pair-fed an isocaloric diet.
Remark : The effects of 15% ethanol-derived calories were attributed to ethanol, while the effects of 25% ethanol-derived calories were attributed partly to decreased caloric intake.
Result : There was decreased caloric intake in the group given 25% ethanol-derived calories and in the pair-fed controls, and in both of these groups there were associated decreases in fetal body weight, organ weights and DNA and protein contents compared to the pair-fed controls of the group given 15% ethanol-derived calories.
Reliability : (4) not assignable
12.11.2004 (356)

Species : Rat
Sex :
Strain : Long-Evans
Route of admin. :
Exposure period : throughout gestation
Frequency of treatm. : Daily
Duration of test :
Doses : 4 or 6g/kg bw
Control group :

Remark : The most frequently reported behavioural teratogenic effect is alteration in motor activity.
Result : There was decreased litter weight but not litter size at birth and increased postnatal mortality. Motor activity of neonates raised by surrogate mothers was impaired at 16 and 20 days of age.
Reliability : (4) not assignable
12.11.2004 (357)

Species : Rat
Sex :
Strain :
Route of admin. :
Exposure period :
Frequency of treatm. :
Duration of test :
Doses :
Control group :

Remark : Behavioural teratogenic effects reported.
Reliability : (4) not assignable
12.11.2004 (358) (359)

Species : Mouse
Sex :
Strain : other: CF-1, CD-1, C3H
Route of admin. :
Exposure period :
Frequency of treatm. :
Duration of test :
Doses :
Control group :

Remark : Some studies in several mouse strains have shown no teratogenic effect,

even at dose levels providing blood ethanol concentrations of 2 g/l or higher. Mice given ethanol orally or in the drinking fluid had pups with minor skeletal variants or decreased fetal body weight, but there was no increase in resorptions or malformations.

Reliability : (4) not assignable
12.11.2004 (360) (361) (311)

Species : Mouse
Sex :
Strain :
Route of admin. :
Exposure period :
Frequency of treatm. :
Duration of test :
Doses :
Control group :

Remark : Studies in mice showed teratogenic effects and resorptions, typically at blood ethanol concentrations in excess of 2 g/l. The effects, such as fetal resorptions, intrauterine growth retardation, cleft palate, altered craniofacial development and exencephaly, limb defects and heart defects, varied with the strain of mice, mode of administration and stage of gestation at which ethanol was administered.
Reliability : (4) not assignable
12.11.2004 (362) (363) (364) (365) (307) (366) (367) (368) (369) (370) (371) (372) (373) (374) (375) (376) (377) (378)

Species : Mouse
Sex :
Strain : Swiss Webster
Route of admin. :
Exposure period :
Frequency of treatm. :
Duration of test :
Doses :
Control group :

Result : Combined administration of ethanol and metronidazole increased the number of resporptions, decreased fetal body weight and had a marginal effect on the incidence of malformations.
Reliability : (4) not assignable
12.11.2004 (379)

Species : Mouse
Sex :
Strain : Swiss Webster
Route of admin. :
Exposure period :
Frequency of treatm. :
Duration of test :
Doses :
Control group :

Result : Ethanol increased the incidence of cleft palate in mice administered methylmercuric chloride and retinyl acetate.
Reliability : (4) not assignable
02.07.2004 (380)

Species : Mouse
Sex :
Strain :

Route of admin. :
Exposure period :
Frequency of treatm. :
Duration of test :
Doses :
Control group :

Remark : Behavioural teratogenic effects reported.
Reliability : (4) not assignable
12.11.2004 (381) (382)

Species : Miniature swine
Sex : Female
Strain :
Route of admin. :
Exposure period : Throughout gestation
Frequency of treatm. :
Duration of test : Throughout gestation
Doses : 3000 and 3600 mg/kg/day
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : In miniature swine given 20% ethanol in drinking-water (>3 g/kg bw/day) as gilts (18 months old) or sows (three years old), there was a significant decrease in mean litter size and in the birth weight of piglets and a significant increase in the incidence of multiple malformations. There was a slight decrease in the number of offspring per litter; there was also a slight decrease in still births. Open-field activity was significantly increased in offspring.
Reliability : (4) not assignable
12.11.2004 (383)

Species : Monkey
Sex : Female
Strain :
Route of admin. :
Exposure period : Days 20-150 of gestation
Frequency of treatm. :
Duration of test :
Doses : 5000 mg/kg/day
Control group : no data specified
Method : other
Year :
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : There was an increase in abortions and stillbirths but no structural or skeletal (facial) deformities.
Reliability : (4) not assignable
12.11.2004 (384)

Remark : Ethanol at high blood levels has caused significant reductions in foetal body weight, increased resorptions and teratogenic effects in a number of species. Some, though not all, studies in mice and rats have demonstrated altered behavioural development following exposure to ethanol in utero. Exposure in utero or during lactation reduced postnatal growth.
Reliability : (4) not assignable

06.07.2004 (18)

Remark : When 20% ethanol in water (v/v) was given as the drinking fluid for 60 days to male Long-Evans rats, which were mated with untreated females one to three weeks after cessation of treatment, the incidence of congenital malformations in the offspring was increased
Reliability : (4) not assignable
12.11.2004 (385)

Remark : In a study to evaluate the role of zinc deficiency in the developmental toxicity of ethanol, CBA/J mice were given a liquid diet, either fortified with zinc or deficient in zinc, and ethanol (15 or 20% of total calories).
Result : Zinc deficiency potentiated the ethanol-induced increase in resorptions and external malformations and the decrease in fetal weight.
Reliability : (4) not assignable
12.11.2004 (386)

Result : Among offspring of Long-Evans rats fed liquid diets containing 35% ethanol-derived calories during gestation days 6-20, there was evidence of behavioural deficits, which persisted until adulthood. Female offspring showed a variety of deficits in maternal behaviour when adult, which may have been related to prenatal hormonal alterations
Reliability : (4) not assignable
12.11.2004 (387)

Result : There was an increase in the incidence of both external and internal malformations in C57Bl/6 mice given a marginally zinc- deficient diet and ethanol during gestation, in comparison with mice given a control diet and with mice treated with ethanol alone.
Reliability : (4) not assignable
12.11.2004 (388)

Remark : When ethanol is given in combination with other chemicals which tend to increase the blood level of ethanol by reducing its metabolism, e.g. 4-methylpyrazole and pyrazole, the teratogenic and fetotoxic effects are increased.
Reliability : (4) not assignable
12.11.2004 (389) (390)

Remark : Administration of ethanol with chemicals that tend to increase the acetaldehyde level, e.g. disulfiram does not increase the teratogenicity of ethanol.
Reliability : (4) not assignable
12.11.2004 (391)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

Endpoint : Neurotoxicity

Study descr. in chapter	:	5.8.2 Developmental Toxicity/Teratogenicity
Reference	:	
Type	:	other: learning and memory following prenatal exposure
Species	:	Rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	Gavage
No. of animals	:	
Vehicle	:	Water
Exposure period	:	
Frequency of treatm.	:	
Doses	:	1 g/kg/day
Control group	:	yes, concurrent vehicle
Observation period	:	9 weeks
Result	:	poor learners
Method	:	Other
Year	:	1998
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4

Method : Breeding and gestation:
Proven 3 month old female breeders (~180g) mated (males ~200g) for 4 days. 12 hour dark/light cycle. Temperature 20-25C, humidity 69-70%. SLH granules chow and water ad libitum. Vaginal sperm-positive plug used as evidence of copulation and defined as day 1. Body weight measured periodically. Ethanol (12.5% in distilled water) administered by peroral intubation from day 1 onwards. Control: equicaloric sucrose.

Cross-fostering:
Litters culled to 11 pups and cross-fostered to develop under approximately identical conditions. Treatment continued during lactation until weaning.
Five treatment groups resulted:
(Pregnant treatment/Lactation treatment)
Control/Control
Control/Ethanol(1)
Ethanol/Control
Ethanol/Ethanol(1)
Control/Control(2)
Notes: (1) lactating dams treated with ethanol but were only feed sucrose pre-natally. (2) Control dams used during lactation period had been fed ethanol pre-natally.

Blood alcohol determination:
Intracardiac puncture from 6 dams on GD14 and PND14, either under light ether anasthesia 1 hour after ethanol administration. Analysis by GC.

Postnatal monitoring:
After weaning (PND23) litters segregated by gender, 10 pups per cage. Survival monitored daily. Weights checked days 3, 10, 20, 30, 45 and at beginning of behavioural studies (63 days).

Behavioural studies:
Two way active avoidance (Shuttle box) test used to assess pharmacological effects on learning and memory. Conditioned stimulus: buzzer and light. Unconditioned stimulus: electric shock (0.2mA) on grid floor in half of box.
Training for 5 days. Memory tested over 12 days.
Positive avoidance recorded if animal avoided unconditional stimulus within 4 secs of conditioned stimulus.

All data assessed using one way ANOVA followed by group individual post-

		hoc comparisons using Scheffe test.
Result	:	No effects on dam bodyweight. Pup body weight showed significant effect following ethanol treatment but growth recovered to control values within 9 weeks. No visible malformations in offspring. Blood ethanol levels in treated dams 350 +/- 57.8 mg/l No dead pups after PND 28 in any group. Significant increase in mortality in offspring exposed to ethanol pre-natally. No effect with postnatal exposure. Highest mortality in group 5. Ethanol treatment did impair learning. In worst case group 4, 60% were poor learners compared to 33% of control group. Effects in females disappeared by 5 months but remained in males.
Reliability	:	(2) valid with restrictions

12.11.2004 (392)

Endpoint	:	Neurotoxicity
Study descr. in chapter	:	
Reference	:	
Type	:	other: Literature review
Species	:	Human
Sex	:	
Strain	:	
Route of admin.	:	
No. of animals	:	
Method	:	
Year	:	1999
GLP	:	
Test substance	:	
Remark	:	Ethanol can evoke a reduction in anxiety and a feeling of pleasure as well as sedation, motor incoordination, aggression, changes in other forms of social interaction and aberrations in cognition. Certain neurotransmitter systems are more or less sensitive to ethanol with both glutamate and GABA systems targets for ethanol's activity. Particularly sensitive components are certain receptor-gated ion channels including GABA(A) and the N-methyl-D-aspartate subtype of the glutamate receptor, the nicotinic cholinergic receptor and the serotonin-3 receptor. Ethanol also elicits an increase of dopamine in the nucleus accumbens. Some of these systems alter their function (adapt) during periods of chronic drinking and such maladaptation may generate manifestations of tolerance, physical dependence and craving on cessation of intake. Maladaptations in systems that gate calcium ion entry into neurons can contribute to brain damage in some alcoholics. See Section 1.13, entry 11.
Reliability	:	(2) valid with restrictions

12.11.2004 (393)

Endpoint	:	Neurotoxicity
Study descr. in chapter	:	
Reference	:	
Type	:	other: literature review
Species	:	
Sex	:	
Strain	:	
Route of admin.	:	
No. of animals	:	
Remark	:	Drinking which results in a blood ethanol concentrations of 50-60mg/l to

900mg/l (20mM) defined as 'moderate'. Such consumption selectively affects the function of the GABA, glutamatergic, serotonergic, dopaminergic, cholinergic, and opioid neuronal systems. Behavioural consequences are dose and time related and can even change on the rising and falling phases of the blood ethanol curve. A number of studies have noted a measurable diminution in neuropsychologic parameters in habitual consumers of moderate amounts of ethanol, but others have not found such changes. Some have even noted positive effects on congnitive effects in aging humans. Consumption by pregnant women can have significant consequences on the developing fetal nervous system.

Reliability : (2) valid with restrictions
A comprehensive review document covering typical dose from drink, pharmacokinetics, implications for nervous system, CNS effects, anxiolytic effects, cognitive effects and developmental effects. Contains 440 references.

12.11.2004 (394)

Endpoint : Behavioural Effects
Study descr. in chapter :
Reference :
Type :
Species : Rat
Sex : Male
Strain : Fischer 344
Route of admin. : inhalation
No. of animals :
Vehicle :
Exposure period :
Frequency of treatm. : see method for details
Doses :
Control group :
Observation period :
Result :
Method : Other
Year : 1991
GLP : no data
Test substance : other TS

Method : Animals: initial body weight 180-210g, housed individually, stainless steel cages.
Inhalation chamber: dynamic inhalation behavioural chamber ex Pradhan & Copeland. Glass chamber over a grid floor fitted with a metal plate accomodating a lever and liquid dipper. Individual animals exposed to multiple concentrations
Lighting: 12hr light/12hr dark
Conditions: temperature 21±1C, Humidity 55±5%
Analytical: 15 minute sampling of chamber atmosphere. Samples analysed by gas chromatography.

Behavioural schedules: Fixed ratio (FR) liquid re-inforcement and self stimulation (SS) behaviour, with 3-4 week training period. Animals then acclimatised to exposure chambers for 3-4 days and stability of response times (<10%) checked before proceeding with exposure.

FR behaviour: based on rats being kept at 80% of starting weight with additional 5% sucrose solution becoming available following pressing of lever 24 or 50x. Re-inforcement only available 5x per session. Exposure regimes: 2 hour exposures to 140, 161, 202 and 398ppm ethanol; five hour exposure to 140ppm; 2 hr exposure to 206ppm daily for 5 days. Control: animal response on previous day in air. Statistical analysis: ANOVA, with significance at $p<0.05$

SS behaviour based on electrical stimulation becoming available via electrodes implanted in posterior hypothalamus following pressing of lever.
Exposure regime: Exposure regimes: 2 hour exposures to 129, 373, 603 and 1287ppm ethanol. Control: animal response on previous day in air.
Statistical analysis: Rate of response broken down into 20 minute periods and consecutive periods compared with comparable control period using ANOVA, with significance at $p<0.05$. For results with significant F, further analysis using Least Signficant difference, Duncan's test and Newman Kuels test.

Blood collected from tail vein afer 2 hrs exposure in SS regimes for blood ethanol level measurements (alcohol dehydrogenase assay).

Result : FR behaviour: reinforcement behaviour dropped by a small but statistically significantly in the 15minute exposure periods from 45mins onward and for exposures of 202ppm and above. There was no cumulative effect (exposure for 5 hours did not produce any effects not seen in the 2hr exposure.) In the daily repeat exposure, effects declined showing a developing tolerance to ethanol.

SS: there was a decline in self stimulation behaviour at exposures of 600ppm and above but these were not statistically significant.

Blood ethanol concentrations following 2 hr exposure: 393ug/ml after 600ppm, 545ug/ml after 1200ppm

Test substance : 100% anydrous ethanol from US Industrials Co.
Reliability : (2) valid with restrictions
Results only presented as small graphs. No indication of total animal numbers used.

19.11.2004 (395)

Endpoint : Neurotoxicity
Study descr. in chapter : 5.8.2 Developmental Toxicity/Teratogenicity
Reference :
Type :
Species : Monkey
Sex :
Strain : Macaca Fascicularis
Route of admin. : in utero
No. of animals : 18

Result : Cognitive impairment score increased as craniofacial linear measurements increased and craniofacial angular measurements decreased, especially in animals exposed to ethanol on gestational days 19 or 20. Incidence and magnitude of cognitive impairment increased with age.
Reliability : (4) not assignable

19.11.2004 (396)

Endpoint : Neurotoxicity
Study descr. in chapter :
Reference :
Type :
Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Gavage
No. of animals :
Vehicle :
Exposure period : 4 day(s)
Frequency of treatm. : Daily
Doses : 2, 4 and 6 g

Control group :
Observation period :
Result : Latency to move, rear and groom + more avoidance response and more correct discriminations in Y maze
Method : Other
Year : 1979
GLP : no data
Test substance : no data

Reliability : (4) not assignable
12.11.2004 (397)

Endpoint : Neurotoxicity
Study descr. in chapter :
Reference :
Type : other: postnatal expression of celltype specific genes.
Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : inhalation
No. of animals : 16
Vehicle : no data
Exposure period : 13 day(s)
Frequency of treatm. : Daily
Doses :
Control group :
Observation period :
Result :
Method : Other
Year : 1993
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Chronic exposure of rats through gestation, in particular through the last 2 weeks of gestation, had little influence on the postnatal expression of cell type specific genes involved in brain development.
Reliability : (4) not assignable
19.11.2004 (398)

Endpoint : Immunotoxicity
Study descr. in chapter :
Reference :
Type :
Species : Human
Sex :
Strain :
Route of admin. :
No. of animals :
Method :
Year : 1989
GLP :
Test substance : other TS

Result : High intakes of alcohol in humans may alter the production and turnover rates of lymphocytes in the thymus or spleen, or both, with a resultant shift in the relative concentrations of the lymphocyte subpopulations, which include the B cells and T cells. Circulating T lymphocyte counts are significantly reduced in alcoholics, as is the ability of these cells to undergo blastic transformation on mitogenic stimulation. Non-specific activation of B lymphocytes occurs in all patients who regularly drink alcohol in excess. In vitro, alcohol alters both the development and the sensitivity of

lymphocytes. Prolonged drinking affects the reticuloendothelial system of the liver by interfering with the mobilization and activation of macrophages and their phagocytic activity. In vitro, alcohol impairs the chemotaxis and adherence of granulocytes to capillary walls. Alcohol inhibits cell mediated immunitiy. It suppresses natural killer cell activity and antibody directed cytotoxicity.

Reliability : (4) not assignable

12.11.2004 (399)

5.10 EXPOSURE EXPERIENCE

Type of experience : Human

Remark : Local effects: As a solvent ethanol can produce dermatitis because of its defatting action. Whilst the majority of contact dermatitis is irritant in nature, both contact urticaria and skin sensitisation have been reported (Adams RM, 1990; Cronin E 1980 ; Fisher AA, 1983)

Reliability : (4) not assignable

29.06.2004 (400) (401) (402)

Type of experience : Human

Remark : Effects of chronic ingestion of alcohol

The study of the effects of long term ethanol ingestion has potentially been confused by potential confounding factors such as smoking and in some cases exposure to toxic alcohol substitutes or contaminants such as methanol.

A wide variety of conditions have been taken to be systemic effects of ethanolism and these include : Nutritional Deficiencies such as of vitamins (B1,B6 and B12)and trace elements such as zinc.

Nervous system disorders such as reduced fine motor skills, impaired cognition, peripheral neuropathies and Wernicke- Korsakoff syndrome. The latter is linked to thiamine deficiency which perturbs normal nerve function.

Gastrointestinal disorders including gastritis and stomach ulceration and atrophy; gut malabsorption and motility problems, pancreatitis, fatty liver, hepatitis, and cirrhosis which may end in hepatic failure with encephalopathy.

Endocrine Disorders. Decreased secretion of testosterone and oxytocin and increased secretion of cortisol, aldosterone and insulin have been associated with chronic
ethanolism.

Haematological Disorders. Bone marrow supression, thrombocytopaenia and signs of abnormal iron metabolism may be observed.

Cardiological Disorders may include cardiomyopathy and in beer drinkers alcoholic beri-beri. The prevalance of hypertension and cerebrovascular accidents is higher in heavy drinkers. Arrhythmias can be induced by acute ethanol ingestion.

Fertility. Ethanolism can be associated with impotence and hypogonadism in males and infertility in women.

Foetal Alcohol Syndrome: Foetal alcohol syndrome was described by Jones and Smith (1973) and named after the presumed cause of a range of anomalies found in the offspring of severely and chronically alcoholic women. The minimum diagnostic criteria have been described by the Research Society of Alcoholism (Rossett HL (1980) as being signs in each of the following categories :

1) pre-natal and/or post-natal growth retardation (weight or length below the 10th percentile).

2) central nervous system involvement (signs of neurological abnormality or developmental delay).

3) characteristic facial dysmorphology with at least two of the following signs
a) microcephaly (head circumference below the 3rd percentile)

b) short palpebral fissures

c) poorly developed philtrum, thin upper lip, and/orflattening of the maxillary area.

Criteria for reporting infants with less extensive dysmorphology suggested to be associated with lower levels of ethanol drinking have been outlined by Hanson et al (1978).

Other abnormalities associated with pre-natal ethanol exposure have been cleft palate, joint and palmar crease abnormalities and septal and other cardiac defects. Dental malocclusions/malalignments and eustachian tube defects are probably secondary to facial hypoplasia. Investigations strongly suggest that maternal ingestion of large doses of ethanol (40 g daily) is teratogenic and foetotoxic.

Studies of the outcome of pregnancy in women who drank heavily during pregnancy indicate infants were small for dates and had growth retardation. A significant decrease in birth weight has been associated with consumption of at least one to two alcoholic drinks per day, although Mills and Graubard (1987) did not find an increased defect rate in offspring of those drinking one to two drinks per day. It appears uncertain if stillbirth is increased amongst heavy drinkers but there is a suggestion that the 2-4% of heaviest drinkers in some studies showed a higher risk of spontaneous abortion.

Some studies have indicated prenatal alcohol results in mental retardation but it is unclear to what degree this might be due to pre-natal alcohol and to what extent this may be attributable to the post-natal environment in which children are raised. It is acknowledged that study of the effects of ethanol drinking on pregnancy outcome is complicated by many potentially confounding factors. (Roman E 1988)

Reliability : (4) not assignable
29.06.2004 (403) (404) (405) (406) (407)

Type of experience : Human

Remark : Acute toxicity: Dermal exposure. Ethanol has a very low octanol: water partition coefficient and this is seen as contributing to the poor dermal uptake of ethanol in intact human skin. Bowers et al (1942) demonstrated the lack of dermal absorption in humans by occluding dressings soaked in 95% ethanol over the legs of subjects with impermeable rubber sheet. Blood ethanol determinations made every three hours over the succeeding 12 hours showed no indication of ethanol absorption through the skin.

On this basis the liklihood of intoxication through intact skin seems very remote. The volatility of ethanol would suggest that inhalation exposure would be a more relevant route of exposure.

Reliability : (4) not assignable

29.06.2004 (408) (409)

Type of experience : Human

Method : Ethanol liquid was vaporised by passing at a known rate through a Rotameter into a tube heated by flame. Air was also passed through the tube at 60l/min and the ethanol laden air cooled before passing into a transparent plastic hood (33cm dia, 25cm high fitted with a cloth skirt) used to place over the head of the volunteer. The hood was oriented so that the outlet of the ethanol laden air was close the the mouth/nose of the volunteer. The ethanol concentration was analysed frequently using a method described elsewhere (Lester, unpublished.)

Remark : Acute toxicity: Inhalation: Lester and Greenberg investigated the effects of ethanol vapour in human volunteers.

At 10-20 mg/l [~5300-10,000ppm] the vapour gave transient coughing and irritation to eyes and nose lasting for 5-10 minutes. Further exposure to this concentration gave some discomfort but could be tolerated.

At 30mg/l [~16,000ppm] ethanol vapour could be tolerated but gave continuous lachrymation and marked coughing.

Exposure to 40mg/l [~20,300ppm] was just tolerable but volunteers declined to be exposed for more than short periods.

Even short excursions into an atmosphere greater than 40mg/l [~20,300] were judged impossible as this concentration was intolerable to the volunteers.

When the ventilation rate was 30l/minute [equivalent of hardwork] an ethanol concentration of 20mg/l [~10,000ppm] was just tolerable.

In more extended exposures of up to 6 hours to ethanol concentrations of 13-17mg/l [7,000-9,000 ppm],blood ethanol concentrations of 15-46mg/100mls were observed. Blood ethanol concentrations were proportional to both the concentration in air and to the respiratory rate. After transient eye and nasal irritation had subsided, there were no reported adverse effects such as weakness, tiredness or intraocular tension.

Measured and nominal concentrations of ethanol in the air were found to be almost identical.

Result : Lester and Greenberg's conclusion that intolerance of the local irritation effects would deter exposure to concentrations that would give rise to systemic intoxication and this premis has been used as a basis for setting workplace exposure limits.

Reliability : (2) valid with restrictions
Whilst old, this study appears to be reliable.

29.06.2004 (410)

Type of experience : Direct observation, clinical cases

Remark : Acute toxicity: Ingestion: The widespread use and abuse of alcoholic beverages has resulted in a large body of data about both the acute and chronic effects of ethanol ingestion. When ingested ethanol is a central nervous system depressant. The degree of effect depends on the amount

ingested and the susceptibility of the individual. Susceptability is dependent upon factors such as genetic background, prior alcohol use, rate of elevation of blood ethanol concentration, coexposure to other xenobiotics eg pharmaceuticals, trauma and the general state of health and nutrition.

Ethanol can induce all stages of anaesthesia. The mechanism of action, as with other anaesthetic agents appears to be interference with ion transport (sodium flux) at the cell membrane. Low concentrations of ethanol affect the frontallobes of the brain resulting in mood changes, alterations in thinking and apparent suppression of inhibitory neurons. The latter appears linked to the excitatory phase of intoxication.

The onset of the effects of ethanol varies, in part depending on whether the individual is habituated to ethanol exposure.

In non-habituated individuals, reduced cognitive ability, motor coordination and sensory perception may be seen at blood ethanol concentrations as low as 50mg/100mls.

Attention, motor coordination and reaction time are significantly affected at blood ethanol concentrations of 135mg/100mls. Slurred speech, ataxia, and drowsiness develop as the concentration increases. These may be associated with a flushed face, dilated pupils, abnormal sweating and gastrointestinal disturbances.

Ethanol can give cardiac arrythmias and result in myocardial depression. Thermoregulation can be inhibited during ethanol induced coma and the resultant hypothermia can contribute to death. Rarely some individuals react to acute ethanol intoxication with severe hypoglycaemia which may follow 12-16 hours after the main ingestion. This appears to be due to inhibition of hepatic gluconeogenesis and may lead to hypoglycaemic coma. In children, particularly those aged under 5 years, ingestion of relatively small amounts of ethanol has resulted in hypoglycaemic convulsions and death.

Blood concentrations of 300-500mg/100mls are generally associated with stupor or coma. Death from respiratory depression occurs at blood ethanol levels of 500mg/100mls and above. The fatal dose has been suggested to be 5-8g/kg body weight for adults and about 3g/kg body weight for children.

Reliability : (4) not assignable

29.06.2004 (411) (412) (413)

Type of experience : Human - Exposure through Food

Remark : Metabolism

The metabolism of ethanol has been reviewed (IARC, 1988). Ethanol is eliminated from the body mainly by metabolism in the liver and only minimally by urinary excretion and pulmonary exhalation. Other tissues such as kidney, stomachand intestines oxidize ethanol to a small extent. The hepatic metabolism of ethanol proceeds in three basic steps. First, ethanol is oxidized within the cytosol of hepatocytes to acetaldehyde; second, acetaldehyde is converted to acetate, mainly in the mitochondria; and third,acetate produced in the liver is released into the blood andis oxidized by peripheral tissues to carbon dioxide, fatty acids and water. The main pathway for ethanol metabolism proceeds via alcohol dehydrogenase. However other pathways for ethanol oxidation have been described including a microsomal ethanol-oxidizing system located in the endoplasmic reticulum and a catalase system located in the peroxisomes. The rate of hepatic metabolism of ethanol is concentration independent except at very low or very high concentrations. Blood ethanol in humans decreases more rapidly at concentrations over 300 mg/dl than at

concentrations below this level, possibly due to oxidation by the microsomal ethanol oxidizing system. The maximum rate of metabolism is 100 - 125 mg/kg body weight/hour, although tolerant individuals may have higher metabolic rates (up to 175 mg/kg/hour) due to enzyme induction. Adults metabolize 7 - 10 g ethanol/hour reducing blood ethanol concentrations at a rate of 15 - 20 mg/100 ml/hour. Ethanol is metabolized more rapidly in chronic alcohol abusers (up to 40 mg/100 ml/hour)and in children (up to 28 mg/100 ml/hour) (Ellenhorn & Barceloux, 1988).

Reliability : (4) not assignable
01.10.2003 (414)

Type of experience : Human

Method : Skin absorption: In order to assess the potential systemic dose, three parameters were measured: the evaporation of [14C]ethanol from the skin surface, the in vitro penetration of [14C]ethanol through excised pig skin and the ethanol concentration in the blood of human volunteers following simulated use of an alcohol based deodorant spray. 20ul labelled ethanol was dosed on to each substrate to give a dose of 2.5ul/cm2. Substrates and residual ethanol were taken at intervals of 10s (up to 90s) and placed in scintillation vials containing 5ml StarcScint and counted on a Beckman LS6000 liquid scintillation counter. In the case of pig skin, the residual skin radioactivity was also assessed (counted for 1 hr). The skin was then removed, solubilised using 5ml Soluene 350 and counted again (after addition of 10ml HionicFluor) to measure activity within the skin. The original vials now without skin, were also recounted. Statistical analysis was performed using JMP v3.1 software.

Remark : These studies provide evidence that a systemic dose of ethanol is likely to be very low after the use of formulations delivering ethanol to the skin.

Result : The rate of evaporation from Benchkote and whole pig skin was similar (t1/2 = 13.6s and 11.7s respectively) whilst that from glass was longer (t1/2 = 24.8s). Ethanol penetration through pig skin in vitro was greater in occluded cells than in non-occluded cells (2.19mg/cm2 and 0.10mg/cm2 in 24 hours respectively). At the maximum flux seen in this experiment under occlusion, the amount of ethanol penetrating from a 1m2 area of skin would give a blood alcohol level of about 4mg% in a 70kg man. In the human use study, none of the blood samples taken from sixteen human volunteers exhibited a detectable level of alcohol.

Reliability : (2) valid with restrictions
16.01.2004 (415)

Type of experience : Human - Medical Data

Remark : Ethanol is excreted in the unmetabolized form in urine, exhaled air and sweat. Its metabolic products are also excreted by exhalation and in the urine. The major route of excretion of ethanol is in the urine.
Excretion: The kidneys and lungs excrete only 5 - 10% of an absorbed dose of ethanol unchanged (Ellenhorn & Barceloux, 1988).

Reliability : (4) not assignable
29.06.2004 (416) (414)

Type of experience : Human – Epidemiology

Method : Epidemiological data published between 1966 and 2000 were subjected to meta-regression analysis in which models were fitted with linear and non-linear effects of alcohol intake.

235 Studies including 117,000 cases were considered and selected covariates were tobacco smoking and gender.

Result : Strong trends in risk were observed for cancers of the oral cavity, pharynx, oesophagus and larynx. Alcohol-related effects were less strong for

cancers of the stomach, colon
and rectum, liver, breast and ovary. For all these increased risks were for found for ethanol intake of 25 g/day. Allowance for tobacco smoke modified association with laryngeal, lung and bladder cancers but not oral, oesophageal or colorectal cancers.

There was no threshold effect for most alcohol-related neoplasms and the inference was limited by absence of distinction between lifelong abstainers and former drinkers.

Reliability : (4) not assignable
29.06.2004 (417)

Type of experience : Human

Remark : Distribution
Once absorbed, alcohols are contained in the water compartment of the body. They are not stored or accumulated to any degree, so that the body burden at any point in time is a result of recent absorption, usually within the previous 12 hours (Conibear, 1988).
Since it is both water and lipid soluble, ethanol easily penetrates the blood-brain barrier and placenta (Ellenhorn & Barceloux, 1988).

Reliability : (4) not assignable
29.06.2004 (418) (419)

Type of experience : Human

Remark : Absorption
The most important routes of exposure in terms of occupational exposure to ethanol are the pulmonary and dermal routes. Ethanol is not well absorbed through intact skin, but is well absorbed by inhalation. Significant intoxication from inhalation is unlikely to occur because ethanol becomes irritating to the eyes and mucus membranes before concentrations that could result in CNS depression are reached. Persistant contact with alcohols can result in the removal of the skin's protective fatty barrier which results in increased absorption (Conibear, 1988).
In man, the small intestine absorbs about 80% of an oral ethanol dose, with the stomach absorbing the remainder. Since ethanol is poorly absorbed from the stomach, factors that delay gastric emptying decrease absorption. In healthy adults, 80 - 90% of absorption occurs within 30 to 60 minutes, but food may delay complete absorption for 4 to 6 hours (Ellenhorn & Barceloux, 1988).

Reliability : (4) not assignable
01.10.2003 (420) (419)

Type of experience : Human – Epidemiology

Remark : Contact sensitivity has been reported in 8 women:

1. Eczema after washing with spirit (Haxthausen, 1944)
2. Eczema after splashing with spirit (Martin-Scott, 1960)
3. Bullous eczema after splashing with alcohol (Fregert et al. 1969).
4. Eczema (Drevets and Seebohm, 1961)
5. Burning and discomfort of the mouth (Fregert et al. 1969).
6. Reaction to allergens diluted in ethanol (Fregert et al. 1963;1969).
7. Post-operative eruption (Ketel and Tan-Lim, 1975).

Reliability : (4) not assignable
22.08.2003 (421)

Type of experience : Direct observation, clinical cases

Remark : Contact dermatitis as a respose to topically applied alcohols is briefly

reviewed. Dermatitis may take the form of eczematous eruption or erythematous flush or contact urticaria. This may follow dermal contact or, in sensitized people, following ingestion of alcoholic beverage. Methods of testing are discussed.

Reliability : (4) not assignable
Flag : Critical study for SIDS endpoint
12.11.2004 (402)

Type of experience : Direct observation, clinical cases

Remark : A case history of contact dermatitis in a 62 yr-old housewife is presented. She gave positive eczematous reactions to topical formulations in ethanol and to ethanol alone.
Reliability : (4) not assignable
12.11.2004 (422)

Type of experience : Direct observation, other

Remark : Personal exposure to solvents including ethanol was studied among hairdressers in 28 salons in 2 regions of Netherlands in 2 seasons and found to be 200 times below the occupational exposure limit. The average exposures differed by an factor of 30 between salons. There were also seasonal and metereological differences.
Reliability : (2) valid with restrictions
12.11.2004 (423)

Type of experience : Direct observation, other

Remark : Drager badge readings from three employees at a liquid inks blending factory gave working day exposure levels of 18, 22 and 51 mg/m^3.
Reliability : (2) valid with restrictions
12.11.2004 (424)

Type of experience : Direct observation, other

Remark : Solvent vapour concentrations in the printing area at BP Chemicals Ltd., Darton Factory 4 taken on mid-summer dates in 1997, 1998 and 1999 showed concentrations (mg/m^3) of ethanol well below the UK 8-hr TWA reference period OES of 1920 mg/m^3.
Reliability : (2) valid with restrictions
12.11.2004 (30)

Type of experience : Direct observation, other

Remark : Organic vapour exposures measured in a BP Group Occupational Hygiene Survey of a flexible packaging area of BP Chemicals Ltd., Darton in 1992 showed exposure values in the range 36 to 337 mg/m^3 for printers and 80 to 250 mg/m^3 during various cleaning activities. These values are well below the 8-hr TWA OES of 1920 mg/m^2.
Reliability : (2) valid with restrictions
12.11.2004 (425)

Type of experience : Direct observation, other

Remark : Concentrations of ethanol in air at the mixing location in 6 Norwegian hairdressing salons ranged from 4 to 36 mg/m^3. The exposure level was significantly lower in salons with local exhaust ventilation than in salons without ventilation.
Reliability : (2) valid with restrictions
12.11.2004 (426)

Type of experience : Direct observation, other

Remark : Both stationary and personal monitoring of ethanol exposure conducted in 6 Norwegain car painting facilities showed very low exposures to ethanol (0.1 to 8.1 ppm versus the Norwegian OES of 500 ppm) and no exposure-related symptoms.
Reliability : (2) valid with restrictions
12.11.2004 (427)

Type of experience : Human – Epidemiology

Remark : In a study of the respiratory hazards associated with exposure to formaldehyde and solvents in acid-curing paints a mean exposure to 17 mg/m^3 of ethanol was recorded. This value is far below the Swedish National Board of Occupational Health and Safety Threshold Value of 1400 mg/m^3.
Reliability : (2) valid with restrictions
12.11.2004 (428)

Type of experience : Direct observation, other

Method : Sampling method : Active sampling using SKC manufactured commercial sorbing Silica Gel tubes. Sampling rate 0,1l.min-1.
Measurement of ethanol: Gas chromatography and FID detector was used as the measurement technique.
Long-term stationary and personal measurement were applied. Time of sampling was>70% of the shift time, the measurement represent the whole shift exposure
Result : Type of company: ELECTRO-TECHNICAL COMPANY

Profession: Operator (assembling department)
Activity: soldering, electric test of technological unit
Chemical identity: Aktivátor 077 (Ethanol 97%, N,N-dimetyl-p-toluidín 3%)
PPE worn? Yes (gloves only)
Method: Personal sampling
Results 1: TWA 3.7, LOD 0.1, LOQ 0.3 (mg/m3)
Results 2: TWA 11.1, LOD 0.1, LOQ 0.3 (mg/m3)

Profession: Operator (repair workplace of technological unit)
Activity: mechanical repair-analyses of technological unit
Chemical identity: Denatured alcohol (Ethanol 100%)
PPE worn? Yes (gloves only)
Method: Personal sampling
Results : TWA 2.3, LOD 0.1, LOQ 0.3 (mg/m3)

Type of company: WOOD PROCESSING

Profession: Painter
Activity: Spraying of wood stain
Chemical identity: Micro Ton SP NK 173-14991 (no further data available)
PPE worn? No
Method: Personal sampling
Results 1: Range 165.02 to 311.34, TWA 200.27, LOD 3.35 to 7.88, LOQ 11.16 to 26.28 (mg/m3)

Profession: Painter
Activity: Using spray gun in spray booth with air evacuation
Chemical identity: Ethanol, Toluene ,Xylene, Butylacetate, Ethylmethylketone, 4-methyl-2-pentanone.
PPE worn? not applicable

Method: Area monitoring of source of emissions
Results 1: Range 69 to 158.08, TWA 110.22, LOD 2.44, LOQ 8.14 (mg/m3)

Type of company: MACHINERY

Profession: Painter
Activity: spraying of paint using spray gun in spray booth with air evacuation
Chemical identity: Sigma Wash (paint) (Ethanol 12,10%, Toluene 14,45%, 1-butanol 2,89%, xylene 0,16%, fenol 0,25% iso-butanol 0,66%, butanone 2,51%, 4-metylpentan-2-one 7,64%, propan-2-ol 22,35%.) Diluent C6000 (Ethanol 5,11%, Ethylacetate 3-7%, Butylacetate 3-7%, Methylacetate 10-15%, Toluene 50-70%.)
PPE worn? Yes (gloves only)
Method: Personal sampling
Result 1: Not detected, LOD 3.7 to 4.5, LOQ 7.0 to 9.0 (mg/m3)
Result 2: Not detected, LOD 3.7 to 4.4, LOQ 7.5 to 8.7 (mg/m3)
Result 3: Not detected, LOD 3.7 to 4.5, LOQ 7.3 to 9.0 (mg/m3)
Result 1: Not detected, LOD 4.1 to 5.4, LOQ 8.3 to 10.9 (mg/m3)

Profession: Technologist
Activity: preparation of paints (mixing)
Chemical identity: not specified
PPE worn? Yes (gloves only)
Method: Personal sampling
Result 1: Not detected, LOD 3.9 to 4.3, LOQ 7.8 to 8.5 (mg/m3)

Type of company: BUS TRANSPORT

Profession: Painter
Activity: spraying of paint using spray gun in spray booth without air evacuation
Chemical identity: Hardener 590 (hardener adding into paint) (Ethanol 10-25%, Toluene 10-25%, Methanol pod 2,5%, Propanol 25-50%, Butanol 25-50%, 4-metylpentan-2-one 10-25%.)
PPE worn? Yes (inhalation only - Respirator 3M model)
Method: Personal sampling
Result 1: Range 19.5 to 579, TWA 190.1, LOD 3.0 to 7.5, LOQ 10.02 to 24.92 (mg/m3)
Result 2: Range 0 to 249.6, TWA 13.0, LOD 10.0 to 19.2, LOQ 33.39 to 64.25 (mg/m3)

Profession: upholsterer
Activity: swabbing adhesive
Chemical identity: Adhesive Chemoprén Univerzál (Ethanol, Xylene, Butylacetate, ethylacetate, Butanol)
PPE worn? Yes (inhalation only - Respirator 3M model)
Method: Personal sampling
Result: not detected, LOD 4.3 to 5.1, LOQ 8.6 to 10.2 (mg/m3)

Type of company: PHARMACEUTICAL BIOTECHNICAL

Profession: Pharmaceutical production chemist
Activity: operator of centrifuge during fractionation with ethanol
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result 1: Range 363.8 to 2539.7, TWA 963.4, LOD/LOQ not available (mg/m3)
Result 2: Range 450.8 to 2383.3, TWA 1435, LOD/LOQ not available

(mg/m3)

Profession: Pharmaceutical production chemist
Activity: operator of equipment during precipitation with ethanol
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result 1: Range 128.9 to 2237.5, TWA 980.0, LOD/LOQ not available (mg/m3)
Result 2: Range 146.3 to 4692.1, TWA 1783.2, LOD/LOQ not available (mg/m3)

Profession: Maintenance engineer
Activity: maintenance
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result: Range 114.4 to 262.5, TWA 204.9, LOD/LOQ not available (mg/m3)

Background measurement
Method: Stationary sampling
Result 1: Range 34.0 to 54.2, TWA 46.2, LOD/LOQ not available (mg/m3)
Result 2: Range 72.0 to 133.5, TWA 99.5, LOD/LOQ not available (mg/m3)

Profession: Chemist operator
Activity: control tasks in regeneration of ethanol
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result: Range 11.3 to 293.7, TWA 63.2, LOD/LOQ not available (mg/m3)

Profession: Pharmaceutical production chemist
Activity: operator, control of drying equipment, homogenisation
Chemical identity: Ethanol 96%
PPE worn? Yes (gloves only)
Method: Personal sampling
Result: Range 175.5 to 405.6, TWA 322.7, LOD 0.72 to 1.29, LOQ 2.398 to 4.292 (mg/m3)

Profession: Pharmaceutical production chemist
Activity: operator of centrifugal, separation of used spirits, washing with ethanol
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result: Range 499.6 to 564.5, TWA 530.1, LOD 0.86 to 1.1, LOQ 2.869 to 3.675 (mg/m3)

Background measurement
Method: Stationary sampling
Result 1: TWA 89.0, LOD 0.89, LOQ 2.953 (mg/m3)
Result 2: Range 43.9 to 51.6, TWA 46.9, LOD 1.77 to 2.81, LOQ 5.895 to 6.699 (mg/m3)

Profession: Chemist operator
Activity: washing of used spirits, pumping of ethanol
Chemical identity: Ethanol 96%
PPE worn? Yes (gloves only)
Method: Personal sampling
Result 1: Range 2504.6 to 4990.2, TWA 3574.6, LOD 1.73 to 2.29, LOQ

5.755 to 7.613 (mg/m3)
Result 2: Range 51.9 to 138.0, TWA 95.9, LOD 2.2 to 2.3, LOQ 7.4 to 7.7 (mg/m3)

Profession: Chemist operator
Activity: pumping of ethanol into colons, distillation of ethanol
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result 1: Range 90.1 to 127.7, TWA 109.1, LOD 2.2 to 2.3, LOQ 7.4 to 7.6 (mg/m3)

Profession: Pharmaceutical production chemist
Activity: operator of fermentation (control, pumping, sampling of ethanol and mixing)
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result 1: Range 58.4 to 6590.9, TWA 3197.7, LOD/LOQ not available (mg/m3)
Result 2: Range 18.8 to 5097.6, TWA 2979.2, LOD 16.7 to 41.8, LOQ 51.9 to 125.5 (mg/m3)

Background measurement
Chemical identity: Ethanol 96%
Method: Stationary sampling
Result: Range 33.0 to 72.8, TWA 53.5, LOD 26.2 to 33.7, LOQ 78.7 to 101.2 (mg/m3)

Profession: Pharmaceutical production chemist
Activity: operator of fractional (adding of ethanol, sampling)
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result: Range 282.6 to 1048.1, TWA 495.1, LOD 22.5 to 39.4, LOQ 67.5 to 118.1 (mg/m3)

Profession: Pharmaceutical production chemist
Activity: operator of fractional (adding of ethanol, sampling) between fractional tank
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result: Range 282.6 to 1048.1, TWA 495.1, LOD 22.5 to 39.4, LOQ 67.5 to 118.1 (mg/m3)

Profession: Pharmaceutical production chemist
Activity: operator of fractional (adding of ethanol, sampling) between fractional tank
Chemical identity: Ethanol 96%
PPE worn? No
Method: Stationary sampling
Result 2: Range 47.7to 1321.6, TWA 437.4, LOD 29.5 to 52.5, LOQ 88.6 to 157.6 (mg/m3)

Profession: Pharmaceutical production chemist
Activity: operator of centrifuge, washing of tubes with ethanol
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result: Range 792.8 to 2636.8, TWA 1369.6, LOD 29.38 to 88.1, LOQ 75.4

to 264.2 (mg/m3)

Background measurement
Chemical identity: Ethanol 96%
Method: Stationary sampling
Result: Range 711.4 to 1634.5, TWA 1003.0, LOD 21.2 to 88.1, LOQ 75.4 to 264.2 (mg/m3)

Profession: Chemist operator
Activity: sampling of ethanol in the processing hall (temperature control)
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result: Range 26.4 to 831.8, TWA 209.6, LOD 11.4 to 18.1, LOQ 34.3 to 54.2 (mg/m3)

Profession: Foreman
Activity: supervision of manufacturing operation
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result: Range 255.5 to 2419.8, TWA 1286.7, LOD 17.8 to 24.6, LOQ 53.3 to 73.8 (mg/m3)

Profession: Maintenance engineer
Activity: repairs
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result: Range 2822.2 to 4686.4, TWA 4063.2, LOD 16.4 to 24.6, LOQ 49.2 to 81.4 (mg/m3)

Background measurement
Chemical identity: Ethanol 96%
Method: Stationary sampling
Result: Range 77.1 to 228.8, TWA 170.5, LOD 16.4 to 34.4, LOQ 52.4 to 103.1 (mg/m3)

Profession: Pharmaceutical production chemist
Activity: adding of material, opening of the processing tank containing ethanol
Chemical identity: Ethanol 96%
PPE worn? Yes (inhalation - face shield respirator)
Method: Personal sampling
Result: Range 11.9 to 102.5, TWA 30.6, LOD 1.6 to 6.0, LOQ 3.1 to 11.9 (mg/m3)

Profession: Foreman
Activity: supervision of manufacturing operation
Chemical identity: Ethanol 96%
PPE worn? No
Method: Personal sampling
Result: Range 9.3 to 344.6, TWA 78.6, LOD 1.6 to 6.0, LOQ 3.2 to 11.9 (mg/m3)

Background measurement from drying equipment
Chemical identity: Ethanol 96%
Method: Stationary sampling
Result: Range 3077.6 to 3223.8, TWA 3151.1, LOD 15.2 to 15.4, LOQ 30.4 to 30.7 (mg/m3)

12.11.2004 (429)

5.11 ADDITIONAL REMARKS

Type : Metabolism

Method : The PBPK model used for this work was modified from that described by Pastino et al. (1997). The parameters describing Michaelis-Menten metabolism of ethanol in the liver (VMAX and KM) were varied using the simulation and optimization software ACSLopt to fit the data. For each exposure scenario the simulation was run for male workers in "sitting awake" and "light exercise" activity levels as designated by ICRP. Mixed venous blood concentration (mM) and area under the curve for the venous blood concentration (mM-hr) are reported.

Result : A reasonably good overall description of the data was obtained). An exact fit would not be expected due to, for example, (a) lack of subject-specific information on body composition (e.g., fat volume, capacity for metabolizing ethanol) and breathing rates throughout the exposure and, (b) lack of data needed for a more realistic, multi-enzyme specification of ethanol metabolism (e.g., MEOSand ADH). The model-predicted time courses of the mixed venous blood concentrations for the 12 exposure scenarios.

The model predictions are that for men exposed to ethanol, at 0.942 and 1.88 mg/L for 8 hr and for the lower breathing rate in men exposed to 9.42 mg/L the liver is able to metabolize ethanol at the rate it enters the body. However,for the higher breathing rate in men exposed to 9.42 mg/L and for men exposed to 37.6 or 63.6 mg/L the rate of ethanol delivery via breathing exceeds metabolic capacity and ethanol blood levels consequently rise for the duration of the exposure. Men exposed to 20 mg/L ethanol for 4 hr also showed a continued accumulation during exposure at the higher breathing rate but little or no accumulation at the lower breathing rate.

Reliability : (4) not assignable

12.11.2004 (430)

Type : Chemobiokinetics general studies

Method : A comparative reprotoxic risk assessment was derived for ethanol exposure due to endogenous generation, alcoholic beverage consumption and food consumption versus a number of occupational and domestic exposure scenarios.

Result : Risk assessment

Reprotoxic effects of ethanol are generally thought to have a no observed effect level below. Although these are not known and are difficult to determine precisely for each individual end point, it can reasonably be assumed that the threshold is related to blood alcohol concentration (BAC), that the critical effect is developmental toxicity and that the recognised safe level of drinking for pregnant women can be used as a benchmark.

Blood alcohol levels from different activities:

Drink driving limit in Europe	500-850mg/l
No effect level for pregnant women	200mg/l
Safe drinking advice from UK Health uthorities (one drink)	80mg/l
100g vanilla ice cream	7.0mg/l
Mouthwash use	3.5-15mg/l
Pharmaceutical use	3.5-15mg/l
Working at 1000ppm	2.9mg/l

Endogenous blood alcohol level 0.035-0.68mg/l
Dermal exposure at work (poor practice) 0.5mg/l
Spray window cleaner 0.2mg/l
Consumer toiletry use 0.12mg/l
Consumer hairspray use 0.09mg/l

From this table it can be seen that the level of risk to pregnant women from either working with ethanol occupationally or using ethanol containing consumer products is negligible. Even assuming the rather absurd case of a female worker maximally exposed to ethanol who, on leaving work, immediately takes a dose of medicine, sets her hair and cleans the windows followed by mouthwash would reach a BAC less than one quarter of the level that would be seen through consumption of a single alcoholic drink. A single drink is recognised as a dose which poses no adverse health risks by the authorities. This latter dose cannot be approached from any conceivable use or misuse of ethanol containing products or any imaginable occupational scenario. The reprotoxic hazard of alcoholic beverages does not exist during the 'normal handling and use' of ethanol and ethanol containing products. Indeed, developing a craving for vanilla ice cream would pose a greater risk for a pregnant woman than working occupationally with ethanol.

Reliability : (4) not assignable
01.10.2003 (431)

Type : Distribution

Remark : The diffusion of ethanol through cell boundaries is slow and is affected by blood flow. Ethanol in the blood passes almost immediately into brain tissue, while its distibution to resting muscle is particularly slow. After oral dosing, ethanol disappeared from the blood of dogs linearly in the post absorption phase, irrespective of the concentration of ethanol in the body; zero-order kinetics were also found in dogs, cats, rabbits, pigeons and chickens after i.v. administration.

Reliability : (4) not assignable
01.10.2003 (18)

Type : Metabolism

Remark : The metabolism of ethanol has been reviewed (IARC, 1988). Ethanol is eliminated from the body mainly by metabolism in the liver and only minimally by urinary excretion and pulmonary exhalation. Other tissues such as kidney, stomach and intestines oxidize ethanol to a small extent. The hepatic metabolism of ethanol proceeds in three basic steps. First, ethanol is oxidized within the cytosol of hepatocytes to acetaldehyde; second, acetaldehyde is converted to acetate, mainly in the mitochondria; and third,acetate produced in the liver is released into the blood andis oxidized by peripheral tissues to carbon dioxide, fatty acids and water. The main pathway for ethanol metabolism proceeds via alcohol dehydrogenase. However other pathways for ethanol oxidation have been described includinga microsomal ethanol-oxidizing system located in the endoplasmic reticulum and a catalase system located in the peroxisomes.

Reliability : (4) not assignable
29.06.2004 (18)

Type : Metabolism

Remark : The activities of alcohol dehydrogenase (ADH), catalase, microsomal ethanol-oxidizing system (MEOS) and aldehyde dehydrogenase (ALDH) were measured in gastric, small intestinal, colonic and rectal mucosal samples of rats fed on a liquid alcohol diet for 1 month.

In the rectum and large intestine of control animals, the activities of ADH, MEOS and catalase were maximal, whereas the activity of ALDH was minimal. After chronic alcohol intoxication, MEOS activity increased significantly in the stomach. An activation of catalase and MEOS and a decrease of the low-K(M) ALDH activity were observed in the rectum. In rats consuming the alcohol diet, hypertrophy of crypts and an increased number of mitoses were noticed in colonic and rectal mucosa. Acute alcohol intoxication (2 g/kg, intragastrically) produced significantly higher acetaldehyde concentrations in the contents of the large intestine and rectum of rats receiving alcohol chronically compared to controls.

Conclusion : After chronic alcohol intoxication, the large intestine regions show a greater imbalance between the activities of acetaldehyde-producing and acetaldehyde-oxidizing enzymes, which results in accumulation of acetaldehyde.

This mechanism can account for the local toxicity of ethanol after its chronic consumption, and relates the development of mucosal damage and compensatory hyper-regenerative processes, and possibly carcinogenesis, in the colonic and rectal mucosae of alcoholics to the effects of acetaldehyde.

Reliability : (4) not assignable

01.10.2003 (432)

Type : Toxicokinetics

Remark : The teratogenic effects of ethanol are reviewed in terms of the differences between direct and indirect effects of ethanol on the developing foetus. Direct effects of ethanol are caused by ethanol interacting with the foetal cell whereas indirect effects of ethanol teratogenicity are defined as any perturbation of the developing fetus resulting from ethanol exposure, but not caused by ethanol's interacting with the fetal cell.

Indirect effects of ethanol teratogenicity include: ethanol-induced maternal undernutrition, ethanol-induced placental dysfunction and acetaldehyde teratogenicity.

Reliability : (4) not assignable

01.10.2003 (433)

Type : other: Percutaneous absorption

Method : The absorption of 14C-ethanol was determined in fresh, metabolically active human breast skin in vitro over 6 hr. Detection of CYP 2E1 was by immunoblotting.

Result : Absorption over 6 hr was low (0.5 to 2.2%) with a 4-fold variation between the 3 individuals. Absorption through occluded skin was higher (5.5 to 13.4%).

Conclusion : Absorption of ethanol through occluded human skin over 6 hr was poor. Occlusion enhanced absorption and increased skin residue.

Reliability : (4) not assignable

01.10.2003 (434)

Type : other: Reviews (II)

Method : Pregnant ICR mice were treated with 100 mg/kg pyrazole, an alcohol dehydrogenase inhibitor, prior to i.p. ethanol injection. In another experiment pregnant mice were housed in an ethanol-vapour box for 3 or 6 days in order to examine the effects of prolonged low level exposure to alcohol. The maternal blood alcohol concentration was maintained at approximately 0.03 mg/mL during inhalation.

Remark : These results suggest that ethanol rather than its

metabolites is mainly responsible for the embryotoxicity.

Result : I.p. treatment with 2 or 4 g/kg ethanol on day 7 of gestation increased the prenatal mortality rate and producedexternal and skeletal malformations in the offspring, and the embryotoxic effects were potentiated by pyrazole pretreatment. The inhalation treatment with ethanol increased the prenatal mortality rate, although teratogenicity was not shown.

Reliability : (4) not assignable

29.06.2004 (435)

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8.1 METHODS HANDLING AND STORING

Safe handling : Do not smoke eat or drink in areas of use and storage.
Fire/exp. protection : Keep away from heat, sources of ignition and incompatible substances. Earth (ground) lines and equipment used during transfer to reduce possibility of static spark initiated fire or explosion.
Storage requirement : Store in tightly closed containers in cool, dry, isolated, well-ventillated area.
Common storage :
Container :
Unsuitable container : Aluminium
Add. information :
Transport code : UN 1170

12.11.2004 (1)

8.2 FIRE GUIDANCE

Hazards : Flammable liquid and vapour
Protective equipment : Approved positive pressure self-contained breathing apparatus with full face mask and full protective clothing.
Extinguishing medium : Dry chemical, alcohol foam, all-purpose AFFF or carbon dioxide.
Unsuit. exting. medium : Water for large volumes of ethanol.
Add. information : Use water to cool fire-exposed containers and to disperse vapour. Avoid vapour flash-back.
Fire class :
Products arising : Oxides of carbon

12.11.2004 (1)

8.3 EMERGENCY MEASURES

Type : accidental spillage

Remark : Keep unecessary people away; isolate hazard area and deny entry.
Stay up wind and keep out of low areas where vapour may accumulate and ignite.
Shut off all sources of ignition.
Stop leak if this can be achieved without risk.
For small spills take up with and or non-combustible absorbant.
For large spills, Dike for later disposal.
11.01.2002 (1)

Type : injury to persons (skin)

Remark : Avoid skin contact by using appropriate chemical protective gloves, face shield, apron and armcovers. Use closed-system transfers wherever possible.
29.06.2004 (1)

Type : injury to persons (eye)

Remark : Prevent eye contact by wearing chemical tight goggles.
Provide an eyewash station in the immediate vicinity of use.
29.06.2004 (1)

Type : injury to persons (oral)

Remark : Accidental ingestion at a level high enough to be dangerous to health is unlikely. Be aware of 'recreational ingestion' and workplace alcoholism.

29.06.2004 (1)

Type : injury to persons (inhalation)

Remark : In circumstances where physical controls cannot be effectively applied use a respirator approved for organic vapours.

11.01.2002 (1)

Type : other: diagnosis of intoxication

Remark : Toxicity from ethanol, methanol, ethylene glycol, and isopropyl alcohol varies widely, and appropriate use of the available laboratory tests can aid in timely and specific treatment. Available testing includes direct measurements of serum levels of these alcohols; however, these levels often are not available rapidly enough for clinical decision making. This article discusses the indications and methods for both direct and indirect testing for ethanol, methanol, ethylene glycol, and isopropanol toxicity. Also discussed are the costs, availability, and turn-around times for these tests.

29.06.2004 (436)

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

Domain : Industry/skilled trades
Process : Recycling
Type of destruction : Incineration

12.11.2004 (1)

Domain : Public at large
Process : Destruction
Type of destruction : Incineration

12.11.2004 (1)

8.5 WASTE MANAGEMENT

Memo : Possibility of recovery/recycling

11.01.2002 (1)

Memo : Possibility of destruction: water purification

11.01.2002 (1)

Memo : Possibility of destruction: incineration

12.11.2004 (1)

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

Memo : Aluminium at higher temperatures.

12.11.2004 (1)

(1) CEFIC Ethyl Alcohol Group (2003)

(2) Ministry of Environment, Prague (2004)

(3) UK Health and Safety Executive, EH40 (2002)

(4) Deutche Forschungsgemeinschaft (2003)

(5) HSDB (2003) Hazardous Substances Databank. US National Library for Medicine.

(6) Centre for Chemical Substances and Preparations, Bratislava

(7) http://www.epa.gov/ttn/atw/orig189.html

(8) IARC Monographs on Evaluation of Carcinogenic Risks to Humans Vol.44 (1988)

(9) Sprung, R., Bonte, W., Rudell, E., Domke, M., Frauenrath, C. (1981). On the problem of endogeneous alcohol. Endogenous ethanol: Further investigations. Alcohol Drugs Behav. 1981;18(2):65-70.

(10) Lerici, C.R., Nicoli, M.C. (1996). Chemical and physico-chemical properties affecting the quality and stability of bakery products. Adv. Food Sci 18(5/6):229-233

(11) Greubet, S. (1997). Versteckter Alkohol in Lebensmittel? Medizische Monatsschrift fur Pharmazeuten 1997;20(8):218-219.

(12) Anonymous (1986). Hearings Before the SubCommittee on Select Revenue Measures of the Committee on Ways and Means, House of Representatives, Ninety-ninth Congress, Second Session, May 12 and 19, 1986.

(13) Association of Industry of Juices and Nectars from Fruits and Vegetables of the EU (1996) Code of Practice for Evaluation of Fruit and Vegetable Juices.

(14) SPIN database http://www.spin2000.net/spin.html

(15) Verschueren, K. (1996) Handbook of environmental data on organic chemicals. 3rd Edition. Van Nostrand Reinhold Company, New York.

(16) http://www.epa.gov/medecotx/searches/aquatic. U.S. EPA online ECETOX database.

(17) http://www.epa.gov/medecotx/searches/terrestrial.

(18) IARC Monographs. (1988) Volume 44. Alcohol Drinking. Lyon, France.

(19) Irvine, L. (2003) Relevance of the Developmental Toxicity of Ethanol in the Occupational Setting: A Review. Appl. Toxicol. 23, 289-99

(20) Phillips P. J., Jenkinson, P. (2001) Is ethanol genotoxic? A review of the published data. Mutagenisis 16, 2, 91-101

(21) Ophaswongse, S., Maibach, H.J. (1994). Alcohol dermatitis and contact dermatitis syndrome. A review. Contact Dermatitis 1994;30:1-6.

(22) Sensible Drinking. The report of an Inter-Departmental Working Group. December 1995. UK Dept. of Health.

(23) Macdonald, I. (Ed). (1999). Health issues related to alcohol consumption. 2nd ed. International Life Sciences Institute. Blackwell Science.

(24) Kalant, H., Poikolainen, K. Moderate Drinking: Concepts, definitions and public health significance.

(25) Corcoran, J., Kruse, H. and Skolnik, S. (1953). Thermal analysis of the systems hydrazine-methanol and hydrazine-ethanol. J. Phys. Chem. 57:435-437.

(26) Merck (1996) The Merck Index 12th edition, Merck & Co., Inc., Rahway, New Jersey.

(27) Ambrose D (1968), Improved boilers for the ebulliometric determination of vapour pressures, J Sci Instrum (J Physics E), series 2, vol 1, p41

(28) Ambrose D, Sprake CHS (1970), Thermodynamic properties of organic oxygen compounds. XXV. Vapour pressures and normal boiling temperatures of aliphatic alcohols. J Chem Thermodynam, 2, 631-45

(29) McKenna, F., Tartar, H., Lingfeiter, S. (1953). Studies of hemiacetal formation in alcohol-aldehyde systems: refraction studies. J. Amer. Chem. Soc. 75.604-607.

(30) BP Chemicals Ltd. Internal data 1997, 1998 and 1999.

(31) Merck (1989) The Merck Index 11th edition, Merck & Co., Inc., Rahway, New Jersey.

(32) Sakurai M, Nakagawa T (1982) Densities of dilute solutions of benzene and methanol at 278.15, 288.15, 298.15, 308.15 and 318.15K. Partial molar volumes Vw and values of dVw/dT for water in benzene and methanol. J Chem Thermodynam, 14, 269-74

(33) Sakurai M, Nakagawa T (1984), Densities of dilute solutions of water in n-alkanols at 278.15, 288.15, 298.15, 308.15 and 318.15K. Partial molar volumes of water in n-alkanols. J Chem Thermodyn, 16, 171

(34) BP Chemicals Ltd, Sales specification (2003)

(35) Howard, P. (1990). Handbook of Environmental Fate and Exposure Data for Organic Chemicals, volume II. Solvents, Lewis Publishers: Chelsea, MI.

(36) International Critical Tables of Numerical Data, Physics, Chemistry and Technology, Vol. III, McGraw-Hill, New York, 1928.

(37) Kavanaugh, M.C; , Stocking, A. (1999). Fate and Transport of Ethanolin the Environment. US EPA Blue Ribbon Panel. URL http://www.epa.gov/oar/caaac/mtbeethan.pdf.

(38) Maher PJ, Smith BD (1979) A new total pressure vapor-liquid equilibrium apparatus. The ethanol+aniline system at 313.15, 350.81 and 386.67K, J Chem Eng Data, 24, 1, p16-22

(39) HSDB (2003) Hazardous Substances Databank. US National Library for Medicine.

(40) Kavanaugh, M.C; , Stocking, A. (1999). Fate and Transport of Ethanol in the Environment. US EPA Blue Ribbon Panel. URL http://www.epa.gov/oar/caaac/mtbeethan.pdf.

(41) Verschueren, K. Handbook of environmental data on organic chemicals. 3rd Edition. Van Nostrand Reinhold Company, New York.

(42) U.S. EPA URL http://www.epa.gov/oar/caaac/mtbeethan.pdf.

(43) Doolittle AK (1935) Lacquer solvents in commercial use, Ind Eng Chem, 27, p1169-79

(44) EU Existing Chemicals Programme HEDSET

(45) US Environmental Protection Agency (2000)

(46) Hine J, Mookerjee PK (1975) The intrinsic hydrophilic character of organic compounds, Correlations in terms of structural contributions, J Org Chem, 40, 3, p292-8

(47) Yanagihara S, Shimada I, Shinoyama E, Chisaka E, Saito K. (1977) Photochemical reactivities of hydrocarbons. Proc. 4th Int. Clean Air Cong. 472 - 477. (See http://esc.syrres.com/efdb/Chemfate.htm)

(48) Hustert, K., Mansour, M., Korte, F. (1978) Reaktionen von essigester und aethanol in gegenwart von umweltkontaminanten (NO2 und SO2) unter simulierten troposphaerischen bedingungen. Chemosphere 1, 35 - 50.

(49) Campbell, I.M. et al. (1976) Rate constants for reactions ofhydroxyl radicals with alcohol vapours at 292 K. Chem. Phys.Letters 38, 362 - 364.

(50) Atkinson R (1989), "Kinetics and Mechanisms of the Gas-Phase reactions of the hydroxyl radical with organic compounds", J Phys Chem ref data, Monograph no 1, pub Am Chem Soc.

(51) Carter, PL (1994) Development of ozone reactivity scales for volatile organic compounts. J Air Waste Man Assoc, 44, 881-899

(52) Carter, W.P.L., The SAPRC-99 Chemical Mechanism and Updated VOC Reactivity Scales (2000). URL http://www.cert.ucr.edu/~carter/reactdat.htm

(53) Andersson-Skold, Y. and L. Holmberg. (2000) "Photochemical Ozone Creation Potentials (POCP) and Replacement of Solvents in Europe". Atmospheric Environment 34:3159-3169.

(54) Derwent RG, Jenkin, ME, Saunders SM (1996). Photochemical ozone creation potentials for a large number of reactive hydrocarbons under European conditions. Atmosp. Env 30, 2, p181.

(55) Lyman, W., Reehl, W., Rosenblatt, D. (1990) Handbook of Chemical Property Estimation Methods: Environmental Behaviour of Organic Compounds. American Chemical Society, Washington, DC.

(56) Anbar, M. & Neta, P. (1967) A compilation of specific bimolecular rate constants for the reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals with inorganic and organic compounds in aqueous solution. Int. J.Appl. Radiation & Isotopes 18, 493 - 523.

(57) Greibel GE, Owens LD (1972). Nature of transient activation of soil microorganisms by ethanol or acetaldehyde. Soil Biol Biochem 4, 1-8

(58) Yasuhara, A. et al. (1981) Analysis of organic substance in highly polluted water by mass spectrometry. Environ. Sci. Technol. 15, 570 - 3.

(59) Cavanagh, L.A; Schadt, C.F; Robinson, E (1969) Atmospheric hydrocarbon and carbon monoxide measurements at Point Barrow, Alaska. Environ. Sci. Technol. 3, 251 - 257.

(60) Sabel GV, Clark TP (1983) Volatile Organic Compounds as indicators of municipal solid waste leachate contamination. Ann Madison Conf Appl Res Pract Munic Ind Waste, p108

(61) Graedel, T.E. Chemical compounds in the Atmosphere. Academic Press, New York, 1978 pp243.

(62) Anon. (1978) Atmospheric hydrocarbons in air during photochemical episodes. Kanagawa-ken taiki osen chosa kenkyuhokoku. 20, 86 - 90.

(63) EPIWIN program published by US Environmental protection agency.

(64) U.S. EPA URL http://www.epa.gov/opptintr/exposure/docs/episuite.htm

(65) Mackay, D. DiGuardo, A., Paterson, S. and Cowan, C. (1996) Evaluating the environmental fate of a variety of types of chemicals using the EQC model. Env. Toxicol. Chem. 15(9):1627-1637.

(66) See http://www.trentu.ca/academic/aminss/envmodel/EQCD.html

(67) Price, K.S. et al. (1974) Brine shrimp bioassay and seawater BOD of petrochemicals. J. Water Pollut. Control Fed. 46, 63 - 77.

(68) Gerhold, R. and Malaney, G. (1966). Structural determinants in the oxidation of aliphatic compounds by activated sludge. J. Water Poll. Control Fed. 38:562-579.

(69) Suflita, J. and Mormile, M. (1993). Anaerobic biodegradation of known and potential gasoline oxygenates in the terrestrial subsurface. Environ. Sci. Technol. 27:976-978.

(70) Birch, R.R., Fletcher, R.J. (1991) The application of dissolved inorganic carbon measurements to the study of aerobic biodegradability. Chemosphere 23(7):855-872.

(71) Young, R.H.F., Ryckman, D.W., Buzzell Jr, J.C. An improved tool for measuring biodegradability. J WPCF, 48(8) Pt. 2:R354-R368. (No year available; possibly 1978)

(72) Corseuil, et al., 1997; Aronson et al., 1997; USGS, 1998 andBarker et al., 1998 (full citations not available).

(73) Kavanaugh, M.C; , Stocking, A. (1999). Fate and Transport of Ethanolin the Environment. US EPA Blue Ribbon Panel.

(74) CERI, Japan (2003). http://qsar.cerij.or.jp/cgi-bin/DEGACC/index.cgi?E

(75) Johnson, W. and Finley, M. (1980). Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. U.S. Dept. of Interior, Fish and Wildlife Service, Washington, DC Resource Publication 137.

(76) Mattson, V., Arthur, J. and Wallbridge, C. (1976). Acute toxicity of selected organic compounds to Fathead Minnows. U.S. EPA Environmental Reasearch Lab., Duluth, Minnesota, EPA 600/3-76-097.

(77) Ewell, W., Gorstch, J., Krnge, R. et al. (1986) Simultaneous evaluation of the acute effects of chemicals on seven aquatic species. Environ. Toxicol. Chem. 5:831-840.

(78) Majewski, H., Klaverkamp, J. and Scott, D (1978) Acute mortality and sub-lethal effects of acetone, ethanol and propylene glycol on the cardiovascular and respiratory systems of rainbow trout (Salmo gairdneri) Water Res. 13:217-221.

(79) Brooke, L.T. et al. (1984) Acute toxicities of organic chemicals to fathead minnows. Vol. 1. University of Wisconsin-Superior.

(80) Juhnke, I. & Luedemann, D. (1978) Ergebnisse der Untersuchung von 200 chemischen Verbindungen auf akute Fischtoxizitaet mit dem Goldorfentest. Z. Wasser Abwasser Forsch. 11, 161 - 164.

(81) Bengtsson, B.-E., et al. (1984) Molecular structure and aquatic toxicity - an example with C1 - C13 aliphatic alcohols. Chemosphere 13, 613 - 622.

(82) In a similar study, a 96-hour LC50 of 10000 - 11500 mg/l wasreported in the bleak (Alburnus alburnus) in a static test at 10 °C. Linden, E. et al. (1979) The acute toxicity of 78 chemicals and pesticide formulations against two brackish water organisms, the bleak (Alburnus alburnus) and the harpacticoid Nitrocra spinipes. Chemosphere 11/12, 843 - 851.

(83) Gillette, L.A., Miller, D.L., Redman, H.E. (1952). Appraisal of a chemical waste problem by fish toxicity tests. Sewage Ind. Wastes 24(11):1397-1401.

(84) Umezu, T. Saponins and surfactants increase water flux in fish gills. Bull Jpn Soc Sci Fish/Nippon Suisan Gakkaishi 1991;57(10):1891-1896.

(85) O'Connor, C.S., Crawshaw, L.I., Bedichek, R.C., Crabbe, J.C.The effect of ethanol on temperature selection in the goldfish, Carassius auratus. Pharmacol Biochem Behav 1988;29(2):243-248.

(86) Takahashi, I.T.,Cowgill, U.M., Murphy, P.G. (1987) Comparison ot ethanol toxicity to Daphnia magna and Ceriodaphnia dubia tested at two differenttemperatures: Static acute toxicity test results. Bull. Environ. Contam. Toxicol. 39:229-236.

(87) Takahashi, I.T., Cowgill, U.M., Murphy, P.G. (1987) Comparison ot ethanol toxicity to Daphnia magna and Ceriodaphnia dubia tested at two different temperatures: Static acute toxicity test results. Bull. Environ. Contam. Toxicol. 39:229-236.

(88) Barahona-Gomariz, M.V., Sanz-Narrera, F., Sanchez-Fortun, S. Acute toxicity of organic solvents on Artemia salina. Bull Environ Contam Toxicol 1994;52(5):766-771.

(89) Rajini, P.S., Krishnakumari, M.K., Majumder, S.K. Cytotoxicity of certain organic solvents and organophosphorus insecticides to the ciliated Protozoan Paramecium caudatum. Microbios 1989;59:157-163.

(90) Bowman, M., Oller, W., Cairns, T. (1981). Stressed bioassay systems foir rapid screening of pesticide residues. Part 1: Evaluation of bioassay systems. Arch. Environ. Contam. Toxicol. 10:9-24.

(91) Calleja, M.C., Persoone, G. Cyst based Toxicity Tests. IV. The potential of ecotoxicological tests for the prediction of acute toxicity in man as evaluated on the first ten chemicals of the MEIC programme. ATLA 20:396-405.

(92) Calleja, M.C., Persoone, G., Geladi, P. Comparative acute toxicity of the firt 50 multicentre evaluation of in vitro cytotoxicity chemicals in aquatic non-vertebrates. Arch Environ Contam Toxicol 1994;26(1):69-78.

(93) Sauvant, M.P., Pepin, D., Groliere, C.A., Bohatier, J. Effects of organic and inorganic substances on the cell proliferation of L-929 fibroblasts and Tetrahymena pyriformis GL Protozoa used for toxicological bioassays. Bull Environ Contam Toxicol 1995;55(2):171-178.

(94) Nalecz-Jawecki, G., Sawicki, J. Sirotox - A new tool for testing the toxicity of volatile compounds. Chemosphere 1999;38(14):3211-3218.

(95) Crisnel, A., Delaunay, L., Rossel, D., et al. Cyst-based ecotoxicological tests using Anastrocans: Comparison of two species of Streptocephalus. Environ Toxicol Water Qual 1994;9(4):317-326

(96) Rayburn, J.R., Fisher, W.S. (1997). Developmental toxicity of three carrier solvents using embryos of the grass shrimp, Palaemonetes pugio. Arch Environ Contam Toxicol 33:217-221.

(97) Rossini, G.D.B., Ronco, A.E. Acute toxicity bioassay using Daphnia obtusa as a test organism. Environ Toxicol Water Qual. 1996;11(3):255-258.

(98) Lilius, H., Hastbacka, T., Isomaa, B. A comparison of the toxicity of 30 reference chemicals to daphnia magna and Daphgnia pulex. Environ Toxicol Chem 1995;14(12):2085-2088`1

(99) Bringmann, G. & Kuhn, R. (1977) Befunde der Schadwirkung wassergefaehrdender Stoffe gegen Daphnia magna. Z. Wasser Abwasser Forsch. 10, 161 - 166.

(100) Baldwin, W.S., Milam. D.L. LeBlanc, G.A. Physiological and biological perturbations in Daphnia magna following exposureto the model environmental estrogen diethylstilboestrol. Environ Toxicol Chem 1995;14(6):945-952.

(101) Office of Pesticide Programs. Environmental Effects Database(EEDB). Environmental Fate and Effects Division, U.S. EPA, Washington, D.C. 1995.

(102) Kuhn, R., Pattard, K., Pernak, K., Winter, A. Results of theharmful effects of selected water pollutants (anilines, phenols, aliphatic compounds) to Daphnia magna. Water Res 1989;23(4):495-499.

(103) Lagerspetz, K.Y.H., Tiiska, A., Senius, K.E.O. Low sensitivity of ciliary activity in the gills of Anodonta to some ecotoxicals. Comp Biochem Physiol 1993;105(C3):393-395.

(104) Lilius, H., Isomaa, B., Holmstrom, T. A comparison of the toxicity of 50 reference chemicals to freshly isolated rainbow trout hepatocytes and Daphnia magna. Aquat Toxicol 1994;30:47-60.

(105) Wu, Z., Chen, G. Studies of acute intoxication by some harmful substances on Penaeus orientalis K. Mar Sci/Haiyang Kexue 1988;4:36-40.

(106) Price, K.S. et al. (1974) Brine shrimp bioassay and seawaterBOD of petrochemicals. J. Water Pollut. Control Fed. 46, 63 - 77.

(107) Schultz, T.W., Arnold, L.M., Wilke, T.S., Moulton, M.P. Relationships of quantitative structure-activity for normal aliphatic alcohols. Ecoptoxicol Environ Saf 1990;19(3):243-253

(108) Schultz, T.W., Tichy, M. Structure-toxicity relationships for unsaturated alcohols to Tetrahymena pyriformis: C5 and C6 analogs and primary propargylic alcohols. Bull Environ Contam Toxicol 1993;51(5):681-688

(109) El Jay, A. (1996) Toxic effects of organic solvents on the growth of Chlorella vulgaris and Selenastrum capricornutum. Bull. Environ. Contam. Toxicol. 57:191-198.

(110) Cowgill, U., Milazzo, D., Landenberger, B. (1991). The senisitivity of Lemna gibba G-3 and four clones of Lemna minor to eight common chemicals using a 7-day test, Res. J. Water Pollut. Control Fed. 63:991-998.

(111) Hess, F. (1980). A Chlamydomonas algal bioassay for detecting growth inhibitor herbicides. Weed Sci. 28(5):515-520.

(112) Cowgill, U.M., Milazzo, D.P., Landenberger, B.D. Toxicity ofnine benchmark chemicals to Skeletonema costatum, a marine diatom. Environ Toxicol Chem 1989;8(5):451-455.

(113) Stratton, G.W. & Smith, T.M. (1988) Interaction of organic solvents with the green alga Chlorella pyrenoidosa. Bull. Environ. Contam. Toxicol. 40:736-742.

(114) Stratton, G.W. (1987) Toxic effects of organic solvents on the growth of blue-green algae. Bull. Environ. Contam. Toxicol. 38:1012-1019

(115) Tadros, M.G., Phillips, J., Patel, H., Pandiripally, V. Differential response of marine diatoms to solvents. Bull Environ Contam Toxicol 1995, 54(6):924-929.

(116) Schmidt C et al (1988) Structure activity relationship of organic substances and bioindicators. Vom Wasser, 70, 21-32.

(117) Krebs, F. (1991) Deutsche Gewasserkundliche Mitteilungen 35(5/6):161-170.

(118) Felix, H.R., Chollet, R., Harr, J. Use of the cell wall-less alga Dunaliella bioculata in herbicide screening tests. Ann Appl Biol 1988;113(1):55-60.

(119) Bringmann, G. & Kuhn, R. (1976) Vergleichende Befunde der Schadwirkung wassergefaehrdender Stoffe gegen Bakterien (Pseudomonas putida) und Blaualgen (Microcystis aeruginosa). gwf-wasser/abwasser 117, 410 - 413.

(120) Bringmann, G. & Kuhn, R. (1980) Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test. Water Res. 14, 231 to 241.

(121) Tadros, M.G., Philips, J., Patel, H., Pandiripally, V. Differential response of green algal species to solvents. Bull Environ Contam Toxicol 1994;52(3):333-337.

(122) Bringmann, G. & Kuhn, R. (1980). Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication inhibition test. Water Res. 14, 231 to 241.

(123) Miles-Richardson, S.R., et al.. Effects of waterbourne exposure to 4-nonylphenol and nonylphenol ethoxylate on secondary sex characteristiucs and gonads of fathead minnows (Pimephales promelas). Environ Res 1999;80(2):S122-S137.

(124) Bennett, W.R., Farrell, A.P. Acute toxicity testing with juvenile white sturgeon (Acipenser transmontanum) Water Qual Res J Can 1998;33(1):95-110.

(125) Cowgill, U.M., Milazzo, D.P. (1991) The sensitivity of Ceriodaphnia dubia and Daphnia magna to Seven Chemicals Utilizing the Three Brood Test. Arch Environ Contam Toxicol 20(2):211-217.

(126) Rayburn, J.R., Fisher, W.S. Developmental toxicity of three carrier solvents using embryos of the grass shrimp, Palaemonetes pugio. Arch Environ Contam Toxicol 1997;33(2):217-221.

(127) Foss, S.S., Rayburn, J.R. Effects of culture duration on toxicity of ethanol to developing embryos of the grass shrimp Palaeomonetes pugio. Bull Environ Contam Toxicol 1997;59:467-471.

(128) Friedman, R.N., Bittner, G.D., Blundon, J.A. Electrophysiological and behavioural effects of ethanol on crayfish. J Pharmacol Exp Ther 1988;246(1):125-131.

(129) Willis, K.J. Acute and chronic bioassays with New Zealand freshwater copepods using pentachlorophenol. Environ ToxicolChem 1999;18(11):2580-2586

(130) Deutsch, U., Oehlmann, J.; Stroben, E. Morphological effectsof tributyl tin (TBT) in vitro on the genital system of the Mesogastropod Littorina littorea (L.)(Prosobranchia) In: J.C. Aldrich (Ed.), Proc 27yth European Marine Biology Symposium, Quantified Phenotypic Responses in Morphology andPhysiology, Sept. 7-11, 1992, Dublin, Ireland:297-300.

(131) Baldwin, W.S., Milam, D.L., LeBlanc, G.A. Physiological and biological perturbations in Daphnia magna following exposure to the model environmental estrogen diethylstilboestrol. Environ Toxicol Chem 1995;14(6):945-952.

(132) Fiskesjo, G. (1985). The Allium Test as a Standard in Environmental Monitoring. Hereditas 102:99-112.

(133) Reynolds, T. Comparative Effects of Aliphatic Compounds on Inhibition of Lettuce Fruit Germination Ann.Bot. 1977;41:637-648.

(134) Meyer, H., A.M. Mayer. Permeation of Dry Seeds with Chemicals: Use of Dichloromethane. Science 1971;171:583-584.

(135) Nashed, R.B., and R.E. Girton. Inhibition by Ethanol of the Growth and Respiration of Maize Roots and Coleoptiles Am J Botany 1958;45:190-193.

(136) Miller, L.P. Further Experiments on the Effect of Halogenated Aliphatic Compounds on the Respiration of Potato tubers Contr Boy T 1935;7:1-17.

(137) Mer, C.L. Growth-Promoting Effect of Ethanol on Oat Seedlings Nature 1958;182:1812-1813.

(138) Rychter, A., H.W. Janes, C.-K. Chin, and C. Frenkel. Effect of Ethanol, Acetaldehyde, Acetic Acid and Ethylene on Changes in Respiration and Respiratory Metabolites in Potato (Solanum tuberosum) Tubers. Plant Physiol 1979;64:108-111.

(139) Reichhart, D., Salaun, J.-P., Benveniste, I., F. Durst. Induction by Manganese, Ethanol, Phenobarbital and Herbicides of Microsomal Cytochrome P-450 in Higher Plant Tissues. Arch Bioch 1979;196:301-303.

(140) Clements, H.F., and E. Akamine. Root Stimulation in Sugar Cane (Saccharum officinarum) with Special Reference to the Efects of Ethyl Alcohol Am J Botany 1940;27:482-487.

(141) Guthrie, J.D. The Effect of Various Chemical Treatments of Dormant Potato Tubers on the Peroxidase, Catalase, pH, and Reducing Properties of the Expressed Juice Contr Boy T 1931;3:499-508.

(142) Davis, D.G., W.P. Wergin, and K.E. Dusbabek. Effect of Organic Solvents on Growth and Ultrastructure of Plant Cell Suspensions. Pesticide Biochem Physiol 1978;8:84-97.

(143) Roberts, B.L., H.W. Dorough. Relative Toxicities of Chemicals to the Earthworm Eisenia foetida. Environ Toxicol Chem 1984;3(1):67-78.

(144) Ewell, W.S. et al. (1986). Simultaneous evaluation of the acute effects of chemicals on seven aquatic species. Environmental Toxicology and Chemistry 5:831-840

(145) Seeber, A., Blaszkewicz, M., Kiesswetter, E., Bandel, T., Golka, K., Heitmann, P., Vangala, R.R., Bolt, H.M. Biomonitoring, Performance and Well-Being under Exposure to Inhalation of Ethanol. Transact German Soc Occup & Environ Med. 34th Ann Meet, Weisbaden, 1994

(146) Kruhoffer, P.W. (1983). Handling of inspired vapourized ethanol in the airways and lungs (with comments on forensic aspects). Forensic Sci Int. 21:1-17.

(147) Jones, A.W., Andersson, L. (1993). Disappearance Rate of Ethanol from the Blood of Human Subjects: Implications in Forensic Toxicology. J Forensic Sci, JFSCA 1993;38(1):104-118.

(148) Crabb, D.W., Bosron, W.F., Li, T.-K. (1987). Ethanol metabolism. Pharmac Ther 34:59-73.

(149) Iwahashi K, Suwaki H (1998) Ethanol metabolism, toxicity and genetic polymorphism, Addict Biol, 3, 249-59

(150) Ueno Y, Fukunaga T, Mizoi Y, Adachi J, Fujuwara S (1990) A pharmacokinetic study of ethanol elimination - first pass metabolism and elimination rate.

(151) Bartsch, W. et al. (1976) Acute toxicity of various solvents in the mouse and rat. Arzneim.-Forsch. 26, 1581 - 1583.

(152) Youssef, A., Madkour, K., Cox, C., Weiss, B. (1992) Comparative lethality of methanol, ethanol and mixtures in female rats. J. Appl. Toxicol. 12(3):193-197.

(153) Wiberg, G., Trenholm, H., Coldwell, B. (1970). Increased ethanol toxicity in old rats: changes in LD50, in vivo and in vitro metabolism, and liver alcohol dehydrogenase activity. Toxicol. Appl. Pharmacol. 16:718-727.

(154) Kimura, E.T., Ebert, D.M., Dodge, P.W. (1971). Acute toxicity limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704.

(155) Kimura, E.T. et al. (1971) Acute toxicity limits of solvent residue for sixteen organic solvents. Toxicol. Appl. Pharmacol. 19, 699 - 704.

(156) Smyth, H.F. Jr. (1941) J. Ind. Hyg. Toxicol. 23, 253 cited in Patty (1982) loc. cit.

(157) Anon. (1935) Am. J. Clin. Pathol. 5, 466 cited in RTECS (1992) loc. cit.

(158) BASF AG Toxicology Department Unpublished report 26-01-1959

(159) Latven, J. (1933) J. Pharm. Exp. Ther. 65, 89. cited in Spector, W.S. (editor), 1965. Handbook of Toxicology. Vol. 1. Acute toxicities of solids, liquids and gases to laboratory animals. Saunders, Philadelphia and London.

(160) Anon. (1967) Gig. Sanit. 32, 31. cited in RTECS (1992) loc. cit.

(161) Bayer EU Existing Chemicals Programme HEDSETAG data February 15 1972.

(162) Smyth, unpublished data, Mellon Inst. cited in Spector (1956) loc. cit.

(163) Takeda, I. (1972) Nichidai Igaku Zasshi 31, 518 cited in Patty (1982) loc. cit.

(164) Munch, J.C. & Schwartze, E.W. (1925) J. Lab. clin. Med. 10, 985. cited in Patty (1982) loc. cit.

(165) Anon. (1936) J. pharmac. exp. Ther. 56, 117 cited in RTECS (1992) loc. cit.

(166) DuJardin-Beaumetz, C. (1875) rend. Acad. Sc. 81, 192. cited in Spector (1956) loc. cit.

(167) BASF AG Toxicology Department Unpublished research 26-01-1959

(168) Smyth, H.F. Jr. (1941) J. Ind. Hyg. Toxicol. 23, 259 cited in RTECS (1992) loc. cit.

(169) Moser, V. and Balster (1985). Acute motor and lethal effects of inhaled toluene, 1,1,1-trichloroethane, halothane and ethanol in mice: effects of exposure duration. Toxicol Appl. Pharmacol 77:285-291.

(170) BASF AG Toxicology Department Unpublished research. (80/30)23-12-1981

(171) Lehmann, K.B. & Flury, F. (1938) Toxikol. Hyg. Tech. Losung.Springer, Berlin. cited in Patty (1982) loc. cit.

(172) BASF AG Toxicology Department Unpublished research (80/95) 19-01-1982

(173) BASF AG Toxicology Department Unpublished research. (80/30) 14-10-1980

(174) BASF AG Toxicology Department Unpublished research. (80/30) 13-11-1980

(175) Unpublished data from the Carnegie-Mellon Institute of Research cited in Patty (1982) loc. cit.

(176) Smyth, H.F. Jr. (1956) Am. ind. Hyg. Assoc. Q. 17, 129. cited in Patty (1982) loc. cit.

(177) Loewy, A. & von der Heide, R. (1918) Ueber die Aufnahme des Aethylalkohols durch die Atmung. Biochem. Z. 86, 125 - 175.

(178) Anon. (1982) Gig. Truda prof. Zabol. 26, 53. cited in RTECS (1992) loc. cit.

(179) Myou S, Fujimura M, Bando T, Saito M, Matsuda T (1994) Aerosolized acetaldehyde, but not ethanol, induces histamine mediated brochoconstriction in guinea pigs. Clin Exp Allergy, 24, 140-3

(180) Zuskin E, Bouhuys A, Saric M (1981) Lung function changes by ethanol inhalation, Clin Allergy, 11, 243-8.

(181) Malik F, Wickramasinghe (1986) Haematological abnormalities in mice continuously exposed to ethanol vapour. Br J. Exp. Path. 67, 831-8

(182) Monick, J.A. (1968) in Alcohols. Their Chemistry Properties and Manufacture. Reinhold 1969, LCCCN 68-23906:p75.

(183) Schechter, M. and Meehan, S. (1995). The lethal effects of ethanol and cocaine and their combination in mice: implications for cocaethylene formation. Pharmacol Biochem Behav 52(1):245-248.

(184) Ho, A and Ho, C. (1979). Toxic interaction of ethanol with other central depressants: antagonism by naloxone to narcosis and lethality. Pharmacol. Biochem. behav. 11:111-114.

(185) Jacobs, G.A. & Martens, M.A. (1992) OECD skin irritation tests on three alcohols. Acute toxicity data. J. Am. Coll. Toxicol. 11, 733.

(186) Phillips, L. II., Steinberg, M., Maibach, H.J., Akers, W.A. (1972) A comparison of rabbit and human skin response to certain irritants. Toxicol Appl Pharmacol 21:369-382.

(187) BASF AG Toxicology Department Unpublished research (78/810- S III) 04-09-1979

(188) BASF AG Toxicology Department Unpublished research TNO Report No R5724 (1978)

(189) Basketter DA, York M, McFadden JP, Robinson MK (2004) Determination of skin irritation potential in the human 4-hour patch test. Contact Dermatitis 51, 1-4

(190) Jacobs, G.A., Guido, A. (1987) OECD eye irritation tests on three alcohols. J. Coll. Am. Toxicol. part A, 9, 56 - 57.

(191) ECETOC Eye Irritation Databank (2nd ed), TR48(2) 1998

(192) Grant, W.M. Toxicology of the eye. Edn 3. (1986). Charles C Thomas, Springfield, USA

(193) BASF AG Toxicology Department Unpublished Research (83/64)06-07-1983

(194) BASF AG Toxicology Department Unpublished research (78/810) 04-09-1979

(195) BASF AG Toxicology Department Unpublished research TNO Report No. R5724 (1978)

(196) Guillot, J.P. et al. (1982) Evaluation of the ocular-irritation potential of 56 compounds. Fd Chem. Toxic.20, 573 - 582.

(197) Descotes, J. (1988). Identification of contact allergens: the mouse ear sensitization assay. J Toxicol -Cut & Ocular Toxicol 7:263-272

(198) BP Chemicals Ltd (1984). Results of the Biological Investigations on Selected Materials of the Breox Polyalkylene Glycol Block Copolymer Range. BP Group Occupational Health Centre Toxicology Report 25-90-0150.

(199) Holmberg, B., Kronevi, T., Ekner, A. (1986). Subchronic toxicity investigation of ethyl alcohol: a test for lowest effective dose (led) to be used in a long term bioassay for carcinogenicity. National Board of Occupational Safety and Health, Solna, Sweden.

(200) National Toxicology Program (1996). NTP Technical Report on Toxicity Studies on Urethane in Drinking water and Urethane in 5% Ethanol Administered to F344 Rats and B6C3F1 Mice. NTP, Research Triangle Park, NC, USA

(201) National Toxicology Program (1996). NTP Technical Report on Toxicity Studies on Urethane in Drinking water and Urethane in 5% Ethanol Administered to F344 Rats and B6C3F1 Mice. NTP, Research Triangle Park, NC, USA

(202) Kanwar, K.C. & Tikoo, A. (1992) Hematological lesions in rat following heavy alcohol ingestion. J. Env. Path. Toxicol. Oncol. 11, 241 - 245.

(203) Coon, R.A. et al. (1970) Animal inhalation studies on ammonia, ethylene glycol, formaldehyde, dimethylamine and ethanol. Toxicol. Appl. Pharmacol. 16, 646 - 655.

(204) Rosin, J. et al. (1988) Studies on the effect of ethanol and/or toluene on rat erythrocytes. J. Appl. Toxicol. 8, 369- 372.

(205) Ainley, C.C. et al. (1988) Is alcohol hepatotoxic in the baboon? J. Hepatol. 7, 85 - 92.

(206) Lieber, C.S. et al. (1975) Sequential production of fatty liver, hepatitis, and cirrhosis in sub-human primates fed ethanol with adequate diets. Proc. Natl. Acad. Sci. USA, 72,437 - 441.

(207) Tsukamoto, H. et al. (1985) Severe and progressive steatosisand focal necrosis in rat liver induced by continuous intragastric infusion of ethanol and low fat diet. Hepatology, 5, 224 - 225.

(208) Simanowski, U.A. et al. (1986) Chronic ethanol consumption selectively stimulates rectal cell proliferation in the rat.Gut 27, 278 - 282.

(209) Smyth, H.F. & Smyth, J.F. Jr. (1928) Inhalation experiments with certain lacquer solvents. J. Ind. Hyg. 10, 261 - 271.

(210) Kager, L. & Ericsson, J.L.E. (1974) Long term toxicity studywith alcohol and 4-methylpyrazole in rat. Acta path. microbiol. scan. Sect. A, 82, 534 - 538.

(211) Mezey, E. et al. (1983) Effect of chronic ethanol feeding onhepatic collagen in the monkey. Hepatology 3, 41 - 44.

(212) Vasdev, S.C. et al. (1975) Myocardial lesions induced by prolonged alcohol feeding in rhesus monkeys. Cardiovascular Res. 9, 134 - 140.

(213) MacDonald, C.M. (1973) The effects of ethanol on hepatic lipid peroxidation and on the activities of glutathione reductase and peroxidase. FEBS Letters 35, 227 - 230.

(214) Marietta CA, Jerrells TR, Meagher RC, Karanian JW, Weight FF, Eckardt MJ (1988) Effects of long term ethanol inhalation on the immune and hematopoetic systems of the rat, Alcoholism 12 (2) 211-4

(215) Di Luzio NR, Stege TE (1979) "Influence of chronic ethanol vapour inhalation on hepatic parenchymal and Kupffer cell function . Alcoholism: Clin Exp Res 3 (3) 240

(216) Rikans, LE, Gonzalez LP (1990) Antioxidant protection systems of rat lung after chronic ethanol inhalation, Alcoholism: Clin Exp Res, 14 (6) 872-7.

(217) Baraona, E. et al. (1974) Small intestinal damage and changes in cell population produced by ethanol ingestion in the rat. Gastroenterology, 66, 226 - 234.

(218) Zeiger, E., Anderson, B., Haworth, S., Lawlor, T, Mortelamns, K. (1992). Salmonella mutagencity tests: V. Results from the testing of 311 chemicals. Environ Mol Mut 19;2-141.

(219) Blevins, R.D., Taylor, D.E. (1982). Mutagencity screening of twenty five cosmetic ingredients with the Salmonella microsome test. J Environ Sci Health 1982;A17(2):217-239.

(220) McCann, J., Choi, E., Yamasaki, E., Ames, B.N. (1975) Detection of carcinogens as mutagens in the Salmonella/microsome terst: Assay of 300 chemicals. Proc Nat Acad Sci 1975;72(12):5135-5139.

(221) Blevins, R.D., Shelton, M.S. (1983). Response of Salmonella typhimurium mutants to delta9-THC and in conjunction with known mutagens. J Environ Sci Health A18(3), 413-443.

(222) De Flora, S., Zanacchi, P., Camoirano, A., Bennicelli, C, Badolati, G.S. (1984). Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutation Res.133;161-198.

(223) De Flora, S., Camoirano, A., Zanacchi, P., Bennicelli, C. (1984). Mutagenicity testing with TA97 and TA102 of 30 DNA-damaging compounds negative with other Salmonella strains. Mutat Res 134:159-165.

(224) Hellmer, L. and Bolcsfoldi, G. (1992) An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay. Mutation Res. 272;145-160.

(225) Hayes, S. (1985). Ethanol-induced genotoxicity. Mutat. Res. 143:23-27.

(226) Hayes, S., Gordon, A., Sadowski, I., Hayes, C. (1984). RK bacterial test for independently measuring chemical toxicity and mutagenicity: Short-term forward selection assay. Mutat Res 130:97-106

(227) Banduhn, N. and Obe, G. (1985). Mutagenicity of methyl 2-benzimidazole, diethylstilboestrol and estradiol: Structural chromosomal aberrations, sister chromatid exchanges, C-mitoses, polyploidies and micronuclei. Mutat. Res. 1985;156:199-218.

(228) Lin, Y-C., Ho, I-C., Lee, T-C. (1989). Ethanol and acetaldehyde potentiate the clastogenicity of ultraviolet light, methane methanesulfonate, mitomycin C and bleomycin in Chinese hamster ovary cells. Mutat. Res. 216:93-99.

(229) Brown, N.M., Trizna, Z., Pathak, S. (1992). Clastogenic interactions between lobeline sulfate and ethyl alcohol: A cytogenetic study. Anticancer Research 1992;12:1467-1470.

(230) Lau, C-F., Vogel, R., Obe, G., Spielmann. H. (1991). Embryologic and cytogenetic effects of ethanol on preimplantation mouse embryos in vitro. Reproductive Toxicology 1991;5:405-410.

(231) Hsu, T.C., Furlong, C., Spitz, M.R. (1991). Ethyl alcohol as a cocarcinogen with special reference to the aerodigestive tract: A cytogenetic study. Anticancer Research 1991;11:1097-1102.

(232) Koenigstein, M., Larisch, M., Obe, G. (1984). Mutagenicity of antiepileptic drugs I. Carbamazepine and some of its metabolites. Mutat. Res. 139;83-86.

(233) Au, W. and Badr, F.M. (1979) Does ethanol induce chromosome damage. In vitro 15, 221

(234) Darroudi, F. & Natarajan, A.T. (1987). Induction of chromosomal aberrations and sister chromatid exchanges in CHO cells by mutagenic metabolites activated by plant microsomal extracts. Biologisches Zentralblatt 106:169-174.

(235) Cadotte, M., Allard, S., Verdy, M. (1973). Lack of effect of ethanol in vitro on human chromosomes. An. Genet 16(1):55-56.

(236) Badr, F.M. et al. (1977) Evaluation of the mutagenic effects of alcohol by different techniques. Adv. Exp. Med. Biol. 85a, 25 - 46.

(237) Wangenheim, J. and Bolcsfoldi, G. (1988). Mouse lymphoma L5178Y thymidine kinase locus assay of 50 compounds. Mutagen. 3(3):193-205.

(238) Amacher, D.E. et al. (1980) Point mutations at the thymidinekinase locus in L5178Y mouse lymphoma cells. II. Test validation and interpretation. Mutation Res. 72, 447 - 474.

(239) Friedrich, U., Nass, G. (1983). Evaluation of a mutation test using S49 mouse lymphoma cells and monitoring simultaneously the induction of dexamethasone resistance, 6-thioguanine resistance and ouabain resistance. Mutat. Res. 1983;110:147-162.

(240) Lasne, C., Gu, Z.W., Venegas, W., Chouroulinkov, I. (1984) The in vitro micronucleus assay for detection of cytogenetic effects induced by mutagen-carcinogens: comparison with the in vitro sister-chromatid exchange assay. Mutat. Res. 130;273-282.

(241) Obe, G., Ristow, H. Acetaldehyde, but not ethanol, induces sister chromatid exchanges in Chinese hamster cells in vitro. Mutat Res 1977;56:211-213

(242) Balansky, R.M., Blagoeva, P.M., Mircheva, Z. I. and de Flora,S. Co-clastogenicity of ethanol with cigarette smoke in rat erythroblasts and anticlastogencity in alveolar macrophages. Cancer Lett 1993;72:183-189.

(243) Tates, A.D. (1980) Cytogenetic effects in hepatocytes, bone-marrow cells and blood lymphocytes of rats exposed to ethanol in the drinking water. Mutation Res. 79, 285 - 288.

(244) Chaubey, R.C., Kavi, B.R., Chauhan, P.S., Sundaram, K. (1977). Evaluation of the effect of ethanol on the frequency of micronuclei in the bone marrow of Swiss mice. Mutation Res. 43:441-444.

(245) Baraona, E., Guerra, M., Lieber, C.S. (1981). Cytogenetic damage of bone marrow cells produced by chronic alcohol consumption. Life Sci. 29, 1797-1802.

(246) Badr, F.M., Badr, R.S., Asker, R.L., Hussain, F.H. (1977). Evaluation of the mutagenic effectsof ethyl alcohol by different techniques. Adv. Exp. Med. Biol. 85A:25-46.

(247) Korte, A., Slacik-Erben, R., Obe, G. (1979). The influence of ethanol treatment on cytogenetic effects in bone marrow cells of Chinese hamsters by cyclophosphamide, aflatoxin B and patulin. Toxicology 12;53-61.

(248) Korte, A., Wagner, H.M., Obe, G. (1981). Simultaneous exposure of Chinese hamsters to ethanol and cigarette smoke: cytogenetic aspects. Toxicology 20;237-246.

(249) Korte, A., Obe, G. (1981). Influence of chronic ethanol uptake and acute acetaldehyde treatment on the chromosomes of bone-marrow cells and peripheral lymphocytes of Chinese hamsters. Mutation Res. 88;389-395.

(250) James, D.A. & Smith, D.M. (1982) Analysis of results from a collaborative study of the dominant lethal assay. Mutation Res. 97:303-314.

(251) Rao, U.N., Aravindakshan, M., Chauhan, P.S. (1994). Studies on the effects of ethanol on dominant lethal mutations in Swiss, C57Bl6 and CBA mice. Mutat Res 1994;311:69-76.

(252) Randall, C.L., Burling, T.A., Lochry, E.A., Sutker, P.B. (1982). The effect of paternal alcohol consumption on foetal development in mice. Drug and Alcohol Dependence 9;89-95.

(253) Berryman, S.H., Anderson Jr., R.A., Weis, J., Bartke, A. (1992). Evaluation of the co-mutagenicity of ethanol and delta9-tetrahydrocannabinol with Trenimon. Mutat. Res. 1992;278:47-60.

(254) Klassen, R.W. & Persaud, T.V.N. (1976) Experimental studies on the influence of male alcoholism on pregnancy and progeny. Exp. Pathol 12, 38 - 45.

(255) Mankes, R.F., LeFevre, R., Benitz, K.-F., Rosenblum, I. et al. (1982). Paternal effects of ethanol in the Long Evans rat. J. Toxicol. Envir. Hlth 10:871-878.

(256) Chauhan, P.S., Aravindakshan, M., Kumar, N.S., Sundaram, K. (1980). Failure of ethanol to induce dominant lethal mutations in Wistar male rats. Mutation Res. 79:263-275.

(257) Machemer, L., Lorke, D. (1975). Experiences with the dominant lethal test in female mice: effects of alkylating agents and artificial sweeteners on pre-ovulatory oocyte stages. Mutation Res. 29;209-214.

(258) Washington, W.J., Cain, K.T., Cacheiro, N.L.A., Generoso, W.M. (1985). Ethanol-induced late fetal death in mice exposed around the time of fertilization. Mutat. Res. 1985;147:205-210.

(259) Badr, F.M. & Badr, R.S. (1975). Induction of dominant lethal mutations in male mice by ethyl alcohol. Nature 253, 134 - 135.

(260) Soffritti M, Belpoggi F, Cevolani D, Guarino M, Padovani M, Maltoni C (2002) Ann N Y Acad Sci, 982 p46-67

(261) Toxicology and Carcinogenesis Studies of Urethane, Ethanol, and Urethane/Ethanol in B6C3F1 Mice (Drinking Water Studies) Draft National Toxicology Program Technical Report NTP TR 510 NIH Publication No. 02-4444, NTP, Research Triangle Park, NC, USA, 2002.

(262) Greim H (1999) Occupational toxicants, vol 12, Critical data evaluation for MAK values and classification of carcinogens, 128-165 (Wiley)

(263) Schmidt, W. et al. (1987) Br. J. Addict. 82, 775.

(264) Abel, E.L. et al. (1987) Prenatal alcohol exposure shortens life span in rats. Teratology 36, 217 - 220.

(265) Kahn, A.J. (1968) Growth 32, 311.

(266) McCoy, G.D. et al. (1981) Cancer Res. 41, 2849.

(267) Castonguay, A. et al. (1984) Cancer Res. 44, 2285.

(268) Medenhall C.L. & Chedid, A. (1980) Dig. Dis. Sci. 25, 587.

(269) Griciute L. et al. (1981) Cancer Lett. 13, 345.

(270) Griciute, L. et al. (1986) Cancer Lett. 31, 267.

(271) Gibel, W. (1967) Arch. Geschwulstforsch. 30, 181.

(272) McCoy, G.D. et al. (1986) Cancer Lett. 33, 151.

(273) Pour, P.M. et al. (1983) J. natl. Cancer Inst. 71, 1085.

(274) Horie, A. et al. (1965) Gann 56, 429.

(275) Kuratsune, M. et al (1971) Gann 62, 395.

(276) Schrauzer, G.N. et al. (1979) J. Stud. Alcohol, 40, 240.

(277) Takahashi, M. et al. (1986) Jpn. J. Cancer Res. 77, 118.

(278) Radike, M.J. (1981) Envir. Hlth. Perspect. 41, 59.

(279) Mandard, A.M. et al. (1981) Bull. Cancer 68, 49.

(280) Schmahl, D. (1976) Cancer Lett. 1, 215.

(281) Kuratsune, M. et al. (1971) Gann 62, 395.

(282) IARC Monographs on the Evaluation of Carcinogenic risks to humans, Vol. 44. Alcohol drinking. IARC, Lyon, France 1988.

(283) George, J., Myers, C., Reel, J. et al. (1985). Ethanol: Reproduction and fertility assessments in CD-2 mice when administered in the drinking water. NTP PB86144979.

(284) Abel, E. (1989). Duration of paternal alcohol consumption does not influence offspring growth and development. Growth Devel. Aging 53:195-199.

(285) Krueger, W.A., Bo, W.J., Rudeen, P.K. Female reproduction during chronic ethanol consumption in rats. Pharmacol Biochem Behav 1982;17:629-631.

(286) Bo, E.J., Krueger, W.A., Rudeen, P.K., Symmes, S.K. Ethanol-induced alterations in the morphology and function of the rat ovary. Anat Rec 1982; 202:235-260.

(287) Van Thiel, D.H., Gavaler, J.S., Lester, R. Alcohol-induced ovarian failure in the rat. J. Clin Invest 1976; 61: 624-632.

(288) Abel, E.L. (1993). Rat Offspring Sired by Males Treated with Alcohol. Alcohol 1993;10:237-242

(289) Rivier C, and Vale W (1983). Influence of Ethanol on Reproductive Functions of the adult Male Rat as a Function of Body Weight. Alcohol: Clin Exp Res 1983; 7(3):210-212

(290) Abel EL, (1995). A Surprising Effect of Paternal Alcohol Treatment on Rat Fetuses. Alcohol 1995;12(1):1-6

(291) Nelson BK, Brightwell WS, Mackenzie-Taylor DR, Burg JR, Massari VJ (1988) Neurochemical but not behavioral deviations in the offspring of rats following prenatal or paternal inhalation exposure to ethanol. Neurotoxicol Teratol 10, 15-22.

(292) Nelson BK; Brightwell, WS, Burg JR (1985) Comparison of Behavioural Teratogenic Effects of Ethanol and n-propanol administered by inhalation to rats. Neurobehav Toxicol Teratol 7, 779-83.

(293) Nelson, B., Brightwell, W., MacKenzie, D., et al. (1985). Teratological assessment of methanol and ethanol at high inhalation level in rats. Fundam Appl Toxicol 5:727-736.

(294) Kieffer, J.D., Ketchel, M.M. Blockade of ovulation in the rat by ethanol. Acta Endocrinol 1970;65:117-124.

(295) Cameron AM, Zahlsen K, Haug E, Nilsen OG, Eik-Ness KB (1985) Circulating steroids in male rats following inhalation of n-alcohols, Arch Toxicol Suppl 8, 422-4.

(296) Mello, N.K., Bree, M.P., Mendelson, J.H., Ellingboe, J. Alcohol self administration disrupts reproductive function in male Macaque monkeys. Science 1983;221:677-679.

(297) Udani, M., Parker, S., Gavaler, J.S., Van Thiel, D.H. (1985) Effects of in utero exposure to alcohol upon male rats Alcoholism Clinical and experimental research 9(4) 355-359.

(298) Parker, S., Udani, M., Gavaker, J.S., Van Thiel, D.H. Adverse effects of ethanol upon the adult sexual behaviour of male rats exposed in utero. Neurobehav Toxicol Teratol 1984;6:289-293.

(299) Oisund, J.F., Fjorden, A-E. and Moerland, J. Is moderate ethanol consumption teratogenic in the rat? Acta Pharmacol Toxicol 1978;43:145-155.

(300) Cicero, T.J., Meyer, E.R., Bell, R.D. Effects of ethanol on the hypothalamic- pituitary-luteinizing hormone axis and testicular steroidogenesis. J Pharmacol Exp Ther 1979; 208:210-215.

(301) Alfonso, M., Parafita, M.A., Mancebo, M.J., Marco, J. Further evidence for effects of ethanol on gonadotrophins and prolactin secretion in female rats. Gen Pharmacol 1985;16:43-47.

(302) Handa, R.J., et al. Exposure to alcohol in utero alters the adult patterns of luteinizing hormone secretion in male and female rats. Life Sci 1985;37:1683-1690.

(303) Thiessen, D.D., Whitworth, N.S., Rodgers, D.A. Reproductive variables and alcohol consumption of the C57Bl/Crgl female mouse. Q J Stud Alcohol 1966;27:591-595.

(304) Vaglenova, J., Petkov, V.V. (1998). Fetal alcohol effects in rats exposed pre- and postnatally to a low dose of ethanol. Alcohol: Clin and Exp Res 22(3):697-703.

(305) Randall, C. and Taylor, W. (1979) Prenatal ethanol exposure in mice: teratogenic effects. Teratol. 19:305-312

(306) Anderson, R., Furby, J., Oswald, C., Zaneveld, L. (1981). Teratological evaluation of mouse fetuses after paternal alcohol ingestion. Neurobehav. Toxicol. Teratol. 3:117-120

(307) Chernoff, G. (1977). The fetal alcohol syndrome in mice: an animal model. Teratol. 15:223-230.

(308) Chernoff, G. (1977). The fetal alcohol syndrome in mice: an animal model. Teratol. 15:223-230. [presumed].

(309) Wier, P., Lewis, S., Traul, K. (1987) A comparison of developmental toxicity evident at term to postnatal growth and survival using ethylene glycol monoethyl ether, ethylene glycol monobutyl ether and ethanol. Teratogen Carcinogen Mutagen 7:55-64.

(310) Schwetz, B.A., Smith, F.A., Staples, R.E. Teratogenic potential of ethanol in mice, rats and rabbits. Teratology 1978; 18:385-392.

(311) Schwetz, B.A., Smith, F.A., Staples, R.E. Teratogenic potential of ethanol in mice, rats and rabbits. Teratology 1978;18:385-392.

(312) Astley, S.J., Magnuson, S.I., Omnell, L.M., Clarren, S.K. Fetal Alcohol Syndrome: Changes in craniofacial form with age, cognition, and timing of ethanol exposure in the macaque. Teratology 1999;59:163-172

(313) Plant, M.L., Abel, E.L., Guerri, C. (1999). Chapter 6: Alcohol and Pregnancy. In: Health Issues Related to Alcohol Consumption. Ed. Macdonald, I. International Life Sciences Inst. 2nd edition. ISBN 1-57881-062-0.

(314) Leichter, J. and Lee, M. Effect of maternal ethanol administration on physical growth of the offspring of rats. Growth 1979;43:288-297.

(315) Lee, M., Leichter, J. Skeletal development of foetuses of rats consuming alcohol during gestation. Growth 1983;47:254-262.

(316) Samson, H.H. Maternal ethanol consumption and fetal development in the rat: a comparison of ethanol exposure techniques. Alcohol Clin Exp Res 1981;5:67-74.

(317) Chen J.J. & Smith E.R. 1979. Horm. Behav. 13, 219

(318) Ihemelandu, E.C. Effect of maternal ethanol consumption on pre- and post-natal muscle development in mice. Growth 1984;48:35-43

(319) Switzer, B.R., Anderson, J.J.B., Pick, J.R. Effects of dietary protein and ethanol intake of pregnant Beagles fed purified diets. J. Nutr. 1986; 116:689-697.

(320) Ellis F.W. et al. 1977. Fed. Proc. 36, 285

(321) Clarren S.K. et al. 1987b. Teratology 35, 66A.

(322) Clarren, S.K., Astley, S.J., Bowden, D.M. Pregnancy outcomesafter weekly oral administration of ethanol during gestation in the Pig-tailed Macaque (Macaca namestrina). Teratology 1987;35:345-354.

(323) Klassen, R.W., Perdsaud, T,V.N. Experimental studies on the influence of male alcoholism on pregnancy and progeny. Exp Pathol 1976; 12: 38-45.

(324) West, J.R., Hodges, C.A., Black,A.C. Jr. Prenatal exposure to ethanol alters the organization of hippocampyl mossy fibres in rats. Science 1981; 211:957-959.

(325) Bond, N.W. Prenatal alcohol exposure and offspring hyperactivity: Effects of para-chlorophenylalanine and methysergide. Neurobehav Toxicol Teratol 1986; 8:667-673.

(326) Rockwood, G.A., Riuley, E.P. Suckling deficits in rat pups exposed to alcohol in utero. Teratology 1986; 33:145-151.

(327) Meyer, L.S., Riley, E.P. Social play in juvenile rats prenatally exposed to alcohol. Teratology 1986; 34: 1-7.

(328) Udani, M., Parker, S., Gavaler, J.S., Van Thiel, D.H. Effects of the in utero exposure to alcohol upon male rats. Alcoholism: Clinical and Experimental Research 1985; 9(4):355-359.

(329) Oisund, J.F., Fjorden, A-E., Moerland, J. Is moderate ethanol consumption teratogenic in the rat? Acta Pharmacol Toxicol 1976;43:145-155,

(330) Sanchis, R., Sancho-Tello, M., Chirivella, M., Guerri, C. The role of maternal alcohol damage on ethanol teratogenicity in the rat. Teratology 1987;36(2):199-208.

(331) McGivern R.F. et al. 1984. Science 224, 896

(332) Meyer L.S. & Riley E.P. 1986. Teratology 34, 1

(333) Barron S. & Riley E.P. 1985. Alcohol. clin. exp. Res. 9, 360

(334) Goad, P.T., et al. The role of maternal diet in the developmental toxicology of ethanol. Toxicol Appl Pharmacol 1984;73:256-267.

(335) Checiu, M. and Sandor, S. The effect of ethanol upon early development in mice and rats. IX. Late effect of acute pre-implantation intoxication in mice. Morphol Embryol 1986;32:5-11.

(336) Mukherjee A.B. & Hodgen G.D. 1982. Science 218, 700.

(337) Rockwood G.A.& Riley E.P. 1986. Teratology 33, 145.

(338) Abel, E.L. Effect of ethanol on pregnant rats and their offspring. Psychopharmacology 1978;57:5-11.

(339) Abel, E.L., Greizerstein, H.B. Ethanol-induced prenatal growth deficiency: Changes in fetal body composition. J Pharmacol Exp Ther 1979;211:668-671.

(340) Weinberg, J. Effects of ethanol and maternal nutrition status of fetal development. Alcohol Clin Exp Res 1985;9:49-55.

(341) Rudeen P.K.et al. 1986. Drug Alcohol Depend. 18, 247.

(342) Fernandez K. et al. 1983. Teratogen. Carcinogen. Mutagen. 3, 457

(343) West J.R. et al. 1981a. Teratology 24, 13

(344) Barron S. et al. 1986. Alcohol. clin. exp.Res. 10, 493.

(345) Zimmerberg B. et al. 1986. Pharmacol. Biochem. Behav. 25, 1021

(346) Washington, W.J.; Cain, K.T., Cacheiro, N.L.A., Generoso, W.M. Ethanol-induced late fetal death in mice exposed aroundthe time of fertilization. Mutation Res 1985; 147:205-210.

(347) Randall, C.L., Burling, T.A., Lochry, E.A., Sutker, P.B. The effect of paternal alcohol consumption on foetal development in mice. Drug Alcohol Res 1982;9:89-95.

(348) Scott W.J. & Fradkin R. 1984. Terotology 29, 46

(349) McLain C.J. & Roe, 1984 (no further details available)

(350) Abel E.L. 1985 Teratology 31, 35

(351) Miller N.W. 1986. Science 233, 1308.

(352) Sharma A. & Rawat A.K. 1986. Alcohol 3, 101.

(353) Mankes, R.F., LeFevre, R., Banitz, K.-F., et al. Paternal effects of ethanol in the Long-Evans rat. J Toxicol Environ Health 1982; 10: 871-878.

(354) Fazakas-Todea, I., Checiu, Sandor, S. The effect of ethanol upon early development in mice and rats. VIII. The effect ofchronic consumption of some beverages upon preimplantation development in rats. Morphol Embryol 1985;31:249-256.

(355) Mankes, R.F., Hoffman, T., LeFevre, R.,, Bates, H., Abraham,R. Acute embryopathic effects of ethanol in the Long-Evans rat. J Toxicol Environ Health 1983; 11:583-590.

(356) Sorette M.P. et al. 1980. Neurobehav. Toxicol. 2, 181.

(357) Abel E.L. & Dintcheff B.A. 1978. J. Pharmacol. exp. Ther. 207, 916

(358) Viirre E. et al. 1986. Neurobehav. Toxicol. Teratol. 8, 615

(359) Vorhees C.V.& Fernandez K. 1986. Neurobehav. Toxicol. Teratol. 8, 23

(360) Hood R.D. et al. 1979. Toxicol. Lett. 4, 79

(361) Lochry E.A. et al. 1982. Neurobehav. Toxicol. Teratol. 4, 15.

(362) Bannigan J. & Burke P. 1982. Teratology 26, 247.

(363) Boggan W.O. et al. 1979. Res. Commun. Chem. Pathol. Pharmacol. 23, 127.

(364) Checiu M. & Sandor S. 1982. Morphol. Embryol. 28, 15.

(365) Chernoff G.F. 1980. Teratology 22, 71.

(366) Daft P.A. et al. 1986. Teratology 33, 93.

(367) Giknis M.L.A. et al. 1980. Neurobehav. Toxicol. 7, 235.

(368) Kronik J.B. 1976. Am. J. Obstet. Gynecol. 124, 676.

(369) Martinez F. et al. 1985. Am. J. Obstet. Gynecol. 151, 428.

(370) Padmanabhan R. & Muawad W.M.R.A. 1985. Drug Alcohol Depend. 16, 215.

(371) Padmanabhan R. et al. 1984. Drug Alcohol Depend. 14, 197.

(372) Randall C.L. et al. 1977. Alcohol clin. exp. Res. 1, 219.

(373) Rasmussen B.B. & Christensen N. 1980. Acta pathol. microbiol. scand., Sect. A. 88, 285.

(374) Stuckey E. & Berry C.L. 1984. J. Pathol. 142, 175.

(375) Sulik K.K. et al. 1981. Science 214, 936.

(376) Sulik K.K. & Johnston M.C. 1983. Am. J. Anat. 166, 257.

(377) Webster W.S. et al. 1980. Neurobehav. Toxicol. 2, 227.

(378) Webster W.S. et al. 1984. Cardiovasc. Res. 18, 335.

(379) Giknis M.L.A. & Damjanov I. 1983. Toxicol. Lett. 19, 37

(380) Lee M. 1985. Teratogen. Carcinogen. Mutagen. 5, 433

(381) Randall C.L. et al. 1986. Alcohol Drug. Res. 6, 351

(382) Yanai J. & Ginsburg B.E. 1976. Q. J. Stud. Alcohol 37, 1564

(383) Dexter, J.D., Tumbleson, M.E., Decker, J.D., Middleton, C.C. Fetal alcohol syndrome in Sinclair (S-1) miniature swine. Alcohol Clin Exp Res 1980;4:146-151.

(384) Scott, W.J. Jr., Fradkin, R. The effects of prenatal ethanol in Cynomolgus monkeys. Teratology 1984; 29:49-56.

(385) Mankes R.F. et al. 1982. J. Toxicol. envir. Hlth 10, 871

(386) Keppen L.D. et al. 1985. Pediat. Res. 19, 944.

(387) Barron S. & Riley E.P. 1985. Alcohol. clin. exp. Res. 9, 360.

(388) Miller S.I. et al. 1983. Pharmacol. Biochem. Behav. 18, 311.

(389) Blakley P.M.& Scott W.J. 1984b. Toxic. appl. Pharmac. 72, 355.

(390) Varma P.K. & Persaud T.V.N. 1979. Res. Commun. chem. Pathol. Pharmacol. 26, 65

(391) Webster W.S. et al. 1983. Teratology 27, 231

(392) Vaglenova, J., Petkov, V.V. (1998). Fetal alcohol effects in rats exposed pre- and postnatally to a low dose of ethanol. Alcohol: Clin and Exp Res 22(3):697-703

(393) Tabakoff, B., Grant, K.A., Hoffman, P.L., Little, H.J. (1998). Adenylyl cylases and alcohol. In Cooper DMF (ed) Advances in secondary messenger and phoshoprotein research, vol 32, Lippincott-Raven, Philadelphia, PA p173-93.

(394) Eckardt, MJ; File, SE; Gessa GL; Grant KA; Guerri, C; Hoffman, P; Kalant, H; Koob, GF; Li TK; Tabakoff, B (1998) Effects of moderate alcohol consumption on the central nervous system. Alcoholism: Clin Exper Res, 22, 5, 998-1041.

(395) Ghosh TK, Copeland JR, Alex PK, Pradhan SN (1991) Behavioral effects of ethanol inhalation in rats, Pharmacol Biochem Behav 38, 699-704

(396) Astley, S.J., Magnuson, S.I., Omnell, L.M., Clarren, S.K. Fetal Alcohol Syndrome: Changes in craniofacial form with age, cognition, and timing of ethanol exposure in the macaque. Teratology 1999;59:163-172

(397) Caul, W.F., Osborne, G.L., Fernandez, K., Henderson, G.J. Open Field and avoidance performance in rats as a function of prenatal ethanol treatment. Addictive behav. 1979;4:311-322.

(398) Maciejewski-Lenoir, D. (1993). Chronic prenatal exposure does not affect the expression of selected genes in rat brain development. Alcohol & Alcoholism 28(4):401-412

(399) Dunne, F.J. (1989) Alcohol and the immune system. BMJ 298, 543 - 544.

(400) Adams R.M. (1990). Occupational Skin Disease. 2nd Edition, W.B. Saunders Company, Philadelphia.

(401) Cronin E. (1980). Contact Dermatitis. Churchill

(402) Fisher, A.A. (1983). Topically Applied Alcohol as a Cause of Contact Dermatitis. Cutis. 31:588-600.

(403) Hanson et al. (1978). J. Pediat. 92, 457-460.

(404) Mills J.L. & Graubard B.I. (1987) Pediatrics 80, 309 -314.

(405) Roman E. et al. (1988). The relationship between Alcohol Consumption and Pregnancy Outcome in Humans. pp 205-235, Chapter 5. In: Issues and Reviews in Teratology, volume 4. Edited by Kalter H., Plenum Publishing, New York.

(406) Rossett H.Ll (1980). Alcoholism 4, 119-122.

(407) Shepard T.H. (1989) Catalog of teratogenic agents. 6th edition, pp. 19 - 29. The Johns Hopkins University Press, Baltimore and London.

(408) Bowers R.V. et al. (1942). Q. J Alc Stud 3, 31-33.

(409) Pohorecky L.A. & Brick J. (1987). Pharmac Ther 36, 335-427.

(410) Lester D., Greenberg L.A. (1951). The Inhalation of Ethyl Alcohol by Man. Q. J Stud Alcohol 12:167-178.

(411) Ellenhorn M.J. & Barceloux D.G. (1988). Medical Toxicology. Elsevier, New York.

(412) Gosselin R.E. et al. (1984). Clinical Toxicology of Commercial Products. 5th Edition, Williams & Wilkins Baltimore.

(413) Rowe V.K. & McCollister S.B. (1982). "Alcohols" in Patty's Industrial Hygiene and Toxicology. 3rd Edition, John Wiley, New York

(414) Ellenhorn, M.J. & Barceloux, D.G.. Medical toxicology - Diagnosis and treatment of human poisoning. Elsevier, New York 1988. p. 782 - 797.

(415) Ruth U., Pendlington,R. U., Whittle, E., Robinson, J. A., Howes, D. (2001) The fate of ethanol topically applied to the skin. Unilever internal study. Food Chem Toxicol, 39, 169-74

(416) Conibear, S.A. (1988) The absorption, metabolism, excretion,and health effects of industrially useful alcohols. Dangerous properties of industrial materials report 8, 2 - 9.

(417) Bagnardi, V., Blangiardo, M., La vecchia, C., Corrao, G. (2001). A meta-analysis of alcohol drinking and cancer risk. Br J Cancer 85(11).

(418) Conibear, S.A. (1988) The absorption, metabolism, excretion and health effects of industrially useful alcohols. Dangerous properties of industrial materials report 8, 2 - 9.

(419) Ellenhorn, M.J. & Barceloux, D.G.. Medical toxicology - Diagnosis and treatment of human poisoning. Elsevier, New York 1988. p. 782 - 797.

(420) Conibear, S.A. (1988) The absorption, metabolism, excretion and health effects of industrially useful alcohols. Dangerous properties of industrial materials report 8, 2 - 9.

(421) Cronin E. (1980). Contact Dermatitis. Churchill Livingstone, Edinburgh.

(422) Fregert, S., Hakanson, R., REorsman, H, Tryding, N. and Ovrum, P. (1963). Dermatitis from alcohols. J Allergy 34(5): 404-408.

(423) W-J van Muiswinkel, H. Kromhout, T. Onos, Kersemaekers, W. (1997). Monitoring and modelling of exposure to ethanol in hairdressing salons. Ann. occup. Hyg. 41(2):235-247.

(424) Coates Inks Data May 2000.

(425) BP Chemicals Ltd (BXL Plastics Ltd) Occupational Hygiene Survey, 1992.

(426) Hollund, B.E. and Moen, B.E. (1998). Chemical exposure in hairdresser salons: Effect of local exhaust ventilation. Ann. occup. Hyg. 42(4):277-281.

(427) Moen, B.E. and Hollund, B.E. (2000). Exposure to organic solvents among car painters in Bergen, Norway. Ann. occup. Hyg. 44(3):185-189.

(428) Alexandersson, R. and Hedenstierna, G. (1988). Respiratory hazards associated with exposure to formaldehyde and solvents in acid-curing paints. Arch Environ Health 43(3);222-227.

(429) Regional Authority of Public health in Banská Bystrica, Slovakia (2004)

(430) Conolly, R. B. (1999) PBPK Modeling of Ethanol Pharmacokinetics in Human Males. BP-AMOCO internal report. Gentry, R (2003) PBPK Modeling of Ethanol Pharmacokinetics in Human Males (in publication).

(431) Ethanol - A comparative risk assessment for reprotoxicity BP-Amoco internal report. (2001)

(432) Pronko, P., Bardina, L., Satanovskaya, V., Kuzmich, A., Zimatkin, S. (2002). Effect of chronic alcohol consumption on the ethanol- and acetaldehyde-metabolizing systems in the rat gastrointestinal tract. Alcohol Alcohol 2002;37(3):229-35.

(433) Shibley, I.A., McIntyre, T.A., Pennington, S.N. Experimentalmodels used to measure direct and indirect ethanol teratogenicity. Alcohol Alcohol 1999;34(2):125-140.

(434) Ashcroft, J-A, Ockendon, M., Hotchkiss, S.A.M. Percutaneous absorption of ethanol and detection of the ethanol-metabolizing cytochrome P450 enzyme CYP 2E1 in human skin. (Abstract).

(435) Ukita, K., Fukui, Y., Shiota, K. Effects of prenatal alcoholexposure in mice: influence of an ADH inhibitor and a chronic inhalation study. Reprod Toxicol 1993;7(3):273-281.

(436) Church, A.S., Witting, M.D. Laboratory testing in ethanol, methanol, ethylene glycol, and isopropanol toxicities. J Emerg Med 1997 Sep-Oct;15(5):687-92.

(437) Anderson RA Jr, Willis BR. and Oswald C, Spontaneous recovery from ethanol-induced male infertility. Alcohol 1985, 2: 479-484.