JACC REPORT

No.34

LIST OF ECETOC PUBLICATIONS (continued inside back cover)

MONOGRAPHS

No.	Title
No. 1	Good Laboratory Practice. Oct 79
No. 2	Contribution to Strategy for Identification and Control of Occupational Carcinogens. Sep 80
No. 2	Definition of a Mutagen, for 6th Amendment. Sep 80
No. 3	Risk Assessment of Occupational Chemical Carcinogens. Jan 82
No. 4	Hepatocarcinogenesis in Laboratory Rodents : Relevance for Man. Oct 82
No. 5	Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology). Dec 83
No. 6	Acute Toxicity Tests, LD_{50}(LC_{50}) Determinations and Alternatives. May 85
No. 7	Recommendations for the Harmonisation of International Guidelines for Toxicity Studies. Dec 85
No. 8	Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment. Feb 86
No. 9	Assessment of Mutagenicity of Industrial and Plant Protection Chemicals. Jun 87
No. 10	Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man. Aug 87
No. 11	Eye Irritation Testing. Jun 88
No. 12	Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity/teratogenicity). Nov 89
No. 13	DNA and Protein Adducts: Evaluation of their Use in exposure Monitoring and Risk Assessment. Oct 89
No. 14	Skin Sensitisation Testing. Mar 90
No. 15	Skin Irritation. Jul 90
No. 16	Mutation Research, Special Issue: Early Indicators of Non-Genotoxic Carcinogenesis. Jun 91
No. 17	Hepatic Peroxisome Proliferation. May 92
No. 18	Evaluation of the Neurotoxic Potential of Chemicals. Sep 92
No. 19	Respiratory Allergy. Aug 93
No. 20	Percutaneous Absorption. Aug 93
No. 21	Immunotoxicity: Hazard Identification and Risk Characterisation. Sep 94
No. 22	Evaluation of Chemicals for Oculotoxicity. Nov 94

JACC REPORTS

No.	Title
No. 1	Joint Assessment of Commodity Chemicals, Melamine. Feb 83
No. 2	Joint Assessment of Commodity Chemicals, 1,4-Dioxane. Feb 83
No. 3	Joint Assessment of Commodity Chemicals, Methyl Ethyl Ketone. Feb 83
No. 4	Joint Assessment of Commodity Chemicals, Methylene Chloride. Jan 84
No. 5	Joint Assessment of Commodity Chemicals, Vinylidene Chloride. Aug 85
No. 6	Joint Assessment of Commodity Chemicals, Xylenes. Jun 86
No. 7	Joint Assessment of Commodity Chemicals, Ethylbenzene. Aug 86
No. 8	Joint Assessment of Commodity Chemicals, Methyl Isobutyl Ketone. May 87
No. 9	Joint Assessment of Commodity Chemicals, Chlorodifluoromethane. Oct 89
No. 10	Joint Assessment of Commodity Chemicals, Isophorone. Sep 89
No. 11	Joint Assessment of Commodity Chemicals, (HFA-132b) 1,2-Dichloro-1,1-Difluoroethane. May 90
No. 12	Joint Assessment of Commodity Chemicals, (HFA-124) 1-Chloro-1,2,2,2-Tetrafluoroethane. May 90
No. 13	Joint Assessment of Commodity Chemicals, (HFA-123) 1,1-Dichloro-2,2,2-Trifluoroethane. May 90
No. 14	Joint Assessment of Commodity Chemicals, (HFA-133a) 1-Chloro-2,2,2-Trifluoromethane. Aug 90
No. 15	Joint Assessment of Commodity Chemicals, (HFA-141B) 1-Fluoro 1.1-Dichloroethane. Aug 90
No. 16	Joint Assessment of Commodity Chemicals, (HCFC-21) Dichlorofluoromethane. Aug 90
No. 17	Joint Assessment of Commodity Chemicals, (HFA-142b) 1-Chloro-1,1,Difluoroethane. Feb 91
No. 18	Joint Assessment of Commodity Chemicals, Vinylacetate. Feb 91
No. 19	Joint Assessment of Commodity Chemicals, Dicyclopentadiene. Jul 91
No. 20	Joint Assessment of Commodity Chemicals, Tris-/Bis-/Mono-(2-ethylhexyl)phosphate. May 92
No. 21	Joint Assessment of Commodity Chemicals, Tris-(2-butoxyethyl)-phosphate. Mar 92
No. 22	Joint Assessment of Commodity Chemicals, Hydrogen Peroxide. Jan 93
No. 23	Joint Assessment of Commodity Chemicals, Polycarboxylate Polymers as Used in Detergents. Nov 93
No. 24	Joint Assessment of Commodity Chemicals, (HFC-125) Pentafluoroethane. May 94
No. 25	Joint Assessment of Commodity Chemicals, (HCFC-124) 1-Chloro-1,2,2,2-Tetrafluoroethane. Jul 94
No. 26	Joint Assessment of Commodity Chemicals, Linear Polydimethylsiloxanes (viscosity 10-100,000 centisokes). Sep 94
No. 27	Joint Assessment of Commodity Chemicals, *n*-Butyl Acrylate. CAS No. 141-32-2. Aug 94
No. 28	Joint Assessment of Commodity Chemicals, Ethyl Acrylate. CAS No. 140-88-5. Sep 94
No. 29	Joint Assessment of Commodity Chemicals, 1,1-Dichloro-1-Fluoroethane (HCFC-141b) CAS No 1717-00-6. Feb 95
No. 30	Joint Assessment of Commodity Chemicals, Methyl Methacrylate. CAS No. 80-62-6. Feb 95
No. 31	Joint Assessment of Commodity Chemicals, 1,1,1 ,2-Tetrafluoroethane (HFC-134a) CAS No. 811-97-2. Feb 95
No. 32	Joint Assessment of Commodity Chemicals, Difluoromethane (HFC-32) CAS No. 75-10-5. May 95
No. 33	Joint Assessment of Commodity Chemicals, 1,1-Dichloro-2,2,2-trifluoroethane (HCFC-123) CAS No. 306-83-2. In press

Joint Assessment of Commodity Chemicals No. 34

Acrylic Acid

CAS No. 79-10-7

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THE ECETOC SCHEME FOR THE JOINT ASSESSMENT OF COMMODITY CHEMICALS

This report has been produced as part of the ECETOC programme for preparing critical reviews of the toxicology and ecotoxicology of selected existing industrial chemicals.

In the programme, commodity chemicals, that is those produced in large tonnage by several companies and having widespread and multiple uses, are jointly reviewed by experts from a number of companies with knowledge of the chemical. It should be noted that in a JACC review only the chemical itself is considered; products in which it appears as an impurity are not normally taken into account.

ECETOC is not alone in producing such reviews. There are a number of organisations that have produced and are continuing to write reviews with the aim of ensuring that toxicological knowledge and other information are evaluated. Thus a Producer, Government Official or Consumer can be informed on the up-to-date position with regard to safety, information and standards. Within ECETOC we do not aim to duplicate the activities of others. When it is considered that a review is needed every effort is made to discover whether an adequate review exists already; if this is the case the review is checked, its conclusions summarised and the literature published subsequent to the review assessed. To assist ourselves and others working in this field we publish annually a summary of international activities incorporating work planned, in hand, or completed on the review of safety data for commodity chemicals. Interested readers should refer to our Technical Report No. 30 entitled "Existing Chemicals: Literature Reviews and Evaluations".

This document presents a critical assessment of the toxicology and ecotoxicology of Acrylic Acid (CAS No. 79-10-7).

Acrylic Acid CAS No. 79-10-7

CONTENTS

1. SUMMARY AND CONCLUSIONS

Acrylic acid (AA) is a clear, colourless, corrosive liquid used in the production of acrylic esters and in the production of polymers and co-polymers for use in paints, coatings, plastics, etc. It is also used as a chemical intermediate. AA can spontaneously polymerise with the evolution of heat. A polymerisation inhibitor, generally the methyl ether of hydroquinone, is therefore added.

The majority of AA released to the environment is expected to enter the aquatic compartment. In water, no hydrolysis will occur but AA will biodegrade rapidly under both aerobic and anaerobic conditions. It is not expected to bioaccumulate. In soils, AA will biodegrade with a half-life of less than one day. In the atmosphere it will photodegrade with a half-life of less than one day.

Protozoa and algae appear to be the more sensitive of the aquatic species examined. AA is moderately toxic to bacteria, fish and crustacea. AA completely inhibited the respiration of soil microflora at 1,000 mg/kg.

Absorption of AA can occur through the skin, lungs and gastrointestinal tract; absorption by the later 2 routes is rapid. Once absorbed, it is quickly and effectively metabolised to CO_2 via normal oxidative catabolic pathways. Although 3-hydroxypropionic acid has been repeatedly identified as a metabolite, the major urinary metabolite(s) is (are) poorly characterised, highly polar material. Only traces of unchanged AA are found in the urine. Metabolism is detoxifying and there is no evidence to suggest that the vinyl moiety undergoes epoxidation.

AA has a low acute toxicity by the oral (rat LD_{50} 1,250-3,200 mg/kgbw), dermal (rabbit LD_{50} 295-750 mg/kgbw) and inhalation (rat 4-h LC_{50} 1,200-6,000 ppm) routes. The main signs of toxicity are irritation and/or corrosion at the site of contact.

AA is irritating and/or corrosive to the skin, eyes, respiratory tract and gastrointestinal tract of laboratory animals. Damage to the eye may be irreversible. Following repeated inhalation exposure, sensory irritation and degeneration of the nasal mucosa were observed. Neat AA or any distilled AA is not a skin sensitiser.

Repeated exposure of rats and mice to AA by gavage causes irritation and ulceration of the stomach; these effects are dose dependent. Repeated administration of AA in drinking water does not cause any systemic adverse effects except for a decrease in body weight gain and changes in the clinical chemistry, both due to decreased water and food consumption. Repeated inhalation exposure

produced sensory irritation and degeneration of the olfactory epithelium which is replaced by respiratory epithelium (respiratory metaplasia), with a minor hyperplasia of the submucosal glands. Depression of respiration has also been reported in both rats and mice (RD_{50} 513 ppm and 685 ppm respectively). Repeated dermal exposure resulted in degenerative and inflammatory changes both of the dermis and epidermis. No systemic effects were seen after exposure by any route.

Results of *in vitro* genotoxicity studies indicate that AA is not mutagenic in the *Salmonella* assay (Ames test) with or without metabolic activation. It does not produce point mutations in the HPGRT-assay in CHO cells, unscheduled DNA synthesis in rat hepatocytes or Syrian hamster embryo (SHE) cells nor does it induce micronuclei in SHE cells. Positive results were obtained for chromosomal aberrations in mouse lymphoma cells (L5178Y) and CHO cells, but not in Chinese hamster lung fibroblasts (4CHL) cells. Equivocal evidence of *in vitro* binding to DNA has also been reported; however, aspects of the study design mean that the study cannot be fully evaluated. *In vivo* genotoxicity studies, show that AA does not produce adducts in DNA of the liver and stomach. A study of DNA adduct formation in the skin produced equivocal results that cannot be fully interpreted. No genotoxic effects were observed in a *Drosophila melanogaster* recessive lethal mutation study or in a mouse dominant lethal study. AA did not cause chromosome aberrations in a rat bone marrow study. Thus all *in vivo* and the majority of *in vitro* genotoxicity studies were negative and AA is considered non-genotoxic.

Systemic effects were not seen in a 12-month chronic toxicity in rats administered AA in drinking water at a concentration of 5,000 mg/l (equivalent dose 331 mg/kgbw/d). Following life-time exposure of rats via drinking water containing up to 1,200 mg AA/l (equivalent dose 78 mg/kgbw/d) no evidence of carcinogenicity was produced. In one, poorly conducted and reported dermal study in mice exposed to AA for their life-time a weak carcinogenic response was attributed to AA exposure. However, in other well performed chronic dermal studies, no such carcinogenic effects were observed, either on the skin or systemically. No chronic studies are available addressing the inhalation route of exposure, however by analogy with the AA esters (which de-esterify in the nasal epithelium) AA is not expected to be carcinogenic by this route of exposure.

In multigeneration reproduction toxicity studies where rats received AA in their drinking water dose dependent signs of general toxicity were observed resulting in reduced food and water intake and lower mean body weight gain in the F_0 generation at 5,000 mg/l (460 mg/kgbw/d) and in the F_1 parental generation at 5,000 and 2,500 mg/l. Retarded growth was exhibited in the F_1 and F_2 pups of the parental groups at 5,000 mg/l and less pronounced at 2,500 mg/l. The no-observed adverse effect level (NOAEL) for reproductive function is 5,000 mg/l (460 mg/kgbw/d) in 2 successive generations. The NOAEL concerning general toxicity is 2,500 mg/l (240 mg/kgbw/d) for the F_0 generation and 500 mg/l (53 mg/kgbw/d) for the F_1 parental generation and F_1 and F_2 offspring.

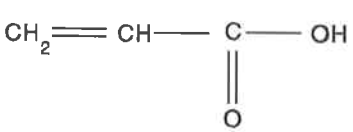
Inhalation exposure to AA does not result in embryotoxicity or teratogenicity even at maternally toxic dose levels. No major effects were observed in a 1-generation reproduction study even at doses toxic for the parents.

Overall, the weight of experimental evidence suggests that AA does not possess genotoxic or carcinogenic potential, or cause reproductive or developmental effects.

No deaths or serious health effects have been reported in humans exposed to acute doses of AA. The main potential for human exposure to AA is by the dermal and inhalation routes. The pungent, characteristic odour is acting as a warning and the irritating properties act as a deterrent to prolonged or repeated exposure. Undiluted AA is corrosive to the skin, eyes and mucous membranes. Industrial exposure to atmospheres containing AA may produce irritation to the eyes, upper respiratory tract and gastrointestinal tract. Pure AA is not a skin sensitiser. Current occupational exposure limit values protect against potential adverse health effects.

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, ANALYTICAL METHODS

2.1 IDENTITY

Name:	Acrylic acid
IUPAC name:	2-Propenoic acid
Synonyms:	Acroleic acid Ethylenecarboxylic acid 2-Propenoic acid Vinylformic acid
D:	Acrylsäure
DK:	Acrylsyre
F:	Acide acrylique
EL:	Ακρυλικό όξύ
I:	Acido acrilico
N:	Akrylsyre
NL:	Acrylzuur
ES:	Acido acrilico
S:	Akrylsyra
SF:	Akryylihappo
CAS name:	2-Propenoic acid
CAS registry No:	79-10-7
EEC No:	607-061-00-8
EINECS No:	201-177-9
Formula:	$C_3H_4O_2$
Molecular mass:	72.06
Structural formula:	

2.2 PHYSICAL AND CHEMICAL PROPERTIES

Acrylic acid (AA) is a clear, colourless, flammable liquid with a pungent odour. It is soluble in water and completely miscible with most organic solvents at any ratio. Data on the physical and chemical properties of AA are given in Table 1.

Table 1: Physical and Chemical Properties

Parameter, units	Value	Reference
Melting temperature, °C	13	Weast *et al*, 1989
Boiling temperature, °C at 1,013 hPa	141.6	Weast *et al*, 1989
Heat of polymerisation, kJ/kg	1,074 1,075 1,079	Elf Atochem, 1992 McCurdy and Laidler, 1964 BASF, 1992
Relative density D_4^{20} (density of water at 4°C is 1,000 kg/m^3)	1.0511	Weast *et al*, 1989
Viscosity, mPa -s at 20°C	1.22 1.3	Elf Atochem, 1992 BASF, 1992
Refractive index n_D at 20°C	1.4224	Weast *et al*, 1985
Vapour pressure, hPa at 20°C	3.8	Thermodynamic Research Center, 1975
Vapour density at 20°C (air=1)	No data	
Threshold odour concentration, ppm	0.094	Amoore and Hautala, 1983
Surface tension, mN/m at 20°C	No data	
Solubility in water, g/kg at 25°C Solubility of water in AA, g/kg at 25°C Miscibility with most organic solvents	Infinite Infinite Infinite	BASF, 1992; Elf Atochem, 1992 BASF, 1992; Elf Atochem, 1992 Weast *et al*, 1989
Fat solubility, mg/100 g at 37°C	No data	
Partition coefficient, log P_{ow} (octanol/water) at 20-25°C at 25°C	0.161[a] 0.38 0.46	GEMS, 1986 as quoted in HSDB, 1995 Korenman and Lunicheva, 1972 BASF, 1988
Partition coefficient, log K_{oc} (soil-sediment/water) at 20°C	0.21-0.63	Archer and Horvath, 1991 (measured)
Henry's Law constant, Pa -m^3/mol at 20°C	1.19×10^{-2} 1.46×10^{-2} 3.24×10^{-2}	Cascieri and Clary, 1993 (calculated) BASF, 1993a (calculated) Singh *et al*, 1984
Flash point, °C, closed cup	46 48.5	Elf Atochem, 1992 BASF, 1994
Auto-flammability, ignition temperature, °C	390 429	BASF, 1992 Elf Atochem, 1992
Explosion limits, % at 47.5-88.5°C	2.4-16	BASF, 1992

a Temperature not specified.

A typical commercial sample of AA has a purity of ≥ 99.0% (w/w) and may contain the following specified impurities: water (< 0.3% w/w) and AA dimer (≤ 0.1%) (BASF, 1992; Elf Atochem, 1992). Glacial AA or any distilled technical AA does not contain water or AA dimer.

AA polymerises readily under the influence of heat, light or by catalysis (e.g. metals), in a strongly exothermic reaction. To prevent polymer formation, the monomer is stabilised by the addition of an inhibitor such as the monomethyl ether of hydroquinone (MeHQ, synonym *p*-methoxy phenol) at levels of 200±20 ppm.

2.3 CONVERSION FACTORS

Conversion factors for AA concentrations in air, calculated at 20°C and 1,013 hPa are:

- 1 ppm = 2.996 mg/m^3
- 1 mg/m^3 = 0.334 ppm

2.4 ANALYTICAL METHODS

2.4.1 Environmental Media

Water

AA levels in water can be determined directly by gas chromatography (GC) using a flame ionisation detector (FID). The detection limit is 1 µg AA/l (Singh and Thomas, 1985).

High-performance liquid chromatography (HPLC) is another technique for separation and quantification of AA in water. The general detection limit is 0.05 µg/l using an UV detector (Brown, 1979).

The amount of AA in water can also be determined by titration (Roth, 1953).

Air

The presence of AA in air can be determined by GC after absorption in water. The detection limit is 0.005 mg/m^3 (0.0017 ppm) (Dmitriev and Komrakova, 1986).

GC can also be used for detecting AA in air by direct sampling. This method makes it possible to detect AA in a small volume (> 5 ml) of workplace air. Using a flame ionisation detector (FID), the detection limit was 2.5 mg/m^3 (0.84 ppm) (Podkorvyrina *et al*, 1981).

Soil and Sediments

No methods have been reported.

2.4.2 Biological Media

AA was determined in blood samples after centrifugation by directly injecting the serum into a GC/FID. A packed column with a fluorocarbon surfactant was used as stationary phase and helium as the carrier gas. The detection limit for AA was approximately 5 µg/ml (Miller *et al*, 1981b).

AA and its urinary metabolites were determined in acidified rat urine using a reversed phase HPLC method with a gradient mixture of 0.01 M triethylamine phosphate (pH 2), water and methanol (De Bethizy *et al*, 1987).

Small amounts of AA were detected in the urine of male Fisher rats after oral (gavage) and i.v. administration of butyl acrylate using an reversed phase HPLC with a dibutylamine phosphate buffer (pH 3.0, 0.01 M) and methanol gradient elution (Sanders *et al*, 1988).

AA was determined in tissue homogenates using HPLC analysis on an carboxylic acid column with 0.1% H_3PO_4 as the mobile phase (Finch and Frederick, 1992).

AA formation from ethyl acrylate was determined in homogenates of different rat tissues using a HPLC method (Frederick *et al*, 1994).

3. PRODUCTION, STORAGE, TRANSPORT AND USE

3.1 PRODUCTION

AA is produced commercially by oxidation of propylene or by a modification of the Reppe process from acetylene. AA can be prepared by the hydrolysis of acrylonitrile, but this method is not commercially viable.

The worldwide production of AA was approximately 1.13 Mt in 1991 (Anonymous, 1992).

3.2 STORAGE

To prevent polymer formation, the AA monomer is stabilised by the addition of an inhibitor such as MeHQ (Section 2.2). The effectiveness of phenolic inhibitors depends on the presence of oxygen. To prevent polymer formation, the monomer must therefore be stored under air (not under inert gases), in the dark at a temperature below 25°C. During long term storage, stabiliser levels should be checked routinely.

Freezing should be avoided as it results in separation of AA from the stabiliser. AA dimerises slowly during storage. This reaction is promoted by elevated storage temperatures and the presence of water, and it cannot be prevented by the addition of chemical stabilisers.

AA is normally stored and shipped in containers made of stainless steel. Containers of mild steel are unsuitable.

3.3 USE

The primary use of AA is as an intermediate in the production of AA esters. In addition, AA is used in the manufacture of water-soluble resins and salts, unsaturated fatty acids and heterocycles, and as a monomer or co-monomer in acrylic polymers. AA forms copolymers with, for example, acrylonitrile, maleic acid esters, vinyl acetate, vinyl chloride, vinylidene chloride, styrene, 1,3-butadiene and ethylene. These polymers are employed in their acid or basic forms as thickening agents, dispersants, flocculants, protective colloids for stabilising emulsions and polymer dispersions, wetting agents, coating and finishing agents, and hygroscopic polymers.

4. ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION

4.1 EMISSIONS

4.1.1 Emissions During Production

AA is normally manufactured in a closed process. AA vapours from vented equipment and tanks are destroyed by flaring, as are vapours resulting from processing. Quantitive information on emissions is not available.

4.1.2 Emissions During Use

No data are available.

4.1.3 Natural Sources

AA has been reported to occur naturally in several species of algae: 9 species of *Chlorophyceae*, 10 of *Rhodophyceae* and 11 of *Phaeophyceae* (IARC, 1979; Grossel and Delesmont, 1986). AA has been found in the housing water of oysters (Brown *et al*, 1977), in the mantela of scallops (Kodama and Ogata, 1983), in crustaceans and in the digestive tract of penguins (Sieburth, 1960), due to the presence of marine algae, in particular *Protogonyaulax* sp. (Kodama and Ogata, 1983) or *Phaedactylum tricornutum* (Brown *et al*, 1977).

AA has been found in the rumen fluid of sheep due to bacterial fermentation of carbohydrates (Noble and Czerkawski, 1973; IARC, 1979).

4.2 ENVIRONMENTAL DISTRIBUTION

AA is soluble in water and has a low Henry's Law constant and a low vapour pressure (Table 1). These would suggest that AA will be poorly adsorbed on soil or sediment (Lyman, 1990) and will have a low vaporation from the aqueous phase and dry surfaces (Thomas, 1990).

A theoretical distribution of AA has been calculated using the fugacity model of Mackay and Paterson (1981). Approximately 97% of AA would be associated to the water phase, 1.6% in air, 1% in sediment and < 1% in soils, suspended solids and biota (Staples, 1993).

4.3 ENVIRONMENTAL FATE AND BIOTRANSFORMATION

4.3.1 Atmospheric Fate

In the atmosphere, AA will undergo rapid abiotic degradation in a reaction with photochemically produced hydroxyl radicals and ozone. The overall atmospheric half-life of AA is estimated to be 6.6 hours (Howard, 1989) to 6.5 days (Atkinson and Carter, 1984). Howard *et al* (1991) estimated a half-life of AA ranging from 2.5 to 23.8 hours, based upon an estimated rate constant for vapour-phase reactions with hydroxyl radicals and ozone in air (Atkinson, 1987).

4.3.2 Aquatic Fate

If released into water, AA will readily biodegrade (CITI, 1992; Douglas and Bell, 1992). Adsorption to sediments or volatilisation will not be significant.

AA was found to be stable to hydrolysis at pH 3, 7 and 11 (Shah, 1990).

Sewage Treatment

In a short-term respiration test of AA with municipal sewage sludge in accordance with current guidelines (ISO, 1986; EEC, 1988; OECD, 1993), the EC_{20} was 900 mg/l. The authors concluded that when AA is appropriately discharged into a sewage treatment plant, no disturbance/interference of the degradation behaviour of the activated sludge is to be expected (BASF, 1993b).

4.3.3 Terrestrial Fate

The adsorption and desorption of AA were examined on five different soils. The adsorption K_{oc} values ranging from 23 to 60 indicate a high mobility of AA through soil (Archer and Horvath, 1991).

The biodegradation of ^{14}C-AA in soil was studied under aerobic conditions after application at a rate of 100 mg/kg. AA was rapidly degraded: within 3 days 72.9% of the applied ^{14}C-AA appeared to have been converted to $^{14}CO_2$; the amount increased to 81.1% over the 28 day study period. The half-life of AA under these conditions was estimated to be less than 1 day (Hawkins *et al*, 1992). When acrylamide was added to soil, the AA formed by hydrolysis was totally degraded within 15 days of its formation (Nishikawa *et al*, 1979).

4.3.4 Biodegradation

Aerobic

The BOD_5 value for glacial AA using acclimated, fresh dilution water, with raw sewage from a local treatment plant as the seeding material, was determined to be 0.315 g O_2/g AA. The COD under the same conditions was 1.48 g O_2/g AA (Flaherty, 1989).

AA has been reported to be 'significantly' degraded (> 30%) in the MITI test and has been classified as 'biodegradable' (Sasaki, 1978).

Biodegradation of AA was up to 68% within 14 days, based on the BOD (biological oxygen demand) test (CITI, 1992). In a closed-bottle test based on the consumption of oxygen, a biodegradation of 81% was achieved within 28 days (Douglas and Bell, 1992). The results of this study showed that AA could be considered as readily biodegraded according to EC criteria.

In a 42-day screening study using a sewage seed inoculum, 71% of AA was mineralised after 19 days (Pahren and Bloodgood, 1961). After acclimatation, 81% was degraded to CO_2 in 22 days (Lin Chou *et al*, 1978).

The rate of biodegradation of AA was found to be >70% in both a standard and modified Zahn-Wellens test (BASF, 1988). AA was completely degraded in a standard Zahn-Wellens test and the authors concluded that AA is biodegradable (BASF, 1993c).

AA was degraded by a strain of *Alcaligenes denitrificans* isolated from a landfill soil. The bacterium degraded AA through the intermediate formation of L-(+)-lactic and acetic acids which are further metabolised (Andreoni *et al*, 1990). *Rhodococcus* bacteria isolated from the effluent of an acrylamide plant, metabolised acrylamide to AA which in turn was converted to ammonia and CO_2 (Arai *et al*, 1981).

Anaerobic

AA is amenable to anaerobic treatment (Speece, 1983) and in an anaerobic screening study utilising 10% sludge from a secondary digester as an inoculum, AA (300 mg/l) was judged to be degradable with >75% of theoretical methane being produced in 8 weeks of incubation (Shelton and Tiedje, 1984).

In another study, AA (3 mM, 0.22 mg/l) was toxic to unacclimated anaerobic acetate enriched cultures (Lin Chou *et al*, 1978).

4.3.5 Bioaccumulation

From the *n*-octanol/water partition coefficient (log P_{ow} = 0.16 to 0.46, Table 1), no bioaccumulation potential is predicted.

Using a regression equation (Bysshe, 1990), a theoretical bioconcentration factor ranging from 0.78 to 1.3 can be estimated. The equation of Veith *et al* (1979) predicts a factor of 1.6 to 2.4.

4.4 EVALUATION

Based on the fugacity model of Mackay and Paterson (1981), the majority of AA released into the environment is expected to enter the aquatic environment. In water, no hydrolysis will occur but AA is readily biodegradable under aerobic conditions and is likely to be degraded by anaerobic treatment. AA is not expected to bioaccumulate. In soils, AA biodegrades rapidly with an estimated half-life of less than one day. In the atmosphere, AA is expected to photodegrade with an estimated half-life in the range 2.5 to 23.8 hours.

5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

5.1 ENVIRONMENTAL LEVELS

5.1.1 Air

No data are available.

5.1.2 Water

AA occurs in waste-water effluents from its production by the oxidation of propylene, at concentrations of less than 0.5 mg/l (Wise and Fahrenthold, 1981).

5.1.3 Soil

No data are available.

5.1.4 Biological Media

Foodstuffs

Fermentation of sugar syrup by *Schizosaccharomyces* sp. has been shown to produce AA (0-3 mg/l) (Fahrasmane *et al*, 1985).

Other Biological Media

The AA content of *Phaeocystis* sp. can be 7.4% (10 mg/g) of dry weight (Sieburth, 1960). Other marine algae contained AA: *Chlorophyceae* (green algae) 0.124-16.5 mg/g dry weight, *Rhodophyceae* (red algae) 0-0.131 mg/g dry weight and *Phaeophyceae* (brown algae) 0-0.02 mg/g dry weight (Glombitza, 1970, 1979).

5.2 OCCUPATIONAL EXPOSURE LEVELS AND HYGIENE STANDARDS

5.2.1 Occupational Exposure (Table 2)

Table 2: Levels at the Workplace at 4 Production Facilities in the USA

(after Cascieri and Clary, 1993)

	No. of samples	TWA[a] (ppm)		STEL[b] (ppm)	Range (ppm)
		Area	Person	Person	
Operations	92	0.02	-	-	<0.01-13.00
(manufacture)	12	-	0.09	-	-
Operations (user)	25	-	0.16	-	0.01-1.00
	7	-	-	2.80	<0.10-63.00
Quality assurance	14	0.06	-	-	<0.01-0.44
	6	-	0.09	-	
	12	-	-	0.10	<0.1 -0.20
Loading/unloading	26	-	0.13	-	0.09-0.66
	2	-	-	1.30	0.40-4.00
Laboratory	1	-	<0.10	-	-
Maintenance	11	-	0.13	-	0.09-0.21

a Full shift > 366 min
b < 15 min

5.2.2 Hygiene Standards

Most industrialised countries have adopted occupational exposure limit values (Table 3).

Table 3: Occupational Exposure Limit Values

Country	TWA (mg/m^3)[a]	STEL (mg/m^3)[a]	TWA (ppm)	STEL (ppm)	Reference
Australia	30	-	10	-	ILO, 1991
Belgium	5.9	-	2	-	ACGIH, 1992
Denmark	30	-	10	-	ILO, 1991
France	5.9	-	2	10	Ministère du Travail, 1995
Germany	-	-	-	-	DFG, 1992
Italy	5.9	-	2	-	ACGIH, 1992
Netherlands	5.9	-	2	-	Arbeidsinspectie, 1993
Norway	30	-	10	-	Arbeidstilsynet, 1994
Sweden	30	45	10	15	AFS, 1993
Switzerland	30	-	10	-	ILO, 1991
UK	30	60[b]	10	20[b]	HSE, 1992
USA	5.9	-	2	-	ACGIH, 1992
	30	-	-	-	OSHA/NIOSH, 1986 as quoted in ILO, 1991

TWA Time-weighted average concentration (8-h working period)
STEL Short-term exposure limit (15 min, unless specified)
a Official values; some countries use different conversion factors and/or other ambient temperature
b 10 min

6. EFFECTS ON ORGANISMS IN THE ENVIRONMENT

6.1 MICRO-ORGANISMS

The influence of AA on the growth of *Pseudomonas putida* has been investigated. The threshold level for inhibition of bacterial growth (EC_3) was 41 mg/l after exposure for 16 h (Bringmann and Kühn, 1980c).

AA had a marked bactericidal activity towards *Escherichia coli* (Brown *et al*, 1977).

Escherichia coli cells in liquid suspension were killed when exposed to 1 mg AA/ml for 17 h, but glass-bead attached organisms remained unharmed from the exposure (Hicks and Rowbury, 1987).

AA is suspected of reducing or eliminating bacterial populations in penguins who ingest AA through phytoplankton in their diet (Herwig, 1978).

6.2 AQUATIC ORGANISMS (Table 4, 5, 6)

Within single cells organisms, the saprozoic flagellate *Chilomonas paramecium* proves to be the most sensitive organism, displaying a threshold level of 0.9 mg/l. Both the holozoic flagellate *Entosiphon sulcatum* and the ciliate *Uronema parduczi* are less sensitive (Table 4).

Table 4: Effect concentrations for acute tests on Single Cell organisms

Organism	Parameter (growth inhibition)	Time (h)	Concentration (mg/l)	Reference
Chilomonas paramecium	EC_5	48	0.90	Bringmann and Kühn, 1981
Entosiphon sulcatum	EC_5	72	20	Bringmann and Kühn, 1980b
Uronema parduczi	EC_5	20	11	Bringmann and Kühn, 1980a

For higher organisms, acute tests have been performed with fish and crustaceans (Table 5). The LC_{50} concentrations for fish range from 27-315 mg/l. The toxicity value (EC_{50}) for *Daphnia magna* was 54-765 mg/l. An LC_{50} value for the saltwater crustacean *Artemia salina* is 600 mg AA/l (Price *et al*, 1974).

Table 5: Effect Concentrations for Acute Tests on Higher Organisms

Organism	Parameter	Time (h)	Concentration (mg/l)	Reference
	Immobility			
Daphnia magna	EC_0	24	175	Bringmann and Kühn, 1977
	EC_{50}	24	270	
	EC_{100}	24	390	
	EC_0	24	51[a]	Bringmann and Kühn, 1982
	EC_{50}	24	54[a]	
	EC_{100}	24	91[a]	
	EC_0	24	156[b]	Bringmann and Kühn, 1977
	EC_{50}	24	765[b]	
	EC_{100}	24	5,000[b]	
	EC_{50}	48	95	Burgess, 1989
	NOEC[c]	48	23	
Artemia salina metanauplia larvae	LC_{50}	48	600	Price *et al*, 1974
	Lethality			
Leuciscus idus golden variety	LC_0	48	210	Juhnke and Lüdemann, 1978
	LC_{50}		315	
	LC_{100}		420	
Oncorhynchus mykiss	LC_{50}	96	27	Bowman, 1990
Cyprinus carpio	LC_{100}	24	100	Nishiuchi, 1975

a Not neutralised
b Neutralised
c No observed effect concentration

The 96-h static EC_{50} of AA for the alga *Selenastrum capricornutum* Printz was 0.17 mg/l (Forbis, 1990). The 72-h EC_{50} for the green alga *Scenedesmus subspicatus* 0.04 mg/l (BASF, 1994) and for *Chlorella vulgaris* 1.53 mg/l (SNF, 1995).

Long-term data are also available for algae (Table 6). *Scenedesmus quadricauda* showed a 5% decrease of growth after exposure to 18 mg AA/l for 8 days. The blue-green algae *Microcystis aeruginosa* is more sensitive, exhibiting an EC_3 for growth-inhibition of 0.15 mg AA/l after 8 days.

Table 6: Effect Concentrations in Chronic Tests of Algal Growth

Organism	Parameter (growth inhibition)	Time (d)	Concentration (mg/l)	Reference
Scenedesmus quadricauda	EC_3	8	18	Bringmann and Kühn, 1978a,b
Microcystis aeruginosa	EC_3	8	0.15	Bringmann and Kühn, 1978a,b
Selenastrum capricornutum	EC_{50}	4	0.17	Forbis, 1990
Scenedesmus subspicatus	EC_{50}	3	0.04a 0.13[b]	BASF, 1994
Chlorella vulagaris	EC_{50}	3	1.53a 63.0[b]	SNF, 1995

a Biomass
b Growth rate

6.3 SOIL ORGANISMS

In a study of the effect of AA on the soil carbon cycle, it was shown that AA had no effect on the respiration of soil microflora at 100 mg/kg, but at 1,000 mg/kg AA completely suppressed respiration (Hossack *et al*, 1992).

6.4 TERRESTRIAL ORGANISMS AND ECOSYSTEMS

No data are available.

6.5 SUMMARY AND EVALUATION

First signs of growth inhibition of *Pseudonomas putida* are seen from a concentration of 41 mg AA/l. Lethality is observed for *Escherichia coli* at 1,000 mg/l.

Protozoa and algae appear to be the more sensitive of the aquatic species examined. They are affected by AA concentrations from 0.04 to 20 mg/l. While for fish and *Daphnia*, the lethal or inhibitory concentration (LC_{50} or EC_{50}) ranges from 27 to 315 mg/l.

The respiration of soil microflora is not affected by 100 mg/kg AA, but completely inhibited at 1,000 mg/kg.

7. KINETICS AND METABOLISM

7.1 ABSORPTION, DISTRIBUTION, AND EXCRETION

7.1.1 Oral

Male Sprague-Dawley rats (3 animals/group) received single oral doses of [2,3-^{14}C]-AA (4, 40 or 400 mg/kgbw, specific activity: 2.43 mCi/mmol) in a 0.5% aqueous methylcellulose solution. Within 8 hours of dosing approximately 35, 55 and 60% of the respective dose was eliminated, most as $^{14}CO_2$; at 24 hours 50-65% had been eliminated and excretion of radioactivity had virtually ceased. After 3 days 44-65% of the radioactivity had been eliminated, while 2.9-4.3% remained in urine, 2.4-3.6% in faeces and 18.9-24.6% in tissues examined (liver, emptied stomach, muscle, blood and plasma). Residual radioactivity was highest in adipose tissue followed by muscle and liver (Rohm and Haas, 1984; De Bethizy *et al*, 1987).

Similar results were obtained by Kutzman *et al* (1982). Following gavage of an aqueous solution of ^{11}C-AA (26 µg/kgbw) to Sprague-Dawley derived female rats, AA was rapidly (within 1 h) absorbed and excreted, mainly as $^{11}CO_2$. Relative retention of the radiolabel ([dpm/g tissue]/[retained dpm/g rat]) after 65 min was ≥ 1% in liver (2.6%), adipose tissue (1.9%), small intestine (1.5%), kidneys (1.2%) and spleen (1.0%); approximately 6% was excreted in urine.

Within 3 days following single oral administration of 400 mg [2,3-^{14}C]AA/kgbw to male Sprague-Dawley rats, 78% of the radiolabel was exhaled as $^{14}CO_2$, while 6.3% was excreted in urine, 1.1% in faeces and 12.8% remained in tissues (4.8% in muscle, 3% in liver, 1.27% in fat, 2% in skin). This excretion pattern was consistent with that of [1-^{14}C]propionate administered in the same manner to another Sprague-Dawley rat as part of the experiment. The radioactivity remaining in the major tissues after 3 days probably represents the incorporation of ^{14}C from the utilisation of radiolabeled acetyl-S-CoA into biosynthetic pathways (Winter *et al*, 1992).

These results were corroborated by a second study in which male Sprague-Dawley rats received by gavage single doses of 400 mg [1-^{14}C]AA/kgbw in distilled water. Excretion of AA-derived radioactivity was determined by collection of urine, faeces and expired air for 3 days following administration of AA. After 3 days, the animals were killed and the distribution of radioactivity was determined in blood and tissues. Approximately 80% of the radioactivity was exhaled as $^{14}CO_2$ within 24 h. Exhalation of volatile organic compounds was negligible (< 0.5% of the dose). Excretion of radioactivity in the urine accounted for approximately 5% of the administered dose; excretion in faeces was 9%. Tissue

concentrations of AA derived radioactivity were generally low, in liver 0.4% of the dose, muscle 0.39% and skin 0.18%; in other tissues below 0.1% (Winter and Sipes, 1993)

Black *et al* (1995) conducted a comparative bioavailability and distribution study in male F344/N rats and male C3HeNCrlBR mice after gavage of single doses of 40 and 150 mg/kgbw of [1-^{14}C]AA (specific activity: 0.1 mCi/mmol) in water. In rats 90.3% of the radioactivity in the low dose animals and 81% in the high dose animals was exhaled within 3 days, with only 0.2% of the dose recovered as organic volatiles. In the high dose group 4.2% and 0.6% of the radioactivity was excreted in urine and faeces respectively. At 3 days 0.3% of the radioactivity was determined in selected tissues; a small portion (1%) of the dose was recovered in the carcass. The content of radioactivity in the stomach lumen dropped from 34% after 1 h to 0.004% after 3 days in the high dose group. Overall recovery of radioactivity was 95.2% of the administered dose for the low dose group and 88.1% for the high dose group. In the low dose animals elimination via exhalation was observed at a faster rate than in the high dose animals. Similar excretion patterns were observed in male mice.

7.1.2 Inhalation

Female Sprague-Dawley rats (39 animals) were exposed to ^{11}C-AA vapour for 1 minute (head/nose, concentration not stated). At selected times after exposure animals were killed by cervical dislocation and the radioactivity in different organs was determined. At 1.5 minutes 18.3% of the delivered dose was retained in the rats. Relatively large amounts of radioactivity were found in the upper respiratory tract: 28% was associated with the snout, 42.9% was found in the head apart from the snout and was considered to be solubilised in the mucous of turbinates and nasopharynx. After 65 minutes the radioactivity in the snout was reduced to 8.1% and approximately 60% of the radiolabel had been expired as $^{11}CO_2$. The elimination of $^{11}CO_2$ was biphasic with $t_{1/2}$ of the α-phase 30.6 min. The amount of radioactivity retained in liver, fat and stomach increased markedly between 1.5 and 65 minutes post exposure. The authors postulate that a portion of AA was ingested after inhalation. Urinary and faecal excretion was estimated to be 15% within 65 minutes (Kutzman *et al*, 1982).

7.1.3 Dermal

In Vivo

Male Sprague-Dawley rats were treated dermally with ^{14}C-labelled AA (5 mg/kgbw) in either phosphate buffer (pH 6 or pH 7.4) or acetone. The appearance of $^{14}CO_2$ in exhaled breath was used as a measure of the rate of absorption. The absorption rate was dependent on the vehicle and decreased in the order acetone > phosphate buffer pH 6 > phosphate buffer pH 7.4. Cumulative absorption after

24 h was 22% from acetone, approximately 19% from phosphate buffer pH 6.0 and 9% from phosphate buffer pH 7.4. The results correlated well with *in vitro* absorption data obtained by the same authors (D'Souza and Francis, 1988).

In vivo absorption of AA was studied in male Sprague-Dawley rats after topical application of 100 µl of a 4% (v/v) solution of [1-^{14}C]AA (specific activity 0.1 mCi/mmol) in acetone (501 µg/cm^2) using a skin mounted, charcoal containing trap covered with fixed aluminium discs to ensure complete recovery of the radiolabel. Excretion of AA derived radioactivity was determined in urine, faeces and expired air over a period of 3 days. After 3 days 73% of the radioactivity had volatilised from the skin and was trapped in the charcoal sorbent; 6% of the radioactivity was detected at the site of application in the skin or on the skin surface. Approximately 16% of the applied dose, representing 75% of the absorbed dose was exhaled as $^{14}CO_2$ within 12 hours. Only 9% of the applied radioactivity (4% of the absorbed dose) was found in urine, while faeces contained negligible amounts of radioactivity. Less than 0.4% of the applied dose was retained in tissues other than skin after 3 days (Winter and Sipes, 1993).

[^{14}C]AA (specific activity: 0.1 mCi/mmol) was applied to the clipped skin of the shoulder and back region of male F344/N rats and male C3H/HeNCrlBR mice at doses of 10 and 40 mg/kgbw in acetone. A non-occlusive frame application was used in the 40 mg/kgbw rat group consisting of a nylon screen skin barrier fixed to the skin surface of the upper back region by bone cement. In the other dose groups activated charcoal impregnated filter papers were applied occlusively and replaced or removed every 1, 6, 8 or 24 hours. Evaporation accounted for the largest fraction of the applied dose in both species and dose groups. In rats the apparent absorption was 19.4% of the applied radioactivity in the low-dose group and 25.6% in the high-dose group. After 3 days 69.5% and 77% of the absorbed dose was recovered as $^{14}CO_2$ in the low- and high-dose group respectively. Approximately 1% of the dose had remained at the site of application at either dose. Urinary excretion accounted for 1-2%, excretion in faeces for less than 1% and 2-3% of the applied radioactivity was found in peripheral tissues and the carcass at the end of the experiment. In mice the apparent absorption was 12.4% in the low-dose and 11.4% in the high-dose animals. In the low-dose group 77.7% of the absorbed radioactivity was exhaled as $^{14}CO_2$, and in the high-dose group 83%. A minor fraction (0.2 to 1.5%) of the dose had remained at the site of application, while 1% of the radioactivity was found in carcass and tissues of both dose groups. After 3 days ^{14}C-levels in fat were higher than levels found at 1 or 8 hours (Black *et al*, 1995).

In Vitro

Dermal penetration capacity of AA was tested *in vitro* in excised human skin by determining the flux of ^{14}C-labelled AA (1 mg/dose cell) dissolved in phosphate buffer pH 6 or pH 7, acetone and ethylene glycol. Dermal absorption decreased in the following order (relative penetrated flux in brackets): acetone (600 µg/cm^2/h) >> phosphate buffer pH 6 (23 µg/cm^2/h) > ethylene glycol (15 µg/cm^2/h) > phosphate buffer pH 7.4 (1 µg/cm^2/h) (D'Souza and Francis, 1988).

Dermal penetration of AA was tested *in vitro* using mouse and human skin. Skin slices were treated in a diffusion chamber with ^{14}C-AA (specific activity: 0.25-1.44 mCi/mmol; dose 100 µl/cm^2) dissolved at 0.01, 0.1, 1 and 4% (w/v) in acetone, water or phosphate buffer pH 6.5. Rates of absorption decreased in the order acetone > water > phosphate buffer pH 6.5. The absorption rate increased as a function of the AA concentration, independent of the vehicle. Permeability coefficients for mouse skin ranged from 0.96-1.73 x 10^{-3} cm/h for water to 1.91-3.1 x 10^{-4} cm/h for phosphate buffer. For human skin permeability coefficients were reported to be between 0.37 and 0.72 x 10^{-3} cm/h for water and 0.47-1.81 x 10^{-4} cm/h for phosphate buffer. Permeability coefficients were not calculated for acetone because of the evaporation of the volatile vehicle during the course of the experiment. Mouse skin was 3 times more permeable than human skin but the authors regarded this difference as not biologically significant. The concentration of AA in both human and mouse skin was highest after application of AA in acetone (1% solution) (49.1 µg/cm$_2$ for the mouse skin, 95.8 µg/cm$_2$ for human skin)), followed by water (26.8 µg/cm$_2$ for mouse skin, 58.1 µg/cm$_2$ for human skin). The respective amounts of AA absorbed in mouse and human skin from phosphate buffer were 4.04 µg/cm$_2$ and 9.34 µg/cm$_2$ (Corrigan and Scott, 1988).

The relative contribution of absorption and evaporation to the disposition of dermally administered ^{14}C-AA was studied in an *in vitro* experiment using freshly obtained clipped dorsal skin of male rats. The skin was placed in a flow-through diffusion cell and 95 µl of 1% (v/v) ^{14}C-AA in acetone was applied to the exposed epidermal surface area. An evaporation trap was fitted over the dosed skin. Air flow at the skin surface was maintained at 600 ml/min and the temperature was adjusted to 32°C. After 6 hours 23.9% of the dose was absorbed into the effluent or remained in the skin. At least 60% had evaporated, the total recovery being 85% (Black *et al*, 1995).

7.1.4 Intravenous

A single dose (10 mg/kgbw) of ^{14}C-labelled AA (specific activity 0.25-1.44 mCi/mmol) in phosphate buffered saline) was injected i.v. into the tail vein of male F344/N rats and male C3H/HeNCrlBR mice. In rats 63% of the ^{14}C-dose was exhaled as $^{14}CO_2$ after 4 hours and 68% after 3 days. The main levels

of radioactivity were recovered in liver (1.57%), kidney (0.17%) and fat (0.012%) at 1 hour; the levels had decreased to 0.15%, 0.027% and 0.005% after 3 days. Overall recovery was only 72.8%. In mice 51% of the radioactivity was exhaled as $^{14}CO_2$ after 3 days, the majority being exhaled at 4 hours. Volatile organic radioactive fractions were approximately 0.6% of the total dose. The radioactivity recovered in liver, kidney, and fat was less than 0.2%. Overall recovery of radioactivity was 58%. ^{14}C concentrations in liver and kidney decreased with time while fat concentrations increased with time (Frantz and Beskitt, 1993).

7.1.5 Intraperitoneal

To assess the influence of AA on the activity of the brain's Creatine Kinase (CK), a single dose of ^{14}C-radiolabelled AA (100 mg/kgbw) was injected i.p. into the tail vein of male Wistar rats. Twenty four hours later the animals were killed and the brain was dissected into 8 regions (cortex, striatum, hypothalamus, hippocampus, midbrain, medulla oblonga, cerebellar hemisphere and cerebellar vermis). The radioactivity of each brain region was measured in a scintillation counter. Only 0.02% of ^{14}C was found in the brain of rats injected with ^{14}C-AA and there were no clear regional differences in ^{14}C distribution (Kohriyama *et al*, 1994).

7.2 METABOLISM

7.2.1 *In Vivo*

After oral administration of [2,3-^{14}C]-AA (specific activity: 2.43 mCi/mmol; 4, 40, 400 mg/kgbw) in methylcellulose (0.5% aq.) to male Sprague-Dawley rats the major part of the radioactivity was exhaled as $^{14}CO_2$. Four metabolites were detected in urine by HPLC analysis. One of the 2 major metabolites co-eluted with 3-hydroxypropionic acid. No peaks associated with 2,3-epoxypropionic acid, lactic acid, glyceric acid or N-acetyl-S-(2-carboxy-2- hydroxyethyl)cysteine were detected suggesting that AA is not epoxidised to 2,3-epoxypropionic acid *in vivo*. According to the authors the *in vivo* metabolism suggests the incorporation of AA in normal cellular metabolism via the propionate pathway, degradation to CO_2 being the major route of elimination. Retention of radioactivity in the adipose tissue may be due to incorporation of acetyl-S-CoA also derived from the propionate pathway (De Bethizy *et al*, 1987).

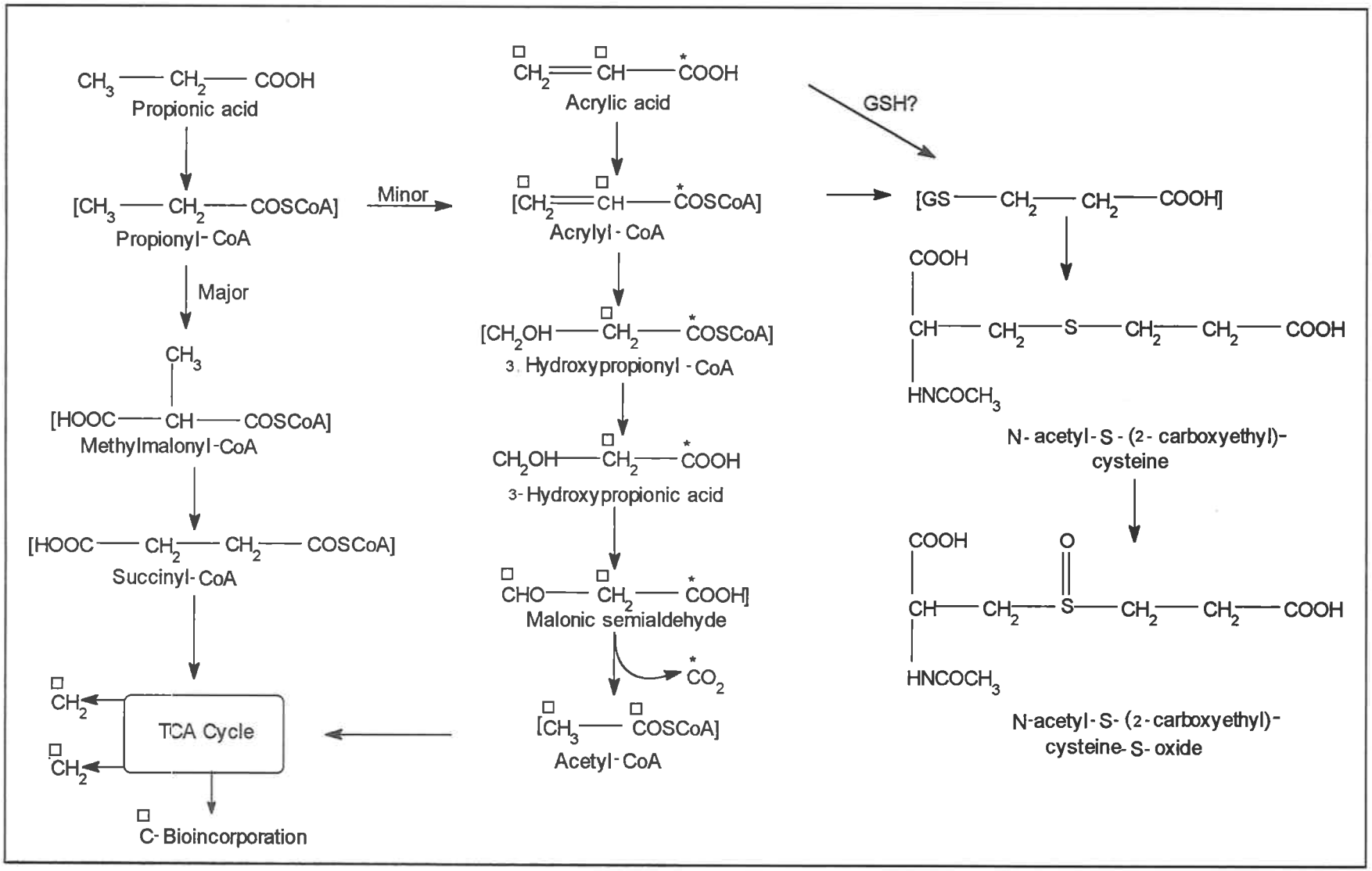
3-Hydroxypropionic acid, N-acetyl-S(2-carboxyethyl)cysteine and N-acetyl-S-(2-carboxyethyl)cysteine-S-oxide were identified (using HPLC and NMR analysis) as metabolites of AA after gavage of single doses of [1,2,3-^{13}C]AA (400 mg/kgbw) in water to male Sprague-Dawley rats (Winter *et al*, 1992). According to the authors the detection of the mercapturates may be a consequence of the high dose

used in this experiment, as a high concentration of the unionised acid is necessary to form the GSH addition products. The metabolic pathways proposed for AA by Winter and Sipes (1993) are summarised in Figure 1.

After gavage of single doses (40 and 150 mg/kgbw) of [1-^{14}C]AA (specific activity: 0.1 mCi/mmol) in water to male F344/N rats urinary metabolites were analysed by HPLC. A major polar metabolite which could not be identified accounted for approximately 2 to 3% of the dose. Trace amounts of a material co-eluting with AA and a metabolite co-eluting with 3-hydroxypropionic acid were also detected. In addition, several other metabolites more polar than AA and 2 metabolites of lower polarity were also present. Plasma and liver were analysed by HPLC for AA and metabolites. A metabolite co-eluting with 3-hydroxypropionic acid was detected in plasma 1 hour after administration. A metabolite corresponding with the major urinary metabolite was detected in the liver of the animals 1 hour after dosing. Neither AA nor metabolites were found in plasma or liver at times later than 1 hour. In kidney no AA or metabolites could be detected. In another experiment, livers collected from C_3H mice dosed by gavage following a similar dosing regime were analysed for AA and metabolites by HPLC. Several metabolites more polar than AA including 3-hydroxypropionic acid and AA itself were detected 1 hour after administration but not at times later than 1 hour (Black *et al*, 1995).

After application of [^{14}C]AA (specific activity: 0.1 mCi/mmol; 10 and 40 mg/kgbw in acetone) to the clipped skin of the shoulder and back region of male F344/N rats and male C3H/HeNCrlBR mice, urine of rats and liver of mice were analysed by HPLC for AA and metabolites. A material co-eluting with 3-hydroxypropionic acid was detected in rat urine, as well as several other metabolites more polar than AA, one co-eluting with the major urinary metabolite and 2 metabolites of lower polarity. Trace amounts of another metabolite not detected after oral administration were found in the 40 mg/kgbw group, but not in the 10 mg/kgbw group. Neither AA nor metabolites were detected in liver of mice (Black *et al*, 1995).

Figure 1: Proposed Metabolic Pathways for AA[a]
(Winter and Sipes, 1993)



a The routes of oxidation of the C-1 (*) and C-2,3 (□) carbons are shown. Intermediates that have not been identified, but the existence of which may be inferred, are enclosed in brackets.

Reactions with Non-protein Sulphydryls (NPSH)

Male Sprague-Dawley rats were dosed orally with single doses of 4, 40, 400 or 1,000 mg/kgbw AA (0.08, 0.8, 8 or 20% w/v) in methylcellulose (0.5% aq.) with and without inhibition of carboxyl esterases by TOCP (tri-*o*-cresyl phosphate, 125 mg i.p.) prior to application of AA. One hour after dosing blood samples were taken by cardiac puncture, the animals were killed and tissue fractions of liver, forestomach and glandular stomach were analysed for NPSH content. A significant depletion of NPSH in the glandular stomach was reported at doses above 0.08%. In the forestomach NPSH depletion occurred at a dose of 20%. No significant effect of AA on NPSH content of blood or liver was observed. TOCP treatment had no effect on the AA dose response. According to the authors the result is somewhat difficult to explain because under normal physiological conditions AA is ionised to 99% (pKa=4.26) and is not expected to react with NPSH, nor are its known metabolites (De Bethizy *et al*, 1987).

Following pretreated with corn oil (1 ml/animal i.p.), inhalation exposure of male Holtzman rats to AA (500, 800 and 1,000 ppm) for 4 hours resulted in a significant decrease in NPSH content of lungs, blood and more markedly of liver and kidney in the 1,000 ppm dose group (Silver and Murphy, 1981; Silver *et al*, 1981).

7.2.2 *In Vitro*

Hepatic microsomal oxidation of AA was studied by incubating [2,3-^{14}C]AA with microsome preparations of male Sprague-Dawley rats. No metabolites could be detected and AA was recovered unchanged from the incubated mixture (De Bethizy *et al*, 1987).

In vitro metabolism of [1-^{14}C]AA was studied using freshly isolated male F344/N rat hepatocytes, liver homogenates and preparations of mitochondria containing essential cofactors. The studies were conducted in sealed vials modified to trap evolved $^{14}CO_2$. Rapid oxidation of AA was observed in all test systems. Addition of equimolar amounts of propionic acid, 3-hydroxypropionic acid or 3-mercaptopropionic acid resulted in a significant inhibition of the oxidation of AA by isolated mitochondria. HPLC analysis of the mitochondrial incubation mixtures revealed a single major metabolite co-eluting with 3-hydroxypropionic acid. The authors suggest that incorporation into the mitochondrial catabolism for propionic acid is the major detoxification route of AA. There was no evidence for toxic intermediates in the mitochondrial metabolism of AA (Finch and Frederick, 1990, 1992).

Black *et al* (1995) studied the kinetics of AA oxidation in slices of 13 different tissues of C_3H mice by incubating tissue slices with [1-^{14}C]AA and collecting $^{14}CO_2$. Preparations of the following organs were examined: liver, kidney, forestomach, glandular stomach, small intestine, spleen, brain, heart, lung, skeletal muscle, fat and skin. All tissues studied oxidised AA to a certain extent, but activity in kidney followed by liver was much higher than in other tissues. In kidney, liver and skin the kinetics followed pseudo first-order Michaelis-Menton kinetics, with a K_m of 0.67 mM for all tissues. Marked differences were observed in V_{max} values, namely 2,890 nmol/h/g for kidney, 616 nmol/h/g for liver, 47.9 nmol/h/g for skin, with respective half-lives of 0.139 h, 0.867 h and 10.2 h. Lung, glandular stomach, heart, spleen, fat and large intestine preparations oxidised AA at rates between 10 and 40% of the rate determined for the liver. Reaction rates in the remaining tissues were less than 10% of those in the liver. The only metabolite determined in kidney slices by HPLC was 3-hydroxypropionic acid. CO_2 in this reaction was supposed to be formed from the C_1 carbon atom of AA, the other reaction product being acetyl-S-CoA derived from C_2 and C_3. This reaction sequence was confirmed by incubating [2,3-^{14}C]AA and [1-^{14}C]acetate with liver and kidney slices and measuring the rate of $^{14}CO_2$ formation. Both substrates were oxidised well by the tissues.

AA oxidation rates and blood tissue partition coefficients were studied in slices of rat tissue using [1-^{14}C]AA. The tissues studied were liver, kidney, forestomach, glandular stomach, small intestine, large intestine, spleen, brain, heart, lung, skeletal muscle, (inguinal) fat and skin obtained from male F344/N rats, liver and kidney also from male Sprague-Dawley rats. AA oxidation followed pseudo first-order Michaelis-Menten kinetics. The rate of oxidation of AA was similar in the 2 rat strains, the highest rate in kidney followed by liver. In kidney a K_m value of 0.625 mM and V_{max} of 3,757 nmol/h/g tissue was reported and in liver a K_m of 0.479 mM and a V_{max} of 2,030 nmol/h/g tissue, resulting in a half-life of 7 minutes in kidney and 10.9 minutes in liver. Oxidation rates in lung, glandular stomach, heart, spleen, small intestine and large intestine were between 10 and 40% of those in liver; the remaining tissues oxidised AA at rates lower than 10%. The absolute rates of AA oxidation in the various tissues (expressed per gram of tissue) were approximately 2 to 3 times higher than the rates in the corresponding mouse tissues except for rates in fat (negligible in rats) and muscle and skin which were comparable in both species. Blood tissue partition coefficients measured in homogenates by micropartitioning showed little variation between tissues with partition coefficients ranging from 0.9 in fat to 1.9 in brain. The authors concluded that due to the relatively small variations of blood tissue partition coefficients compared to the marked differences of oxidation rates in the different tissues, the rate of AA detoxification of a tissue *in vivo* is likely to depend on the intrinsic oxidation rate and the proportion of cardiac output reaching the tissue. Thus from the above study it could be predicted that liver and kidney are the most important tissues for systemic detoxification of AA as they showed the highest oxidation rates and are well perfused *in vivo*. Rapid oxidation by these tissues may explain the

low systemic toxicity of AA, while low rates of AA oxidation in other tissues may eventually explain toxicity of AA to portal of entry tissues as skin, stomach and respiratory tract (Black and Finch, 1995).

Segal *et al* (1987) studied the reaction of AA (70 mM) with 2'-deoxynucleotides (1.3 mM) and calf thymus DNA after incubation for 40 days at 37°C and pH 7.0-7.2. Reaction products were separated using paper chromatography and the extracted bands analysed by HPLC. Michael adducts of deoxyadenosine, deoxycytidine, deoxyguanidine and deoxythymidine were identified after incubation with the single nucleotides. Incubation with calf thymus DNA yielded adducts of adenine, guanine and thymidine. Thus the authors demonstrated that under extreme reaction conditions (high surplus concentration of AA and long incubation times) AA is able to react with nucleophiles *in vitro*. However, these results are not relevant to *in vivo* exposure.

In contrast to the results of Segal *et al* (1987), Frederick and Reynolds (1988) found that incubation of the negatively charged acrylate anion with 2 representative nucleophiles, methylamine and imidazole, did not result in the formation of adducts of the acrylate ion with the nucleophile. The formation of Michael products *in vitro* via the non-ionised form of AA investigated as an alternative was found to be theoretically possible. In the opinion of the authors this is unlikely to occur *in vivo* because of the rapid metabolism and excretion of AA.

Molecular modelling (MOLY molecular graphics/modelling package) suggests that binding of AA to cellular nucleophiles might be due to small amounts of undissociated acid in the equilibrium between the acrylate anion and AA at cellular pH. The ratio of acrylate anion to undissociated acid was calculated to be 1:580 at pH 7. Based on calculated reaction paths and charge distributions the authors conclude that Michael addition to nucleophiles cannot occur through the predominant anion at pH 7 but must be due to AA itself, the rate of reaction being a function of the concentrations of AA and nucleophiles, and the rate constant which can be derived from the energy of activation (Frederick and Reynolds, 1989).

The reaction of AA with reduced glutathione (GSH), with and without enzymatic activation, and its effect on the non-protein sulphydryl level of rat blood *in vitro* was studied by Miller *et al* (1981b). Incubation of 4 mM AA with 2 mM GSH in phosphate buffer for 30 minutes resulted in a GSH depletion of only 6%. The amount of depleted GSH was not increased by adding an aliquot of a soluble enzyme fraction (liver 10^5 g fraction). Incubation of 8 mM AA had only a minimal effect on blood NPSH. The results suggest that the reaction of AA with NPSH is negligible (Miller *et al*, 1981a).

Similar results were obtained by Silver and Murphy (1981) who found that GSH was depleted by 3% following incubation of 5 mM AA with 5 mM GSH in a 0.1 mM phosphate buffer for 5 min at 37°C and pH 7.3.

The rate constant for the reaction of sodium acrylate with GSH at 37°C and pH 7.3 was reported to be 0.014 l/mol/min again indicating a negligible reactivity with glutathione (Hashimoto and Aldridge, 1970).

7.3 SUMMARY

AA is rapidly absorbed in experimental animals after oral or inhalation administration. Dermal absorption is strongly dependent on the vehicle and the pH of the solution. AA is rapidly metabolised by normal oxidative pathways to CO_2. The main metabolic pathway of AA seems to be a secondary, non vitamin-B_{12} dependant pathway of propionic acid metabolism consisting of reactions similar to fatty acid β-oxidation which yields CO_2 and acetyl-S-CoA. Major urinary metabolites are poorly characterised substances more polar than AA. A small amount of 3-hydroxypropionic acid was identified as one of the urinary metabolites. There is no evidence to suggest that epoxide intermediates are formed during the metabolism of AA. At high local tissue concentrations, where tissue damage would be expected, AA may react with glutathione or other non-protein sulphydryl groups.

Bioavailability studies with radiolabelled AA via the oral, dermal and i.v. route of exposure suggest that route-dependent differences in metabolism may occur. After dermal and i.v. administration, ^{14}C levels in adipose tissues increased with time. No such increases were observed following oral administration, suggesting a possible first pass metabolism for the oral route. The increased ^{14}C levels in adipose tissues are probably the result of the incorporation of acetyl-S-CoA derived from AA into anabolic pathways.

8. EFFECTS ON EXPERIMENTAL ANIMALS AND *IN VITRO* TEST SYSTEMS

8.1 ACUTE TOXICITY

The acute toxicity of AA after oral, dermal and inhalation exposure is relatively low (Table 7-9). The main symptoms of intoxication are local irritant and corrosive effects, and general non-characteristic symptoms. Microscopic changes at autopsy after single administration were in accordance with the irritant properties of AA.

8.1.1 **Oral** (Table 7)

Table 7: Acute Oral Toxicity

Species	LD_{50} (mg/kgbw)	Remark	Reference
Rat	193	glacial AA	Union Carbide, 1977 as quoted in IARC, 1979
Rat	340	glacial AA	Carpenter *et al*, 1974
Rat	1,350		Majka *et al*, 1974
Rat	1,500		BASF, 1958
Rat	2,500		Smyth *et al*, 1962
Rat	2,100-3,200		Miller, 1964 as quoted in IARC, 1979
Rat	1,250		Klimkina *et al*, 1969
Mouse	2,400[a]		Boyland, 1940
Mouse	1,200		BASF, 1958
Mouse	830	glacial AA	Klimkina *et al*, 1969
Rabbit	250		Klimkina *et al*, 1969

a Reported as 60 mg/animal

The oral LD_{50} values in rats and mice vary considerably with rat LD_{50} values ranging from 1,250 to 3,200 mg/kgbw for technical AA and 193 to 340 mg/kgbw for glacial AA. This wide variation appears to be related to the forms in which the AA was administered, i.e. undiluted, as an aqueous solution at various concentrations, or as neutralised solution.

8.1.2 Inhalation (Table 8)

Table 8: Acute Inhalation Toxicity

Species	Time (h)	Exposure concentration (ppm)	Remark	Reference
Rat	4	1,200[a]	LC_{50}	Majka *et al*, 1974
Rat	4	>1,740[b]	LC_{50}	BASF, 1980
Rat	5	6,000[c]	1/4 dead	Gage, 1970
Rat	3.5	saturated atmosphere	No deaths	Union Carbide, 1977 as quoted in Clayton and Clayton, 1982
Rat	8	saturated atmosphere	No deaths	Smyth *et al*, 1962
Rat	4	4,000[d]	No deaths	Union Carbide, 1977 as quoted in Clayton and Clayton, 1982
Rat	4	2,000	0/6 dead	Carpenter *et al*, 1974
Rat	1	saturated atmosphere, no deaths		Carpenter *et al*, 1974
Rat	0.5 1 3	atmosphere with maximum possible enrichment	0/12 dead 1/6 dead 6/6 dead	BASF, 1979a
Rat	1	1,442 and 1,394 (static)[e] 2,253 (dynamic)[f]	No deaths. Irritation of eye and nose. Body weight reduction over 8 days.	Nachreiner and Dodd, 1988

a Reported as 3,600 µg/l
b Reported as >5.1 mg/l
c Reported as 19 mg/l
d Reported as 11.5 g/m^3
e Animals exposed to atmospheres in sealed chambers
f Animals exposed to atmosphere passing through chamber

Inhalation exposure was followed by eye irritation with damage to the cornea, respiratory tract irritation, dyspnoea and pulmonary oedema and, at high concentrations, skin lesions (LC_{50} approximately 1,200 ppm).

8.1.3 Dermal (Table 9)

Table 9: Acute Dermal Toxicity

Species	LD_{50} (mg/kgbw)	Reference
Rabbit	750	Union Carbide, 1977 as quoted in IARC, 1979
Rabbit	295	Carpenter *et al*, 1974
Rabbit	640 (approximately)	BASF, 1979b

Dermal application resulted in dyspnoea, diarrhoea and blood in the urine.

8.1.4 Intraperitoneal

The LD_{50} following i.p. administration of AA to the rat has been evaluated at 22.5 µl/kgbw (23.6 mg/kgbw) (Singh *et al*, 1972).

8.1.5 Evaluation

AA has a relatively low toxicity following oral administration (LD_{50} rat 1,250-3,200 mg/kgbw for technical AA, 193-340 mg/kgbw for glacial AA), dermal application (LD_{50} rabbit 295-750 mg/kgbw) and inhalation exposure (4-h LC_{50} rat 1,200 ppm). The main signs of toxicity are irritation and/or corrosion at the site of contact.

8.2 SUBCHRONIC TOXICITY

8.2.1 Oral

Groups of 10 male and 10 female Wistar rats were dosed (5 d/wk) by gavage with AA in water at doses of 150 or 375 mg/kgbw/d for 3 months. Six males and 9 females from the 375 mg/kgbw/d dose group died during the course of the study. Clinical examination of those animals surviving to 3 weeks revealed tympanites (flatulent distension) of the gastrointestinal tract, cyanosis and dyspnoea. Gross pathological and histopathological examination revealed irritation of both the forestomach and the glandular stomach. The irritation was manifested as thickening of the plica marginata and hyperaemia or bloody erosions/ulcerations of the gastric mucosa. Other observed effects included elevations of the diaphragm, lung oedema and emphysema, alveolar hyperaemia and dystelectases (insufficient

ventilation of parts of the lung). In some animals, catarrhal or catarrhal-purulent rhinitis were also detected. Animals dying during the study exhibited necrotising nephrosis as a secondary effect, primarily due to the tympana compressing the renal vessels (BASF, 1987). In the 150 mg/kgbw/d dose group, 5 males and 5 females died during the study. The clinical signs and histopathological findings were substantially the same as those observed in the higher dose group, but in general were observed in fewer animals and were less severe. A no-effect level was not established in this study (Hellwig *et al*, 1993).

In a parallel study, groups of 10 male and 10 female Wistar rats received drinking water containing 0, 120, 800, 2,000 or 5,000 mg AA/l (equivalent doses 0, 9, 61, 140 or 331 mg/kgbw/d respectively) for 3 months. No deaths were recorded in either sex at any dose during the study. Water intake was reduced in both sexes and at all dose levels, however the effects were most pronounced in the males from the 2,000 and 5,000 mg/l dose groups. No significant effects were observed on body weight gain, with the exception of the high dose male group which showed a slight reduction. There was no obvious treatment-related pattern in any of the clinical chemical or haematological parameters. There were no gross or histopathological changes attributable to exposure to AA. The no-observed adverse effect level (NOAEL) for body weight change was 331 mg/kgbw/d (Hellwig *et al*, 1993).

In a range-finding study F344/N rats (5 of either sex/group) received AA in drinking water at concentrations of 0, 0.15, 0.3 or 0.6% (equivalent dose for males 0, 210, 420 or 680 mg/kgbw/d; for females 0, 220, 400 or 760 mg/kgbw/d) for 7 days. A significant reduction in body weight gain was seen in the high dose males on days 4 and 7, and a slight reduction in high-dose females on day 1. Water intake decreased by 26% in the males and by 19% in the females. Food consumption was not affected. No adverse signs were observed in animals from the 2 lower doses. The NOAEL was calculated at 410 mg/kgbw/d (DePass *et al*, 1983).

In a subsequent drinking-water study F344/N rats (15 of either sex/group) were administered 0, 83, 250 or 750 mg AA/kgbw/d for 3 months. No gross or microscopic lesions due to the treatment with AA were observed at any of the dose levels. The 750 mg/kgbw dose caused a decrease in body weight gain, a decrease in food and water consumption, increased relative kidney weights in males and females and increased relative testes weights in males, an increase in blood urea nitrogen and alkaline phosphatase and an increase in urinary protein concentration. The 250 mg/kgbw/d dose caused a decrease in water consumption and serum cholesterol and increased relative kidneys and testes weights. The changes in various organs weights and clinical chemistry parameters observed in this study were possibly related to the decrease of food and water consumption because of the bad palatability problems with the drinking water. The NOAEL was 83 mg/kgbw/d (DePass *et al*, 1983).

8.2.2 Inhalation

Alderley-Park strain rats (4 males, 4 females) were exposed by inhalation (6 h/d) to AA at 1,500 ppm on 4 consecutive days. The animals displayed inflammatory changes to the nose, apathy and body weight loss; histological investigation revealed congestion in the kidneys (this is considered a secondary effect). Exposure (6 h/d, 5 d/wk) of Alderley-Park rats (4 males, 4 females) to 300 ppm AA for 4 weeks caused irritation of the nose, apathy, and retarded body weight gain. No changes of the internal organs were found at autopsy. A concentration of 80 ppm was considered to be the no-observed effect level (NOEL) (Gage, 1970).

Wistar rats (number/sex not stated) were exposed (4 h/d, 6 d/wk) to atmospheres containing AA vapours at 700 mg/m^3 (234 ppm) for 5 weeks. Body weight gain was reduced in comparison with the controls; after 29 and 35 days the excretion of phenol red in the urine (an indicator of kidney disfunction) was significantly increased ($p \geq 0.05$ and 0.001). The specific gravity of the urine was significantly lower ($p \geq 0.05$). Inflammation of the upper respiratory tract and mucosal lesions in the stomach were also observed (Majka *et al*, 1974).

In a range-finding study F344/N rats (4 males, 4 females/group) and B6C3F$_1$ mice (6 males, 4 females/group) were exposed (6 h/d, 5 d/wk) to atmospheres containing 0, 25, 75 or 225 ppm AA (measured by IR spectrophotometry) for 2 weeks. At 225 ppm there were obvious signs of nasal irritation in rats and mice. After 4, 7 and 10 days there was a significant reduction in body weight gain of the treated rats and mice in comparison with the controls. The fat stores in female rats were reduced, but the absolute and relative weights of the organs (brain, heart, liver, kidneys and testes) were unchanged in both species. Histologically, there were inflammatory degenerative lesions of the nasal mucosa, with focal metaplasia. At the lower concentrations of 25 and 75 ppm in comparison with the controls, bodyweight gain was unaffected, as were the absolute and relative organ weights. Macroscopically, no clear lesions were observed in the nasal mucosa. Histologically, a slight lesion of the nasal mucosa was detected in mice exposed to 75 ppm and a very slight lesion of the nasal mucosa in some mice exposed to 25 ppm. The distribution of the lesions showed that the olfactory epithelium was more sensitive than the respiratory epithelium. The NOEL was determined to be 25 ppm for the rat (Miller *et al*, 1981a).

Groups of F344/N rats and B6C3F$_1$ mice (15 of either sex/group/species) were exposed (6 h/d, 5 d/wk) to 0, 5, 25 or 75 ppm AA (monitored 2-3 x/h) for 13 weeks. Body weight gain was significantly decreased in female mice in the 25 and 75 ppm groups after 12 weeks of exposure. Haematological and clinical chemical parameters were studied in 10 rats and 10 mice from each exposure group, and urine analysed with 10 male and 10 female rats. A slight reduction of haemoglobin was observed in

mice of the 25 and 75 ppm exposure groups as well as in female mice of the 75 ppm group. No adverse effects on the other parameters were detected in any dose group. Histopathological examinations of the nasal mucosa in rat in the 75 ppm exposure group and in some or all mice at each treatment level showed some slight dose-related focal degeneration. Histopathological examinations were conducted in the 5 and 25 ppm groups on target organs identified in the 75 ppm group. Nasal lesions were also observed. The NOEL was determined to be 25 ppm in the rat and < 5 ppm in the mouse (Miller *et al*, 1981a).

Rats and mice (number, age and sex not stated) were exposed (6 h/d) to an atmosphere containing 75 ppm AA for 5 days. They were dosed with radioactive thymidine 18 hours after the last exposure. The aim of the investigation was to determine the effect of AA on olfactory cell proliferation using DNA synthesis as the marker. This study showed that AA caused a 17-fold increase of cell proliferation in mice and a 4-fold increase in rats, compared to the corresponding controls (Swenberg *et al*, 1987).

8.2.3 Dermal

AA was irritating to the skin of male C3H/HeJ mice at a dose of 5% in acetone but not at 1% when applied daily for 2 weeks. AA produced no observable systemic toxic effects in male C3H/HeJ mice at a dose of 1% AA in acetone applied (3 x/wk) for their life-time (DePass *et al*, 1985).

The skin of 30 female ICR mice, 30 male C3H mice and 30 female B6C3F_1 mice was treated (3 x/wk) for 13 weeks with AA at 0%, 1% and 4% in acetone. Little or no gross irritation was observed in the solvent control or the 1% AA level, and minimal histopathological proliferative changes were observed at the 1% AA level. All strains of mice showed significant skin irritation at 4% in acetone; skin shedding, fissuring and sloughing of necrotic tissue were evident. Microscopic effects at the 4% level included proliferative, degenerative and inflammatory changes of the epidermis and dermis. Similar effects were occasionally observed in the 1% dose group, but rarely in the control group (Tegeris *et al*, 1988).

8.2.4 Evaluation

Repeated administration of AA by gavage may cause irritation and ulceration of the stomach; these effects are dose dependent. Other effects include lung oedema and emphysema. Repeated administration of AA in drinking water does not cause any systemic adverse effects except for a decrease in body weight gain and changes in the clinical chemistry, probably due to decreased water and food consumption. The NOAEL in drinking water ranges from 83 to 410 mg AA/kgbw/d.

Repeated inhalation exposure to AA for 13 weeks caused no changes in the body weight gain of rats exposed to 75 ppm, but a significant decrease in female mice in the 25 and 75 ppm exposure groups. No adverse effects on haematological and clinical chemistry parameters were observed at any dose. Slight focal degeneration of the olfactory epithelium was observed in rats and mice. The NOEL was determined to be 25 ppm for rats and < 5 ppm for mice.

Repeated dermal application of AA (4% in acetone) to mice resulted in degenerative and inflammatory changes both of the dermis and epidermis. No systemic effects were seen at any dose. No NOEL was determined.

8.3 SKIN, RESPIRATORY AND EYE IRRITATION, SENSITISATION

8.3.1 Skin Irritation

Moderate to severe necrosis was observed after application of undiluted AA to the unoccluded skin of rabbits for 24 hours (Carpenter *et al*, 1974).

In a series of studies, local corrosive damage occurred within 1 minute of a semi-occlusive application of undiluted AA to the shaved dorsal skin of "white rabbits". At a concentration of 50% in water, AA produced erythema and oedema, scaling, crusts and scars. Slight reddening of the skin was observed with a 20% aqueous solution after exposure for 1 minute. There were no irritation effects when the rabbits were exposed to a 10% aqueous solution for 15 minutes (BASF, 1958).

Repeated dermal application (3x/wk) of AA in acetone to the skin of mice for 13 weeks caused skin irritation, skin degeneration and ulceration (Section 8.2.3: Tegeris *et al*, 1988).

8.3.2 Eye Irritation

After instillation of one drop (approximately 50 µl) of undiluted AA into the conjunctival sac of the rabbit eye immediate spontaneous corrosion of the conjunctivae and of the cornea were observed; these lesions were irreversible (BASF, 1958).

Undiluted AA caused severe damage to the eyes of rabbits 18-24 hour after 0.5 ml of the liquid was applied to conjunctival sac of the eyes (Carpenter *et al*, 1974).

Severe irritation and corneal burns were observed after exposure to 0.5 ml of a 10% aqueous solution of AA (Smyth *et al*, 1962).

The lowest concentration to produce visible damage to the eye of rabbits was reported to be a 1% solution in water (Union Carbide, 1977 as quoted in IARC, 1979).

8.3.3 Respiratory Tract Irritation

Groups of 5 male Holtzmann rats were exposed for 1 hour to AA at concentrations of 100, 300 and 500 ppm. Depending on the severity of local irritation of the respiratory tract the respiratory frequency and minute ventilation volume were reduced. In the high dose group the respiration frequency was approximately 80% of the control level (Silver *et al*, 1981).

In sensory irritation studies the single AA exposure needed for a 50% reduction of the respiratory rate (RD_{50}) was 513 ppm in F344/N rats and 685 ppm in $B6C3F_1$ mice. During exposure to 75 ppm of AA for 6 hours a 20-30% decrease in minute volume was observed in both species (Buckley *et al*, 1984).

Single exposure (1 h) of Sprague-Dawley rats (5 males, 5 females/group) to atmospheres of AA (generated by a bubbler method) at concentrations of 1,394 and 1,442 ppm AA (static method) or 2,352 ppm (dynamic method) led to signs of ocular and respiratory irritation such as lacrimation, perioral and perinasal wetness and incrustation and abdominal breathing in all dose groups. These clinical signs were reversible during the 14 day post-exposure observation period. In the 2,552 ppm (dynamic) exposure group cloudy corneas were an additional finding which was not reversible within 14 days post exposure (Nachreiner and Dodd, 1989).

Nasal and eye irritation and respiratory difficulty occurred in Alderley-Park rats (2 males, 2 females) following a single inhalation exposure (5 h) to a saturated AA atmosphere (19 mg/l, 6,000 ppm). Rats (4 of either sex) exposed (6 h/d) to AA at 1,500 ppm on 4 consecutive days showed nasal discharge. Rats (4 males, 4 females) exposed (6 h/d, 5 d/wk) to 300 ppm AA for 4 weeks showed some nasal irritation. No toxic sign was seen in rats (4 males, 4 females) exposed to 80 ppm AA following the same exposure regime (Gage, 1970).

In a whole body inhalation study groups of 15 $B6C3F_1$ female mice were exposed (6 or 22 h/d) to 0, 5 or 25 ppm AA for 2 weeks. One group of animals was designated for exposure at 25 ppm for 4.4 h to provide for a concentration x time (C x T) product equal to 5 ppm for 22 h. Upon termination of exposure the nasal cavity was collected from 10 animals per group. The remaining 5 animals per dose group were retained for a 6 weeks recovery period prior to histopathological analysis. At 5 ppm for

6 h/d no adverse effect resulting from this exposure was detected on histopathological examination. At 25 ppm for 22 h/d a high incidence of severe olfactory damage was observed in the dorsal meatus region extending onto the ethmoid turbinates. In the recovery animals of this dose group the damaged epithelium was replaced by resistant respiratory-like epithelium. In the other 3 groups (5 ppm x 22 h/d, 25 ppm x 4.4 h/d and 25 ppm x 6 h/d) where a very similar C x T product (110, 110, 150 ppm -h/d) was administered the olfactory disorganisation observed at the termination of the exposure period was fully reversible at the end of the 2 weeks recovery period (Lomax *et al*, 1994).

In a 13-week study F344/N rats and B6C3F$_1$ mice (15 animals/sex/group/species) were exposed (6 h/d, 5 d/wk) to AA at concentrations of 0, 5, 25 and 75 ppm. Histopathological examination of the nasal mucosa showed slight focal degeneration of the olfactory epithelium in the higher dosed rats only. In the mouse study the nasal change was seen in all exposure groups (concentration-dependent). Additionally in the high concentration group slight cell infiltration, slight hyperplasia of the submucosal glands and in some cases replacement of olfactory epithelium by respiratory epithelium were observed. The NOEL was 25 ppm for the rat and < 5 ppm for the mouse (Miller *et al*, 1981a).

The different "sensitivities" to toxic effects of AA on the nasal mucosa of rats and mice seen by Miller *et al* above (NOEL rat 25 ppm, mice < 5 ppm) can be explained by differences in dosimetry. Buckley *et al* (1984) and Barrow *et al* (1987) describe studies designed to determine the dose of AA delivered to the nasal passages of mice and rats exposed to 75 ppm. After 4 exposures, the breathing rate and tidal volumes were measured on the fifth day and the delivered dose (µg/min/cm^2) calculated from the results for these parameters and the surface area of the nasal passages (2.77 cm^2 for mice and 13.44 cm^2 for rats). It was found that the delivered dose was roughly 2 times higher for the mouse than for the rat, supporting the above view that the differences in effect might be related to differences in dosimetry, rather than to species-specific sensitivities. While sensory irritants can reduce respiratory rate and minute volume to different degrees in different species, AA induced a similar reduction in both rats and mice. The differences in dosimetry were, therefore, a reflection of the differences between rats and mice with respect to respiratory parameters and the surface area of the nasal passages.

8.3.4 Gastrointestinal Tract Irritation

AA was administered orally to Sprague-Dawley rats (4 males/group) at concentrations of 0.08, 0.8, 8 or 20 % (w/v) in methylcellulose (0.5% aq.) in a constant volume of 5 ml/kgbw, equivalent to doses of 4, 40, 400 and 1,000 mg/kgbw. The animals were killed 1 hour after dosing and a histopathological examination was performed. Doses higher than 0.8% had caused an increase in weight of both the glandular and forestomach accompanied by oedema and haemorrhage (De Bethizy *et al*, 1987).

Four hours after oral administration of AA (144 mg/kgbw, 3% in corn oil) to 7 male F344/N rats a slight increase in stomach size and superficial mucosal necrosis in the glandular stomach were observed in 2 animals. The local irritation was not increased in severity by increasing the dose to 290 mg/kgbw or following the observation period of 24 hours (Ghanayem *et al*, 1985).

Wistar rats (10 males, 10 females/group) were administered, by gavage (5 d/wk), AA dissolved in water (3% and 7% in a constant volume of 5 ml) at doses of 150 or 375 mg/kgbw for 3 months (equivalent to daily doses of 2,000 or 5,000 mg AA/l drinking water). Pathological examination revealed a dose-dependent pronounced irritation in both glandular and forestomach, including thickening of the plica marginata, hyperaemia or bloody erosions/ulcerations of the gastric mucosa. A NOEL was not determined (Hellwig *et al*, 1993).

In a parallel study Wistar rats (10 males and females/group) were given drinking water containing 120, 800, 2,000 and 5,000 mg AA/l (equivalent to respective doses of approximately 9, 61, 140 or 331 mg/kgbw/d) for 3 months. There were no gross or histopathological changes attributable to AA exposure (Hellwig *et al*, 1993) (Section 8.5.1).

The results of the studies of Hellwig *et al* clearly demonstrate a difference in the tissue response with local tissue concentrations of AA produced by varying the mode of administration. Bolus exposure produced pronounced irritation on the glandular and forestomach at 150 and 375 mg/kgbw, whereas an equivalent daily exposure in drinking water produced no histopathological changes in the stomach.

8.3.5 Skin Sensitisation

AA (0.1 ml of a 20% aqueous solution) was applied (1 x/d) to the skin of guinea pigs until definite skin irritation occurred. A challenge application was performed 11 days later with a 2% aqueous AA solution. No evidence of skin sensitisation was seen 8, 12 and 24 hours after the challenge. The AA was considered pure but no analytical data were provided (BASF, 1958).

In a comparative study using the Guinea Pig Maximisation Test (GPMT) and the Landsteiner Draize Test (LDT) the sensitisation potential of AA was tested in female guinea pigs. For the GPMT intradermal (i.d.) injections of AA and Complete Freund's Adjuvant (CFA) and AA in CFA were performed on the back of the animals followed 1 week later on the same site by a topical application of AA in petrolatum. After a 2 weeks rest period a 24 hours challenge application of AA at a non-irritating concentration was applied on one flank. The reading of the skin reactions performed 24 and 48 hours after the removal of the patch showed a positive reaction in 21 out of 25 animals. For the LDT 10 injections of AA (0.1 ml, 0.1% in saline) were performed in the back of the animals. Two weeks

after the last injection an i.d. injection of 0.05 ml AA (0.1% in saline) was made in a fresh skin area. One out 25 animals showed a positive reaction. According to the grading used, AA was considered to be an extreme sensitiser by the GPMT and a weak sensitiser by the LDT. The AA was considered pure but no analytical data were provided (Magnusson and Kligman, 1969).

AA (0.1 ml) of the highest non-irritating concentration was applied (4 x/10 d) to the skin of male guinea pigs. After the third application, an i.d. injection of 0.2 ml CFA was given at one point adjacent to the application site. When challenged 2 weeks after the last application none of the 10 animals showed evidence of skin sensitisation. The AA was considered pure but no analytical data were provided (Rao *et al*, 1981).

A group of 6 guinea pigs (of either sex) was injected (4 x) subcutaneously (s.c.) with 0.1 ml of a mixture of AA (2 mg/ml) and FCA in the footpad and once in the nape of the neck. One drop (0.02 ml) of AA (5% in acetone/olive oil 4/1) was applied (1 x/wk) to the shaved flank of the animals for up to 12 weeks (Polak method). Three out of 6 guinea pigs exhibited a weak positive reaction. The AA was considered pure but no analytical data were provided (Parker and Turk, 1983).

A positive reaction was seen in female guinea pigs treated with a sample of AA which "contained an impurity of 45%" as analysed by GC. When challenged with the same concentration of AA purified by distillation none of the animals showed a positive skin reaction (Van der Walle *et al*, 1982). The positive reaction was attributed to α,β-diacrylopropionic acid (DAPA) which, historically, was present as an impurity in non-distilled commercial AA (Waegemaekers and Van der Walle, 1984).

8.3.6 Evaluation

AA is irritating and/or corrosive to the skin, eyes, respiratory tract and gastrointestinal tract. Damage to the eye may be irreversible. Repeated inhalation exposure produced sensory irritation and degeneration of the nasal mucosa (predominantly the olfactory epithelium), with a minor hyperplasia of the submucosal glands. Depression of respiration has also been reported in both rats and mice (respective RD_{50} 513 ppm and 685 ppm).

Neat AA or any technical AA prepared by a process which includes distillation is not a skin sensitiser. The sensitisation potential previously attributed to AA was due to the presence of an impurity, α,β-diacryloxypropionic acid.

8.4 GENETIC TOXICOLOGY

In vitro genetic toxicology assays are routinely used as the first screen for genotoxic activity of chemicals. These assays, however, provide information only on the intrinsic potential of these chemicals to cause damage to the DNA. To determine whether or not this intrinsic potential is expressed in whole animals it is therefore necessary to conduct *in vivo* genetic toxicology assays which take account of absorption, distribution, metabolism and excretion of the chemical and its metabolites. The results of *in vivo* assays therefore often overrule results obtained *in vitro*.

8.4.1 *In Vitro* Bacterial Gene Mutation Assays

AA has been tested both in the presence and absence of auxiliary metabolic activation (S9-mix) in a number of Ames bacterial gene mutation assays which have reproducibly shown that it is not mutagenic to bacteria even when tested up to cytotoxic concentrations.

Lijinsky and Andrews (1980) tested AA in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 using a range of concentrations up to 1,000 µg/plate and 250 µg/plate in standard plate incorporation and liquid pre-incubation assays respectively. Both assays were conducted in the presence and absence of both Aroclor-1254 induced rat and hamster liver S9-mix. Although negative responses were reported in all strains no cytotoxicity was observed at the concentrations tested. The study is not defendable because of the range of concentrations was inadequate being below the cytotoxic dose.

Zeiger *et al* (1987) reported AA as non-mutagenic in 2 pre-incubation assays using *S. typhimurium* strains TA1535, TA1537, TA98 and TA100. AA was tested over a range of concentrations from 3.3 to 3,333 µg/plate in the presence and absence of both Aroclor-1254 induced rat and Syrian hamster liver S9-mix. No significant increases in the numbers of revertant colonies were observed at concentrations some of which resulted in cytotoxicity.

Cameron *et al* (1991) reported AA as non-mutagenic in a standard plate incorporation assay using *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 in the presence and absence of both Aroclor 1254-induced rat and Syrian hamster liver S9-mix. AA was tested over a range of concentrations between 33 and 5,000 µg/plate; cytotoxicity was observed at the highest dose level but there were no significant increases in the number of revertant colonies.

Oesch (1977) tested AA in a plate incorporation assay over a range of concentrations from 3.1 to 1,000 nl/plate (3.26-1,051 ng/plate) using *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 in the presence and absence of rat liver S9-mix. No mutagenic or cytotoxic effects were observed. Based on the hypothesis that AA may be metabolised via an epoxide metabolite, AA was retested in *S. typhimurium* strain TA98 in the presence of 1,1,1-trichloropropene-2,3-oxide, an epoxide hydrolase inhibitor. Again no mutagenic or cytotoxic effects were observed (Oesch, 1977).

Negative results for AA have also been reported for an Ames bacterial gene mutation assay conducted by Litton Bionetics (1979) over a range of concentrations from 0.005 to 10 µl/plate (0.0053-10.5 µg/plate) using *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 in the presence and absence of rat liver S9-mix. Cytotoxicity was observed but there were no significant increases in the number of revertant colonies.

8.4.2 *In Vitro* Mammalian Cell Gene Mutation Assays

AA has been tested in both the CHO/HGPRT and L5178Y $TK^{+/-}$ mammalian cell gene mutation assays and has been shown to induce increases in numbers and frequencies of mutant colonies.

AA was reported as non-mutagenic when tested (5 h) in the CHO-K_1-BH_4/HGPRT mutation assay at a range of concentrations from 0.3 to 1.9 µl/ml (0.32-2.0 µg/ml) and 1.0 to 2.4 µl/ml (1.05-2.52 µg/ml) in the absence and presence of Aroclor-1254 induced rat liver S9-mix respectively. All concentrations of AA were adjusted to pH 7.5 before addition to the cell cultures. Dose-related cytotoxicity was observed but there were no significant increases in absolute counts or frequencies of mutant colonies in the treated cultures (McCarthy *et al*, 1992). This assay type is now generally considered to be insensitive to genotoxic agents. The same data was previously reported by Yang (1988).

Moore *et al* (1988) reported dose-dependent increases in numbers and frequencies of mutant colonies when AA was tested over a range of concentrations up to 600 µg/ml in the mouse lymphoma L5178Y $TK^{+/-}$ assay in the absence of S9-mix. Dose-related cytotoxicity was observed and the majority of induced mutant colonies were classified as small colonies which are considered to be indicative of a clastogenic (chromosome breakage) mechanism of action. These data were confirmed in a repeat experiment and are also referred to by Moore and Doerr (1990). The authors do not state if there was any correction for pH in these assays.

Comparable results have been reported by Cameron *et al* (1991) who tested AA in mouse lymphoma L5178Y cells in the presence and absence of Aroclor-1254 induced rat liver S9-mix. AA was tested over a range of concentrations between 1.62 and 5.44 x 10^{-3} M (117 and 392 µg/l) in the absence of

S9-mix and between 4.41 x 10^{-3} and 2.65 x 10^{-2} M (318 and 1,890 µg/l) in the presence of S9-mix. Dose related cytotoxicity was observed as were increases in the number and frequencies of mutant colonies in the presence and absence of S9-mix. The assay was not repeated and the authors do not state if there was any correction for pH.

8.4.3 *In Vitro* Chromosome Damage Assays

AA has been tested in a series of *in vitro* chromosomal aberration assay and in the definitive assays has been shown to induce chromosomal aberrations *in vitro*.

AA has been reported as positive in a cytogenetic assay in Chinese Hamster Lung (CHL) cells (Ishidate *et al*, 1988). AA induced significant increases in the frequencies of chromosomal aberrations following treatment at 750 µg/ml in the absence of S9-mix for periods of 24 and 48 hours. The report does not state if there was any correction for pH.

Wiegand *et al* (1989) tested AA in a micronucleus assay in Syrian Hamster Embryo (SHE) fibroblasts. AA was reported to be negative when tested up to 10 µg/ml in the absence of S9-mix only; SHE cells were reported to possess intrinsic metabolic capacity. However, no measure of cytotoxicity was provided in the data and so the maximum concentration tested cannot be adequately defended.

Moore *et al* (1988) have reported AA as positive in a cytogenetic assay in mouse lymphoma L5178Y cells following a 4-hour treatment period in the absence of S9-mix. AA was tested at concentrations of 300, 450 and 500 µg/ml but the authors do not state if there was any correction for pH. Dose-related increases in chromosomal aberrations were recorded in this single assay.

McCarthy *et al* (1992) tested AA in CHO-K_1 cells in the presence and absence of Aroclor-1254 induced rat liver S9-mix. CHO cultures were treated for a period of 4 hours at a range of concentrations of AA (adjusted to pH 7) from 2,846 to 5,000 µl/ml and 1,615 to 2,846 µl/ml in the absence and presence of S9-mix respectively. Dose related increases in chromosomal aberrations were observed both in the presence and absence of S9-mix. These data were previously reported by McCarthy *et al* (1988).

8.4.4 Other *In Vitro* Genotoxicity Endpoints

Wiegand *et al* (1989) tested AA in an unscheduled DNA synthesis (UDS) assay and a cell transformation assay in SHE cells (Section 8.4.3). Although both assays showed no genotoxic activity of AA the data cannot be interpreted due to many deficiencies in the studies.

McCarthy *et al* (1992) tested AA in a UDS assay in isolated primary rat hepatocytes. The hepatocyte cultures were treated with AA (adjusted to pH 7.2-7.4) at a range of 8 concentrations between 0.01 and 0.6 µl/ml (0.01 and 0.63 µg/ml) for a period of 18 to 20 hours. Dose related cytotoxicity, as measured by release of lactic acid dehydrogenase, was observed in the absence of any induction of UDS. These data were previously reported by Curren (1988).

AA has been reported to form 2-carboxyethyl adducts when incubated in the absence of S9-mix for 40 days with 2'-deoxynucleosides or calf thymus DNA (Segal *et al*, 1987). The relevance of the results of this study is questionable because appropriate control treatments were not conducted and the 2-carboxyethyl adducts were formed after an exceptionally long treatment period.

In contrast to the results of Segal *et al* (1987), Frederick and Reynolds (1989) found via molecular modelling that incubation of the negatively charged acrylate anion with 2 representative nucleophiles, methylamine and imidazole, would not be expected to result in the formation of adducts of the acrylate ion on the nucleophile. The formation of Michael products via the non-ionised form of AA was investigated as an alternative and was found to be theoretically possible. In the opinion of the authors, this is unlikely to occur *in vivo* because of the rapid metabolism and excretion of AA.

Klimkina *et al* (1988) published a paper on the effect of acrylates on primary immune response and mutagenesis under experimental conditions but this publication was not available for review.

8.4.5 *In Vivo* Chromosome Damage Assays

Groups of 5 male and 5 female Sprague-Dawley rats were treated with a single oral dose of AA in water (dose levels of 100, 333 or 1,000 mg/kgbw) and the bone marrow was sampled at 6, 12 and 24 hours post-dose. Other groups of 5 male and 5 female rats were given AA in drinking water *ad libitum* at concentrations of 2,000 or 5,000 mg/l for a period of 5 days with the bone marrow again being sampled at 6, 12 and 24 hours post-dose. The maximum dose levels used in these 2 assays were based on previous experience with AA, body weight decreases, clinical observations and mortality observed in a preliminary study. No reduction of mitotic index or any increase in chromosomal aberrations was observed in the animals treated with AA via either the acute or sub-acute dosing regime (McCarthy *et al*, 1992). These data were previously reported by McCarthy *et al* (1988).

8.4.6 Dominant Lethal Assay

Male CD-1 mice received either a single oral dose of AA dissolved in water (adjusted to pH 6) at 32, 108 or 324 mg/kgbw or 5 doses of 16, 54 or 162 mg/kgbw every 24 hours. The dose levels were based on preliminary toxicity tests to determine body weight changes, mortality, mating index and fertility index. No dominant lethal effects were observed in the female mice mated with the treated males (McCarthy *et al*, 1992).

8.4.7 *Drosophila* Sex-Linked Recessive Test

McCarthy *et al* (1992) tested AA in a *Drosophila melanogaster* sex-linked recessive lethal (SLRL) test. The AA was administered by feeding with 2% AA (in 5% sucrose, pH 6) for 3 days and by injection (0.3 µl/fly) at base of the wing with 2% AA (in 0.7% saline, pH 6). The maximum dose level was determined from the results of previous studies. No significant increases in sex-linked recessive lethal mutations were observed. The assay system was validated using N-nitrosodimethylamine as a positive control.

8.4.8 DNA Damage/Adducts *In Vivo*

Sagelsdorff *et al* (1988) conducted preliminary studies to explore the potential of AA to bind to DNA *in vivo*. Two male Sprague-Dawley rats received a single oral dose of 233 or 257 mg/kgbw of a solution of [2,3-^{14}C]AA (specific radioactivity 1.1 mCi/mmol [15.3 µCi/mg]) in a buffered (pH 5.5) aqueous solution. All of the radioactivity present in the DNA co-eluted with the natural nucleotides. On this basis the authors concluded that no DNA adducts were observed in the livers or stomachs of these rats 24 hours after administration. Groups of 2 female ICR mice were treated topically (13 and 15 mg AA/mouse on a shaved skin area of 8 cm^2) with 13-15% unbuffered solutions of [2,3-^{14}C]AA in acetone. Some of the mice received a 7 day pretreatment with a 5% unlabelled solution of AA. DNA labelling was observed in the skin (epidermis) of these mice 24 hours after administration of the radiolabelled AA and was slightly greater in the mice without pretreatment. Based on the observation that parts of the radioactivity did not co-elute with the natural nucleotides, the authors concluded that DNA adduct had been formed. However, no attempt was made to identify the chemical identity of the "adducts". In addition, due to the limitations of the experimental procedure, it was not possible to determine whether or not the binding occurred in DNA of viable or non-viable cells. The biological significance of these findings cannot therefore be determined.

8.4.9 Summary and Evaluation

AA is not mutagenic to bacteria when tested up to cytotoxic concentrations. Neither does it induce any significant increases in mutant colonies in a CHO/HGPRT assay, although this assay is now generally considered insensitive to the detection of *in vitro* genotoxic compounds. The data from a series of *in vitro* mammalian cell gene mutation and chromosomal aberration assays are contradictory and confounded by the potential problems of artifactual gene mutation and chromosomal damage that can be induced in such *in vitro* genetic toxicology assays by changes in pH (Scott *et al*, 1991). However, the *in vitro* cytogenetic assay conducted by McCarthy *et al* (1992) clearly showed AA to be an *in vitro* clastogen even when the dosing solutions were neutralised prior to treatment of the cells. This result would therefore tend to support the observations by Moore *et al* (1988) and Cameron *et al* (1991) that AA induces L5178Y small colony mutants.

AA did not induce chromosomal aberrations, dominant lethal mutations or sex-linked recessive lethal mutations in a series of *in vivo* tests, confirming that the chromosomal damage induced by AA *in vitro* is not expressed in the whole animal.

8.5 CHRONIC TOXICITY AND CARCINOGENICITY

8.5.1 Oral

Groups of 20 male and 20 female Wistar rats were given AA in drinking water at concentrations of 0, 120, 800, 2,000 or 5,000 mg AA/l (equivalent to doses of 0, 9, 61, 140 or 331 mg/kgbw/d) for 12 months. Reduced water intake and/or food consumption and reduced body weight gains were observed in the 2,000 and 5,000 mg/l dose groups. Reduced water intake was attributed to palatability problems with AA. There were no treatment related changes in organ weights. Some statistically significant differences were observed in urinalysis and haematology. These effects were marginal, inconsistent or lacking a dose response relationship and were not regarded as treatment related. Gross and histopathological examination of the tissues and organs revealed no changes attributable to AA exposure. The NOEL for this study was 800 mg/l (140 mg/kgbw/d) (Hellwig *et al*, 1993).

In a drinking water carcinogenicity study, groups of 50 males and 50 females Wistar rats were administered AA via their drinking water at concentrations of 0, 120, 400 or 1,200 mg/l (equivalent to 0, 8, 27 and 78 mg/kgbw/d) for 26 (males) or 28 (females) months. Water consumption in both sexes from the 120 and 400 mg/l group showed a marginal increase in most animals. In the 1,200 mg/l group water intake was slightly reduced, in both sexes from day 42 onwards. Mean body weights of the

treated males were almost the same as the controls, whereas in females, mean body weights were higher than controls. There were no clinical signs of toxicity and no increase in mortality in any of the treatment groups. No haematological changes attributable to AA exposure were observed. With the exception of a slight increase in hepatocellular fatty deposits where it was not possible to exclude the involvement of AA, no histopathological changes attributable to AA exposure were observed. The incidence and organ distribution of tumours in the groups treated with AA did not differ from the controls (Hellwig *et al*, 1993).

8.5.2 Dermal

AA (25 µl of a 1% solution in acetone) was applied (3x/wk) to the shaved skin of the back of 40 male C3H/HeJ mice for their life-time. Aliquots of 25 µl of acetone and 25 µl of 0.1% 3-methylcholanthrene in acetone were applied as vehicle and positive control respectively. No epidermal tumours were observed in either the AA or acetone exposed groups. In the positive control group, 39 of the 40 exposed mice had tumours, 33 of which were malignant epitheliomas. There were no effects on survival and no gross signs of irritation that could be attributed to AA exposure. One AA treated mouse displayed epithelial hyperplasia (DePass *et al*, 1985).

In a submission to the US-EPA, the Hoechst-Celanese Corporation reported the results of a chronic skin painting study with AA in C3H and ICR strains of mice (50 males and 50 females/strain). The mice were treated with 25 or 100 µl of 1% AA in acetone for either 6 weeks or approximately 21 months. Mice treated for 6 weeks only were retained for the 21 months period with the skin being shaved at the same intervals as for those dosed throughout the 21 months period. There was no evidence of skin tumours in either strain of mouse. In the male C3H and both sexes of the ICR mouse there was no evidence of tumours in any internal organs. In female C3H mice a statistically significant increase over concurrent control values for lymphosarcomas was observed. However, the concurrent control group had an unusually low incidence (0%) of lymphosarcomas. A re-evaluation of this study (Frith, 1991) concluded that the increased incidence seen in the study was a "spurious finding" and was not related to the administration of AA for the following reasons: (i) no relationship was found between non-neoplastic lesions and dose, (ii) lymphomas were found in the con-current control groups, whereas the original report reported none, (iii) the spontaneous frequency of lymphosarcomas reported in the literature in untreated female C3H mice over 18 months old was approximately 10%, similar to the results observed in the high dose group of the EPA reported study (Cote *et al*, 1986b).

In a study reported in abstract form only the possibility of AA being a complete carcinogen and/or a 2-stage promoter was investigated (Cote *et al*, 1986a). AA in acetone was applied (3 x/wk) to the shaved skin of the backs of 30 female ICR/Ha mice for 1.5 years. The exact dose is unclear but was probably

1 mg AA/0.1 ml acetone (Sivak, 1986, below). Additional groups were treated (3 x/wk) with 7,12-dimethylbenzanthracene (DMBA) and then with AA (single dose of 20 μg DMBA/0.1 ml acetone followed by 1 mg AA/0.1 ml acetone) or with 0.1 ml acetone. At the end of the study, the AA only group contained 2 epithelial cell carcinomas; the DMBA initiated group, promoted with AA showed 3 papillomas and 1 epithelial cell carcinoma and the third group, initiated with DMBA and then promoted with acetone showed no tumours. On the basis of these data the authors concluded that AA was a weak complete carcinogen. In the summary that accompanied the abstract, it was suggested that there was also an increase of leukaemia in the AA treated animals (Cote *et al*, 1986b). An independent review of the study was conducted by A.D. Little Inc. (Sivak, 1986) who concluded that the results were invalid for the following reasons. There was no written protocol for the study. Descriptions of the dose employed in the study were inconsistent being described at different times as 4.0 mg AA/0.1 ml acetone; 4 mg AA/0.25 ml acetone and 1 mg AA/0.1 ml acetone. The results of pilot studies on skin toxicity mentioned in the original abstract were not available. There was a lack of histopathological data for the leukemias. Since Sivak did not confirm the incidence and distribution of tumours it is suggested that they were not treatment related.

8.5.3 Injection

A group of 30 female Hsd:(ICR)Br mice received 20 μmol (1.4 mg) AA in 0.5 μl trioctanoin (glycerol tricapronate) by s.c. injection (1 x/wk) for 52 weeks and was then observed for a further 93 days. A control group was injected with trioctanoin only. Two sarcomas were observed in the AA treated group, the first being observed after 323 days (49.5 weeks). There were no tumours in the control group (Segal *et al*, 1987). The relevance of this route of administration for human carcinogenic risk assessment is questionable since Grasso (1987) for example induced local sarcomas with repeated injections of distilled water or saline.

In an experiment to determine the possible induction of hepatic ornithine decarboxylase activity (considered to be correlated with tumour-promoting activity), male Wistar-derived rats were injected i.p. with 0. 0.8, 1.6 or 2.4 mg AA/kgbw in DMSO. No increase in hepatic ornithine decarboxylase activity was observed (Van de Zande *et al*, 1986).

8.5.4 Inhalation

No studies have been reported of the carcinogenic activity of AA following inhalation exposure. Frederick *et al* (1994) demonstrated that the simple acrylate esters are rapidly hydrolysed to AA and the corresponding alcohol in the respiratory tract. This being the case, the inhalation studies conducted on methyl and butyl acrylates in the rat, at atmosphere concentrations of 0, 5, 25 or 75 ppm for 2 years

(Reininghaus *et al*, 1991) and on ethyl acrylate in both the rat and the mouse, at atmosphere concentrations of 0, 5, 25 or 75 ppm for 2 years and 225 ppm for 6 months followed by a 21 month observation period (Miller *et al*, 1985) are relevant to the assessment of the chronic toxicity of AA. With all these esters, high exposure produced evidence of irritation and degeneration of the olfactory mucosa, similar to those seen in the 13-week inhalation study with AA (Section 8.2.2: Miller *et al*, 1981a). No tumours developed with any of the 3 esters.

8.5.5 Evaluation

AA was not carcinogenic in the rat following oral exposure (drinking water). In one, poorly conducted and reported mouse dermal study a weak carcinogenic response was attributed to AA exposure. However, in other well performed dermal studies, no such carcinogenic effects were observed, either on the skin or systemically. No studies are available addressing the inhalation route of exposure, however by analogy with the AA esters (which de-esterify in the nasal epithelium) AA is not expected to be carcinogenic by this route of exposure.

8.6 REPRODUCTIVE TOXICITY, EMBRYOTOXICITY AND TERATOGENICITY

8.6.1 Oral

F334/N rats (10 males, 20 females) received AA (purity >99%) in their drinking water at dose levels of 0, 83, 255 or 750 mg/kgbw/d for 13 weeks. Each male was then mated with 2 females, and AA exposure continued for both sexes until the end of lactation. Dose related reductions in body weight gain and in food and water consumption were observed in the F_0 animals from the 250 and 750 mg/kgbw/d groups. Treatment related effects were restricted to the 750 mg/kgbw/d group. Pups of both sexes showed decreased body weight gain. Reduction in absolute and relative liver weights was observed in both sexes, however no histological changes were observed in the livers. Female pups also showed a reduction in both absolute and relative spleen weights, again no histological changes were apparent. In the high dose group the fertility index of the F_0 generation, the number of pups born alive and the percentage of pups weaned was reduced (DePass *et al*, 1983). Although the authors report that the effects on fertility index were not statistically significant, the fertility index and litter size of the control group were atypically low.

AA was administered in drinking water continuously throughout the study to Wistar rats at doses of 0, 500, 2,500 and 5,000 mg/l. Twenty five males and 25 females in the F_0 and F_1 parental generation were mated 70 days after the beginning of treatment and the F_2 litter was killed 21 days after birth. The

amount of AA administered during premating F_0/F_1 parental generation was approximately 53 mg/kgbw/d in the 500 mg/l group, 240 mg/kgbw/d in the 2,500 mg/l group and 460 mg/kgbw/d in the 5,000 mg/l group. Dose-dependent signs of general toxicity resulting in reduction in food and water uptake, lower mean body weights were observed in the F_0 at 5,000 mg/l and in the F_1 parental generation at 2,500 mg/l dose levels. Gross and histopathological changes in the fore and glandular stomach indicative of the irritating reactions were observed in the higher dose group animals. No adverse substance related effects were observed at 500 mg/l. Signs of developmental toxicity (retarded growth) were exhibited in the F_1 and F_2 pups of the parental groups of 5,000 mg/l and less pronounced at 2,500 mg/l. The authors concluded that the NOAEL for reproductive function was 5,000 mg/l (460 mg/kgbw/d) in 2 successive generations. The NOAEL concerning general toxicity was 2,500 mg/l (240 mg/kgbw/d) for the F_0 generation and 500 mg/l (53 mg/kgbw/d) for the F_1 parental generation and F_1 and F_2 offspring. The NOEL for reproduction was determined at 255 mg/kgbw/d (Hellwig *et al*, 1993).

8.6.2 Inhalation

Groups of 20 pregnant Sprague-Dawley rats were exposed (6 h/d) to atmospheres containing AA at 0, 40, 120 and 360 ppm on days 6 to 15 of gestation. After exposure the dams were observed up to day 20 of gestation. Irritation of the respiratory tract and the eyes was observed in the 360 ppm exposed animals. A dose related reduction in the intake of food and water resulting in a decrease in bodyweight gain was observed in the exposed dams. This was statistically significant in females exposed at 360 ppm and in females exposed at 120 ppm when corrected for uterus weight. The slight effects observed in animals exposed at 40 ppm is indicative of a slight maternal toxicity. No effects on reproductive performances and on the foetuses were observed. It was concluded that at concentrations inducing maternal toxicity in Sprague-Dawley rats (40 to 360 ppm) AA showed no adverse effects on reproduction. The NOEL for reproductive effect was 360 ppm, the highest dose tested (Klimisch and Hellwig, 1991).

Pregnant New Zealand white rabbits (16/group) were exposed (6 h/d) to 25, 75 and 225 ppm AA vapours during days 6-18 of gestation. The overall pregnancy rate was equivalent for all groups and ranged from 94-100%. Exposure related clinical signs were observed in the 225 ppm group and periodically in the 75 ppm group. The clinical signs were perinasal/perioral wetness and nasal congestion. Reduced bodyweight gain and food consumption were observed in the 75 and 225 ppm exposure groups. Ulceration of the nasal turbinates was observed at scheduled sacrifice in one animal from the 225 ppm group. No dose related effects were recorded in the 25 ppm group animals. No dose related effects were observed in the reproduction function of the dams. No evidence of developmental toxicity including teratogenicity were observed at any dose levels in the offspring. The

NOEL for maternal toxicity was 25 ppm. The NOEL for this study for developmental effects was 225 ppm the highest dose tested (Neeper-Bradley and Kubena, 1993).

8.6.3 Other routes of exposure

Groups of 5 pregnant female Sprague-Dawley rats were injected i.p. with 0, 2.3, 4.5 or 7.5 µl AA/kgbw (0, 2.4, 4.7 or 7.9 mg/kgbw) on day 5, 10 and 15 of gestation. Foetuses from all treatment groups were smaller than controls. Foetuses from the high and medium dose groups showed dose dependant gross and skeletal abnormalities including heamangiomas of the neck and/or at different places of the body, elongated and fused sternebrae and frontal ribs. In the high dose group three foetuses were found dead (6% of the total). In the low dose group no gross abnormalities were observed but 3 of 31 obtained foetuses had elongated and fused sternebrae and frontal ribs (Singh *et al*, 1972). This study is difficult to interpret; the route of exposure is not a relevant route of exposure which may bypass significant metabolic/detoxification pathways. In addition, the control group treated with distilled water, normal saline and cotton-seed oil also showed gross and skeletal abnormalities.

Laparotomies were carried out on pregnant Sprague-Dawley rats on day 13 of pregnancy under anaesthetic and half of the developing foetuses were injected with 10, 100 or 1,000 µg AA/foetus. The foetuses were examined for compound related effects on day 20 of gestation. One foetus injected with 100 µg AA showed slight hydrocephalus and micrognathia. Doses of 10 or 100 µg/foetus showed no foetotoxic effects. A dose of 1,000 µg/foetus resulted in 78% resorptions (Slott and Hales, 1985). Studies of this type represent grossly atypical routes of potential human exposure, and are not relevant for human risk assessment.

8.6.4 Evaluation

In 2 reproductive studies in drinking water, AA produced some signs of foetotoxicity predominantly decreased bodyweight gain, following the exposure of the parental generation. Gross and histopathological changes were seen in the fore and glandular stomach of animals exposed to 460 mg/kgbw/d. No gross abnormalities were observed in the offspring in either study. A NOAEL for reproductive function was established at 5,000 mg/l (460 mg/kgbw/d) in 2 successive generations. A NOAEL for general toxicity was established at 2,500 mg/l (240 mg/kgbw/d) for the F_0 generation and 500 mg/l (53 mg/kgbw/d) for the F_1 generation.

Inhalational exposure of pregnant rats and rabbits to atmospheres containing AA at concentrations upto 360 ppm (rats) and 225 ppm (rabbits), produced no evidence of developmental toxicity in either

species. Maternal toxicity was observed in the low dose groups in both species (40 ppm in rats and 25 ppm in rabbits).

8.7 NEUROTOXICITY

The inhibition of Creatine Kinase (CK) activity of the brain of male Wistar rats was tested, *in vitro* and *in vivo*, in a comparative study with acrylamide. The *in vitro* activity of CK (µmol/mn/mg protein) was determined by incubation (30 min at 37°C) of homogenised central hemisphere with solutions of different concentrations of AA (0.1, 0.2, 0.4, and 0.8% neutralised with NAOH). The results showed a dose dependent decrease of CK activity. *In vivo*, 50 mg/kgbw AA (in isotonic and neutralised saline) was injected i.p. to rats for 8 consecutive days. The animals were killed 24 hours after the last injection. The brain was dissected to isolate the cortex, striatum, hypothalamus, hippocampus, midbrain, medulla oblonga, cerebellar hemisphere and cerebellar vermis. Each brain region was homogenised and the CK activity (µmol/mn/mg protein) determined. Except for mild suppression of aspartase aminotransferase (ASAT) in hippocampus, no suppression of CK, lactate dehydrogenase (LDH) or ASAT activities was found in any brain region of rats treated with AA (Kohriyama *et al*, 1994). The absence of modified brain Creatine Kinase activity is in good agreement with the lack of any nervous system effects seen in all short and long-term toxicity studies described in the previous sections.

9. EFFECTS ON HUMANS

9.1 ACUTE AND SUBCHRONIC TOXICITY

No data are available.

9.2 IRRITATION AND SENSITISATION

9.2.1 Eye and Respiratory Tract Irritation

Undiluted AA is corrosive to the human eye and mucous membranes (Gosselin *et al*, 1976; Kühn-Biret, 1980). In the occupational setting AA is irritant to eyes, upper respiratory tract and gastrointestinal tract (Rycroft, 1977).

9.2.2 Skin Irritation and Sensitisation

Undiluted AA is corrosive to the skin (Gosselin *et al*, 1976; Kühn-Biret, 1980). During 48-hour covered patch tests AA (0.1% in petrolatum) proved non-irritant to most subjects. Of 6 workers with occupationally induced contact allergic dermatitis to various acrylate and methacrylate esters none reacted to 0.1% AA in petrolatum in a 48-hour diagnostic patch test (Condé-Salazar *et al*, 1988).

A laboratory worker developed acute generalised urticaria while working with acrylic resins and AA. Immediate hypersensitivity testing yielded a severe local reaction to AA. Reexposure in the workplace to vapours of AA resulted in generalised urticaria. The purity of the AA was not specified (Fowler, 1990).

9.2.3 Summary and Evaluation

No deaths or serious health effects have been reported in humans exposed to acute doses of AA. The main potential for human exposure to AA is by the dermal and inhalation routes. The pungent, characteristic odour is acting as a warning (threshold 0.094 ppm, Table 1) and the irritating properties act as a deterrent to repeated exposure.

Undiluted AA is corrosive to the skin, eyes and mucous membranes. Industrial exposure to atmospheres containing AA may produce irritation to the eyes, upper respiratory tract and gastrointestinal tract. Based on animal experiments (Section 8.3) and present human experience, pure

AA does not appear to be a skin sensitiser. Current occupational exposure limit values protect against potential adverse health effects.

9.3 HAZARD ASSESSMENT

The critical effects of AA are the nasal irritation and olfactory epithelium degeneration observed in mice and rats in a 13-weeks study (Section 8.2.2-3: Miller *et al*, 1981a). Mice were more affected than rats, the difference being a sequel of differences in dosimetry. Barrow *et al* (1987) were able to predict from air flow rates and nasal cavity surface area that the delivered dose ($\mu g/min/cm^2$) in mice is 88% higher than in rats (Section 8.3.3). This was supported by histopathology and cell proliferation rates: mice showed a higher cell proliferation rate (factor 17 versus controls) than rats (factor 4 versus control) (Section 8.2.2: Swenberg *et al*, 1987).

In addition, rodents show a nasal anatomy and respiratory physiology different from man. The irritant effects of a chemical are amplified by the complexity of the nasal turbinates of the rat and the relative nasal surface areas compared with the nasal volume (8 times more in the rat than in man) (DeSesso, 1993). The nasal anatomical and physiological differences between rodent and man indicate that the mouse model is not relevant and that the rat as a model for the inhalation hazard due to inhalation of chemicals provides an additional safety factor for a risk evaluation for man. It is concluded that the NOEL of 25 ppm observed in the 13-weeks rats study by inhalation (Miller *et al*, 1981a) is the basis of risk evaluation for man.

10. FIRST AID AND SAFE HANDLING ADVICE

10.1 FIRST AID AND MEDICAL TREATMENT

There is no specific treatment or antidote for over-exposure to AA. Supportive medical treatment as indicated by the patient's condition is recommended.

10.1.1 Skin and Eye Injuries

Clothing contaminated with AA should be removed and either discarded and laundered before reuse. Affected areas of skin must be washed with copious quantities of water. The skin must be rinsed for at least 10 min. If the eyes are splashed, they should be irrigated immediately with eye-wash solution or clean water, holding the eyelids apart for at least 10 min. A physician should then be consulted.

10.1.2 Inhalation

The patient must be taken into fresh air, kept warm and at rest if he experiences difficulty in breathing after inhaling AA fumes. If the patient stops breathing, artificial respiration should be administered until qualified medical personnel is able to take over. Medical aid should be summoned immediately.

10.1.3 Ingestion

If AA has been swallowed, do not induce vomiting. Never give anything by mouth to an unconscious person. A physician should be consulted immediately.

10.2 SAFE HANDLING

10.2.1 Safety at Work

The main risk of injury stems from AA's irritating action on the skin and mucous membranes. Contact with the skin and eyes should therefore be avoided as should inhalation of high concentrations of AA vapour. AA should be used only in well ventilated areas. AA vapour is denser than air; pits and confined spaces should be avoided.

Suitable respiratory equipment must be worn on occasions when exposure to AA vapour above the recommended exposure limit is likely.

The following protective clothing must be worn when handling AA: eye-face protection and rubber gloves (preferably nitrile) which should be changed regularly to avoid permeation. Rubber boots should also be worn when handling large quantities.

10.2.2 Storage Safety

AA is stable in the presence of a polymerisation inhibitor. It is susceptible to polymerisation initiated by prolonged heating or a catalyst. Therefore, the following precautions must always be observed when storing AA.

- AA must be stored under air as the stabiliser (hydroquinone monomethylether) is only effective in the presence of oxygen
- Heat and direct sunlight must be excluded, as these promote polymerisation
- AA must be stored at temperatures preferably not exceeding 25°C
- Care should be taken to prevent contamination, as contaminants can render the stabiliser ineffective or can react with AA and promote polymerisation.

10.2.3 Fire Safety and Extinguishants

AA is classified as a highly flammable liquid. It can form an explosive mixture in air; adequate ventilation should be provided and smoking prohibited. Precautions should be maintained to eliminate all sources of ignition of AA when in contact with air. AA may polymerise on heating. Sealed containers may rupture if hot. Heat, UV-light, peroxide, azo-compounds, alkalis and oxidising agents may cause rapid polymerisation resulting in explosion. Fires can be extinguished with water, alcohol-resistant foam, dry powder or CO_2.

If fire does break out, neighbouring tanks and pipelines must be kept cool with plenty of water, otherwise the heat generated by the fire will cause their contents to polymerise.

10.2.4 Protection against Fire and Explosion

To avoid ignition, the following precautions are recommended.

- All plant and equipment should be explosion-proof as laid down in national standards
- All containers must be earthed
- All sources of ignition must be excluded
- No smoking is allowed

- No welding should be done until all tanks and pipelines have been drained and thoroughly flushed with water or hot caustic soda.

10.3 MANAGEMENT OF SPILLAGE AND WASTE

In all cases of spillage naked flames should be extinguished. Smoking and sparks must be avoided. Small spills of a few litres can be soaked up with suitable absorbent materials such as sand or earth. AA should not be absorbed onto sawdust or other combustible materials. Larger spills must be prevented from spreading by the use of earth or sand and the material should be pumped into containers.

Surfaces contaminated with AA should be washed well, first with alcohol and then with soap and water. All wastes should be sealed in vapour-tight plastic bags for eventual disposal.

AA should not be allowed to drain into domestic sewers as serious explosion hazards could result. Local authorities should be informed immediately if spilt liquid AA has entered surface water drains.

Waste quantities of AA can be incinerated in accordance with local, state or national regulations. Empty storage drums must be thoroughly rinsed and washed before recycling.

When aqueous waste containing AA is discharged to adapted biological waste-water treatment plants it is expected to be mineralised. No disturbance of the bacterial activity of sewage treatment plants is expected if AA is properly diluted.

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