European Chemicals Bureau

European Union Risk Assessment Report
acrylonitrile

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RISK ASSESSMENT

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ACRYLONITRILE

CAS No: 107-13-1

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RISK ASSESSMENT

Final Report, 2004

The risk assessment of acrylonitrile has been prepared by Ireland on behalf of the European Union. The scientific work contained in the report has been prepared by the Hazardous Substances Assessment Unit of the Health and Safety Authority.

Contact:

Hazardous Substances Assessment Unit
Health and Safety Authority,
10, Hogan Place,
Dublin 2

Mrs Roisin McEneany, Dr Majella Cummins
Tel: 353-1-6147000
Fax: 353-1-6147017
E-mail: majella_cummins@hsa.ie

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Foreword

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

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

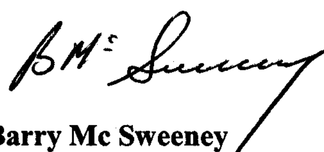
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each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

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

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

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

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



Barry Mc Sweeney
Director-General
DG Joint Research Centre

Catherine Day
Director-General
DG Environment

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

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

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

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS-No.: 107-13-1
EINECS-No.: 203-466-5
IUPAC name: 2-propenenitrile
Synonyms acrylonitrile

Environment

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

This conclusion is reached because of:

- concerns for effects on the local aquatic sphere as a consequence of exposure arising from production of acrylic fibres at a particular site.

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

This conclusion applies to:

- the aquatic compartment including sediment and microorganisms, for production of acrylonitrile and further processing to fibres and other plastics, with the exception of processing to acrylic fibres at one site only. It also applies to the terrestrial compartment, secondary poisoning and to the atmospheric compartment, the major compartment of distribution of acrylonitrile, for production of acrylonitrile and further processing to fibres and other plastics.

Summary of results

Acrylonitrile monomer released to the environment as a consequence of production or further processing will distribute primarily to the atmosphere and to the aqueous environment. Redistribution to other environmental compartments is anticipated to be negligible. There is rapid photodegradation of acrylonitrile, while in the aquatic environment acrylonitrile, while not readily biodegradable based on available information, appears to degrade rapidly in wastewater treatment plants following acclimation, and also degrades in surface water. Up to 99% biodegradation has been reported in simulation tests.

Since acrylonitrile is toxic to aquatic organisms and is not readily biodegradable, release into the aquatic environment could present some risk to aquatic species in the vicinity of plants producing or further processing acrylonitrile. However, the data for virtually all sites involved in production and processing of acrylonitrile in Europe in 1995/96, numbering 43 in all, most of which have industrial WWTPs, indicate PEC:PNEC ratios of less than 1 for surface water, using a PNEC of 17 µg/l. PEC:PNEC ratios for sediment for these sites are similarly below 1, indicative overall of low concern for the aquatic environment. It should be noted, however, that this conclusion applies only at a particular point in time to 42 out of the total of 43 European sites existing at that time and which provided aquatic release data relating to the period 1994-1996, and cannot be extrapolated generally for the aquatic environment. The specific risk reduction measures (e.g. wastewater treatment) or particular characteristics of the assessed sites (e.g. high dilution factors due to effluent emissions into very large rivers or estuaries) cannot be

extrapolated to sites not covered by this risk assessment, for example new sites starting up after the data for this assessment were gathered, or sites located outside the European Union.

In this risk assessment it was established that one site, located in a coastal position, had a PEC:PNEC ratio of 3.1, and the levels of acrylonitrile in effluent were comparatively high compared with other sites, at 35 mg/l. It is concluded that there are concerns for possible effects on the local aquatic environmental sphere as a consequence of exposure arising from production of acrylic fibres at this site.

In relation to risk assessment for microorganisms in wastewater treatment plants, PEC:PNEC ratios were in general below 1, indicative of little risk for microorganisms in WWTP.

All 43 production and further processing companies provided data on atmospheric emissions. These showed that emissions were generally low, being reduced by scrubbing of gaseous and volatile wastes before discharge to the atmosphere. Predicted atmospheric concentrations ($PEClocal_{air}$) of acrylonitrile in the vicinity of acrylonitrile production facilities and facilities involved in further processing of acrylonitrile into acrylonitrile-containing polymers and other monomers were between 0.001 and 0.240 mg/m3. Results of monitoring have indicated average concentrations of below 1 µg/m3 at the perimeter of acrylonitrile plants. There is a paucity of data about the effects of these low levels of acrylonitrile on species exposed via the atmospheric environment, although results of the mammalian toxicology reported in Section 4 would indicate a low level of concern. Derivation of PEC:PNEC ratios for the atmospheric environment provided values of below 1.0 for all sites. Acrylonitrile is also rapidly photodegraded. In addition, information regarding a catastrophic event which happened outside the EU and during which the contents of a large storage tank containing acrylonitrile were released very rapidly, showed damage to vegetation observed within a 100 m zone of the spill. No damage to vegetation was observed greater than 100 m from the spill where acrylonitrile concentrations of up to 20 ppm were measured, a concentration far greater than the expected fence-line value.

Risk characterisation for the terrestrial compartment has excluded the possibility of sludge application to land, given information from industry that little industrial sludge from acrylonitrile production and processing facilities is spread on land in Europe. The majority of companies providing information on this aspect indicated that contaminated sludge is incinerated together with other wastes. Risk characterisation has therefore been based on the values obtained from EUSES (European Union System for the Evaluation of Substances, 1997) for $PECregional_{soil}$, which results in a very low PEC:PNEC value for soil, indicating that there is little risk for the soil compartment. This conclusion is based on the assumption that sludge from the WWTP is not applied to soil, however, an assumption which is supported for the European Union, based on the data supplied. It cannot be extrapolated to sites not covered by this risk assessment. The estimate of $PECregional_{soil}$ reflects primarily point source emissions from production or further processing, and diffuse emissions from car exhausts etc. have not been taken into account. However, even with a significant contribution to $PECregional_{soil}$ from such sources, the PEC:PNEC ratio will still be well below 1.

Exposure of species relevant for the food chain to low levels of acrylonitrile in the environment is theoretically possible. Physicochemical considerations and experimental evidence suggest that acrylonitrile is unlikely to bioaccumulate in exposed biota, and toxicity studies in mammalian species provide little evidence of cumulative toxicity in a range of species. Concentrations of acrylonitrile in biota are expected to be very low, and it is therefore concluded that the potential for secondary poisoning is very small. Estimates of the regional and continental PEC for acrylonitrile would also indicate little or no concern for the environment.

Human health

Human health (toxicity)

Workers

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

This conclusion is reached because of:

- concerns for general systemic effects and carcinogenicity as a consequence of exposure arising during the production and processing of the substance.

In relation to conclusion (iii) for repeated dose (systemic) toxicity by the inhalation or, (by route-to-route extrapolation) the dermal route, this primarily reflects the toxicity seen in chronic studies in rats and the relatively low Margins of Safety (MOSs) between anticipated exposure levels and doses producing toxicity. Many of the findings in the animal repeated dose studies are mirrored in reported findings in workers. Overall, however, the human data are difficult to assess in relation to establishment of a dose-response relationship. The EU Working Group on Classification and Labelling agreed that acrylonitrile should not be classified with R 48 (risk of serious damage to health on prolonged exposure) based on the information available. Nevertheless, for the purposes of this risk assessment, given the difficulties in assessing the human data and the low MOSs achieved, it is recommended that conclusion (iii) be applied to the repeated dose (systemic) toxicity end point.

In relation to carcinogenicity, it is recognised that there is a low risk at any level of exposure, given that acrylonitrile is currently regarded as a carcinogen for which a threshold cannot be reliably identified. The magnitude of this risk has been estimated to lie between $1.3 \cdot 10^{-4}$ to $1.8 \cdot 10^{-2}$ for workers exposed to 2 ppm (the current OEL in a number of EU countries) for 8 hours a day, 5 days a week and a working life of 40 years. A Margin of Exposure (MOE) of 57.5 has been derived, based on a T_{25} of 16.1 mg/kg/day in the male rat obtained from the 2-year inhalation study carried out by Quast (1980).

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

This conclusion applies to:

- the end points of acute toxicity, skin, eye and respiratory irritancy, skin sensitisation, corrosivity, repeated dose (local) toxicity by the inhalation route, neurotoxicity. mutagenicity and reproductive toxicity.

Consumers

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

This conclusion is reached because of:

- concerns for carcinogenicity.

Risks cannot be excluded for all exposure scenarios, as the substance is identified as a non-threshold carcinogen. The adequacy of existing controls and the feasibility and practicability of

further specific measures should be considered. However, the risk assessment indicates that risks are already low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

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

This conclusion applies to:

- the end points of skin sensitisation, repeated dose toxicity by the inhalation or (by route-to-route extrapolation) the dermal route, mutagenicity and reproductive toxicity.

Humans exposed via the environment

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

This conclusion is reached because of:

- concerns for carcinogenicity after highest predicted atmosphere concentrations at a local level.

There could be some concern for carcinogenicity for humans exposed via air, with respect to the immediate vicinity of plants, based mainly on potential for local exposure to a carcinogen for which a threshold cannot be reliably identified. This conclusion however should be qualified indicating that risks are already very low. This should be taken into account when considering the adequacy of controls feasibility and practicability of further specific risk reduction measures.

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

This conclusion applies to all other endpoints.

Human health (risks from physico-chemical properties)

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

This conclusion is reached because:

- the risk assessment shows that risks to workers, consumers and humans exposed via the environment related to physico-chemical properties are not expected. Risk reduction measures already being applied are considered sufficient.

CONTENTS

1 GENERAL SUBSTANCE INFORMATION 7

1.1 IDENTIFICATION OF THE SUBSTANCE 7

1.2 PURITY/IMPURITIES, ADDITIVES 7

1.3 PHYSICO-CHEMICAL PROPERTIES 7
1.3.1 Physical state at standard temperature and pressure 7
1.3.2 Melting / solidifying point 8
1.3.3 Boiling point 8
1.3.4 Relative density 8
1.3.5 Vapour pressure 8
1.3.6 Surface tension 8
1.3.7 Water solubility 9
1.3.8 Partition coefficient 9
1.3.9 Flash-point 10
1.3.10 Autoflammability 10
1.3.11 Explosivity 10
1.3.12 Oxidising properties 10
1.3.13 Other physico-chemical properties 10
1.3.14 Summary of physico-chemical properties 10

1.4 CLASSIFICATION 11

2 GENERAL INFORMATION ON EXPOSURE 13

2.1 PRODUCTION 13

2.2 USES 14
2.2.1 Fibres 14
2.2.2 ABS and SAN plastics 14
2.2.3 Adiponitrile and acrylamide synthesis 14
2.2.4 Nitrile rubbers 15
2.2.5 Other uses 15

2.3 EXPOSURE CONTROLS 15

3 ENVIRONMENT 16

3.1 ENVIRONMENTAL EXPOSURE 16
3.1.1 General discussion 16
3.1.2 Environmental releases 17
3.1.2.1 Releases of acrylonitrile during production 17
3.1.2.2 Releases during processing of acrylonitrile to polymers 19
3.1.2.3 Regional and continental releases due to point sources 23
3.1.2.4 Regional and continental releases due to point and diffuse sources 25
3.1.3 Environmental fate 27
3.1.3.1 Degradation in the environment 27
3.1.3.1.1 Atmospheric degradation 27
3.1.3.1.2 Aquatic degradation 28
3.1.3.1.3 Degradation in soil and sediment 36
3.1.3.1.4 Summary of environmental degradation data on acrylonitrile 36
3.1.3.2 Distribution 37
3.1.3.2.1 Modelling of distribution 37
3.1.3.2.2 Distribution to soil and sediment, adsorption:desorption 38
3.1.3.2.3 Volatilisation 38

3.1.3.2.4 Bioconcentration and bioaccumulation 38
3.1.3.2.5 Degradation products, identity and environmental fate 39
3.1.4 Aquatic compartment (including sediment) 39
3.1.4.1 Predicted local environmental concentrations (PEC_{local}) for the aquatic compartment ... 39
3.1.4.1.1 Calculation of PEC $local_{water}$ for acrylonitrile production 39
3.1.4.1.2 Calculation of $PEClocal_{water}$ due to processing of acrylonitrile to polymers, acrylamide and adiponitrile 42
3.1.4.1.3 Calculation of $PEClocal_{water}$ for wastewater treatment plants 43
3.1.4.1.4 Calculation of PECsediment 43
3.1.4.1.5 Calculation of regional and continental concentrations in the aquatic environment ($PECregional_{water}$ and $PECcontinental_{water}$) 44
3.1.4.2 Measured levels of acrylonitrile in water 45
3.1.4.3 Comparison of PEC_{water} with measured data 46
3.1.5 Atmospheric compartment 46
3.1.5.1 Calculation of $PEClocal_{air}$ 47
3.1.5.2 Calculation of PEC_{air} for regional and continental scenarios 49
3.1.5.3 Measured levels of acrylonitrile in air 49
3.1.6 Terrestrial compartment 51
3.1.6.1 Calculation of PEC_{soil} 52
3.1.6.2 Measured levels in soil 52
3.1.7 Secondary poisoning 53

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION)-RESPONSE (EFFECT ASSESSMENT) 54
3.2.1 Aquatic compartment 54
3.2.1.1 Toxicity test results 54
3.2.1.1.1 Fish 54
3.2.1.1.2 Aquatic invertebrates 57
3.2.1.1.3 Algae and aquatic plants 60
3.2.1.1.4 Microorganisms 60
3.2.1.2 Calculation of PNEC for aquatic organisms 61
3.2.1.3 Calculation of PNEC for microorganisms in wastewater treatment plants 62
3.2.1.4 Calculation of PNEC for sediment 62
3.2.2 Terrestrial compartment 62
3.2.2.1 Toxicity test results 62
3.2.2.1.1 Plants 62
3.2.2.1.2 Non-soil dwelling arthropods 62
3.2.2.2 Calculation of PNEC for terrestrial species 63

3.3 RISK CHARACTERISATION 64
3.3.1 Aquatic compartment (including sediment) 64
3.3.1.1 Water 64
3.3.1.2 Microorganisms in wastewater treatment plants 66
3.3.1.3 Sediment 66
3.3.2 Atmosphere 67
3.3.3 Terrestrial compartment 67
3.3.4 Secondary poisoning 68

4 HUMAN HEALTH 69

4.1 HUMAN EXPOSURE (TOXICITY) 69
4.1.1 Exposure Assessment 69
4.1.1.1 General discussion 69
4.1.1.2 Occupational exposure 69
4.1.1.2.1 Occupational exposure during acrylonitrile production and polymer production and processing 70
4.1.1.2.2 Measured levels in the workplace 83
4.1.1.2.3 Biological indicators of exposure to acrylonitrile 89
4.1.1.2.4 Modelling of exposure 89

4.1.1.2.5 Summary of occupational exposure 92
4.1.1.3 Consumer exposure 93
4.1.1.3.1 Skin contact with fibres 94
4.1.1.3.2 Food packaging 100
4.1.1.3.3 Other sources of contamination 102
4.1.1.3.4 Summary of consumer exposure 102
4.1.1.4 Humans exposed via the environment 103
4.1.2 Effects assessment: hazard identification and dose (concentration) – response (effect) assessment 105
4.1.2.1 Toxicokinetics, metabolism and distribution 105
4.1.2.1.1 Studies in animals 105
4.1.2.1.2 Studies in humans 115
4.1.2.1.3 Adduct production as a dosimeter of exposure to acrylonitrile 116
4.1.2.1.4 Summary of toxicokinetics, metabolism and distribution 118
4.1.2.2 Acute toxicity 120
4.1.2.2.1 Studies in animals 120
4.1.2.2.2 Studies in humans 127
4.1.2.2.3 Summary of acute toxicity 128
4.1.2.3 Irritation 129
4.1.2.3.1 Studies in animals 129
4.1.2.3.2 Studies in humans 131
4.1.2.3.3 Summary of irritation 132
4.1.2.4 Corrosivity 132
4.1.2.5 Sensitisation 132
4.1.2.5.1 Skin 132
4.1.2.5.2 Respiratory tract 133
4.1.2.5.3 Summary of sensitisation 133
4.1.2.6 Repeated dose toxicity 133
4.1.2.6.1 Inhalation studies in animals 133
4.1.2.6.2 Oral studies in animals 142
4.1.2.6.3 Dermal studies in animals 153
4.1.2.6.4 Summary of repeated dose toxicity in animals 153
4.1.2.6.5 Studies in humans 155
4.1.2.6.6 Summary of repeated dose toxicity in humans 157
4.1.2.7 Mutagenicity 158
4.1.2.7.1 Mutagenicity studies *in vitro* 158
4.1.2.7.2 Mutagenicity studies *in vivo* 168
4.1.2.7.3 Evidence of mutagenicity in humans 171
4.1.2.7.4 Cell transformation studies 172
4.1.2.7.5 Summary of the mutagenic profile of acrylonitrile 173
4.1.2.8 Carcinogenicity 174
4.1.2.8.1 Oral carcinogenicity studies in rats 174
4.1.2.8.2 Inhalation carcinogenicity studies in rats 183
4.1.2.8.3 Dermal carcinogenicity studies in animals 187
4.1.2.8.4 Summary of carcinogenicity studies in animals 188
4.1.2.8.5 Mechanism of carcinogenicity 189
4.1.2.8.6 Epidemiological studies 190
4.1.2.8.7 Summary of carcinogenicity studies 217
4.1.2.9 Toxicity for reproduction 219
4.1.2.9.1 Effects on fertility 219
4.1.2.9.2 Developmental toxicity 223
4.1.2.9.3 Summary of toxicity for reproduction 227
4.1.3 Risk characterisation 228
4.1.3.1 General aspects 228
4.1.3.2 Workers 230
4.1.3.2.1 Acute toxicity 231
4.1.3.2.2 Irritation and corrosivity 232
4.1.3.2.3 Sensitisation 232
4.1.3.2.4 Repeated dose toxicity 233

4.1.3.2.5 Carcinogenicity 238
4.1.3.2.6 Mutagenicity 238
4.1.3.2.7 Toxicity for reproduction 240
4.1.3.2.8 Summary of the risk characterisation for workers 240
4.1.3.3 Consumers 241
4.1.3.3.1 Skin sensitisation 242
4.1.3.3.2 Repeated dose toxicity 242
4.1.3.3.3 Summary of repeated dose toxicity for consumers 244
4.1.3.3.4 Carcinogenicity 244
4.1.3.3.5 Mutagenicity 244
4.1.3.3.6 Toxicity for reproduction 245
4.1.3.3.7 Summary of risk characterisation for consumers 245
4.1.3.4 Humans exposed via the environment 246

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES) 248
4.2.1 Exposure assessment 248
4.2.1.1 Workers 248
4.2.1.2 Consumers 248
4.2.1.3 Humans exposed via the environment 248
4.2.2 Effects assessment: Hazard identification 248
4.2.2.1 Explosivity 248
4.2.2.2 Flammability 249
4.2.2.3 Oxidising potential 249
4.2.3 Risk characterisation 249
4.2.3.1 Workers 249
4.2.3.2 Consumers 249
4.2.3.3 Humans exposed via the environment 250

5 RESULTS 251

5.1 ENVIRONMENT 251
5.1.1 Aquatic compartment (incl. sediment) 252
5.1.2 Atmosphere 253
5.1.3 Terrestrial compartment 253
5.1.4 Secondary poisoning 253

5.2 HUMAN HEALTH 253
5.2.1 Human health (toxicity) 253
5.2.1.1 Workers 255
5.2.1.2 Consumers 255
5.2.1.3 Humans exposed via the environment 256
5.2.2 Human health (risks from physico-chemical properties) 257

6 REFERENCES 258

ABBREVIATIONS 282

Appendix A.1 Local PEC and other emission parameters for surface water for acrylonitrile production plants in Europe 287
Appendix A.2 Local PEC and other emission parameters for surface water for acrylonitrile processing plants in Europe 288
Appendix A.3 Local PEC and other emission parameters to air for acrylonitrile production plants in Europe 290
Appendix A.4 Local PEC and other emission parameters to air for acrylonitrile processing plants in Europe 291
Appendix B 293
Appendix C Evaluation of the dose-response of microscopic brain tumour incidence in F344 rats, exposed to acrylonitrile via drinking water 296
Appendix D Modelling of exposure 306

TABLES

Table 1.1 Solubilities of acrylonitrile in water 9
Table 1.2 Physico-chemical properties of acrylonitrile 11
Table 3.1 Aquatic and atmospheric releases of acrylonitrile from production sites in the EU 18
Table 3.2 Production data, aquatic and atmospheric releases of acrylonitrile from acrylic fibre production facilities in the EU 20
Table 3.3 Production data, aquatic and atmospheric releases of acrylonitrile from ABS/SAN production facilities in the EU 21
Table 3.4 Production data, aquatic and atmospheric releases of acrylonitrile from nitrile:butadiene copolymer production facilities in the EU 22
Table 3.5 Production data, aquatic and atmospheric releases of acrylonitrile from acrylamide and adiponitrile production facilities in the EU 23
Table 3.6 Summary of estimated regional and continental releases of acrylonitrile from point and diffuse sources 27
Table 3.7 Rate constants for reaction of acrylonitrile with hydroxyl radical and ozone and derived trophospheric lifetimes 28
Table 3.8 Biodegradation of acrylonitrile in aqueous systems 29
Table 3.9 First-order rate constants and degradation half-lives of acrylonitrile in the troposphere, surface water and soil 37
Table 3.10 Mackay level 1 environmental distribution of acrylonitrile based on the OECD EQC model V.1.0.. 37
Table 3.11 Summary of $PEClocal_{water}$ for acrylonitrile production facilities 41
Table 3.12 Summary of $PEClocal_{water}$ for acrylonitrile fibre production facilities 42
Table 3.13 Summary of PEC $local_{water}$ for ABS-SAN production facilities 42
Table 3.14 Summary of $PEClocal_{water}$ for nitrile:butadiene copolymer production facilities 43
Table 3.15 Summary of PEC $local_{water}$ due to processing of acrylonitrile to acrylamide and adiponitrile 43
Table 3.16 Measured levels of acrylonitrile in surface waters and in effluents from manufacturing facilities..... 46
Table 3.17 Calculation of $PEClocal_{air}$ for acrylonitrile production facilities 47
Table 3.18 Calculation of $PEClocal_{air}$ for acrylonitrile fibre production facilities 47
Table 3.19 Calculation of $PEClocal_{air}$ for ABS SAN production facilities 48
Table 3.20 Calculation of $PEClocal_{air}$ for nitrile:butadiene copolymer production facilities 48
Table 3.21 Calculation of $PEClocal_{air}$ dur to processing of acrylonitrile to acrylamide and adiponitrile 48
Table 3.22 Levels of acrylonitrile in the air in the vicinity of manufacturing plants and in urban air 51
Table 3.23 $PECregional_{soil}$ and $PECcontinental_{soil}$ for acrylonitrile production and processing 52
Table 3.24 Acute toxicity of acrylonitrile to fish 55
Table 3.25 Acute toxicity of acrylonitrile to aquatic invertebrates 58
Table 4.1 Data on impervious materials for acrylonitrile protection 83
Table 4.2 Summary table of mean exposure data from European-based industry 84
Table 4.3 Occupational exposure data for acrylonitrile production and further processing plants 85
Table 4.4 Specific exposure data from one European fibre processing plant 85
Table 4.5 Personal exposures relating to the manufacture of acrylic fibre in Europe 86
Table 4.6 Measurement results (8-hr time-weighted averages for acrylonitrile) 89
Table 4.7 Residual levels of acrylonitrile monomer in PAN fibres 95
Table 4.8 Acrylonitrile levels in food contained in ABS polymers 101
Table 4.9 Tissue levels of radioactivity in rats given a single oral dose of 46.5 mg/kg [1-^{14}C]-acrylonitrile..... 107
Table 4.10 Comparison of the formation of CEO, the conjugation of acrylonitrile and CEO with glutathione and the steady state level of cyanoethylene oxide (CEO) in rats, mice and humans expressed as a ratio of the activity in mouse or rat to that in human 114
Table 4.11 Acute toxicity of acrylonitrile in the rat following inhalation exposure 122
Table 4.12 Acute toxicity of acrylonitrile in the rat following inhalation exposure 123
Table 4.13 LC_{50} values for acrylonitrile in the rat 123
Table 4.14 Acute toxicity of acrylonitrile by different routes of administration in a range of species 124
Table 4.15 Cumulative mortality data for rats exposed to acrylonitrile via inhalation for 2 years 139
Table 4.16 Treatment-related histopathological changes in the nasal passages of rats exposed to acrylonitrile by inhalation for up to 2 years 141
Table 4.17 Summary of mortality data in rats given acrylonitrile in drinking water over a 2-year period 147
Table 4.18 Cumulative mortality data in rats given acrylonitrile in drinking water over a 2-year period 148
Table 4.19 Mortality of rats maintained for 2 years on drinking acrylonitrile 151

Table 4.20 Tumours observed in rats administered acrylonitrile in drinking water for up to 2 years (study 1) ... 176
Table 4.21 Tumours observed in rats administered acrylonitrile in drinking water for up to 2 years (study 2) ... 179
Table 4.22 Incidence of tumours observed in male rats administered acrylonitrile in drinking water for up to 2 years ... 182
Table 4.23 Brain tumours and pre-neoplastic changes in the brain in rats following inhalation exposure for up to 2 years ... 186
Table 4.24 Summary of the findings of 8 carcinogenicity studies carried out with acrylonitrile in rats... 188
Table 4.25 Meta-analysis of cancer mortality in workers exposed to acrylonitrile... 195
Table 4.26 Exposure characteristics of selected plants ... 196
Table 4.27 Mortality of UK workers involved in the polymerisation of acrylonitrile and production of pan fibres... 199
Table 4.28 Comparison of mortality of UK workers in polymerisation of acrylonitrile and production of pan fibres – Comparison of factory 5 with other factories... 199
Table 4.29 Mortality related to level of exposure ... 200
Table 4.30 Acrylonitrile use in the 8 companies in the Swaen et al. (1998) study and number of exposed workers employed ... 202
Table 4.31 Total mortality in workers exposed to acrylonitrile grouped into 3 exposure cumulative categories and latency periods... 203
Table 4.32 Lung cancer mortality in workers exposed to acrylonitrile by 3 exposure cumulative categories and latency periods... 204
Table 4.33 Estimated ranges for each acrylonitrile exposure level category ... 205
Table 4.34 Exposed workers by year of first exposure and plant ... 206
Table 4.35 Standardised mortality ratios fot the acrylonitrile exposed workers with US and DuPont mortality rates as reference... 207
Table 4.36 Summary description of studies included in meta-analysis... 208
Table 4.37 Relative risk to be detected from the expected number of cases in the Collins et al. meta-analysis study (1988) ... 211
Table 4.38 Observed and expected cases of prostate cancer, DuPont company rates... 214
Table 4.39 Expected causes of death in the Blair et al. (1998) study... 216
Table 4.40 Results of histopathological examination of the testis and epididymis from male rats exposed to acrylonitrile by inhalation for up to 2 years ... 222
Table 4.41 Calculation of margins of safety (MOS) for workers in relation to local, systemic or reproductive toxicity following inhalation exposure... 237
Table 4.42 Calculation of margins of safety (MOS) for consumers in relation to repeated dose toxicity and toxicity for reproduction... 246
Table A.1 Local PEC and other emission parameters for surface water for acrylonitrile production plants in Europe ... 287
Table A.2 Local PEC and other emission parameters for surface water for acrylonitrile processing plants in Europe ... 288
Table A.3 Local PEC and other emission parameters to air for acrylonitrile production plants in Europe ... 290
Table A.4 Local PEC and other emission parameters to air for acrylonitrile processing plants in Europe ... 291
Table B.1 Risk characterisation using different methods... 295
Table C.1 Mortality data of rats exposed to acrylonitrile via drinking water ... 297
Table C.2 Incidence of brain tumour in rats exposed to acrylonitrile via drinking water... 298
Table C.3 Calculation of the Kaplan-Meier probability for having a tumour at death for a population of 10 animals ... 299
Table C.4 Calculation of the Kaplan-Meier probability for microscopic brain tumours ... 299
Table C.5 Quast inhalation study in Sprague Dawley rats... 304
Table C.6 Quast inhalation study in Sprague Dawley rats (95% upper confidence limit)... 304

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS-No.: 107-13-1
EINECS-No.: 203-466-5
IUPAC name: 2-propenenitrile
Synonyms: Vinyl cyanide, cyanoethylene, acrylonitrile
Molecular weight: 5.06
Molecular formula: C_3H_3N
Structural formula: $CH_2 = CH - CN$

1.2 PURITY/IMPURITIES, ADDITIVES

Information provided from a number of producers indicates that acrylonitrile is marketed as an inhibited liquid product containing 99.5% w/w acrylonitrile and water (0.2-0.5%) as polymerisation inhibitor. A typically stabilised product contains 30-50 ppm ammonia and 0.4-0.5% w/w water, or 30-50 ppm hydroquinone monomethylether (MHQ) and 0.4-0.5% w/w water.

Based on the product specifications provided by a number of acrylonitrile producers, the following impurities can be found in marketed acrylonitrile in individual amounts up to a maximum of 500 mg/kg: acetone (300 mg/kg), acetonitrile (500 mg/kg), aldehydes as acetaldehyde (50 mg/kg), propionitrile (30 mg/kg), acrolein (5 mg/kg), methanol (5 mg/kg), isopropanol (5 mg/kg), hydrogen cyanide (5 mg/kg), peroxides as hydrogen peroxide (0.2 mg/kg), iron (0.1 mg/kg), copper (0.1 mg/kg), water (0.2-0.5% wt).

The purity of acrylonitrile is determined by gas chromatographic methods with flame ionisation detection (PCK AG, 1992), although a nitrogen-specific detector (PND) may also be used because of higher selectivity. Spectroscopic methods including ultra-violet, infrared and mass spectrometry are also used to characterise acrylonitrile.

1.3 PHYSICO-CHEMICAL PROPERTIES

Physico-chemical properties of acrylonitrile are summarised in a number of reference texts (Merck Index, 1996; CRC Handbook, 1995-1996) and comprehensively reviewed by American Cyanamid (1959), Groet et al. (1974), and Kirk-Othmer (1991).

1.3.1 Physical state at standard temperature and pressure

Acrylonitrile is a clear colourless liquid with a characteristic, slightly pungent odour (Kirk-Othmer, 1991). A yellow coloration may develop in the presence of light. In the absence of stabilisers, spontaneous polymerisation may occur at elevated temperatures, or in the presence of light, acid or alkali (EC Erdölchemie, 1994).

1.3.2 Melting / solidifying point

The solidifying point of acrylonitrile has been reported as -83.55°C ± 0.5°C (American Cyanamid, 1959), this value being derived from three separate reports in the literature. One such report was that of Davis and Wiedeman (1945) who measured a value of -83.6°C ± 0.5°C on a toluene-in-glass thermometer standardised by reference to anhydrous ethyl acetate. A value of -83.6°C has been used in the assessment of environmental exposure using EUSES (European Union System for the Evaluation of Substances, 1997).

1.3.3 Boiling point

The boiling point at 1,013 hPa is 77.3°C, as reported in Kirk-Othmer (1991), Davis and Wiedeman (1945), the IUCLID data set and other reference texts. Groet et al. (1974) quote a range of boiling points at reduced pressure as follows: 64.7°C at 666.5 hPa; 45.5°C at 333.25 hPa; 23.6°C at 133.3 hPa; 8.7°C at 66.65 hPa; -20.3°C at 13.3 hPa. A value of 77.3°C has been used in the assessment of environmental exposure using EUSES.

1.3.4 Relative density

American Cyanamid (1959) reported densities of 0.8060 at 20°C and 0.8004 at 25°C, these figures being drawn from company data and a number of reports in published literature. These values are reported in the IUCLID data sheet and in secondary reference material such as the Merck Index. BASF (1989) have determined a value of 0.8066 using a pycnometre method.

1.3.5 Vapour pressure

The vapour pressure of acrylonitrile at 20°C has been reported variously as 106.7 hPa (Baxter, 1979), 115 hPa (Kirk-Othmer, 1991), 116 hPa (BASF AG, 1994) 120 hPa (EC Erdölchemie, 1994) and 124 hPa (BG-Chemie, 1990). Davis and Wiedeman (1945) measured the vapour pressure by the static method. Their paper provided a curve of vapour pressure dependency on temperature, and Bayer (1949) subsequently published values for the vapour pressure of acrylonitrile at different temperatures based on this experimental work. A value of 85 mm Hg (113.3 hPa) was quoted by Bayer for the vapour pressure at 20°C. The IUCLID data sheet cites the values of BG-Chemie (1990) and BASF AG (1994). The value of 115 hPa cited by Kirk-Othmer has been accepted as valid.

In addition to the work of Davis and Wiedeman (1945), the review paper of Groet et al., (1974) provided information on the effect of increasing temperature on the vapour pressure of acrylonitrile. The following values were quoted: 13.3 hPa at -20.3°C; 66.65 hPa at 8.7°C; 133.3 hPa at 23.6°C; 333.25 hPa at 45.5°C; 666.5 hPa at 64.7°C; 1,013 hPa at 77.3°C. Verschueren (1983) cites a value of 133.3 hPa at 22.8°C, and this value has been used in the assessment of environmental exposure using EUSES.

1.3.6 Surface tension

Vogel et al. (1952) measured the surface tension of acrylonitrile using the capillary rise method. The experimental details of this non-Annex V test method are described in full and the result of

24.8 mN/m at 15.1°C (units used in the paper were dynes/cm^2) is accepted as valid. American Cyanamid (1959) quote a value of 27.3 mN/m at 24°C, based on the experimental data of Vogel and company data, while Groet et al., (1974), Langvardt (1985) and Kirk-Othmer (1991) all cite a value of 26.6 mN/m at 25°C. American Cyanamid additionally provided a formula for the calculation of surface tension of aqueous solutions of acrylonitrile as follows:

$C = 0.233d - 0.018d^2 + 0.00013d^3$ where C = 0-6 weight percent and d = dynes/cm

1.3.7 Water solubility

Langvardt (1985) provided the data reproduced in **Table 1.1** for the solubilities of acrylonitrile in water, the data illustrating the high solubility of the chemical in water.

Table 1.1 Solubilities of acrylonitrile in water

Mass fractions %		
T °C	Acrylonitrile in water	Water in acrylonitrile
0	7.15	2.10
10	7.17	2.55
20	7.30	3.08
30	7.51	3.82
40	7.90	4.85
50	8.41	6.15
60	9.10	7.65
70	9.90	9.21
80	11.10	10.95

Similar values of 72 g/l at 0°C, 73.5 g/l at 20°C and 79.0 g/l at 40°C are reported by American Cyanamid (1959). Methodology is not reported, but given the high solubility the figures given in these reference texts are accepted as valid. A value of 73 g/l has been used in the assessment of environmental exposure using EUSES.

1.3.8 Partition coefficient

Log P_{ow} for acrylonitrile has been measured experimentally by a number of investigators, and ranges from -0.14 to 0.3 at 25°C, as cited in the IUCLID data sheet. Pratesi et al. (1979) used the shake flask method with analysis of both phases by HPLC and reported a value of 0.25, while Fujisawa and Masuhara (1981), using similar methodology, reported a value of 0. Tanii and Hashimoto (1984) provided a value of 0.09, with analysis of the aqueous phase only, by GLC, this estimate being consistent with a value of 0.08 reported by BASF (1988). Sangster (1989) reviewed the cited values in these three papers and concluded that the value of 0.25 measured by Pratesi et al. was the most reliable (recommended) value, with an estimated uncertainty of $\pm$ 0.2. This value is comparable with one of 0.3 reported by Tonogai et al. (1982). Additionally, the KOWWIN vl.35a, Log Octanol-Water Partition Coefficient Estimation Programme, Syracuse Research Corporation (1994), which is based on the method of Hansch and Leo, has provided a

calculated value of 0.209. A value of 0.25 has been taken for risk assessment purposes, and has been used in the assessment of environmental exposure using EUSES.

1.3.9 Flash-point

Acrylonitrile is a highly flammable liquid. Flash point values of 0°C and -5°C have been reported using the Open Cup method, with a value of -1°C using the Closed Cup method. These results indicate that acrylonitrile should be classified as highly flammable according to the EU classification criteria.

1.3.10 Autoflammability

A very small experimental range of 480-481°C is reported.

1.3.11 Explosivity

Although the standard Annex VB tests for explosivity have not been performed, vapours of acrylonitrile form explosive mixtures with air (EC Erdölchemie, 1994). The explosive substance:air ratio of acrylonitrile stabilised with 30-40 ppm ammonia has been reported by Nabert and Schön (1980) to lie between 2.8-28 vol/vol. American Cyanamid (1959), Nabert and Schön (1970) and Groet et al. (1974) had earlier reported the explosive limits to lie between 3.05% and 17%, and these figures have also been cited by Langvardt (1985). The IUCLID data sheet uses the figures of Nabert and Schön (1980).

1.3.12 Oxidising properties

On structural grounds, acrylonitrile will not have oxidising properties.

1.3.13 Other physico-chemical properties

The reference texts and reviews cited above, e.g. American Cyanamid (1959), Groet et al. (1974) and Kirk-Othmer (1991) provide a range of other properties of acrylonitrile, including refractive index (n_D^{25} = 1.3888), dielectric constant (38), ionisation potential (10.75 electron-volts), heat of combustion (-1,761.89 kJ/mol, 25°C), heat of vaporisation (32.65 kJ/mol) and heat of polymerisation (72.4 + 2.1 kJ/mol). None of these properties are of specific relevance for risk assessment purposes.

1.3.14 Summary of physico-chemical properties

The data provided by American Cyanamid (1959) are drawn from published data in existence in 1959 and are reproduced in **Table 1.2** below. Although few methodological details are available for comparison with current Annex V test methods, they are considered to represent a valid data set. Other values are cited in **Table 1.2** where considered to be more reliable. Despite the absence of methodological detail in the papers cited above, the consistency of the data would indicate that remeasurement of the physicochemical properties of acrylonitrile using current Annex V test methods is not necessary.

Table 1.2 Physico-chemical properties of acrylonitrile

Parameter	Value	Reference
Physical state	Colourless liquid	American Cyanamid (1959)
Solidifying Point	- 83.55°C * ± 0.5°C	American Cyanamid (1959)
Boiling point	77.3°C *	Kirk-Other (1991)
Relative Density	0.8060 at 20°C	American Cyanamid (1959)
Vapour Pressure	115 hPa at 20°C 133.3 hPa at 22.8°C *	Kirk-Other (1991) Verschueren (1983)
Surface tension	27.3 mN/m at 24°C	American Cyanamid (1959)
Water solubility	73.5 g/l at 20°C *	American Cyanamid (1959)
Partition coefficient (log Pow)	0.25 *	Pratesi et al. (1979)
Flash point	0°C (open cup method) -5°C (open cup method)	American Cyanamid (1959) Langvardt (1985)
Autoflammability	481°C	American Cyanamid (1959)
Explosive limits	2.8-28 vol/vol.	Nabert and Schön (1980)

* value used in the assessment of environmental exposure in this report

1.4 CLASSIFICATION

Classification and labelling according to the 26th ATP of Directive 67/548/EEC[4]:

Classification

F; R11	Highly flammable
Carc. Cat.2; R45	May cause cancer
T; R23/24/25	Also toxic by inhalation, in contact with skin and if swallowed
Xi; R37/38-41	Irritating to respiratory system and skin. Risks of serious damages to eyes
R43	May cause sensitisation by skin contact
N; R51-53	Dangerous for the environment, toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

Notes: D, E

[4] The classification of the substance is established by Commission Directive 2000/32/EC of 19 May 2000 adapting to technical progress for the 26th time Council Directive 67/548 on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

Labelling

F; T; N
R 45-11-23-/24/25-37/38-41-43-51/53
S 9-16-53-45-61

Specific concentration limits

C ≥ 20%	T; R45-23/24/25-37/38-41-43
10%≤C<20%	T; R45-23/24/25-41-43
5%≤C<10%	T; R45-23/24/25-36-43
1%≤C< 5%	T; R45-23/24/25-43
0.2%≤C< 1%	T; R45-20/21/22
0.1%≤C<0.2%	T; R45

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

World production of acrylonitrile in 1985 exceeded 3,000,000 tonnes per annum, with economic forecasts at that time predicting a slow (1-2% per annum) in growth. Estimated world capacity in 1991 was 4,200,000 tonnes per annum, while world demand in 1993 was 3,846,000 tonnes (source PCI World Acrylonitrile Report, 1994). Current production volume in the EU is in excess of 1,250,000 tonnes per annum, US production is approximately 1,500,000 tonnes per annum, Japan produces approximately 600,000 tonnes per annum, and the rest of the world accounts for the balance. There is a paucity of data for the former Soviet Union and Eastern European countries. In addition to a production volume of greater than 1,250,000 tonnes per year, it is estimated that the European Union imports a further 100,000-300,000 tonnes per annum from outside Europe. **Figure 2.1** shows the 1993 world acrylonitrile demand by regions in more detail, while **Figure 2.2** shows breakdown of the total tonnage into various end uses. Approximately 52% of the total EU production of acrylonitrile is used in production of fibres, 15% in production of ABS and SAN resins, 15% in the production of acrylamide and adiponitrile and 18% for other uses (source PCI World Acrylonitrile Report, 1996).

Figure 2.1 1993 World demand by regions

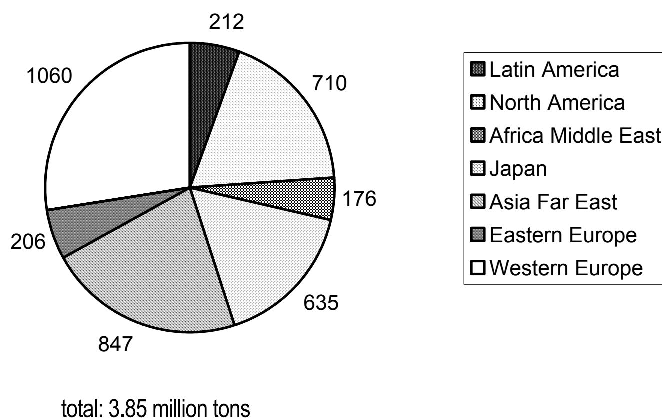
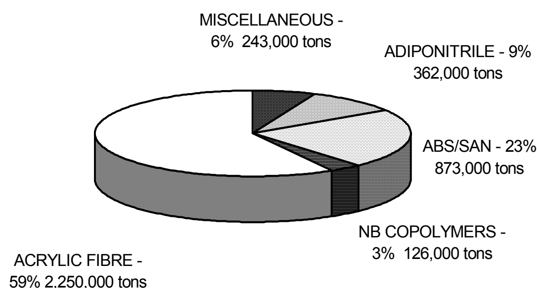


Figure 2.2 World acrylonitrile end use breakdown 1993



Acrylonitrile is produced in a closed system by catalytic ammoxidation of ammonia and propylene (Weissermel and Arpe, 1988). Fractional distillation of the crude (85%) product following scrubbing to remove ammonia yields 99.9% pure acrylonitrile.

2.2 USES

Acrylonitrile is now used almost exclusively as a monomer in the production of polymeric materials, with some use as a precursor for acrylamide and adiponitrile. Acrylonitrile can therefore be regarded as an industrial intermediate.

2.2.1 Fibres

As shown in **Figure 2.2**, the largest use of acrylonitrile is the production of acrylic and modacrylic textile fibres, some 60% of total production being dedicated to this end use. These fibres are used in clothing, domestic furnishings and other industrial purposes such as a precursor to carbon fibres, concrete reinforcement fibre and asbestos replacement. The use of acrylonitrile in production of acrylic fibre worldwide in 1993 was approximately 2,250,000 tonnes, while the average use in Western Europe over the period 1991-1995 was approximately 700,000 tonnes (source PCI World Acrylonitrile Report, 1996). Acrylic fibres are predominantly manufactured from acrylonitrile (>85%), with other monomers such as acrylates, methacrylates or vinyl acetate being used as minor constituents of the fibre, while modacrylic fibres are copolymers of acrylonitrile (35-85%) with vinyl chloride, vinylidene chloride and other vinyl monomers. The polymerised product in solution is extruded using either a wet or dry spinning process to give a bulk fibre product for end use.

2.2.2 ABS and SAN plastics

The second largest use of acrylonitrile is in the production of acrylonitrile-butadiene-styrene (ABS) and styrene-acrylonitrile (SAN) plastics. Approximately 20% of total volume or 660,000 tonnes were produced in Western Europe in 1995, with utilisation of approximately 200,000 tonnes of acrylonitrile (PCI World Acrylonitrile Report, 1996). Reaction of acrylonitrile with butadiene and/or styrene in emulsion or solution polymerisation systems and further processing of the polymer gives a rigid plastic product which has a wide variety of uses including automotive parts, household appliances, pipe fittings, products likely to come in contact with food, and other end products.

2.2.3 Adiponitrile and acrylamide synthesis

A relatively large proportion of the total acrylonitrile production in Europe is used in the synthesis of these two monomers, amounting to approximately 100,000 tonnes of each in 1995 (source PCI World Acrylonitrile Report, 1996). The predominant use of both monomers is also in the production of polymeric materials. Acrylamide is produced from acrylonitrile by a catalytic hydration process in solution in which unreacted acrylonitrile is recovered and recycled.

2.2.4 Nitrile rubbers

Acrylonitrile can also be co-polymerised with butadiene to produce nitrile rubber, nitrile rubber latex and elastomers. Production in this sector in Western Europe in 1995 amounted to 134,000 tonnes (46,000 tonnes of acrylonitrile) (source PCI World Acrylonitrile Report, 1996). Although some traditional uses for nitrile rubber are in decline, these polymers are still widely used in products which are likely to come in contact with petroleum products, solvents, oil, etc. and in personal protective equipment, due to their low permeability and resistance. An increasing use is in nitrile latices.

2.2.5 Other uses

Other uses include the synthesis of novel polymeric materials, production of fatty amines and fatty alcohols and other miscellaneous uses. The former use of acrylonitrile as a fumigant and pesticide in agriculture and in flour milling has now been discontinued in the European Union.

2.3 EXPOSURE CONTROLS

The toxicity profile, including potential carcinogenicity, of acrylonitrile and potential for exposure due to the high volatility of the substance has led to stringent controls on exposure. Occupational exposure is controlled via engineering controls and adherence to occupational exposure limits (2 ppm or 4.5 mg/m^3 in most areas of the world), together with use of personal protective equipment (PPE). Exposure of the environment is controlled via controls on air and water emissions, existing in the majority of countries involved in the production or further use of acrylonitrile.

Controls on emissions necessitate incineration of wastes and/or post-stripping of both gaseous and aqueous effluents. In Germany, the Technische Anleitung zur Reinhaltung der Luft (TA-Luft) restricts installation emissions to 5 mg/m^3 with a mass flow in excess of 25 g/h, and acrylonitrile in emissions from incinerators should not exceed 0.2 mg/m^3. Specific limits are also applied to emissions from e.g. the drying stage of fibre production and from ABS plastic and nitrile rubber production. Similar restrictions exist on acrylonitrile emissions in many other countries.

In respect of consumer exposure, regulatory controls on maximum levels of acrylonitrile permissible in products coming in contact with food products also exist in the majority of countries using acrylonitrile copolymers.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

Acrylonitrile does not occur naturally in the terrestrial environment, although it has been detected in interstellar space (Gardner and Winnewisser, 1975). Anthropogenic acrylonitrile can potentially be released to the environment during (1) synthesis of the monomer, (2) polymer production, (3) end product usage. Releases of acrylonitrile may also occur as a result of (4) combustion of hydrocarbon fuels and (5) cigarette smoking. The incomplete combustion during incineration of municipal wastewater sludge has been identified as a minor source of release of acrylonitrile to the environment. The major compartments of release are water and air.

Sources (1) and (2) can be regarded as point sources of release and, given the physico-chemical characteristics of acrylonitrile, represent the major industrial sources of release. Production of acrylonitrile and further reaction to polymeric products may occur within the same facility, with consequent releases due to both processes, or production and further processing may take place at separate facilities. Emissions as a result of (3) can be considered as diffuse sources, but given the solid polymeric nature of these products and the low concentrations of free monomer detectable in them, represent a relatively minor source of release of acrylonitrile. Cigarettes also represent diffuse emission sources of acrylonitrile, but are again a relatively minor source compared with production and further processing.

Diffuse releases as a result of (4) and (5) may contribute more significantly to the overall releases of acrylonitrile into the environment. Benjey (1993) examined annual emissions for dominant point, area and mobile source categories for acrylonitrile, based on the 1990 interim emission inventory for volatile organic compounds in the USA. He attributed the proportion (as a percentage) of the estimated total emissions of 160,000 tonnes to various source category codes (SCC), and concluded the following:

- 11% of acrylonitrile emissions were attributable to light duty gas vehicles, urban roads (vehicle exhaust);
- 7% to hazardous waste treatment and storage;
- 7% to miscellaneous non-industrial solvent use;
- 6% to production of acrylonitrile;
- 5% to light duty gas vehicles, rural roads (vehicle exhaust);
- 4% to off-highway gasoline vehicles (vehicle exhaust);
- 4% to light duty gas trucks, urban roads (vehicle exhaust);
- 3% to waste gas flares
- 3% to miscellaneous general plastics production
- 3% to gasoline marketing
- 47% to other uses

More recently, however, Benjey has concluded (personal communication to Environment Canada, also made available to the authors of this report) that mobile source (vehicle exhaust) contribution to total acrylonitrile emissions is considerably less than he reported in 1993. Instead of 24% it is likely to be in the range of 5–10%, primarily from diesel car exhausts. Environment

Canada (personal communication) has concluded that emissions from vehicle exhaust are unlikely to be significant, due to improvements in catalyst technology together with stoichiometric control of engine operation.

3.1.2 Environmental releases

3.1.2.1 Releases of acrylonitrile during production

Acrylonitrile is now produced from ammonia and propylene via catalytic ammoxidation in a closed system. The predominant process used is the Sohio process, which achieves greater than 85% conversion rates from stoichiometric quantities of ammonia and propylene in the presence of air at 400-500°C at 20-200 kPa (e.g. Groet et al., 1974; Weissermel and Arpe, 1988; Langvardt, 1985). Fractional distillation of the crude product following scrubbing to remove ammonia results in 99.9% pure acrylonitrile, which may be used on site for the production of acrylonitrile-containing polymers or transported by road, rail or ship in stabilised monomeric form to end users for the production of acrylonitrile polymers and copolymers. By-products from the reaction include acetonitrile and hydrogen cyanide.

Although production takes place in closed systems in a largely continuous process, start-up, shut-down, product recovery and purification steps result in some release of acrylonitrile to waste. However, wastes are either incinerated, or treated by for example gas scrubbing of emissions followed by release of scrubber washes to wastewater, thus significantly reducing the environmental emissions.

At the time that data were collected for the purposes of this risk assessment, in 1994–1996, acrylonitrile was produced by 7 manufacturers at 8 sites in the European Union, located in Germany, Italy, Netherlands, Spain and the United Kingdom. The production volume at that time was approximately 1,250,000 tonnes per annum. Production facilities in Austria, France and a second production facility in the UK halted production in 1990-1992. **Table 3.1** presents individual production data for the currently operational sites, obtained from the IUCLID data sheets submitted by the companies and the PCI World Report (1996). The majority of companies have independently confirmed production data provided in the PCI World Report, with minor changes to tonnages in some cases. For reasons of confidentiality the sites have been coded to avoid identification of individual plants.

Specific emission data for releases to water and air have been provided for all eight sites. This information is summarised in **Table 3.1** and in Appendices 1.1 and 1.2.

On a total EU production tonnage in 1996 of 1,250,500 tonnes, maximum releases to water totalled approximately 14 tonnes, 0.01 kg per tonne or 0.001% (**Table 3.1** and Appendix A.1). All of the sites except site 5 have industrial WWTP facilities, while site 5 discharges directly into the marine environment. These releases can be compared with figures for estimated total releases from acrylonitrile plants in the US in 1976, which indicated a figure of approximately 2% of production or 20 kg per tonne. The introduction of stricter emission controls in the US reduced estimated releases significantly. By 1993, total releases had fallen to approximately 2,380 tonnes per annum (0.16% or 1.6 kg per tonne), of which approximately 630 tonnes were released to air (fugitive or non-point emissions plus stack emissions), 1.4 tonnes to surface water and approximately 1,740 tonnes disposed by underground injection (1993 US EPA Toxics Release Inventory). Estimates of total discharges into the aquatic environment for the Federal

Republic of Germany in 1990/91 indicated less than 520 kg per annum as a result of acrylonitrile production (less than 0.0002%) (BUA Report, 1995).

On the 1996 production tonnage of 1,250,500 tonnes, emissions to air in the EU totalled approximately 280 tonnes, 0.22 kg per tonne or 0.02% (**Table 3.1**, Appendix A.2). In the Federal Republic of Germany in 1990, atmospheric releases from acrylonitrile production and from further processing were estimated as 6 tonnes and 56 tonnes, respectively (BUA Report, 1995). This is based on a total production figure of 340,000 tonnes (approximately 0.02% overall, 0.002% for production), and represents a release of 0.02 kg per tonne for acrylonitrile production and 0.16 kg per tonne for further processing. The substantially lower figure for acrylonitrile production compared with further processing reflects the fact that production of acrylonitrile is carried out in effectively closed systems, while production of the acrylonitrile-containing polymeric products is, in part, open system use.

Table 3.1 Aquatic and atmospheric releases of acrylonitrile from production sites in the EU

Site	Production in 1996 (t/year)	Release to water /maximum measured effluent levels	Comments	Atmospheric release /maximum emission levels	Comments
1	120,500 (1997)	< 100 kg/yr, Acrylonitrile not detected in waste effluent, detection limit 0.1 ppm (100 µg/l)	Industry data. Wastewater treatment by distillation and stripping with steam. Emissions to sea	1.235 t/yr	Industry data
2	190,000	65 t/yr to WWTP 3.8 mg/l in influent to WWTP (1996), max. 1.3 µg/l in effluent, flow rate 360m^3/hr, estimated release from WWTP 4.0 t/yr	Industry data. Emissions from this site are combined emissions for a production facility and a ABS/SAN facility	12.4 t/yr Measured average fenceline concentration 0.6 µg/m^3 95% C.L 2.5 µg/m^3 (1995)	Industry data
3	85,000	< 2.5 mg/l in influent to WWTP, flow 360 m^3/d, max, emission to WWTP 330 kg/yr, estimated 43 kg/yr from WWTP	Industry data. Emissions to sea	5 t/yr	Industry data (point and fugitive emissions)
4	300,000	< 31 kg/yr (1991) Acrylonitrile not detected in WWTP effluent, detection limit 2 µg/l	Industry data. Combined emissions for a production facility, a fibre facility and an ABS/ SAN polymer facility	3.2 t/yr (1995)	Source, Emissionsminderung Germany (1996)
5	280,000	9.3 t/yr 5.8 mg/l in effluent (1995)	Industry data. Emissions to large marine estuary, dilution at discharge point >> 490	259 t/yr	Industry data. Release represents 197 tonnes from storage (modelled), 62 tonnes from production (monitored)
6	60,000	< 40 kg/yr (1996)	Industry data	54 kg/yr (1996)	Industry data
7	110,000	< 24 kg/yr (1996) < 50 µg/l in effluent from WWTP	Industry data. Site-specific biodegradation data from industry indicate 93.9% removal in WWTP.	2.3 t/yr	Industry data

Table 3.1 continued overleaf

Table 3.1 continued Aquatic and atmospheric releases of acrylonitrile from production sites in the EU

Site	Production in 1996 (t/year)	Release to water /maximum measured effluent levels	Comments	Atmospheric release /maximum emission levels	Comments
8	105,000	2.5 mg/l in influent to WWTP, flow 360 m^3/d, estimated release to WWTP 330 kg/yr, estimated release from WWTP 53 kg/yr. AN not detectable in effluent from WWTP	Industry data. Emissions to sea	2 t/yr	Industry data (point and fugitive emissions)

Source PCI World Acrylonitrile and Derivatives Supply/Demand Report (1996); BUA Report (1995) and industry information.

3.1.2.2 Releases during processing of acrylonitrile to polymers

As outlined in Section 2.2, acrylonitrile is predominantly used as a monomer in the production of a range of polymeric materials, using broadly similar polymerisation processes. Following polymerisation, unreacted monomer is recovered and recycled to the reactor. The subsequent processing of the initial polymers varies according to the nature of the final product (fibres, plastics, nitrile rubbers). Approximately 52% of the total EU production of acrylonitrile is used in the production of fibres, 15% in the production of ABS and SAN resins, 15% in the production of acrylamide and adiponitrile and 18% for other uses.

Data provided by the PCI World Acrylonitrile and Derivatives Supply/Demand Report (1996) indicated that at that time there were 11 major facilities producing acrylic fibres throughout Western Europe, two of which ceased production in 1996/97, 13 facilities producing ABS/SAN plastics, 10 facilities producing nitrile:butadiene copolymers (one company shown in **Table 3.4**, III, has since ceased production), 3 facilities producing acrylamide and 1 producing adiponitrile. Information provided by industry indicates that limited (drum) quantities of acrylonitrile are used by an unquantifiable number of small companies in Europe. The estimate for drummed product for the whole European Union market is less than 1,000 tonnes, on a total production tonnage of 1,250,500 tonnes, or less than 0.1%. The fraction of total acrylonitrile usage accounted for by these companies is thus considered to be very small relative to that used by the 38 major facilities identified above, and has not been taken into account in this assessment of environmental exposure. The PCI Report detailed production capacity and use data for all of the major facilities, as reproduced in **Tables 3.2, 3.3, 3.4** and **3.5**, with minor changes to tonnages based on updated data from industry. The sites have been coded to avoid identification of individual plants, which are or were located in France, Germany, Belgium, the Netherlands, Spain, Portugal, Italy, the United Kingdom and Ireland.

The location of facilities processing acrylonitrile to polymers was established prior to the accession of Austria, Finland and Sweden to the European Union, and processing facilities in these countries have not therefore been taken into consideration in this assessment of environmental exposure. Similarly, a small number of additional facilities involved in the production of acrylonitrile polymers have opened elsewhere in the European Union since data were first gathered for this risk assessment in 1994–1996. These have also not been taken into consideration in this risk assessment.

Atmospheric and aquatic releases data have been provided by industry for all currently operational sites, are also shown in the tables. These industry-derived data have been used to

derive local PECs in Sections 3.1.1 and 3.1.2. For the 3 sites which have now ceased production and for which emission data were not provided the default release estimates provided by the TGD were used to model theoretical releases in **Tables 3.2 and 3.4**. In calculating default emissions for processing acrylonitrile to other products, Table A.3.10 of the TGD was applied for processing of acrylonitrile to polymers or copolymers such as fibres, ABS and SAN resins and nitrile rubbers. Type 1 use is assumed (monomer, UC 43, process regulator), in a "wet" polymerisation reaction, providing the following emission factors:

Air 0.05; Wastewater 0.01 ; Soil 0 ; Emission days 300.

However since production at these sites has now ceased, these estimates have not been used further in derivation of local or regional PECs in Sections 3.1.1 and 3.1.2.

Table 3.2 Production data, aquatic and atmospheric releases of acrylonitrile from acrylic fibre production facilities in the EU

Site	Acrylonitrile processed t/year (1996)	Release to water and /or maximum measured effluent levels	Comments	Release to atmosphere or maximum emission levels	Comments
A	23,000 [1)]	60 kg/yr, 0.04 ppm average 1994-1995	Maximum 0.7 ppm	1,150 t/yr[3]	Default value
B	70,000	5.75 t/yr Emissions to sea [2)]	Industry data	14 t/yr	Industry data
C	40,000	< 200 kg/yr 0.2 mg/l in effluent	Industry data No WWTP	20.4 t/yr	Industry data
D	112,000	< 31 kg/yr (1991) [3)] Undetectable in WWTP effluent	Detection limit 2 µg/l	85 kg/yr (1995)	Source Emissionsminderung Germany (1996)
E	78,000	294 t/yr, 35 mg/l in effluent (1995)	Industry data	154 t/yr	Industry data
F	130,000	< 235 kg/yr, <0.25 mg/l in effluent of WWTP (below detection limit)	Industry data	26.2 t/yr	Industry data
G	62,000	Not detectable in effluent from WWTP, Estimated annual release 200 kg/yr	Industry data Detection limit 0.1 mg/l,	5 t/yr	Industry data
H	40,000	2.13 t/yr (1996) < 0.5 mg/l in effluent of WWTP (below detection limit)	Industry data	41.2 t/yr	Industry data
I	58,000	580 t/yr [4)]	Default value	2900 t/yr	Default value
J	49,000	< 8 kg/yr Undetectable at detection limit of 0.1 mg/l	Industry data	13.3 t/yr	Emissionserklärung Germany (1996)
K	78,000	< 350 kg/yr, <0.25 mg/l in effluent of WWTP	Industry data	16 t/yr	Industry data

Source PCI World Acrylonitrile & Derivatives Supply/Demand Report (1996), BUA Report (1995) and industry information

1) This facility ceased production in 1997, and has not been included in PEC calculations

2) This site will complete a WWT facility by 2000

3) Emissions from this site are combined emissions for a production facility, a facility producing acrylic fibres and a facility producing ABS/SAN polymers

4) This facility ceased production in 1996, and has not been included in PEC calculations

Table 3.3 Production data, aquatic and atmospheric releases of acrylonitrile from ABS/SAN production facilities in the EU

Site	Acrylonitrile processed in t/year (1996)	Release to water and or maximum measured effluent levels	Comments	Release to atmosphere or maximum emission levels	Comments
AA	10,300	3.6 t/yr (1997). Mean effluent concentration 1.16 mg/l	Industry data. Direct release to sea, no WWTP	35 t/yr	Industry data
BB	26,000	65 t/yr to WWTP [1)], estimated release from WWTP 4.0 t/yr	Industry data	23.5 t/yr Av. emissions 0.6 µg/m³	Industry data 95 % C.L 2.5 µg/m³ (1995)
CC	18,000	25 t/yr to municipal WWTP, <1 t/yr from municipal WWTP	Industry data	20 t/yr.	Industry data
DD	5,000	WWTP effluent < 0.1 mg/l, estimate for annual release 500 kg/yr.	Industry data	3 t/yr.	Industry data
EE	30,000	< 31 kg/yr (1991) [2)] Acrylonitrile not detected in WWTP effluent, detection limit 2 µg/l	Industry data	<3.1 t/yr (1995)	BUA Report Incineration of exhaust gases.
FF	4,000	0.5 t/yr. Average effluent concentration 0.2 mg/l	Industry data	4.3 t/yr	Industry data
GG	16,000	4 kg/yr	Industry data.	73 t/yr	Industry data
HH	25,000	4 kg/yr Effluent concentration < 1µg/l	Industry data	1.45 t/yr	Industry data
II	27,000	< 100 kg pa before WWTP, <10 kg/yr post WWTP	Industry data	585 kg/yr	Industry data
JJ	12,000	Nil. Undetectable in effluent, processing at pH 10.00 results in complete hydrolysis	Industry data	17 t/yr	Industry data
KK	4,500	Influent to WWTP < 6.2 mg/l, estimated release 5.72 t/yr.	Industry data	11 t/yr	Industry data
LL	48,000	13,2 t/yr WWTP effluent < 100 µg/l [3)]	Industry data	5.5 t/yr	Industry data
MM	16,000	WWTP effluent < 0.05 mg/l, estimated < 100 kg/yr post WWTP	Industry data	Not detected	Incineration of all volatile emissions

Source PCI World Acrylonitrile & Derivatives Supply/Demand Report (1996), BUA Report (1995) and industry information

1) Emissions are combined emissions for a production facility and a facility producing ABS/SAN polymers

2) Emissions are combined emissions for a production facility, a facility producing acrylic fibres and a facility producing ABS/SAN polymers

3) Emissions also include emissions from a small facility producing NB latices

Table 3.4 Production data, aquatic and atmospheric releases of acrylonitrile from nitrile:butadiene copolymer production facilities in the EU

Site	Acrylonitrile processed in t/year (1996)	Release to water and or maximum measured effluent levels	Comments	Release to atmosphere or maximum emission levels	Comments
AAA	4,500 NB copolymer	8.14 t/yr (1995) 11.8 mg/l in effluent	Industry data No WWTP	21.6 t/yr (1995)	Industry data
BBB	1,200 NB latex	5.26 t/yr to municipal WWTP	Industry data. Initial physicochemical treatment of WW containing <50 mg/l	<17.5 kg/yr	Industry data Off-gas streams burnt off by TAREX system
CCC	1,100 NB latex	1,100 kg/yr to WWTP, 28 kg/yr post WWTP	Industry data	660 kg/pa	Industry data
DDD	1,500 NB latex	≤ 90 kg/yr, concentration in effluent <1 mg/l (limit of detection)	Industry data	< 1 t/yr	Industry data
EEE	10,450 NB copolymer	3.15 t/yr	Industry data	0.6 t/yr	Industry data
FFF	9,000 NB copolymer 3,000 NB latex	Initial release to WWTP 60 tpa, (1989) max release to hydrosphere approx. 2.7 tonnes (1991), undetectable in WWTP effluent, limit of detection 50 µg/l	BUA Report (1995)	1.1 t/yr	BUA Report (1995) Incineration of waste gases
GGG	4,400 NB copolymer, NB latex	3.2 t/yr	Industry data	20.2 t/yr	Industry data
HHH	600 NB latex	13.2 t/yr, concentration in WWTP effluent <100 µg/l [1)]	Industry data	5.5 t/yr [9]	Industry data
III	3,000 NB copolymer	30 t/yr [2)]	Default value	150 t/yr	Default value
JJJ	10,000 NB latex	< 4 t/yr 2.5 mg/l into WWTP, estimated < 0.25 mg/l in effluent, assuming 90% biodegradation	Industry data	4 t/yr	Industry data

Source PCI World Acrylonitrile & Derivatives Supply/Demand Report (1996), BUA Report (1995) and industry information

1) Emissions are combined emissions for a facility producing ABS/SAN polymers and this facility, producing NB latices

2) This facility has now ceased production, and has not been included in PEC calculations

Table 3.5 Production data, aquatic and atmospheric releases of acrylonitrile from acrylamide and adiponitrile production facilities in the EU

Site	Acrylonitrile processed in t/year (1996)	Release to water and or maximum measured effluent levels	Comments	Release to atmosphere or maximum emission levels	Comments
L	39,000 acrylamide and acrylic acid	Nil (not detectable in effluent) (1995)	Industry data	4.5 t/yr (1995)	Industry data
M	40,000 acrylamide	<30 kg/yr (1997) Acrylonitrile not detectable in effluent (limit of detection 1 mg/l)	Industry data	<1.5 t/yr (1997)	Industry data
N	161,000 adiponitrile	Nil (not detectable in effluent)	Industry data	95 t/yr	Industry data The total release represents 41 tonnes from production (monitored) and 54 tonnes from storage (modelled)
O	23,000 acrylamide	Nil (not detectable in effluent)	Industry data	53 kg/yr	Industry data

Source PCI World Acrylonitrile & Derivatives Supply/Demand Report (1996), BUA Report (1995) and industry information.

The data in **Table 3.2** and Appendix A.2 show that for processing of acrylonitrile to fibres, an approximate total of 300 tonnes acrylonitrile per annum were released to water on a total acrylonitrile consumption of 659,000 tonnes, excluding sites A and I that are no longer in production. This represents a release of 0.46 kg/tonne, which can be compared with a figure of 0.01 kg per tonne for production facilities. However, 294 tonnes of this release were related to site E, and if this marine site is excluded, together with its production figure, the figure becomes 0.015 kg per tonne. The figure for processing to ABS/SAN polymers was 0.1 kg/tonne and to NB copolymers was 0.87kg/tonne (**Tables 3.3, 3.4** and Appendix A.2). Releases to water for processing to acrylamide and adiponitrile were extremely low (**Table 3.5**).

A total of 290 tonnes acrylonitrile were released to air during processing of acrylonitrile to fibres, on a total acrylonitrile consumption of 659,000 tonnes, excluding sites A and I, representing a release of 0.44 kg/tonne. The figure for processing to ABS/SAN polymers was 0.82 kg/tonne, for processing to acrylamide and adiponitrile was 0.38 kg/tonne and to NB copolymers was 1.2 kg/tonne. In the Federal Republic of Germany in 1990, total atmospheric releases from further processing were estimated as 56 tonnes (BUA Report, 1995), based on a total production figure of 340,000 tonnes (0.16 kg per tonne). Of this, 36.6 tonnes related to production of polyacrylonitrile and fibres by three large producers. Comparable figures for the United Kingdom for 1995 were approximately 615 tonnes to air on an annual ACN processing capacity of approximately 250,000 tonnes, or 2.46 kg/tonne.

3.1.2.3 Regional and continental releases due to point sources

The total releases from all currently operational production and processing plants, as summarised in **Tables 3.1 to 3.5** and Appendices A.1, A.2, A.3, A.4, provide a figure of 393 tonnes per annum release to water, including the emission of 294 tonnes related to site E. The releases to air

for continental releases at a European level are 900 (rounded to the nearest 100) tonnes per annum to air.

It should be noted that these figures reflect the total releases from all currently operational production and processing plants, wherever their location. However, the TGD focus on releases to the land-based aquatic environment in the consideration of emissions to water on a regional basis and in derivation of $PECregional_{water}$ and $PECcontinental_{water}$ using EUSES. At least 13 of the 43 production and further processing facilities in Europe are located in marine or estuarine locations, and if the emissions from these 13 sites are excluded from consideration, a figure of 43 tonnes per annum release to water is derived.

In considering the impact of releases from marine or estuarine locations to atmosphere, the validity of excluding such sites in the consideration of atmospheric emissions, both regional and continental, can be questioned, despite the absence of guidance on this aspect in the TGD. For this reason, although a figure of 184 tonnes per annum can be derived if these sites are excluded, the figure of 900 tonnes per annum derived from totalling emissions from all sites is considered to represent a more appropriate worst-case analysis.

The continental release figures can be used as a basis for the estimation of regional emissions, following the “10% rule”. Using the release figure of 43 tonnes per annum to surface water derived following exclusion of the marine or estuarine locations and the figure of 900 tonnes derived from the atmospheric emissions of all the sites, the corresponding figures for regional releases are 4.3 tonnes per annum to water and 90 tonnes to air.

An alternative approach to the derivation of typical regional emissions of acrylonitrile is to total the estimated emissions for both the production and processing scenarios in (1) Germany, (2) the Netherlands and Belgium. These two regions of the European Union have been chosen because of the preponderance of marine sites in other major regions of production and processing. Using this approach, the figures for Germany are 18.1 tonnes per annum released to the hydrosphere and 75.5 tonnes released to the atmosphere, on a production volume of 470,000 tonnes acrylonitrile (37.6% of total European production). The figures for the Netherlands and Belgium are 5.1 tonnes to the hydrosphere and 60.1 tonnes to the atmosphere, on a production volume of 190,000 tonnes (15.2% of total European production).

The figures for releases to the hydrosphere for both regions are comparable to those derived using the 10% rule on emissions excluding those of the marine or estuarine locations, when adjustment is made for the actual production tonnages in the regions. The estimates for atmospheric emissions are however substantially lower than those derived by application of the “10% rule” to the estimated continental releases. This is not unexpected, since a number of sites in other countries have relatively high emissions to air. Application of the 10% rule to continental air emissions therefore may be considered a worst-case approach to estimation of regional releases.

As an alternative to the 10% rule in relation to atmospheric emissions, the total emissions from production and processing facilities in the United Kingdom can be taken as a worst-case approach. This provided a figure of 608 tonnes per annum for regional releases to atmosphere, which is considerably higher than the 90 tonnes per annum derived using the 10% rule.

3.1.2.4 Regional and continental releases due to point and diffuse sources

The estimates in Section 3.1.2.3 only represent releases from major point sources. They do not take into account regional/continental releases due to minor point or diffuse sources such as further processing of acrylonitrile-containing polymers and diffuse sources such as waste disposal, transport and storage of acrylonitrile and vehicle emissions, as considered in (A) to (C) below.

(A) Releases during further processing and use of acrylonitrile polymers

Subsequent processing steps involving acrylonitrile in polymerised form, e.g. drying, dyeing of fibres or use in textile manufacture, shaping of acrylonitrile plastics and rubbers, are assumed to result in relatively minor releases of free acrylonitrile compared with the initial polymerisation and processing steps, given the low content of residual monomer. Levels of free monomer generally in the range 1-10 ppm were reported in articles made from acrylonitrile-containing polymers (Page and Charbonneau, 1983; Vaz, 1983), see also Section 4.1.1.3.2, although higher levels have also been cited in studies carried out in the 1970’s. Assuming a reasonable worst-case scenario of a level of 100 ppm in plastics before moulding or extrusion into the wide range of articles derived from acrylonitrile-containing polymers, a reduction in the level of residual free monomer after moulding or extrusion to 10 ppm, with no emissions controls in place and a total production volume of approximately 1,000,000 tonnes per annum, including fibres, a release of 90 tonnes per annum to air can be estimated from this source. Total release of residual monomer from the articles during use and/or subsequent disposal would contribute an additional 10 tonnes per annum, giving a worst-case total of 100 tonnes per annum from this source. This contribution to the total diffuse emissions is relatively small in comparison with the total estimated emissions to air of approximately 900 tonnes per annum taken from the company data in **Tables 3.1, 3.2, 3.3, 3.4** and **3.5**.

Forrest et al. (1995) sampled the working environment in the immediate vicinity of ABS and SAN injection moulding, with a detection limit of $1 \cdot 10^{-4}$ mg/m^3 and was able to detect acrylonitrile during purging of the machine, but not during normal operation, indicating that releases during thermoprocessing of acrylonitrile plastics are very low.

A worst-case scenario for the release of acrylonitrile from acrylonitrile polymers following disposal in landfills has been modelled for ABS polymers. Assuming a maximum concentration of 100 ppm of acrylonitrile in the polymer, polymer dimensions of 0.5 cm $\cdot$ 1 m^2, a polymer:soil ratio of 1:10 (1 m^3 ABS in 10 m^3 soil) and using the AMEM OECD program, it can be estimated that 0.0372 g acrylonitrile will be released from a 5 kg sheet of ABS polymer over a period of 10 years, 0.7 g per tonne per annum. Assuming a EU production of 250,000 tonnes per annum of ABS and SAN resins and a 1% disposal to landfill, this represents a release of 1.86 kg acrylonitrile per annum.

While not directly relevant to emissions to the wider environment, Section 4.1.1.3.1 provides information on acrylonitrile release from acrylic fibres which further confirms the very low levels of free monomer in acrylonitrile polymers and the view that disposal of such products by e.g. landfill will not contribute substantially to overall environmental releases of acrylonitrile.

(B) Releases during storage and transport of acrylonitrile

Releases of acrylonitrile during storage and transport have been assumed to be negligible, reflecting the use of dedicated, closed-system containers.

(C) Release of acrylonitrile from wastes and other sources

BUA (1995) reported that in Germany aqueous and atmospheric wastes from production and further processing plants are routinely pretreated by incineration, gas scrubbing and wastewater treatment as applicable. Waste residues are also in the main incinerated, with limited landfill for low-level acrylonitrile wastes. The method of disposal of acrylonitrile wastes by deep-well injection used in the US before 1977 was not used in Western Europe. It is unlikely, therefore that a significant quantity of acrylonitrile will be released from waste.

Baker et al. (1984) reported that acrylonitrile could be detected in the emissions from cigarette burning tests at levels of 13-17 µg per standard cigarette (70 mm in length and 25 mm in diameter, containing 1g of tobacco). Given a total of 606,756 tonnes of tobacco smoked in EU Member States excluding Greece (no data available) in 1994 (Statistiska centralbyran, Sweden, 1995), with an average of 15 µg acrylonitrile produced per cigarette, total emissions of acrylonitrile in Europe (excluding Greece) from this source would amount to 9.1 tonnes. Again, this contribution to the total diffuse emissions is small in comparison with the total estimated emissions to air of approximately 900 tonnes per annum taken from production and processing data. It must be recognised, however that the exposure may be high for the smoker themselves and for those in the vicinity of smokers.

Vehicle exhausts were reported by Benjey (1993) to represent a significant source of diffuse emissions of acrylonitrile to air. Benjey estimated that 24% of the total US emissions of 160,000 tonnes per year were attributable to vehicle exhausts, while 10% were due to acrylonitrile production and miscellaneous general plastics production, and 47% to other (unspecified) sources. He further estimated that point sources accounted for only 24% of total emissions. Benjey’s data were largely based on default assumptions, and only a very rough estimate of diffuse emissions due to non-point or, more specifically, vehicle exhausts can be derived, using these data as a basis.

An assumption has been made that emissions due to vehicle exhausts in the EU may be 2.5 times those attributable to production and further processing of acrylonitrile. Taking the estimate for continental releases to air of approximately 900 tonnes per annum which has been derived in Section 3.1.2.3 by totalling the reported releases from all currently operational production and processing plants, an estimate of approximately 2,300 tonnes per annum (to the nearest 100) can be derived for vehicle emissions. However, as already stated in Section 3.1.1, more recently Benjey has concluded (personal communication to Environment Canada, then communicated to the authors of this report) that mobile source (vehicle exhaust) contribution to total acrylonitrile emissions is considerably less than he reported in 1993. Instead of 24%, it is likely to be in the range of 5-10%, primarily from diesel car exhausts. If a figure of 10% is assumed, then using Benjey's assumptions, emissions from this source would be roughly similar to those from production and further processing, approximately 900 tonnes per annum.

The estimated release of the higher figure of 2,300 tonnes per annum of acrylonitrile from vehicle exhausts, together with an additional 110 tonnes from other diffuse sources (release from fibres, plastics and cigarette smoke) can be added to the estimate of 900 tonnes per annum for continental releases from point sources to give a total of 3,310 tonnes per annum to air. If the lower figure for vehicle exhaust emissions were used, the total emissions to air would be in the region of 1,910 tonnes per annum. Emissions to water remain at an estimated 43 tonnes per annum (excluding marine sites). Regional releases derived from these figures using the 10% rule are 330 tonnes per annum to air and 4.3 tonnes to water. These estimates are summarised in

Table 3.6, which also includes the regional releases derived for Germany and the Netherlands/Belgium and the releases to atmosphere estimated for the United Kingdom.

Table 3.6 Summary of estimated regional and continental releases of acrylonitrile from point and diffuse sources

	Regional emissions to surface water (t/yr)	Regional emissions to air (t/yr)	Continental emission to surface water	Continental emission to air (t/yr)
Derived from total point source emissions	4.3 (using 10% rule excluding marine sites)	90 (using 10% rule on total emissions)	43 (excluding marine sites)	900
Derived from total point source emissions for Germany	18.1	75.5	181 (x 10 regional emissions)	755 (x 10 regional emissions)
Derived from total point source emissions for the Netherlands and Belgium	5.1	60.1	51 (x 10 regional emissions)	601 (x 10 regional emissions)
Derived from total point source emissions for the United Kingdom (atmosphere only	NA	608	NA	6,080
Derived from total point source and diffuse emissions	4.3 (using 10% rule excluding marine sites)	330 (using 10% rule on total emissions)	43 (excluding marine sites)	3,310

It should be noted that the estimated total release of 3,310 tonnes per annum to air shown in **Table 3.6** is likely to represent a overestimate, given that the estimated 2,300 tonnes per annum of acrylonitrile from vehicle exhausts may be significantly lower in practice, e.g. 900 tonnes per annum, giving a total release of 1,910 tonnes.

3.1.3 Environmental fate

3.1.3.1 Degradation in the environment

3.1.3.1.1 Atmospheric degradation

Acrylonitrile is labile in the atmosphere, due to photodegradation processes. Studies of photooxidation of acrylonitrile by ozone and hydroxyl radicals ($OH^{\cdot}$) by several groups (Atkinson et al., 1982; Hansen et al.; 1982, Edney et al., 1982; Munshi, 1989) under simulated atmospheric conditions indicate that reaction with $OH^{\cdot}$ is the major loss process in the troposphere for acrylonitrile. The reaction with ozone is slow and is not likely to constitute a major route of degradation. Munshi (1989) determined a rate constant of $1.38 \cdot 10^{-19}$ cm^3/mol/s for the reaction of acrylonitrile with O_3, giving a trophospheric lifetime of 84 days, while Atkinson et al. (1982) determined a rate constant of $< 1.0 \cdot 10^{-19}$ cm^3/mol/s at an O_3 concentration of $\leq 2.4 \cdot 10^{13}$ mol/cm^3. Both Hansen et al. (1982) and Edney et al. (1982) have published a rate constant of $3.2 \cdot 10^{-12}$ cm^3/mol/s for the reaction of acrylonitrile with $OH^{\cdot}$, giving an estimated

half-life of 5 days in the troposphere, based on an estimated hydroxyl radical concentration of $5 \cdot 10^5$ mol/cm^3.

Theoretical estimation of the photo-oxidation of acrylonitrile using the AOPWINvl.55a Atmospheric Modelling Programme (Hoechst, 1994) has provided similar results, giving an overall predicted rate constant of $3.945 \cdot 10^{-12}$ cm^3/mol/s for the reaction of acrylonitrile with $OH^\cdot$, and a half-life of 4.067 days, based on a hydroxyl radical concentration of $5 \cdot 10^5$ mol/cm^3. The rate constant for the reaction of acrylonitrile with O_3 is predicted as $0.87 \cdot 10^{-19}$ cm^3/mol/s, giving a half-life of 130.971 days at an O_3 concentration of $7 \cdot 10^{11}$ mol/cm^3. Both experimental results (Hashimoto et al., 1984) and theoretical modelling (Hoechst, 1994) suggest that addition of $OH^\cdot$ to the olefinic double bond represents the initial degradation reaction.

Harris and co-workers (1981) showed that the reaction of acrylonitrile with $OH^\cdot$ was independent of temperature in the range studied but showed a small increase with increasing pressure. Formaldehyde has been demonstrated as a primary reaction product following reaction of acrylonitrile with $OH^\cdot$ in the presence of NO by Edney at al. (1982), Hashimoto et al. (1984) and Spicer et al. (1985). CO, HCN, formyl cyanide (HCOCN) and formic acid have also been reported as degradation products (Edney et al., 1982; Hashimoto et al., 1984).

Table 3.7 summarises the published rate constants and trophospheric half-lives for the reaction of acrylonitrile with $OH^\cdot$ and O_3. The estimated half-life for reaction with $OH^\cdot$ is sufficiently long to allow redistribution of acrylonitrile to the aqueous compartment and to soil, with associated exposure of populations in the vicinity of the emission source, but is unlikely to be long enough to allow redistribution to the stratosphere.

Table 3.7 Rate constants for reaction of acrylonitrile with hydroxyl radical and ozone and derived trophospheric lifetimes

Species	Rate constant cm3/mol/s	Trophospheric lifetime/half-life (days)	Reference
O_3	$1.38 \cdot 10^{-19}$	Lifetime 84 *	Munshi et al. (1989)
O_3	$<1.0 \cdot 10^{-19}$	Lifetime >115 *	Atkinson et al. (1982)
O_3	$0.87 \cdot 10^{-19}$	Half-life 131 **	Hoechst (1994)
$OH^\cdot$	$3.2 \cdot 10^{-12}$ ****	Half-life 5 ***	Hansen et al. (1982) Edney et al. (1982)
$OH^\cdot$	$3.95 \cdot 10^{-12}$	Half-life 4.1 ***	Hoechst (1994)

* Value assumes an O_3 concentration of $1 \cdot 10^{12}$ mol/cm^3
** Value assumes an O_3 concentration of $0.7 \cdot 10^{12}$ mol/cm^3
*** Value assumes an $OH^\cdot$ concentration of $5 \cdot 10^5$ mol/cm^3
**** Value used in EUSES

3.1.3.1.2 Aquatic degradation

Abiotic degradation

Acrylonitrile is relatively hydrolytically stable, with no hydrolysis reported to occur in distilled water over the pH range 4-10 (Going et al., 1978). Knoevenagel and Himmelreich (1975) reported photo-oxidation of acrylonitrile in the presence of water to occur under experimental conditions, approximately 25% degradation being reported in a 24-hour period. It appears that

elevated temperatures were used in this study, and the results may be of little relevance for normal environmental conditions. The authors suggest that this process of abiotic aquatic degradation will occur in surface waters, in the layers accessible by light. Randall (1980) reported 99.0% wet air oxidation of acrylonitrile after 1 hour at 275°C and pressures of 70-140 kg/cm^3.

Going et al. (1978) also demonstrated decomposition of acrylonitrile over a period of 23 days at a concentration of 10 mg/l in Mississipi River water at different pHs. Concentrations of acrylonitrile in river water at unadjusted pH fell linearly to undetectable levels by day 6, decomposition was slower at pH 4.0 and pH 10.0, although levels at pH 10 were also below the limits of detection by day 23. The degradation seen in this study may be due to a combination of biodegradation and volatilisation of acrylonitrile from the test medium rather than abiotic degradation.

Biodegradation

The biodegradation of acrylonitrile in aqueous systems has been extensively studied using a range of experimental systems. Much of the earlier literature relates to experimental simulation tests, acclimation studies and BOD/COD tests, rather than assessment of biodegradability using current Annex V or OECD test methods. Although two recent ready biodegradability tests carried out indicate that acrylonitrile cannot be regarded as readily biodegradable based on the results of the Annex V ready biodegradation test, a third ready biodegradability study in seawater has demonstrated almost 80% degradation over a 28-day period. The majority of the earlier studies in the literature show extensive biodegradation by acclimated microbial populations. When the emission data from the majority of sites are also taken into account, it can be concluded that acrylonitrile is rapidly biodegradable in situations where an adapted microbial population can be expected, such as in an industrial wastewater treatment plant, as discussed further in the conclusion to this Section. **Table 3.8** summarises the results of a large number of biodegradation studies carried out on acrylonitrile, using a variety of experimental approaches including the ready biodegradability tests mentioned above. Individual studies are described in more detail in the text.

Table 3.8 Biodegradation of acrylonitrile in aqueous systems

Method	Experimental details	Results	Comment	Reference
Ready biodegradability, closed bottle test, OECD 301D	2 mg/l acrylonitrile effluent from laboratory wastewater treatment plant	0% degradation at 28 days	Valid for risk assessment purposes	BASF (1996)
Ready biodegradability, modified MITI test OECD 301 C	100 mg/l acrylonitrile and 30 mg/l suspended solids	14.7% degradation in 28 days	Valid for risk assessment purposes	Chemicals Inspection and Testing Institute, Japan (1992)
Ready biodegradability in seawater, closed bottle test, OECD 306 (1992)	2.45 mg/l acrylonitrile, details of seawater not specified	78.9% degradation in 28 days, 45% within 10-day window	Use results with caution	AN Group (1996)
Modified BOD	Inoculum municipal sludge, acrylonitrile 10 mg/l, 30-day study	12-day lag period before biodegradation. BOD_5 0, BOD_{30} 1.21, ThOD 3.17, BOD_{30}/ThOD 38%.	Additional information	Buzzell et al. (1968) Young et al. (1968)

Table 3.8 continued overleaf

Table 3.8 continued Biodegradation of acrylonitrile in aqueous systems

Method	Experimental details	Results	Comment	Reference
Modified MITI test, inherent biodegradability OECD 302 C	30 mg/l acrylonitrile and 100 mg/l suspended solids	41-74% degradation over 28 days	Valid for risk assessment purposes	Chemicals Inspection and Testing Institute, Japan (1992)
Modified BOD test	Inoculum laboratory seed aeration culture fed industrial effluent sludge, 200-1,200 ppm acrylonitrile, 10-day study	50% inhibition of expected BOD at 400 mg/l, 10-day BOD 0.7, theoretical (COD) 2.3	Additional information	Mills and Stack (1954)
Modified BOD test	Inoculum laboratory seed aeration culture maintained on industrial effluent sludge	100% degradation of 10 mg/l acrylonitrile after acclimation period of 10 days with 10 mg/l. BOD 721 mg/g, COD 1612.5 mg/g, 44.7% biodegradation	Additional information	Cherry et al. (1956)
Modified BOD test	Inoculum Ohio River water, 10 mg/l acrylonitrile	Lag period of approx. 1 week followed by rapid degradation. BOD 0.5 on days 10-20, 1.3 on day 25	Additional information	Ludzack et al. (1959)
Inherent biodegradability/ acclimation study	Inoculum municipal activated sludge Study of removal of acrylonitrile in a continuous flow reactor over a 60-day period	Following acclimation, 97-98% removal of acrylonitrile based on BOD and 99.9% based on analysis of acrylonitrile	Valid for risk assessment purposes	Kinncannon et al. (1983) Stover and Kinncannon (1983)
Inherent biodegradability/ acclimation study	Inoculum industrial activated sludge. Study of removal of acrylonitrile in a bench scale activated sludge unit following initial spiking with 200 mg/l acrylonitrile	Following acclimation period, >99.97% removal of acrylonitrile at earliest evaluation period of 2 days	Valid for risk assessment purposes	Freeman and Schroy (1984)
Inherent biodegradability/ acclimation study	Inoculum weak settled sewage sludge. Study of removal of acrylonitrile in a bench scale activated sludge unit over 6 weeks with increasing concentrations of acrylonitrile (22-89 mg/l)	Following the initial 14-day acclimation period, >90-97% removal of acrylonitrile over subsequent 4 weeks. Efficiency decreased at >177 mg/l	Valid for risk assessment purposes	Ludzack et al. (1961)
Inherent biodegradability/acclimation study under anaerobic conditions	Inoculum municipal sewage sludge. Study of removal of acrylonitrile in a continuous flow volume digester system with increasing concentrations of acrylonitrile (1-20 mg/l)	Following attainment of equilibrium 20 mg/l AN had no effect on levels of effluent BOD.	Additional information	Lank and Wallace (1970)
Sludge respiration test EPA internal method	Respiration of unadapted anaerobic activated sludge over 6 hours	Inhibition of respiration at 50 mg/l and above	Additional information	Hovious et al. (1973)

Ready biodegradability studies

The Chemicals Inspection and Testing Institute, Japan, (1992) has examined the ready biodegradability of acrylonitrile using the OECD 301C, Ready Biodegradability: Modified MITI Test (I). The study was carried out over 28 days at 25$\pm$1°C with a test concentration of 100 mg/l acrylonitrile and 30 mg/l suspended solids. Degradation was measured by determination of Biological Oxygen Demand (BOD), Dissolved Organic Carbon (DOC) and specific chemical analysis. Degradation of acrylonitrile at 28 days was determined by comparison of the measured BOD with the calculated Theoretical Oxygen Demand (ThOD).

The results of replicate flasks provided biodegradation figures of 24%, 5%, 15% based on BOD/$ThOD_{NO2}$ (mean 14.7%). The figures based on BOD/$ThOD_{NH4}$ were 37%, 8% and 24%, mean 23%. Percentage degradation was also estimated by means of specific analysis of test substance (D_t at 28 days), giving values of 61%, 29%, 41%, mean 46.3%. The higher values for degradation using the latter method may in part be due to some loss of acrylonitrile from the test flasks. Degradation at earlier time points in this study was negligible (0% at 7 days, 0.3% at 14 days).

The ready biodegradability of acrylonitrile has also been determined by BASF AG (1996) in the Closed Bottle Test, OECD Test Guideline 301D. The study used a test concentration of 2 mg/l nominal and an inoculum of effluent from a laboratory wastewater treatment plant fed with municipal and synthetic sewage (5 mg/l). The measured Biological Oxygen Demand (BOD) was compared with the calculated Theoretical Oxygen Demand (ThOD), dissolved oxygen content was monitored in replicate flasks maintained in the dark (2 per time period for blank controls, 2-4 for test substance flasks) at 0, 2, 4, 7, 11, 14, 19, 21, 25, 28 days.

Levels of O_2 in the test flasks were comparable to those in the blank controls at all monitoring times, indicating that no biodegradation of acrylonitrile had occurred over the 28-day period of the test (-2% at 4 days, 5% at 7 days, -3% at 11 days, 4% at 14 days, -1% at 21 days, -13% at 28 days). 80% degradation of the reference compound (sodium benzoate) occurred within 21 days, indicating satisfactory performance of the test. A toxicity test (2 mg/l sodium benzoate + 2 mg/l acrylonitrile) run under the same conditions showed 39% degradation after 14 days, indicating that acrylonitrile was not inhibitory to the microbial inoculum.

These two studies are considered to be valid for risk assessment purposes, and the results indicate that acrylonitrile cannot be regarded as degradable in standard ready biodegradation tests. The absence of biodegradability in the closed bottle test is unexpected, given that other studies described below indicate at least a degree of inherent biodegradability and that some degradation was seen in the somewhat more stringent conditions of the MITI test (100 mg/l acrylonitrile compared with 2 mg/l in the closed bottle test). It is however accepted as a valid result.

A third study has been carried out (AN Group, 1996) on the ready biodegradability of acrylonitrile in seawater using OECD Test Guideline 306, 1992, (closed bottle test) and a test concentration of 2.45 mg/l acrylonitrile. The source of the seawater was the east coast of Scotland (Eyemouth), a surface sample (0.5-1 foot) being taken. Dissolved organic carbon was not determined on the sample, but it was aged for 6 days before use. In this study 78.9% degradation of acrylonitrile was achieved over the 28-day period of the test, indicating a high degree of degradability. An estimated 45% degradation was achieved within a 10-day period following start of degradation (10% on day 3). 97.4% degradation of the control substance, sodium benzoate, had occurred by day 4 of this study, and little or no inhibition of degradation was observed in a toxicity control (1.54 mg/l sodium benzoate and 2.45 mg/l acrylonitrile) monitored over a 4-day period.

It can be concluded from this study that acrylonitrile will be degraded rapidly if discharged into a marine environment. The conflicting results obtained in the three ready biodegradation studies described above can be attributed to differences in the test environments and the microbial populations contained in each.

BOD/COD tests

Mills and Stack (1954) reported a 10-day BOD for acrylonitrile of 0.7 (COD 2.3), using a dispersed seed aeration system which had been maintained in the laboratory for over 1 year on a replica of process effluents from the Carbide and Carbon Chemicals Company, South Charleston, Va. The experimental protocol involved a "shock" loading with acrylonitrile diluted with the process effluent feedstock to give a concentration range of 200-1,200 ppm. However, in a further study Mills and Stack (1955) produced an acclimated population of sewage organisms by re-spiking with acrylonitrile over a 27-day period, and demonstrated 70 % degradation over 5 days. They suggested that only 70% was achieved due to bacterial metabolism and transfer losses, and commented that biological oxidation of acrylonitrile using domestic sewage was unlikely, the nitrile group would have to be hydrolysed first, either chemically or by using acclimatised bacteria.

Cherry et al. (1956), using the same dispersed seed aeration system as Mills and Stack (1954), reported that 100% of the COD of acrylonitrile at a concentration of 10 mg/l was removed following an initial acclimation period of approximately 10 days. These authors determined a BOD_5 of 721 mg/g and an experimentally derived COD of 1,612.5 mg/g. Stover and Kincannon (1983) reported a 5-day BOD of 0.3, although the experimental basis for this figure is not obvious.

Buzzell et al. (1968) (also reported in Young et al., 1968) used a modified BOD test involving a two-bottle, single dilution, re-aeration system at 20 ± 0.5°C and municipal sewage sludge as seed (2 ml/l dilution water). O_2 utilisation was monitored daily over 30 days. Acrylonitrile at 10 mg/l showed a lag period of 12 days before degradation commenced, with a BOD_5 of 0 and a BOD_{30} of 1.21 (ThOD 3.17), representing 38% biodegradation by day 30. Nitrification was not detected until after day 20.

The results of these studies would argue against the ready biodegradability of acrylonitrile, but support the contention that it is inherently biodegradable.

Inherent biodegradability studies

The Chemicals Inspection and Testing Institute, Japan, (1992) has also carried out an "OECD 302 C, Inherent Biodegradability: Modified MITI test (II)", using 100 mg/l suspended solids and 30 mg/l acrylonitrile over a test period of 14 days. Degradation was measured by determination of BOD, DOC and specific chemical analysis.

Degradation of acrylonitrile at 14 days and 25 ± 1°C was determined as 74%, 67%, 41% based on $BOD/ThOD_{NO2}$ (mean 60.7%). At 7 days the mean degradation was 25.7%. The figures based on $BOD/ThOD_{NH4}$ at 14 days were 117%, 107% and 65% (mean 96%). Percentage degradation determined by means of specific analysis of test substance (D_t at 28 days), gave values of 100%, 100%, 100%. MITI concluded in its Gazette that acrylonitrile is a biodegradable substance, substantiated by the results of this study.

Acclimation studies

Watson (1993) carried out a study of the effects of acclimation on aerobic biodegradability using a single flask method compared with an enrichment procedure in a series of flasks. In the single flask method seed microorganisms from an industrial wastewater treatment plant (10% Mixed Liquor Suspended Solids) were exposed to gradually increasing concentrations of acrylonitrile (2, 4, 6, 10, 15 mg/l as organic carbon) over the period 2-7 days. Loss of Dissolved Organic Carbon reached 79% by day 4 in this system. When the acclimated microorganisms were used as the source of inoculum in a subsequent biodegradation study, using a test concentration of 20 mg/l acrylonitrile, 60% biodegradation was achieved by day 21 based on CO_2 evolution. In the enrichment procedure, a series of 1% transfers of the test medium including MLSS was made through a series of 9 flasks, each containing higher concentrations of acrylonitrile. After a period of 21 days, ≥70% loss of DOC was achieved.

Tabak et al. (1981), in an earlier study, had used a similar protocol to Watson. This involved a static culture, flask screening procedure incorporating settled domestic wastewater as microbial inoculum, incubated with 5 or 10 mg/l acrylonitrile for a 7-day period, followed by 3 weekly subcultures (28-day incubation in total). These authors reported 100% biodegradation of acrylonitrile tested at all subculture stages.

Acclimation of microbial species to acrylonitrile

A number of authors have reported microbial strains isolated from acclimated cultures which were able to use acrylonitrile as a carbon/nitrogen source, with consequent degradation of high concentrations of acrylonitrile. In 1972, Worne reported isolation of a *Pseudomonas* species that was able to achieve 100% degradation of 500 mg/l acrylonitrile in 4 hours, following a period of acclimation (21 days). Yamada et al. (1979) isolated from municipal sludge a bacterium of the genus *Arthrobacter* which was able to utilise acrylonitrile and other nitriles as a sole source of carbon and nitrogen. This organism rapidly degraded acrylonitrile (70 mM) to acrylic acid and ammonia over a 1-3 hour time period. Thiery et al. (1986) reported a mutant strain of *Brevibacterium sp.* R312, which could again utilise acrylonitrile as a sole source of carbon and nitrogen, while growth of the wild type bacterium was inhibited. Yang et al. (1984) isolated a bacterial culture from activated sludge contaminated with wastewater from polyacrylonitrile polymerisation. Species identified included *Arthrobacter* and *Thiobacillus,* and the culture rapidly degraded up to 120 mg/l acrylonitrile. Wenzhong et al. (1991) isolated a number of species from nitrile polluted soil, including *Corynebacterium boffmanii, Arthrobacter flavescens* and reported complete degradation of 4,000 mg/l AN by these bacteria (at a microbial concentration of 20 g/l).

Simulation tests

A number of authors have studied the biodegradation of acrylonitrile in laboratory scale, continuous flow, activated sludge systems. Kinncannon and his group (Kinncannon et al., 1983; Stover and Kinncannon, 1983) used a 3 litre internal recycle reactor system initially seeded with municipal activated sludge and operated at mean residence times of 2, 4 or 6 days, with hydraulic retention time being maintained at 8 hours. Following acclimation, influent, effluent, mixed liquor and off-gas samples were collected over a 60-day testing period. Stover and Kincannon showed 96.6 to 98.1% treatment efficiency based on BOD_5 and 99.9% based on specific compound analysis. They also showed no stripping of acrylonitrile from the test system in the absence of biological activity.

Freeman and Schroy (1984) similarly reported over 99.9% removal by biological action in a continuous flow bench scale oxidation system operated over 45 days. The systems were designed to operate at 50%, 100% or 150% recycle rates at an influent water flow rate of 10 ml/min and were initially seeded with recycle sludge obtained from Monsanto's Port Plastics plant in Addyston, Ohio. The sludge had already been acclimated to the plant wastewater containing acrylonitrile, phenol and styrene. The reactor was initially spiked with 200 mg/l acrylonitrile and then fed over a 4-week period with acrylonitrile at 0.284 g/hr. Sludge was periodically wasted to maintain steady Mixed Liquor Volatile Suspended Solid (MLVSS) concentrations, which averaged 1,715 mg/l over the period of the experiment. Dissolved Oxygen Concentrations were generally maintained at 2 mg/l, and acrylonitrile was analysed in both the aqueous and atmospheric phases.

The results of Freeman's study showed greater than 99.97% removal of acrylonitrile by biological action at the earliest sampling period (2 days) and thereafter throughout the 4-week period of the study. There was little (0.01%) stripping of acrylonitrile to air in the biological system. In all cases acrylonitrile concentrations in the effluent from the system was less than the detection limit for the system of 0.5 mg/l. Freeman used a mathematical computation of the equilibrium relationship between the liquid concentration and the concentration in the aeration bubbles to derive the actual concentration of acrylonitrile in the effluent phase. The study also included a sterile system operated over a 5-day period under similar conditions but with no seed sludge. Acrylonitrile concentrations in the reactor were analysed at 367 mg/l on day 5. The material balance for the sterile system (3.2% error) showed 18% stripping to air of the acrylonitrile entering the reactor.

Ludzack et al. (1961) examined the effect of increased concentrations of acrylonitrile in a bench-scale activated sludge unit (5.5 l) seeded with weak settled sewage (BOD 50-75 mg/l). A semi-continuous flow system was used with compensatory removal of sludge solids in addition to aqueous effluent. Gradually increasing concentrations of acrylonitrile (22-89 mg/l) during the acclimation period showed 40% of influent nitrogen detectable as effluent ammonia during days 1-4. By days 10-13, 90% influent nitrogen was detectable as effluent oxidised material, and during four subsequent weeks of normal operation 90%-97% of the acrylonitrile theoretical BOD was removed. Efficiency decreased on acrylonitrile overload with 177 mg/l and was materially reduced at 266 mg/l.

Lank and Wallace (1970) examined the effect of acrylonitrile on anaerobic sewage digesters, using a controlled residence 10 l working volume digester system fed on a daily basis with acrylonitrile and raw sludge from a municipal treatment plant, spent sludge being wasted from the system to maintain steady state sludge concentrations. Hydraulic residence time was 20 days. Gradually increasing dosages of acrylonitrile (1, 2, 4, 10, 20 mg/l) were fed and the performance of the digester was compared with a control digester operated under identical conditions but without acrylonitrile feed. No effect on effluent COD was detected at the highest concentrations, indicating that at these concentrations acrylonitrile was not harmful to an anaerobic digester.

Biodegradation of acrylonitrile in industrial biotreatment plants

Data from a number of the European sites involved in production or further processing of acrylonitrile have confirmed the results of simulation tests such as those of Freeman and Schroy (1984) and other work on the biodegradation of acrylonitrile reported in the literature. Namely, acrylonitrile is rapidly biodegradable in situations where an adapted microbial population can be expected, such as in industrial biotreatment plants. Analytical determination of acrylonitrile in influent and effluent from a dedicated biotreatment plant principally handling effluent from an

acrylonitrile production facility with a production capacity of approximately 100,000 tonnes per annum indicated a concentration of 0.82 mg/l in the influent to the biotreatment plant and a concentration of less than 0.05 mg/l in the effluent after treatment. The analytical method used was purge trap gas chromatography (EPA method SW-8260B) with a detection limit of 5 µg/l and a quantitation limit of 50 µg/l. On the basis of these results, the biotreatment plant removed greater than 93.9% of the acrylonitrile load. Information from another production facility indicated influent concentrations to the biotreatment plant of 300-500 mg/l and effluent concentrations of 0.5-1.3 mg/l, giving a removal of greater than 99%, while data from a fibre production facility could not detect acrylonitrile in the effluent from their biotreatment plant at a detection limit of 0.25 mg/l (influent level 35 mg/l), again a removal rate of greater than 99%. In general, all data for emissions to surface water provided for this report by companies with biotreatment plants indicate similar removal rates.

Data were also provided for a US facility producing 172,000 tonnes/year acrylonitrile production with a biotreatment plant with mean flow of 5 million gallons (US)/day (18,900 m^3/day). In 1996 aqueous emissions of acrylonitrile to the biotreatment plant were 28.5 tonnes (78.2 kg/day). The average influent concentration to the biotreatment plant was 0.44 mg/l and acrylonitrile was not detectable in the effluent from the biotreatment plant with an analytical limit of detection of 10 µg/l. On this basis biodegradation in the wastewater treatment plant was >97.7%. Higher acrylonitrile loadings to the biotreatment plant in 1993 of 39.7 tonnes (109 kg/day) still showed no detectable acrylonitrile in the effluent.

One company cited in this report has experience of intermittent operation of their biotreatment plant, and reported that after reconstituting the biomass with activated sludge from a municipal sewage plant, the industrial biotreatment plant operates at 99% removal rates within a few days of start-up.

Degradation in river water

Ludzack et al. (1959), using Ohio River water taken from the intake of the Cincinnati Water Works in a 5 gallon oxidation unit, reported marked inhibition of CO_2 production by spiking with 10 mg/l acrylonitrile in week 1 of a 110-day study. BOD increased from approximately 0.5 during days 10-20 to approximately 1.3 on day 25, at which time period respiking with 10 mg/l again produced transient marked inhibition of CO_2 production. Oxidation recommenced rapidly, with no evidence of a lag period or the plateau effect seen between days 10-20, and a similar pattern was seen on subsequent serial dosings.

The observation of Going et al. (1978) that concentrations of acrylonitrile in river water at unadjusted pH fell to undetectable levels by day 6 is also relevant. However the relative importance of biodegradation, abiotic degradation and volatilisation of acrylonitrile from the test medium cannot be ascertained.

Inhibition of sludge respiration

Hovious et al. (1973), in a study carried out for the US EPA, used a Warburg respirometer procedure over 6 hours and showed inhibition of sludge anaerobic respiration by 50 mg/l acrylonitrile and above in non-substance-limited conditions. Inhibition was not as marked in substance-limited conditions.

Conclusion on aquatic degradation

Three independent studies of acceptable quality indicate that acrylonitrile does not meet the criteria for ready biodegradability. Reflecting this fact and the effects assessment in Section 3.2.1, classification as “dangerous for the environment” with N; R51-53 is appropriate. Acrylonitrile has an initial inhibitory effect on activated sludge systems and other microbial populations, and an acclimation period appears to be necessary before biodegradation is established. However, extensive biodegradation is seen in such acclimated systems, with greater than 95% biodegradation being reported in a number of studies which simulated conditions in a wastewater treatment plant.

It can be concluded that acrylonitrile will be extensively degraded following a short acclimation period if emitted to WWTP from industrial point sources, either primary production or secondary processing plants. The experimental evidence also indicates that it will be rapidly degraded following discharge into a marine environment, and that it will be degraded, although at a slower rate, if released directly into the freshwater environment. In relation to the assessment of exposure at local and regional level, acrylonitrile has been treated as readily biodegradable in deriving PECs for individual sites that are known to have wastewater treatment plants. It has however been treated as inherently biodegradable in deriving regional and continental PECs using EUSES (see Section 3.1.4.1.5).

3.1.3.1.3 Degradation in soil and sediment

Donberg et al. (1991; 1992) showed, in a study investigating the biodegradation of [14C]-acrylonitrile in a variety of surface soils, that concentrations of up to 100 ppm were degraded in under 2 days. Over 50% of radioactivity was recovered as $C0_2$ after 6 days, with transient formation of acrylamide and acrylic acid. Higher concentrations (500 and 1,000 ppm) were degraded only slowly, and this correlates with experimental evidence that these levels inhibit respiration of soil microbes, with gradual acclimation.

Wenzhong et al. (1991) isolated 2 strains of bacterium, *Corynebacterium hoffmanii* and *Arthrobacter flavescens*, from nitrile-polluted soils. Aqueous cultures of these bacteria were able to degrade 5g/l acrylonitrile. There is also evidence (Giacin et al., 1973) that acrylonitrile-butadiene-methyl methacrylate polymers and acrylic fibres can be slowly broken down by soil fungi (*Penicillium, Bacillus, Aspergillus, Cladosporium*). Similar breakdown by microbial populations present in sediments is likely. Overall significant accumulation in the soil or sediment compartments is not anticipated.

3.1.3.1.4 Summary of environmental degradation data on acrylonitrile

The data summarised in this section show that acrylonitrile is degraded in the atmosphere, in water and in soil. Half-lives for the different compartments are summarised in **Table 3.9**. Photolytic degradation in water has also been reported. In relation to atmospheric degradation the published rate constant for the reaction of acrylonitrile with $OH^{\cdot}$ of $3.2 \cdot 10^{-12}$ cm/mol/s has been used in EUSES, representing an estimated half-life of 5 days in the troposphere.

A first-order rate constant, k (d^{-1}), of $4.62 \cdot 10^{-3}$ is derived for biodegradation in surface water, representing a half-life of approximately 150 days. This may be compared with the experimental data for biodegradation, which provide values for the half-life ranging from 2 days in a fully

acclimated system (e.g. Watson, 1993) to 15 days in a ready biodegradability test in seawater (AN Group, 1996) and 30 days in an OECD 301C, Ready Biodegradability: Modified MITI Test (I).

A rate constant of 0.00231 (d^{-1}) and a half-life of 300 days can be derived in soil using the approach in Section 2.3.6 and Table 6 of the TGD. The half-life in the oxic zone of sediment can be assumed to be similar. The work of Donberg et al. (1992) indicates a half-life of approximately 6 days.

Table 3.9 First-order rate constants and degradation half-lives of acrylonitrile in the troposphere, surface water and soil

Compartment	Rate constant	Half-life (days)
Troposphere	$3.2 \cdot 10^{-12} cm^3/mol/s$ ($OH^{\cdot}$)	5
Surface Water	k (d^{-1}) of $4.62 \cdot 10^{-3}$	150 [1)]
Soil	0.00231 (d^{-1})	300 [2)]

1) Experimental values range from 2-30 days
2) Experimental value of 6 days reported

3.1.3.2 Distribution

3.1.3.2.1 Modelling of distribution

Given the volatility and high water solubility of acrylonitrile, distribution to the aquatic and atmospheric compartments can be predicted. Environmental distribution at 20°C has been modelled with the Mackay level 1 six compartment fugacity model using the OECD EQC Model v. 1.0, measured values for vapour pressure, water solubility, log P_{ow} and other physicochemical properties as identified in Table 1.2 and a value of 9.62 $Pa/m^3/mol$ for Henry's law constant. The output, as shown in **Table 3.10**, indicates that the predicted major environmental compartment for acrylonitrile is air, with a smaller proportion entering the aqueous compartment and negligible quantities being predicted for other compartments.

Table 3.10 Mackay level 1 environmental distribution of acrylonitrile based on the OECD EQC model V.1.0

Compartment	Distribution (%)
Air	66.3
Water	33.6
Soil	0.053
Sediment	0.00118
Suspended sediment	0.0000368
Fish	0.00000299

(courtesy of the Danish EPA)

3.1.3.2.2 Distribution to soil and sediment, adsorption:desorption

Acrylonitrile potentially can be redistributed to soil from the atmospheric or aqueous compartments, by the spreading of acrylonitrile-contaminated sewage sludge or as a result of accidental spills. An experimental study (Zhang et al., 1990) of adsorption:desorption of acrylonitrile to montmorillonite clay (K, Na, Ca, Mg) provided no evidence of adsorption to soil, the adsorption:desorption processes being in equilibrium. This was supported by calculation of the Koc (soil-sorption coefficient) using QSAR (Koch and Nagel, 1988) or from the water solubility of acrylonitrile (Kenaga, 1980), when values of 11.5 and 9.0 respectively were derived, showing little potential for adsorption to soil. A similar conclusion can be reached regarding the potential of acrylonitrile to adsorb to sediments.

The EUSES output[5] provides a calculated value of 2.0 for Koc, based on QSAR for a hydrophobic substance. Acrylonitrile could more appropriately be considered as a non-hydrophobic substance, for which a log Koc of $0.52 \cdot \log Kow + 1.02 - 1.14 = 1.15$ can be calculated, giving a Koc of 14.1, which is reasonably consistent with the values published in the literature. The EUSES model was run using both Koc values, and the output did not change significantly. For the purposes of this risk assessment the value of 1.15 calculated for a non-hydrophobic substance has therefore been used in the EUSES model.

3.1.3.2.3 Volatilisation

Transfer of acrylonitrile from the aqueous to the atmospheric compartment can potentially arise as a consequence of volatilisation and air stripping from WWTP, given the relatively high vapour pressure of acrylonitrile. However this is counterbalanced by its high water solubility. Acrylonitrile can be regarded as a moderately volatile compound, based on the calculated Henry's Law constant of 9.6 $Pa/m^3/mol^{-1}$. There is little experimental evidence available to prove or disprove the possibility of volatilisation, and in practice direct transfer of acrylonitrile from water to air is likely to be minimal, particularly in WWTP where rates of biodegradation are likely to far exceed the potential volatilisation. This is supported by the results of Freeman and Schroy (1984) and Stover and Kincannon (1983).

3.1.3.2.4 Bioconcentration and bioaccumulation

Bioaccumulation of acrylonitrile is not anticipated, given experimentally-derived values of 0-0.3 for the log Kow, and a bioconcentration factor (BCF) of 1.0 has been calculated from the known water solubility of acrylonitrile. EUSES calculates a BCF for fish and aquatic biota of 1.41 l/kg. In contrast an experimentally-derived bioconcentration factor of 48 has been reported (Barrows et al., 1980) in *Lempomis machrochirus*, measured as ^{14}C uptake and determined over an experimental period of 28 days at a concentration of 10 µg/l acrylonitrile. The apparent discrepancy between the results of this study and the calculated bioaccumulation factor may be due to uptake of ^{14}C-labelled degradation products in addition to acrylonitrile and to cyanoethylation of macromolecules, as has been observed in pharmacokinetic studies in rodents and other species. Potentially therefore, the carcinogenic effects observed in long-term studies with acrylonitrile in rodents could also be expressed in fish. This possibility has not been extensively investigated, but in a study carried out by Walker et al. *(in press*) no carcinogenic

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[5] **Euses Calculations** can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it

effects were detected in the fish *Oryzias latipes* or in *Poecilia reticulata* in a short-term (24 weeks) carcinogenicity bioassay.

3.1.3.2.5 Degradation products, identity and environmental fate

Acrylonitrile polymers may ultimately be disposed to landfill at the end of their life cycle or may be incinerated. In the former case, the polymers are assumed to have infinite stability, although residual acrylonitrile is released (see Section 3.1.2.4) and experimental evidence has indicated that soil bacteria can slowly degrade certain acrylonitrile-containing polymers (Giacin et al., 1973). In the latter case, incineration in a high-performance incinerator (>850°C) results in complete combustion to CO_2, H_2O, NH_3, NO_x and N_2. Waste products resulting from acrylonitrile production or further processing are similarly incinerated. In conditions of incomplete combustion, for example in a fire involving acrylic fibres or plastic copolymers, carbon monoxide and hydrogen cyanide are the initial products of combustion, although these can be converted further to CO_2, H_2O, NH_3 and N_2 at high temperatures in the presence of oxygen. Photodegradation products of acrylonitrile itself (formaldehyde, CO, HCN, formyl cyanide (HCOCN) and formic acid) have already been discussed in Section 3.1.3.1.1 above. Complete biodegradation of acrylonitrile results in formation of CO_2 and NH_3, with subsequent nitrification.

3.1.4 Aquatic compartment (including sediment)

Exposure of the aquatic compartment to acrylonitrile may occur as a result of release to water during production of monomer, polymerisation of monomer to give acrylonitrile polymers, further processing of polymer to give polymeric products, use and, ultimately, disposal of the polymeric products. Exposure on a local scale will be to a limited (multiple) number of point sources involved in the large-scale production of acrylonitrile and/or further processing of acrylonitrile polymers. Exposure to acrylonitrile as a result of disposal of polymeric products to landfill for example, with subsequent release to the aquatic environment, will be insignificant compared with manufacturing point sources. Emissions from vehicle exhausts and other diffuse sources, already discussed in Section 3.1.2.4 will largely be to the atmosphere rather than the aquatic compartment.

Following release into the aquatic environment, acrylonitrile will be primarily removed by biodegradation. Photolysis will represent an additional minor removal mechanism in surface waters, and volatilisation from water to air is also theoretically possible.

3.1.4.1 Predicted local environmental concentrations (PEC_{local}) for the aquatic compartment

3.1.4.1.1 Calculation of PEC $local_{water}$ for acrylonitrile production

In general the $PEClocal_{water}$ was calculated using the approach outlined in the TGD, Section 2.3.8.3, and assuming initial on-site STP treatment followed by release to a municipal STP or surface water. The calculations involved the following steps:

1. Derivation of the local daily emission rate ($Elocal_{water}$) by acceptance of the emission data provided by industry.

2. Conversion of the local emission rate to a concentration in untreated wastewater ($Clocal_{inf}$) by the effluent volume (default 2,000,000 l/day) with conversion to mg/l (Equation 17 of the TGD).

3. Derivation of a concentration in the STP effluent ($Clocal_{eff}$) by assumption of a fraction of 0.116 (from EUSES) of the STP influent going to effluent. This accounts for a fraction of 0.850 being biodegraded (EUSES, ready biodegradable substance), 0.0324 being stripped to air, and 0.00013 going to sludge.

   This calculation was only applied to sites known to have a STP, and where a site provided actual analytical data for $Clocal_{eff}$ this value was used in preference. For sites without STP, a worst-case assumption of no biodegradation has been made ($Clocal_{inf} = Clocal_{eff}$).

4. Derivation of the local concentration in surface water ($Clocal_{water}$) from the following equation: $Clocal_{water} = Clocal_{eff}/D$.

   The default value of 10 was used for D, the dilution factor, unless specific information on dilution factors for individual sites was supplied, as shown in Appendices A.1 and A.2.

5. The average annual local concentration in surface water was derived by multiplication of $Clocal_{water}$ by 300/365, assuming 300 emission days per year for acrylonitrile (Equation 32 of the TGD).

6. The $PEClocal_{water}$ was derived by addition of the $PECregional_{water}$ to the calculated value of $Clocal_{water}$ (Equation 33 of the TGD). $PECregional_{water}$ was taken from EUSES, the value being 0.00281.

7. The $PEClocal_{water,ann}$ was derived by addition of the $PECregional_{water}$ to the calculated value of $Clocal_{water,ann}$ (Equation 34 of the TGD).

In relation to exposure assessment for the aquatic environment for the currently operational production and further processing facilities identified in **Tables 3.1 to 3.5**, the companies can be divided into three groups:

1. facilities with dedicated industrial wastewater treatment plants,
2. two facilities, sites CC and BBB, discharging into a municipal wastewater treatment plant following preliminary physicochemical treatment to recover acrylonitrile from waste,
3. facilities discharging directly into the aquatic environment (river/marine/estuarine) without biotreatment.

In the case of group (1), which represents a large proportion of total sites (33 out of a total of 43), given the results of laboratory-scale simulation studies and information provided regarding the biodegradation of acrylonitrile in individual industrial biotreatment plants as described in Section 3.1.3.1.2 (biodegradation), ready biodegradability has been assumed (85% degradation) in deriving $PEClocal_{water}$ although in many cases an actual concentration (or a maximum value) for acrylonitrile in effluent to surface water was provided, which formed the basis for subsequent calculations.

In the case of the two facilities in group (2), it could be argued that an assumption of ready biodegradability cannot be made, since total acclimation of the microbial population of the municipal wastewater treatment plant to acrylonitrile waste may not occur. It was however

concluded, on the basis of information provided by the site operators regarding the frequency of emissions and the detailed information available on the biodegradation of acrylonitrile, that it was valid to make an assumption of ready biodegradability for these sites also. In the case of group (3) facilities, numbering 8 in all, an assumption of no biodegradation in the receiving water has been made. However the majority of these sites provided analytical data for acrylonitrile in the plant effluent, which again formed the basis for subsequent calculations.

$PEClocal_{water}$ has been calculated for each of the 8 production facilities in Europe, release data for which have been presented in **Table 3.1**. All but one of these facilities have dedicated wastewater treatment facilities, site 5 being the exception, and site-specific dilution factors were supplied for a number of them, as shown in Appendix A.1. Results are summarised in **Table 3.11** and the results of all the calculations are shown in Appendix A.1. For virtually all the sites, excluding sites 1 and 5, the calculated concentration in surface water ($Clocal_{water}$) after application of the site-specific dilution factor provided was extremely low, < 0.1 µg/l, and the $PEClocal_{water}$ shown in **Table 3.11** and Appendix A.1 therefore approximates the PECregional, derived from EUSES.

In relation to derivation of the local concentration in surface water ($Clocal_{water}$) using site-specific dilution factors rather than the default value of 10, information was sought by and provided on the justifications for these site-specific dilution factors. Information received included detailed studies of estuarine tidal and river flow in the case of 3 of the sites located on estuaries, including site 5 above and data on river flow including seasonal peaks and depressions in the case of sites located on large rivers, and capacities of WWTP receiving acrylonitrile-containing effluent.

Table 3.11 Summary of $PEClocal_{water}$ for acrylonitrile production facilities

Site	$PEClocal_{water}$ (mg/l)	$PEClocal_{water, ann}$ (mg/l)
1	0.0128	0.011
2 (+BB) [1)]	0.0029	0.0028
3	0.0320	0.031
4 (+ D + EE) [2)]	0.003 [3)]	0.003 [3)]
5	0.0144	0.0123
6	0.0030	0.0030
7	0.003 [3)]	0.003 [3)]
8	0.0032	0.032

1) Emissions from this site are combined emissions for a production facility and a facility producing ABS/SAN polymers
2) Emissions from this site are combined emissions for a production facility, a facility producing fibres and a facility producing ABS/SAN polymers
3) Value represents PECregional (EUSES)

3.1.4.1.2 Calculation of $PEClocal_{water}$ due to processing of acrylonitrile to polymers, acrylamide and adiponitrile

Table 3.12 Summary of $PEClocal_{water}$ for acrylonitrile fibre production facilities

Site	$PEClocal_{water}$ (mg/l)	$PEClocal_{water, ann}$ (mg/l)
B	0.0092	0.0081
C	0.003 [2)]	0.003 [2)]
D (+EE + 4) [1)]	0.003 [2)]	0.003 [2)]
E	0.053	0.044
F	0.0029	0.00029
G	0.0078	0.0069
H	0.0078	0.0069
J	0.003	0.0029
K	0.0070	0.0063

1) Emissions from this site are combined emissions for a production facility, a facility producing fibres and a facility producing ABS/SAN polymers
2) Value represents PECregional (EUSES)

Table 3.13 Summary of PEC $local_{water}$ for ABS-SAN production facilities

Site	$PEClocal_{water}$ (mg/l)	$PEClocal_{water, ann}$ (mg/l)
AA	0.0036	0.0035
BB (+2) [1)]	0.003 [2)]	0.003 [2)]
CC	0.0086	0.0076
DD	0.0035	0.0034
EE (+D+ 4) [3)]	0.003 [2)]	0.003 [2)]
FF	0.0029	0.0029
GG	0.003 [2)]	0.003 [2)]
HH	0.003 [2)]	0.003 [2)]
II	0.0030	0.0030
JJ	0.003 [2)]	0.003 [2)]
KK	0.0060	0.0054
LL (+ HHH) [4)]	0.0031	0.0031
MM	0.0032	0.0031

1) Emissions from this site are combined emissions for a production facility and a facility producing ABS/SAN polymers
2) Value represents PECregional (EUSES)
3) Emissions from this site are combined emissions for a production facility, a facility producing fibres and a facility producing ABS/SAN polymers
4) Emissions from this site are combined emissions for a facility producing ABS/SAN polymers and a facility producing NB copolymers

Table 3.14 Summary of $PEClocal_{water}$ for nitrile:butadiene copolymer production facilities

Site	$PEClocal_{water}$ (mg/l)	$PEClocal_{water, ann}$ (mg/l)
AAA	0.0122	0.011
BBB	0.0030	0.003 [2)]
CCC	0.0034	0.0033
DDD	0.0046	0.0043
EEE	0.0032	0.0031
FFF	0.0078	0.0069
GGG	0.0078	0.0069
HHH (+ LL) [1)]	0.0035	0.0033
JJJ	0.0036	0.0035

1) Emissions from this site are combined emissions for a facility producing ABS/SAN polymers and a facility producing NB copolymers
2) Value represents PECregional (EUSES)

Table 3.15 Summary of PEC $local_{water}$ due to processing of acrylonitrile to acrylamide and adiponitrile

Site	$PEClocal_{water}$ (mg/l)	$PEClocal_{water, ann}$ (mg/l)
L	0.003 [1)]	0.003 [1)]
M	0.003 [1)]	0.003 [1)]
N	0.003 [1)]	0.003 [1)]
O	0.003 [1)]	0.003 [1)]

1) Value represents PECregional (EUSES)

3.1.4.1.3 Calculation of $PEClocal_{water}$ for wastewater treatment plants

The initial compartment of release for the majority of the acrylonitrile production and processing plants is to a site wastewater treatment plant. Local concentrations will lie between $Clocal_{inf}$ and $Clocal_{eff}$ (TGD 17 and 18). For the purposes of the risk assessment, the PEC_{STP} can be considered to be equal to the effluent concentration (TGD 23), and values calculated for those sites for which site-specific emission data were available or for which actual analytical data were provided for $Clocal_{eff}$ range from 0 to 5.8 mg/l, with the majority lying well below 1 mg/l (Appendices A.1 and A.2).

3.1.4.1.4 Calculation of PECsediment

The calculated Koc for acrylonitrile is low, indicating little potential for adsorption of acrylonitrile to sediment. $PEClocal_{sediment}$ has been derived from $PEClocal_{water}$, assuming a thermodynamic equilibrium between the two, on the basis of the following equation:

$$PEClocal_{sediment} = (Ksusp_{water} / RHO_{susp}) \cdot PEClocal_{water} \cdot 1{,}000$$ (Equation 35 of the TGD)

Based on the site-specific data provided, the calculated $PEClocal_{sediment}$ for sites involved in the production of acrylonitrile lie in the range of $2.3 \cdot 10^{-3}$ to 0.01 mg/kg wwt. For processing sites the range was $2.3 \cdot 10^{-3}$ to 0.04 mg/kg wwt. Results for all sites are given in Appendices A.1 and A.2.

Measured levels in sediment

Monitoring results indicated that acrylonitrile was undetectable in sediment samples taken in the vicinity of 11 acrylonitrile manufacturing or processing plants in the US (US EPA data, 1978, limit of detection < 50 µg/m^3). A survey carried out in 1987 for the Japanese Environment Agency (Japanese Environment Agency Office of Health Studies, 1991) detected acrylonitrile in 4 out of 66 sediment samples at levels of between $14 \cdot 10^{-3}$ and $114 \cdot 10^{-3}$ mg/kg (limit of detection $7 \cdot 10^{-3}$ mg/kg). A recent report (Hoke et al., 1993), using GC-MS, indicated acrylonitrile levels of 0.1-4.2 mg/kg in sediment (expressed on a dry weight basis) from the Grand Calumet River - Indiana Harbour, US, (a designated area of concern, International Joint Commission) over the period 1988-1989. Comparable figures for sediment pore water were < 0.1-1.8 µg/l. Acrylonitrile was one of 63 chemicals (out of a total 104 analytes) detected in the sediment samples, and one of 44 to be detected in pore water. The authors concluded from the results of toxicity tests on pore water and sediment samples that the chief toxic contaminants were petroleum hydrocarbons, metals, ammonia and metals.

3.1.4.1.5 Calculation of regional and continental concentrations in the aquatic environment ($PECregional_{water}$ and $PECcontinental_{water}$)

Estimates of $PECregional_{water}$ and $PECcontinental_{water}$ due to acrylonitrile production and further processing have been made by means of EUSES using the release data provided by industry for non-coastal sites. The contribution of emissions from marine or estuarine sites to $PECregional_{water}$ and $PECcontinental_{water}$ is assumed to be low and is not addressed in the TGD. For this reason, the emissions of sites 1, 3, 5, 8, C, E, AA, GG, JJ, KK, AAA, GGG and M have been omitted from the input into EUSES, although they have been included in the assessment of $PEClocal_{water}$ as described in Sections 3.1.4.1.1 and 3.1.4.1.2 above.

In relation to derivation of $PECregional_{water,}$ releases to surface water for sites located in Germany were chosen as input into EUSES, since Germany represents a discrete European region having few coastal sites. In relation to derivation of $PECcontinental_{water}$, input into EUSES was taken as the total releases to surface water for all non-coastal European sites. Further details of the input into and output from the model can be seen as part of the report at the website of the European Chemicals Bureau (http://ecb.jrc.it). The algorithms in EUSES are mainly identical with the estimating procedures according to the TGD. The following results were obtained for regional and continental PECs:

$PECregional_{water}$ 2.81 µg/l

$PECcontinental_{water}$ 0.41 µg/l

It should be noted that acrylonitrile has been treated as an inherently biodegradable substance within EUSES, rather than as readily biodegradable. This is despite the fact that a large proportion of the emissions data input into the model are for sites with WWTP, where ready biodegradability has been assumed on the basis of the evidence presented by industry. However this is not the case for all sites, even when the marine sites are excluded from the EUSES input. The treatment of acrylonitrile as inherently biodegradable within EUSES is therefore considered

valid, although it results in higher estimates of $PECregional_{water}$ and $PECcontinental_{water}$ as shown above.

Environmental levels have also been estimated using the Mackay level 3 model (ECETOC Special Report, 1994) using the geophysical parameters for Germany. The following results were obtained:

Concentration in surface water	$2.37 \cdot 10^{-3}$ µg/l
Concentration in groundwater	$0.30 \cdot 10^{-3}$ µg/l
Concentration in drinking water	$2.37 \cdot 10^{-3}$ µg/l

Results of a similar modelling exercise carried out for the United Kingdom by Nielsen et al. (1993) gave very similar results (concentration in surface water $0.25 \cdot 10^{-3}$-$4.89 \cdot 10^{-3}$). These values are considerably lower than the EUSES output cited above.

3.1.4.2 Measured levels of acrylonitrile in water

The presence of acrylonitrile in water systems has been reported by a number of investigators, particularly at sites of production or further processing, as summarised non-exhaustively in **Table 3.16.**

Going et al. (1978) carried out measurements in the vicinity of production or processing facilities in 11 industrial areas of the US, and detected concentrations ranging from 0 to 4,300 µg/l. High levels of 3,500 µg/l and 4,300 µg/l were only reported in the vicinity of two plants, producing PAN fibres and nitrile elastomers respectively, the levels in the vicinity of the remaining sites being 0-19.7 µg/l. The limit of detection was reported to be 0.1-1.3 µg/l. Cantoni and Senati (1979) reported levels of up to 25,000 µg/l in effluents from a nitrile elastomer production plant, pre-wastewater treatment.

Tables 3.2, 3.3, 3.4 and 3.5 of this report provide information provided by industry on levels of acrylonitrile in water in the vicinity of European production and processing plants. The site of measurement included influent into a wastewater treatment plant, effluent from the site into a tidal estuary, and effluents from wastewater treatment plants. Acrylonitrile could not be detected in the effluents from wastewater treatment plants from a number of sites, at limits of detection ranging from 0.1-1,000 µg/l.

In addition to studies carried out in the vicinity of production or processing facilities several investigators have carried out measurements in municipal and surface waters, although little published information is available for European water courses. Ramstad and Nicholson (1982, quoted in Nielsen et al., 1993) reported levels of $0.07 \cdot 10^{-3}$ µg/l in municipal water in Michigan, US, although the validity of this figure must be questioned, given the limit of detection of approximately 0.1 µg/l. Hall et al. (1987) were unable to detect acrylonitrile in the Potomac river, West Virginia, US, at a detection limit of 10 µg/l. Krill and Sonzogni (1986), using GC-MS, were also unable to detect acrylonitrile in an examination of water from approximately 1,800 wells in Wisconsin, US. The specific detection limit for acrylonitrile was not given, but a generally applicable limit of 0.1-3 µg/l was cited for the range of organic compounds under investigation. A survey carried out in 1987 did not detect acrylonitrile in 75 surface water samples at a limit of detection of 2 µg/l (Japanese Environment Agency Office of Health Studies, 1991).

Table 3.16 Measured levels of acrylonitrile in surface waters and in effluents from manufacturing facilities

Location	Acrylonitrile level (µg/l)	Comment	Reference
USA, acrylonitrile production	< 0.1	Limit of detection approx.1µg/l	Going et al. (1978)
USA, acrylamide production	0.8	Limit of detection approx.1 µg/l	Going et al. (1978)
USA, PAN fibre facility	3,500	Limit of detection approx.1µg/l	Going et al. (1978)
USA, nitrile elastomer facility	up to 4,300	Limit of detection approx.1 µg/l	Going et al. (1978)
Germany, acrylonitrile production	2		Industry information
NL, ABS-SAN	< 100	Limit of detection 100 µg/l	Industry information
Germany, nitrile copolymers	Not detected	Limit of detection 50 µg/l	Industry information
Germany, acrylic fibres	2,000	Level refers to influent into WWTP	Industry information
UK, acrylic fibres	35,000	Discharge into large estuary	Industry information
Australia, SAN polymer production	<100		Worksafe Australia, personal communication
Michigan, USA	$0.07 \cdot 10^{-3}$		Ramstad and Nicholson (1982)
Potomac river, USA	Not detected	10 µg/l	Hall et al. (1987)
Wisconsin well water	Not detected	Limit of detection 0.1-3 µg/l	Krill and Sonzogni (1986)
Japan, surface water samples (75)	Not detected	Limit of detection of 2 µg/l	(Japanese Environment Agency, 1991)

3.1.4.3 Comparison of PEC_{water} with measured data

The monitoring data reported in Section 3.1.4.2 indicate that concentrations of acrylonitrile in surface waters are generally below the limit of detection of 1 - 2 µg/l. Higher levels are detected in the vicinity of production and processing plants, typically in the range of 0-100 µg/l, although levels as high as 35 mg/l have been reported. Values for $PEClocal_{water}$ calculated as described in Section 3.1.4.1.1 for the identified production and processing sites in Europe range from 3 µg/l, with the majority of values lying below 100 µg/l.

3.1.5 Atmospheric compartment

Atmospheric exposure to acrylonitrile may occur as a result of emissions during production of monomer, polymerisation of monomer to give acrylonitrile polymers, further processing of polymer to give polymeric products, use and, ultimately, disposal of the polymeric products. In addition, as previously stated in Section 3.1.1, other diffuse emission sources include vehicle exhausts as a result of combustion of hydrocarbon fuels, cigarette smoke and incomplete combustion of municipal wastewater sludge.

3.1.5.1 Calculation of $PEClocal_{air}$

(Equation 25 of the TGD)

Table 3.17 Calculation of $PEClocal_{air}$ for acrylonitrile production facilities

Site	$PEClocal_{air}$ (mg/m^3)	$PEClocal_{air, ann}$ (mg/m^3)
1	0.001	0.001
2	0.001	0.001
3	0.005	0.005
4	0.003	0.003
5	0.240	0.240
6	0.0	0.0
7	0.002	0.002
8	0.002	0.002

Table 3.18 Calculation of $PEClocal_{air}$ for acrylonitrile fibre production facilities

Site	$PEClocal_{air}$ (mg/m^3)	$PEClocal_{air,ann}$ (mg/m^3)
B	0.013	0.011
C	0.019	0.016
D	0.0001	0.0001
E	0.143	0.117
F	0.024	0.020
G	0.005	0.004
H	0.038	0.031
J	0.012	0.010
K	0.015	0.012

Table 3.19 Calculation of $PEClocal_{air}$ for ABS SAN production facilities

Site	$PEClocal_{air}$ (mg/m³)	$PEClocal_{air,ann}$ (mg/m³)
AA	0.033	0.027
BB	0.0009	0.0007
CC	0.001	0.0008
DD	0.003	0.002
EE	0.003	0.002
FF	0.014	0.010
GG	0.068	0.056
HH	0.0014	0.0012
II	0.0006	0.0005
JJ	0.016	0.013
KK	0.010	0.0085
LL	0.0052	0.0043
MM	0.0001	0.0001

Table 3.20 Calculation of $PEClocal_{air}$ for nitrile:butadiene copolymer production facilities

Site	$PEClocal_{air}$ (mg/m³)	$PEClocal_{air,ann}$ (mg/m³)
AAA	0.020	0.0165
BBB	0.0001	0.0001
CCC	0.0001	0.0001
DDD	0.001	0.0008
EEE	0.0006	0.0005
FFF	0.0011	0.0009
GGG	0.0188	0.0155
HHH	0.0052	0.0043
JJJ	0.0038	0.0031

Table 3.21 Calculation of $PEClocal_{air}$ dur to processing of acrylonitrile to acrylamide and adiponitrile

Site	$PEClocal_{air}$ (mg/m³)	$PEClocal_{air,ann}$ (mg/m³)
L	0.0042	0.0035
M	0.0015	0.0012
N	0.0881	0.0724
0	0.0001	0.0001

3.1.5.2 Calculation of PEC_{air} for regional and continental scenarios

Estimates of $PECregional_{air}$ and $PECcontinental_{air}$ due to acrylonitrile production and further processing have been made by means of EUSES using the release data provided by industry. In relation to derivation of $PECregional_{air}$, releases to atmosphere for sites located in the United Kingdom were chosen as a worst-case input into EUSES. In relation to derivation of $PECcontinental_{air}$, input into EUSES was taken as the total releases to atmosphere for all European sites. For further details of the input into and output from the model, refer to the website of the European Chemicals Bureau (http://ecb.jrc.it).

The following results were obtained:

$PECregional_{air}$ $7.08 \cdot 10^{-5}$ mg/m^3 (0.071 µg/m^3)

$PECcontinental_{air}$ $2.49 \cdot 10^{-5}$ mg/m^3 (0.025 µg/m^3)

Concentrations in air have also been estimated using the Mackay level 3 model (ECETOC Special Report, 1994) using the geophysical parameters for Germany, giving a value of $0.049 \cdot 10^{-5}$ mg/m^3, while results of a similar modelling exercise carried out for the United Kingdom by Nielsen et al. (1993) gave results in the range 0.008-$0.016 \cdot 10^{-5}$ mg/m^3. These values are 10-fold lower than the EUSES output cited above.

Concentrations in air have also been estimated using the Mackay level 3 model (ECETOC Special Report, 1994) using the geophysical parameters for Germany, giving a value of $0.049 \cdot 10^{-5}$ mg/m^3, while results of a similar modelling exercise carried out for the United Kingdom by Nielsen et al. (1993) gave results in the range 0.008 - $0.016 \cdot 10^{-5}$ mg/m^3. These values are 10-fold lower than the EUSES output cited above.

As indicated in Section 3.1.2.4, diffuse emissions such as use and, ultimately, disposal of acrylonitrile polymers, acrylonitrile in vehicle exhausts as a result of combustion of hydrocarbon fuels, cigarette smoke and incomplete combustion of municipal wastewater sludge will also contribute to the regional and continental PEC_{air}. The contribution to PEC_{air} from these sources is estimated to be approximately 2-3 times that of the contribution from point production and further processing sources. This may be an overestimate if vehicle emissions are not as significant a source of acrylonitrile as was previously thought. Nevertheless, an initial estimate of the impact of these diffuse emissions on overall $PECregional_{air}$ and $PECcontinental_{air}$ has been made by applying a factor of 3.5 to the above, based on the rationale outlined in Section 3.1.2.4 that total releases from point and diffuse sources on a continental (EU) scale can be estimated as 3,310 tonnes per annum, of which 900 tonnes may be attributable to point sources - 3,310/900 = 3.68. Using this approach, an estimate of $PECregional_{air}$ and $PECcontinental_{air}$ due to emissions from all sources is derived, as follows:

$PECregional_{air}$ $2.61 \cdot 10^{-4}$ mg/m (0.26 µg/m^3)

$PECcontinental_{air}$ $9.16 \cdot 10^{-5}$ mg/m (0.09 µg/m^3)

3.1.5.3 Measured levels of acrylonitrile in air

Going et al. (1978) monitored air and water emissions in the vicinity of acrylonitrile production or processing facilities in 11 industrial areas of the US, and reported levels in air ranging from <0.1-325 µg/m^3 (detection limit 0.3 µg/m^3). Cicollella et al. (1981) carried out measurements of acrylonitrile in air at different locations and during a range of activities within a number of

French production or processing facilities. These authors found levels from 5-48.4 mg/m^3 in the vicinity of drains and tanks of ABS, ABS-SAN and nitrile rubber facilities. Loading and unloading of raw materials and products gave rise to levels of 4.1-6.1 mg/m^3 at an acrylonitrile production facility, while levels as high as 540 mg/m^3 were detected as a consequence of minor leaks.

It should be noted that the levels reported by Going and by Cicollella relate to emission levels pertaining at least 15 years ago. Since that time, increasingly stringent controls on emissions have reduced reported atmospheric levels in the vicinity of production or processing facilities significantly. The Japanese Environmental Protection Agency in 1987 monitored air and water emissions in the vicinity of Japanese plants (Japanese Environmental Protection Agency, 1991) and found levels of between 0.042 and 2.4 µg/m^3 (detection limit 0.04 µg/m^3). Information supplied by industry for an acrylonitrile production and ABS-SAN polymerisation site (**Table 3.1**) showed mean levels of 0.6 µg/m^3 acrylonitrile (but with a highest detected level of 240 µg/m^3). Comparable figures for an ABS plastics polymerisation facility were 0.2 µg/m^3 mean level (limit of detection), with a highest detected level of 15 µg/m^3, while no acrylonitrile was detected in fenceline monitoring carried out at an acrylonitrile fibre manufacturing facility in 1994. BUA report (1995) results of monitoring carried out in the vicinity of 2 acrylonitrile production and processing facilities between May and October, 1985. Acrylonitrile was not detectable in 401 out of a total of 430 samples at a limit of detection of 1 µg/m^3, and a mean level of 0.9 µg/m^3 was calculated, assuming a value of 0.5 µg/m^3 for those samples in which acrylonitrile was not detected.

In relation to the wider atmospheric environment, Schenck (1986) carried out measurements of acrylonitrile in urban German air over the period 1977-1984. Levels ranged from 0.01-10.4 µg/m^3, while clean (rural) air contained less than 0.002 µg/m^3. Wiersema et al. (1989) did not detect acrylonitrile over a 6-month monitoring period of urbanised and industrialised air in the Gulf Coast of Texas (limit of detection 0.122 µg/m^3). The US EPA (1986) reported on a study of acrylonitrile levels in urban air in the US, in which the maximum level detected in Santa Clara County, California in October, 1984, was 2.5 µg/m^3, mean levels of 0.35-0.46 µg/m^3 were found in 3 cities in New Jersey in July-August, 1981, and a mean level of 0.46 µg/m^3 was reported for Texas cities sampled between October, 1985 and February, 1986. These reported levels are reasonably comparable to the regional and continental $PEClocal_{air}$ of 0.26 and 0.09 µg/m^3, respectively estimated above for combined point and diffuse emissions. Acrylonitrile has been detected in interstellar space (Gardner and Winnewisser, 1975), the source is however thought to be gas phase chemical reactions in interstellar clouds.

Table 3.22 summarises non-exhaustively the published values for atmospheric acrylonitrile in urban air and in the vicinity of manufacturing plants.

Table 3.22 Levels of acrylonitrile in the air in the vicinity of manufacturing plants and in urban air

Location	Acrylonitrile level (µg/m3)	Comment	Reference
USA, ABS-SAN production	< 0.2 - 325	Limit of detection: 0. 3 µg/m^3	Going et al. (1978)
USA, acrylamide production	<0.1 - 15.9	Limit of detection: 0. 3 µg/m^3	Going et al. (1978)
USA, PAN fibre facility	< 0.1 - 1.1	Limit of detection: 0. 3 µg/m^3	Going et al. (1978)
USA, nitrile elastomer facility	< 0.1 - 3.1	Limit of detection: 3 µg/m^3	Going et al. (1978)
Netherlands, ABS-SAN	0.6 average		Industry information
Germany, acrylonitrile production and processing	0. 9 average	Limit of detection: 1.0 µg/m^3	BUA (1995)
Japan, vicinity of manufacturing plants	0.042 - 2.4	Limit of detection: 0.04 µg/m^3	Japanese Environmental Protection Agency (1991
France, vicinity of tanks and drains in manufacturing facility	5,000 – 48,400		Cicollella et al. (1981) Krill and Sonzogni (1986)
Germany, urban air	0.01 - 10.4		Schenck (1986)
Germany, clean air	0.002		Schenck (1986)
USA, Texas	Not detected	Limit of detection: 0.122 µg/m^3	Wiersema et al. (1989)
USA, California	0 - 2.5		US EPA (1986)
USA, New Jersey, urban air	0.35 - 0.46		US EPA (1986)
USA, Texas	0.46 average		US EPA (1986)

3.1.6 Terrestrial compartment

Acrylonitrile can potentially be redistributed to soil from the atmospheric or aqueous compartments, by the spreading of acrylonitrile-contaminated sewage sludge or as a result of accidental spills. Acrylonitrile is anticipated to be relatively mobile in soil, and this was supported by the results of an adsorption:desorption study of acrylonitrile to montmorillonite clay which provided no evidence of adsorption, the adsorption:desorption processes being in equilibrium. This was supported by calculation of the Koc (soil adsorption coefficient) using QSAR or from the water solubility of acrylonitrile when values of 11.5 and 9.0, respectively were derived, showing little potential for adsorption to soil.

Information provided by industry indicates that little industrial sludge from acrylonitrile production and processing facilities is spread on land in Europe. Analytical data were provided for sludge from one facility where the local authority has authorised the spreading of sludge from the facility on land, showing acrylonitrile to be undetectable in the sludge. The majority of companies providing information on this aspect indicated that contaminated sludge is incinerated together with other wastes. The main source of release of acrylonitrile to soil will therefore be deposition from the atmospheric compartment. Acrylonitrile entering the terrestrial compartment in small quantities will be degraded (Section 3.1.3.1.2, Conclusion on aquatic degradation). Run-off from the soil will be released to groundwater, where acrylonitrile will also undergo biodegradation. The above considerations would therefore indicate that levels of acrylonitrile in soil will be extremely low.

3.1.6.1 Calculation of PEC_{soil}

In accordance with Section 2.3.8.5 of the TGD, the $PEClocal_{soil}$ was calculated assuming sludge from a WWTP containing acrylonitrile was applied to soil once a year for 10 years. To this was added the (lesser) loads from wet and dry deposition. Accounting for the effects of loss through leaching, volatilisation, and biodegradation involves (1) a series of mass transfer coefficients and rate constants between soil, air and water, and (2) default data on rainfall, soil depth and density, and sludge application rate. No measured field data were available for these parameters. Thus the results of EUSES are taken for PEC_{soil} at all levels.

The result for $PEClocal_{soil}$ for processing sites was 3.86 µg/kg wet weight (wwt) averaged over 30 days or 180 days. The value for production was 0.17 mg/kg wet weight averaged over 30 days or 0.046 mg/kg wet weight averaged over 180 days, while PEC_{local} in soil pore water (agricultural soil) was 0.3 mg/kg wwt. It should be noted, however, that this is a worst-case exposure scenario, and appears unrealistic, given the information from industry that little industrial sludge from acrylonitrile production and processing facilities is spread on land in Europe. The majority of companies providing information on this aspect indicated that contaminated sludge is incinerated together with other wastes

EUSES[6] provides estimates of $PECregional_{soil}$ and $PECcontinental_{soil}$ for acrylonitrile production and further processing. Results are shown in **Table 3.23**.

Table 3.23 $PECregional_{soil}$ and $PECcontinental_{soil}$ for acrylonitrile production and processing

	$PECregional_{soil}$	$PECcontinental_{soil}$
Agricultural soil (mg/kg wwt)	$5.0 \cdot 10^{-5}$	$8.21 \cdot 10^{-6}$
Agricultural soil pore water (mg/l)	$1.36 \cdot 10^{-4}$	$2.24 \cdot 10^{-5}$
Natural soil (mg/kg wwt)	$8.12 \cdot 10^{-6}$	$2.88 \cdot 10^{-6}$
Industrial soil (mg/kg wwt)	$2.15 \cdot 10^{-4}$	$2.41 \cdot 10^{-5}$

3.1.6.2 Measured levels in soil

Limited monitoring data are available. Going (1978) detected no acrylonitrile in soil samples taken in the vicinity of 10 out of 11 acrylonitrile manufacturing and processing plants in the US (limit of detection of approximately 0.5 mg/kg). Acrylonitrile was detected in soil of one site at a concentration of approximately 0.5 mg/kg. It appears from information provided by industry that industrial sludge from acrylonitrile production or processing plants in Europe is incinerated, although specific information is not available for plants at this time. If it can assumed that contribution to soil concentrations via application of industrial sewage sludge from acrylonitrile production or processing sites will be negligible, PEC_{soil} will be negligible. The main contribution will be via wet or dry deposition from the atmosphere in the immediate vicinity of acrylonitrile plants.

[6] **Euses Calculations** can be viewed as part of the report at the website of the European Chemicals Bureau: http://ecb.jrc.it

3.1.7 Secondary poisoning

Exposure of aquatic species to low levels of acrylonitrile in the aquatic environment is theoretically possible. Section 3.1.3.2.4 indicates that acrylonitrile is unlikely to bioaccumulate in exposed biota, and this is supported by toxicity studies in mammalian species (Section 4), where there was little evidence of cumulative toxicity in a range of species. An experimentally-derived BCF of 48 in fish was probably in part attributable to binding of acrylonitrile to tissue macromolecules, as also demonstrated in rodents, rather than to true bioaccumulation. EUSES calculates a BCF for fish of 1.41. Concentrations of acrylonitrile in biota are therefore expected to be very low, and there are no reports in the literature of detectable levels of acrylonitrile in aquatic biota.

Similarly, given the very low levels of acrylonitrile anticipated to be present in the terrestrial environment, no significant levels are expected in plants or other terrestrial species, potential routes of exposure being deposition from air or contaminated effluents or surface waters.

EUSES provides values for exposed biota, as follows:

Regional concentration in wet fish, mg/kg:	$3.96 \cdot 10^{-3}$
Regional concentration in plant root tissue, mg/kg:	$1.3 \cdot 10^{-4}$
Regional concentration in plant leaves, mg/kg:	$1.66 \cdot 10^{-5}$
Regional concentration in grass, mg/kg:	$1.66 \cdot 10^{-5}$
Regional concentration in meat (wet weight), mg/kg:	$1.30 \cdot 10^{-7}$
Regional concentration in milk (wet weight), mg/kg:	$1.30 \cdot 10^{-6}$

EUSES also provides values for daily human intake from the environment, as follows:

Daily intake through drinking water (mg/kg/day):	$8.01 \cdot 10^{-5}$
Daily intake through consumption of fish (mg/kg/day):	$6.51 \cdot 10^{-6}$
Daily intake through consumption of leaf crops (mg/kg/day):	$2.84 \cdot 10^{-7}$
Daily intake through consumption of root crops (mg/kg/day):	$7.12 \cdot 10^{-7}$
Daily intake through consumption of meat (mg/kg/day):	$5.61 \cdot 10^{-10}$
Daily intake through consumption of milk (mg/kg/day):	$1.04 \cdot 10^{-8}$
Daily intake through intake of air (mg/kg/day):	$1.52 \cdot 10^{-5}$
Regional total daily intake for humans (mg/kg/day)	$1.03 \cdot 10^{-4}$

It is concluded from these results that the potential for secondary poisoning is very small. As discussed in Section 3.1.5.2, relating to the derivation of $PECregional_{air}$ and $PECcontinental_{air}$, however, these results are based on the point source emissions from the various production and processing plants in Europe as the input into EUSES. Diffuse emission sources such as cigarette smoke, loss of monomer from plastics and fibres during use and, in particular, vehicle exhausts will contribute additionally to the above levels in various biota and to daily human intake from these biota. This is in part counterbalanced by the fact that the input into EUSES includes some default emission scenarios, and the values cited above can be regarded as a reasonable estimate of indirect exposure via the environment.

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

3.2.1 Aquatic compartment

The acute toxicity of acrylonitrile for aquatic organisms has been assessed in a variety of fish and aquatic invertebrate species (**Tables 3.24 and 3.25**). A number of chronic toxicity studies have been carried out, and the effects on algal growth have also been examined. The majority of studies are published in the scientific literature rather than being available as comprehensive study reports, and experimental detail including information related to adherence of current principles of GLP, is lacking. The publications span 45 years, are of variable quality, and none of the studies comply totally with the requirements of current OECD or Annex V testing protocols. Monitoring of acrylonitrile in test media was not reported for most of the published studies, nor did experimental design take account of the volatility of acrylonitrile. Actual exposure concentrations in studies carried out under static conditions in open vessels can therefore be anticipated to be somewhat lower than those cited. A number of key studies, carried out in general compliance with current OECD/Annex V testing protocols are however available for risk assessment purposes, as follows:

- Acute toxicity in *Cyprinodon variegatus* (AN Group, 1997a),
- Early life stage test in *Pimephales promelas* (Analytical Bio Chemistry Laboratories Inc., 1980),
- 30-day study chronic toxicity study in *Pimephales promelas* (Henderson et al., 1961),
- Algal growth inhibition test in *Scenedesmus subspicatus* (Bayer, 1995) and *Skeletonema costatum* (AN Group, 1997b).

3.2.1.1 Toxicity test results

3.2.1.1.1 Fish

<u>Acute toxicity to fish</u>

As shown in **Table 3.24** acrylonitrile is moderately toxic to fish, with 96-hour LC_{50} for fresh water fish generally lying in the range of 10-20 mg/l (nominal). A study carried out by the AN Group (1997a) in the marine species Sheepshead Minnow (*Cyprinodon variegatus*) determined the 96-hour LC_{50} of 8.6 mg/l (mean measured concentration 0-24 h.) This result can be compared with a 96-hour LC_{50} of 14.0 mg/l in another marine species, *Gobius minutus* (Adema, 1976). Zhang et al. (1996a; 1996b) has reported a 96-hour LC_{50} of 5.16 mg/l for *Ctenophayngodon idellus,* and suggests that this species may be more sensitive to the toxic effects of acrylonitrile than North American fish species. Toxicity increases with time, as would be anticipated, with little decline in the slope of the response curve over a 24-96 exposure period. Reported 48-hour LC_{50} values lie between 14.3 mg/l and 33.5 mg/l. In the AN Group study the 96-hour LC_{50} of 8.6 mg/l can be compared with a 72-hour LC_{50} of 15.9 mg/l, a 48-hour LC_{50} of 15.9 mg/l and a 24-hour LC_{50} of 28.2 mg/l. The ratio of the 24-hour LC_{50} to the 96-hour LC_{50} in this study is 3.3, indicating the potential chronic toxicity of acrylonitrile.

In addition to the AN Group study, the publications of Zhang et al. (1996a) on acrylonitrile toxicity in *Cyprinus carpio* and *Ctenophayngodon idellus*, those of Buccafusco et al. (1981) in

Lempomis machrochirus and Henderson et al. (1961) in *Lempomis machrochirus, Lesbistes reticulatus* and *Pimephales promelas,* together with the Adema (1976) study on *Gobius minutus* are considered to be sufficiently valid for the risk assessment, although full adherence to current OECD or EU Annex V test methods cannot be established.

The comparative study of Henderson et al. (1961) in *Pimephales promelas* using static conditions for short-term exposure and continuous flow conditions for prolonged exposure showed the toxicity of acrylonitrile to be greater in continuous flow conditions (96-hour LC_{50} static 18.1 mg/l, continuous flow 10.1, 48-hour LC_{50} static 21.5 mg/l, continuous flow 14.8), indicating some loss of acrylonitrile from static solutions, as might be expected for this volatile substance. Henderson also reported that the toxicity of acrylonitrile in this species was slightly greater in hard water (96-hour LC_{50} of 14.3 mg/l) compared with soft water (96-hour LC_{50} of 18.1 mg/l).

The AN Group study (1997a) provides a 96-hour No-Observed-Effect-Concentration (NOEC) of 5.4 mg/l (mean measured concentration). Other published information on NOEC for acute toxicity in fish is regarded as less reliable. A 48-hour LC_0 for *Leuciscus idus* was established by von Juhnke and Lüdemann (1978) as 8-16 mg/l and a 24-hour LOEC for *Oncorhynchus mykiss* has been quoted by Slooff in 1979 as 5 mg/l. The NOEC over 1-4 days appears to lie in the 5-10 mg/l range.

Table 3.24 Acute toxicity of acrylonitrile to fish

Species	Experimental conditions	Effect concentration (mg/l)	Comment	Reference
Cyprinodon variegatus	pH 7.8-8.1, temp. 21$\pm$1C Diss. O_2 86-95% Salinity 34-35% Semi-static test Fish 0.55-0.75g	24-hr LC_{50}, 28.2 48-hr LC_{50}, 15.9 72-hr $LC_5$0, 15.9 96-hr LC_{50}, 8.6 96-hr NOEC, 5.6 Measured concentrations	Considered in risk assessment	AN Group (1997a)
Lepomis machrochirus	pH 6.5-7.9, temp. 21-23°C Diss. O_2 0.3-9.7 mg/l Hardness 32-48 mg/l $CaCO_3$ Static test Fish 0.3-1.2 g	24-hr LC_{50}, 28.0 96-hr LC_{50}, 10.0	Considered in risk assessment	Buccafusco et al. (1981)
Lepomis machrochirus	Fish approx. 2g. Static test	24-hr LC_{50}, 25.5 48-hr LC_{50}, 14.3 96-hr LC_{50}, 11.8	Considered in risk assessment	Henderson et al. (1961)
Lepomis machrochirus	Fish 3.5-7.5cm. Static test	96-hr NOEC, 10.0	Supporting information	Buzzell et al. (1968)
Cyprinus carpio	pH 7.0+0.5, temp. 23$\pm$2°C Diss. O_2 8.0$\pm$0.34 mg/l Hardness 1.86 mg/l $CaCO_3$ Semi-static test Fish 31.8$\pm$3.4mg	96-hr LC_{50}, 19.64	Considered in risk assessment	Zhang et al. (1996a)
Cyprinus carpio	Fish 3g.	24-hr LC_{50}, 37.4 48-hr LC_{50}, 24.0	Supporting information	Marcoci and Ionescu (1978)
Cteno-phayngodon idellus	pH 7.0$\pm$0.5, temp. 23$\pm$2°C Diss. O_2 8.0$\pm$0.34 mg/l Hardness 1.86 mg/l $CaCO_3$ Semi-static, fish 3.14$\pm$0.61g	96-hr LC_{50}, 5.16	Considered in risk assessment	Zhang et al. (1996a)

Table 3.24 continued overleaf

Table 3.24 continued Acute toxicity of acrylonitrile to fish

Species	Experimental conditions	Effect concentration (mg/l)	Comment	Reference
Gobius minutes marine	pH 8.0 15±1C Static test Fish 6.04±1.5 cm	24-hr LC_{50}, 20.0 48-hr LC_{50}, 15.0 72-hr LC_{50}, 14.0 96-hr LC_{50}, 14.0	Considered in risk assessment	Adema (1976)
Oncorhynchus mykiss	No details available	48-hr LC_{50}, 7.0	Supporting information	Jackson and Brown (1970)
Oncorhynchus mykiss	No details available	96-hr LC_{50}, 24	Supporting information	Neilsen et al. (1993)
Lebistes reticulatus (fry)	Fish approx. 0.1g. Static test	24-hr LC_{50}, 44.6 48-hr LC_{50}, 33.5 96-hr LC_{50}, 33.5	Considered in risk assessment	Henderson et al. (1961)
Leuciscus idus	No details available	LC_{50}, 16-28 LC_{100} 20-48 LC_0 8-16	Supporting information	von Juhnke and Lüdemann (1978)
Leuciscus idus	No details available	LC_{100}, 50.0 LC_0, 5.0	Supporting information	Wellens (1982)
Pimephales promelas	Fish approx. 1.5g. Static test 20mg/l $CaCO_3$	24-hr LC_{50}, 34.3 48-hr LC_{50}, 21.5 96-hr LC_{50}, 18.1	Considered in risk assessment	Henderson et al. (1961)
Pimephales promelas	Fish approx. 1.5g. Static test 380mg/l $CaCO_3$	24-hr LC_{50}, 32.7 48-hr LC_{50}, 16.7 96-hr LC_{50}, 14.3	Considered in risk assessment	Henderson et al. (1961)
Phoxinus phoxinus	No details available	24-hr LC_{50}, 38.2 48-hr, 17.6	Supporting information	Marcoci and Ionescu (1978)
Rhodeus sericeus	No details available	48-hr LC_{50}, 25.7	Supporting information	Marcoci and Ionescu (1978)
Leucaspius delineatus	No details available	48-hr LC_{50}, 22.7	Supporting information	Marcoci and Ionescu (1978)
Brachydanio rerio	pH 8.0±0.2, 20±2°C Diss. O_2 90±0.5 mg/l Flow through test	48-hr LC_{50}, 15.0	Supporting information	Slooff (1979)
Brachydanio rerio	No details available	48-hr LC_{50}, 25 48-hr LC_0, 3 48-hr LC_{100}, 40	Supporting information	Wellens (1982)
Lagadon rhomboides	13.7-20.4°C Fish length 5.7-11.3 mm	24-hr LC_{50}, 24.5 24-hr LC_0, 20 24-hr LC_{100}, 30	Supporting information	Daugherty and Garrett, (1951)
Carrassius sp.	No details given	48-hr LC_{50}, 40.0	Supporting information	Paulet and Vidal (1975)
Alburnus alburnus	12.3-18.2°C pH 7.3-7.5	Survival time @ 40mg/l = 47 hours, @ 25 mg/l = 16 days, @ 20 mg/l = > 20 days	Supporting information	Bandt (1953)
Leuciscus rutilus	12.3-18.2°C pH 7.3-7.5	Survival time @ 40mg/l = >6 days, @ 30 mg/l = >11 days	Supporting information	Bandt (1953)

Note: all concentrations cited are nominal concentrations unless otherwise stated

Chronic toxicity to fish

Information on the chronic toxicity of acrylonitrile in fish is limited, but two separate studies indicate an LC_{50} of approximately 2 mg/l on prolonged exposure (30-day LC_{50} in *Pimephales promelas* 2.6 mg/l, 100-day LC_{50} in *Oncorhynchus mykiss* 2.2 mg/l). The 30-day study of Henderson et al. (1961), in *Pimephales promelas* using flow through conditions was judged to be valid for risk assessment purposes. The study of Jackson et al. (1970) in *Oncorhynchus mykiss* provides no information concerning experimental conditions and cannot be considered as valid for the risk assessment.

A 30-day early life stage toxicity test using *Pimephales promelas* was sponsored by Monsanto Chemical Company and carried out by Analytical Bio Chemistry Laboratories Inc. (1980) in accordance with the American Society for Testing and Material Guideline (1979), using flow-through conditions. The study showed a significantly reduced survival rate at mean measured test concentrations $\geq$0.86 mg/l acrylonitrile and significantly reduced growth rate at test concentrations $\geq$0.34 mg/l. The mean measured concentration of 0.34 mg/l represented a nominal concentration of 0.25 mg/l and was the lowest concentration used in the study. No conclusive No Observable Effect Concentration can therefore be established from this study. However the effect on growth rate was only seen in two of the four replicate test chambers, and the report concludes that 0.34 mg/l represents the upper limit of the Maximum Acceptable Toxicant Concentration (MATC). For risk assessment purposes, this can either be accepted as the NOEC, or perhaps more correctly as a LOEC. In accordance with the TGD, if the LOEC is 10% of the EC10 or 20% of the EC20 then the NOEC can be taken as LOEC/2, i.e. 0.34/2 = 0.17 mg/l. This can be regarded as a worst-case analysis, with the NOEC realistically probably being approximately 0.3 mg/l.

Hawkins et al. (1991) examined the chronic toxicity and carcinogenicity of acrylonitrile in the medaka (*Oryzias latipes*). The experimental procedure involved either continuous exposure to test concentrations of 2.5 ppm or 5 ppm nominal acrylonitrile over a 28-day period or a multiple pulsing exposure to 8 ppm once or twice per week for a 4-week period. Groups of fish were then examined 6, 9 or 12 months following the initiation of exposure. The study revealed no evidence of carcinogenicity or significant chronic toxicity in the acrylonitrile-exposed fish.

3.2.1.1.2 Aquatic invertebrates

Acute toxicity to aquatic invertebrates

Acrylonitrile is also moderately toxic to aquatic invertebrates (**Table 3.25**). The 48-hour EC_{50} in *Daphnia magna* reported in four separate studies lies between 7.6 mg/l and 22 mg/l. Of these, the studies of LeBlanc (1980) and Zhang et al. (1996a; 1996b) can be regarded as valid for risk assessment purposes, and provided very similar values of 7.6 mg/l, 8.7 mg/l and 10 mg/l (nominal concentrations), respectively. A low short-term NOEC of 0.78 mg/l was established in the LeBlanc study, carried out in hard water.

Zhang et al. (1996a) also examined the acute toxicity of acrylonitrile to other aquatic invertebrates, *Chironomus sp., Limnodrilus hoffmeisteri* and *Artemia salina*, and reported 48-hour EC_{50} values of between 14.2 and 16.9 mg/l. Similar acute toxicity results were obtained in a study of reasonable quality carried out by TNO in *Crangon crangon* (48-hour EC_{50} 20 mg/l) (Adema, 1976). Acrylonitrile was reported by Erben and Beader (1983a; 1983b) to be substantially more toxic in *Gammarus fossarum*, *Asellus aquaticus* and *Radix peregra* with 48-hour EC_{100}'s of

<0.1 mg/l and in *Lymnaea stagnalis*, with a 48-hour LC_{50} > 0.16 mg/l. Few experimental details were provided in these papers, for which only a summary in English is available, and the results are not considered valid for risk assessment purposes. There is doubt about the concentrations quoted by the authors in their experiments with acrylonitrile, which may in fact be greater by a factor of 10. Further justification for this view is given by the fact that the authors in the same experiment determined 48-hour EC_{50}'s in *Asellus* of < 30 mg/l for acetone and 1.0 mg/l for cumene, with similarly low values for these chemicals in the other three species. Acetone and cumene are not generally regarded as highly toxic to aquatic species. Zhang et al. (1996a) determined a 48-hour EC_{50} of 40.00 mg/l for *Radix pliculata*, with a 96-hour EC_{50} of 17.9 mg/l.

Table 3.25 Acute toxicity of acrylonitrile to aquatic invertebrates

Species	Experimental conditions	Effect concentration (mg/l)	Comment	Reference
Daphnia magna	pH 7.0±0.5 Diss. O_2 7.5 - 8.0 mg/l 24 ± 1°C Hardness1.86 mg/l $CaCO_3$ *Daphnia* < 24 hrs old	48-hr EC_{50}, 10.0	Considered in risk assessment	Zhang et al. (1996b)
Daphnia magna	pH 7.0±0.5, 25±1°C Diss. O_2 8.0±0.34 mg/l Hardness1.86 mg/l $CaCO_3$ *Daphnia* < 24 hrs old	48-hr EC_{50}, 8.70	Considered in risk assessment	Zhang et al. (1996a)
Daphnia magna	pH 8.0±0.2 22±1°C Hardness 173±13mg/l $CaCO_3$ *Daphnia* < 24 hrs old	24-hr LC_{50}, 13.0 48-hr LC_{50}, 7.6 NOEC 0.78	Considered in risk assessment	LeBlanc (1980)
Daphnia magna	No information available, US EPA unpublished data	48-hr LC_{50}, 22.0	Supporting information	Nielsen et al. (1993)
Limnodrilus hoffmeisteri	pH 7.0±0.5 Diss. O_2 8.0±0.34 mg/l 15±2°C Hardness1.86 mg/l $CaCO_3$ Animals 1-2 cm	96-hr EC_5, 16.90	Considered in risk assessment	Zhang et al. (1996a)
Chironomus sp.	pH 7.0±0.5 Diss. O_2 8.0±0.34 mg/l 20±1°C Hardness 1.86 mg/l $CaCO_3$ < 24 hrs old	48-hr EC_5, 14.21	Considered in risk assessment	Zhang et al. (1996a)
Artemia salina	pH 7.0±0.5, 25±1°C Diss. O_2 8.0±0.34 mg/l Hardness 1.86 mg/l $CaCO_3$ < 24 hrs old	48-hr EC_5, 14.34	Considered in risk assessment	Zhang et al. (1996a)
Gammarus	22°C	Survival time @ 50mg/l = <22 hours	Supporting information	Bandt (1953)
Gammarus fossarum	pH 7-8 Diss. O_2 3.5-4.7 mg/l 17-20°C Hardness 300 mg/l $CaCO_3$	4-hr LC_{50}, <0.024 4-hr LC_{100}0.04 48-hr LC_{100} <0.024, NOEC 96-hour 0.012	Supporting information	Erben and Beader (1983a)

Table 3.25 continued overleaf

Table 3.25 continued Acute toxicity of acrylonitrile to aquatic invertebrates

Species	Experimental conditions	Effect concentration (mg/l)	Comment	Reference
Asellus aquaticus	pH 7-8 Diss. O_2 7.8-8.6 mg/l 17-20°C Hardness 300 mg/l $CaCO_3$	24-hr LC_{50}, <0.04 48-hr LC_{100} .04-0.07 72-hr LC_{100} 0.064 96-hr LC_{100} 0.016	Supporting information	Erben and Beader (1983a)
Radix peregra	pH 7-8 Diss. O_2 7.0-8.3 mg/l 20°C Hardness 300 mg/l $CaCO_3$	24-hr LC_{50} 0.04-0.16 24 hr.LC_{100} 0.16 48-hr LC_{50} < 0.04 72-hr LC_{100}, 0.04	Supporting information	Erben and Beader (1983b)
Radix pliculata	pH 7.0±0.5 Diss. O_2 8.0±0.34 mg/l 22±1°C Hardness 1.86 mg/l $CaCO_3$ 3-4 day old juveniles	48-hr EC_{50} 40.00 96-hr EC_{50} 17.94	Considered in risk assessment	Zhang et al. (1996a)
Crangon crangon	15°C	24 hr. LC_{50} 10-33	Supporting information	Portman (1970)
Crangon crangon	pH 8.0 15±1°C Static test Shrimp 5.5±0.5 cm	24-hr LC_{50}, 25.0 48-hr LC_{50}, 20.0 72-hr LC_{50}, 6.0 96-hr LC_{50}, 6.0	Considered in risk assessment	Adema (1976)
Ophryotrocha diadema	Seawater 21°C	48-hr LC_{50}, 10-33	Supporting information	Parker (1984)

Note: all concentrations cited are nominal concentrations unless otherwise stated

Chronic toxicity to aquatic invertebrates

Zhang et al. (1996b) carried out a 14-day and a 21-day chronic toxicity study in *Daphnia magna* using the OECD 1987 Testing Guidelines with daily renewal of test solutions but without measurement of acrylonitrile. They reported that the results of the two studies were identical, with a NOEC for survival of 2 mg/l nominal (LOEC > 4 mg/l) and a NOEC for reproduction of 0.5 mg/l nominal. It is difficult to establish how these values were derived from the experimental data presented in the paper, which show little or no dose-response for both survival and reproduction in the concentration range used. While the paper provides useful information for risk assessment purposes, in that the NOEC based on nominal concentrations is in the same range as that established in the fish early life study described in Section 3.2.1.1.1 (chronic toxicity), its validity in respect of deriving a PNEC based on an assessment factor of 10 can be questioned, as discussed further in Section 3.2.1.2. However, given the consistency of the aquatic toxicity data for acrylonitrile overall and the fact that the results of the Zhang et al. study are in line with those of the fish early life study and the algal growth inhibition tests, an assessment factor of 10 is overall considered to be appropriate.

The US EPA (1980) reported a no adverse effect level of 3.6 mg/l in a life cycle study (21 days) in *Daphnia*. No further information is available on this study, the quality of the study cannot be judged, and results are provided for information only.

3.2.1.1.3 Algae and aquatic plants

In a study carried out by Bayer AG (1995) the 72-hour EC_{50} (biomass) for algae *(Scenedesmus subspicatus*) was 3.1 mg/l nominal (approximately 2.5 mg/l actual concentration), and for growth rate was > 7.1 mg/l (nominal). The calculated NOEC was 0.8 mg/l, and the LOEC 1 mg/l.

The AN Group (1997b) have examined the effect of acrylonitrile on the growth of *Skeletonema costatum,* a unicellular chain forming marine diatom over a 72-hour period in accordance with the 1990 PARCOM guidelines for testing of offshore chemicals and drilling muds. In this study the 72-hour EC_{50} (biomass) was 1.63 mg/l (mean measured concentration at t_0, with 96% loss in concentration over 72 hours), and for growth rate was 14.1 mg/l. The NOEC for effects on biomass was 0.41 mg/l and for effects on growth rate was 3.0 mg/l. The mean concentrations to which the organisms were exposed over the experimental period were undoubtedly lower than those cited. The 72-hour NOEC for effects on biomass can be considered to represent a NOEC in a long-term study for algae, and is thus relevant for the purpose of deciding on an appropriate assessment factor for derivation of a PNEC.

Garrison (1978) showed that 100 mg/l acrylonitrile caused total inhibition of photosynthesis and respiration in the sea grass *Ruppia maritima*. Lower acrylonitrile levels (10-100 ppm) caused inhibition of growth rate (node number, leaf number and biomass), although at levels below 1 ppm a stimulation of growth was observed. However this work is described in abstract form only, with no description of experimental conditions, and the results cannot be considered valid for risk assessment purposes.

3.2.1.1.4 Microorganisms

Studies on the toxicity of acrylonitrile to microorganisms are of variable quality, and have yielded somewhat conflicting results, in part due to differences in the length of the exposure period and the acclimation which occurs over extended exposure periods. Results of biodegradation testing, as previously outlined, shows that acrylonitrile has an initial inhibitory effect on non-acclimated microorganisms, but following acclimation EC_{50}’s for microorganisms are generally in excess of 100 mg/l.

A detailed study carried out for the US EPA (Hovious et al., 1973) indicated at least 50% inhibition of anaerobic respiration of unadapted activated sludge over a 5-hour period in the presence of 150-500 mg/l acrylonitrile in substrate-limited conditions and 100-mg/l in non-substrate limited conditions. In the same study, 7.5 mg/l of acrylonitrile produced initial inhibition but was later tolerated, indicating acclimation. An EC_{50} of 400 mg/l was cited for aerobic activated sludge over a 24-hour period (Buzzell et al., 1968), although recent data (BASF, 1992) on effects of acrylonitrile on respiration of activated sludge indicated a 3-hour EC_{20} of greater than 1,792 mg/l. Another recent study using an anaerobic toxicity test (ATA) adapted for aerobic bacteria gave an EC_{50} over 15 hours of 52 mg/l (Blum and Speece, 1991), while methanogens in the same test over a 48-hour exposure period provided an EC_{50} of 90 mg/l.

Xu and Dutka (1987) derived an EC_{50} of 254 mg/l over 30 minutes using the ^{R}Microtox test (*Photobacterium phosphoreum*). Substantially lower EC_{50}'s (range 1-10 mg/l) have however been established for *E. coli* and *Saccharomyces cervisiae* (Loveless et al., 1954) and for *Nitrosomonas sp.* (Blum and Speece, 1991) in isolated culture. Walton et al. (1989) showed in a 6-day study on soil microorganisms that 1 mg/g acrylonitrile inhibited soil respiration at all time periods (50% for silt loam on day 4). Respiration had not returned to normal by day 6. Overall

the data suggest that acrylonitrile, while having an initial inhibitory effect on microorganisms, is of relatively low toxicity at concentrations below 100 mg/l.

3.2.1.2 Calculation of PNEC for aquatic organisms

The data set for acrylonitrile includes a wide range of information on short and long-term toxicity in fish, *Daphnia* and other aquatic invertebrates. The results of short-term tests are relatively consistent, both inter-species and intra-species, and while only a limited number of studies can be regarded as totally valid for risk assessment purposes, in that they contain adequate information regarding methodology and have been carried out according to internationally-accepted protocols, other information in the literature is consistent with the results of these studies and can be used as supporting information. It should be noted, however, that analysis of acrylonitrile was not carried out in the majority of studies and results given referred to nominal concentrations of acrylonitrile. Actual exposure levels in these studies were likely to have been somewhat lower than those cited. The majority of studies regarded as valid for risk assessment purposes were conducted using flow-through conditions and actual exposure levels are taken to be equivalent to nominal concentrations. For static and semi static exposure conditions results in the main have been expressed as mean measured concentrations over the exposure period.

Valid short term $L(E)C_{50}$'s for acrylonitrile have been reported for fish, Daphnia, algae and microorganisms. The most reliable result in fish is considered to be that in the saltwater species *Cyprinodon variegatus* in which a 96-hour LC_{50} of 8.6 mg/l was reported. The absolute validity of the lower figure of 5.2 mg/l for *Ctenophayngodon idellus* cannot be ascertained. The lowest 48-hour EC_{50} for Daphnia was 7.6 mg/l (nominal concentration, *Daphnia magna*). Two valid results are available for the 72-hour EC_{50} (biomass) in algae, 2.5 mg/l in *Scenedesmus subspicatus* and 1.63 mg/l (measured concentration at t_0) in the saltwater diatom *Skeletonema costatum*.

Long-term toxicity data are available in fish, Daphnia and algae. The fish early life stage toxicity test in *Pimephales promelas*, using flow-through conditions, provided a LOEC/NOEC of 0.34 mg/l, while a 30-day flow through test in mature fish of the same species provided a long-term LC_{50} of 2.6 mg/l. As discussed in Section 3.2.1.1.1 (chronic toxicity), if the value of 0.34 mg/l is taken as a LOEC, a NOEC may be derived by application of a safety factor of 2, giving a NOEC of 0.17 mg/l. The 14/21 day life cycle study in *Daphnia magna* provided a NOEC of 0.5 mg/l (nominal), while NOEC's for effects on biomass in algae are reported as 0.8 mg/l (*Scenedesmus subspicatus*) and 0.41 mg/l (*Skeletonema costatum*).

As indicated in Section 3.2.1.1.2 (chronic toxicity), although the validity of the prolonged toxicity study in *Daphnia* by Zhang et al. (1996b) can be questioned in respect of reaching the conclusion that there are three valid long-term NOEC's, the NOEC determined in the study was quite consistent with those established in the fish early life study and the algal toxicity study. For risk characterisation purposes, a PNEC has therefore been derived using an assessment factor of 10. Applying this factor to the NOEC derived from the fish early life stage toxicity test in *Pimephales promelas* gives a PNEC of 17 µg/l.

In respect of aquatic plant life, the data on *Ruppia maritima* would suggest a NOEC in the region of 1 ppm (1 mg/l) and an estimated EC_{50} of 10 mg/l. Application of an assessment factor of 1,000 to the estimated EC_{50} would give a PNEC of 10 µg/l. However this estimate must be regarded as

very speculative, as the data are not considered to be valid for risk assessment purposes (Section 3.2.1.1.3).

3.2.1.3 Calculation of PNEC for microorganisms in wastewater treatment plants

The results of microbial toxicity tests and biodegradation studies on acrylonitrile indicate a potential effect on microorganisms in wastewater treatment plants, at least on start-up. The reported EC_{50}'s range from 1- >1,800 mg/l. Results with acclimated microbial populations and in simulation tests have indicated no inhibitory effect of acrylonitrile at levels as high as 200 mg/l, and a conservative estimate of 50 mg/l for a NOEC in such populations has been assumed. Application of a factor of 10 to this NOEC derives a PNEC of 5 mg/l for microorganisms in acclimated wastewater treatment plants handling acrylonitrile on a continuous basis. A factor of 10 is considered justified given the relatively large body of data on microbial toxicity of acrylonitrile.

Little information is available on NOEC in microbial populations newly exposed to acrylonitrile, but several authors report no effects at 10 mg/l. The lowest EC_{50} for specific bacterial populations were in the range 1-10 mg/l. Reflecting also the results of the MITI ready/inherent biodegradation studies reported in Section 3.1.3.1.2 a conservative value of 1 mg/l has been assumed for NOEC in newly exposed populations and applying a factor of 10 to this derives a PNEC of 100 μg/l.

3.2.1.4 Calculation of PNEC for sediment

$PEC_{sediment}$ and $PNEC_{sediment}$ can be derived from the results for water, assuming a thermodynamic equilibrium with water and Foc = 0.1. A PNEC for sediment of 0.0126 mg/kg (based on PNEC (aquatic) of 17 μg/l) was derived using the above approach.

3.2.2 Terrestrial compartment

3.2.2.1 Toxicity test results

3.2.2.1.1 Plants

Little information is available on the effects of acrylonitrile on higher plants. Burg and Burg (1967) reported in abstract form that acrylonitrile had no effect on pea stem sections *in vitro* (the pea straight growth test) at a concentration of 9 mg/l. No other data are available.

3.2.2.1.2 Non-soil dwelling arthropods

The fumigant effect of acrylonitrile has been investigated in a number of insect species known to infest food products. An LC_{50} (exposure in air) of between 0.7-2.8 mg/l was reported by Bond and Buckland (1976) for three species, *Sitophilus granarius*, *Tribolium confusum*, *Tenebrioides mauritanicus*, following an 8-hour exposure period. In a study on 8 different insect species, *Sitophilus oryza*, *Zabrotes pectoralis*, *Stegobium paniceum*, *Acanthoscelides obtectus*,

Oryzaephilus surinamensis, *Rhyzopertha dominica*, Lindgren et al. (1954) reported the LC_{50} over a 2-hour period to range between 2-6.5 mg/l and over a 6-hour period the range was between 0.8-2.0 mg/l.

The study of Adu and Muthu (1985) on the effects of acrylonitrile on four life forms of *Callosobruchus chinensis* showed that the pupae were more resistant than eggs, larvae or adult forms, with a 24-hour LC_{50} of 1.26 mg/l, compared with approximately 0.1 mg/l for the other forms. In adults and larvae of *Tribolium castaneum* and larvae of *Trogoderma granarium* the LC_{50} was reported to be between 0.8-1.1 mg/l in air (Rayendran and Muthu, 1981). In the latter study, LC_{50} concentrations of acrylonitrile significantly inhibited the enzymes trehalase and phosphorylase in both *Tribolium castaneum* and *Trogoderma granarium.* Sublethal concentrations had similar effects on *Tribolium castaneum* adults but had less effect on these enzymes at the larval stage in either species, and had no effect on acetylcholinesterase.

3.2.2.2 Calculation of PNEC for terrestrial species

In the absence of toxicity data for soil-dwelling organisms, PNEC for soil was derived using the equilibrium partition method:

$$PNEC_{soil} = \frac{Ksoil_{water}}{RHO_{soil}} \cdot PNEC_{water} \cdot 1{,}000$$

where $Ksoil_{-water}$ = 0.268 m^3/m^3, RHO_{soil} = 1,700 kg/m^3, $PNEC_{water}$ = 17 µg/l

A PNEC for soil of 0.00268 mg/kg (based on PNEC (aquatic) of 17 µg/l) can be derived using the above approach. The reported NOEC for soil microorganisms is comparatively high, at approximately 100 mg/kg, providing a PNEC of 100 µg/kg (assessment factor 1,000). Data available for derivation of a PNEC for non-soil-dwelling terrestrial organisms are limited and of unknown quality. Using the LC_{50} data generated in a number of insect species and assuming a conservative figure of 0.5 mg/l for the LC_{50} gives a PNEC of 0.5 µg/l air.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment (including sediment)

3.3.1.1 Water

A PNEC of 17 µg/l has been derived for aquatic species exposed to acrylonitrile, as outlined in Section 3.2.1.2. This proposed PNEC is based on application of an assessment factor of 10 to the lowest NOEC established in the 3 long-term tests in fish, *Daphnia* and algae available for acrylonitrile. PECs for the aquatic environment for production of acrylonitrile and further processing to polymers, acrylamide and adiponitrile have been calculated using the approach outlined in the TGD, as laid out in Section 3.1.4.1, **Tables 3.11, 3.12, 3.13, 3.14** and **3.15**.

Appendices A.1 and A.2 summarise the PEC data for the aquatic compartment for all acrylonitrile producers and facilities processing and compare them with the PNEC of 17 µg/l to derive a risk characterisation ratio. The results show that only 1 of the 43 sites, site E, has a PEC:PNEC ratio above 1, the value for this site being 3.1. This site is located on a large estuary, does not have a wastewater treatment plant and the levels of acrylonitrile in effluent are relatively high compared with the majority of other sites, at 35 mg/l. Initially, no site-specific dilution factor was provided for the site and application of the default dilution factor of 10 gave a PEC:PNEC ratio in excess of 200. The company has since commissioned a modelling study of the estuary in question. They have provided data indicating that the average freshwater flow into the estuary is 217.5 m^3/s (1984 to 1993 data), and that an appropriate dilution factor due to freshwater inflow is 781.2, based on an effluent discharge of 1,000 m^3/hour. They further provided an estimated dilution factor due to saltwater cycling of 96,744. The company was requested to apply the model developed by Germany in the risk assessment of 4,4'-methylene dianiline for seawater dilution (TM III/98). Using a radius of 100 m from the end of the effluent discharge pipe, an average depth of outflow of 4.4 m (difference between average high and low water depths), and tidal water flow of 15.3 km/day (estimated from current meter measurements at the point of outflow) they derived a dilution factor of 701 from this model. This is the figure that has been used to derive the PEC:PNEC ratio of 3.1 given in Appendix A.2.

The PEC:PNEC ratios for 2 other sites, B and C, merit further discussion.

In the case of site B, aquatic emissions are initially to a small canal having a flow rate of 0.75 m^3/s, for which the Local Authority has accepted a dilution factor of 29, giving a PEC:PNEC ratio of 14.8. However, the canal joins a larger river within a distance of less than 100 metres. Flow rate in this larger river is 30 m^3/s, giving a dilution factor of 1,123, and if this dilution factor is applied in derivation of the PEC_{water}, rather than the dilution factor of 29, a PEC:PNEC ratio of 0.54 is derived. This is the scenario that has been used in the risk characterisation for site B, as shown in Appendix A.2. Additionally, information was provided by the operator of site B indicating that a wastewater treatment plant has been commissioned and will be in operation by 2000. It is concluded that this site presents a low risk to the aquatic environment, and that risk reduction measures are in train to further minimise risk to the environment.

Company C also discharges to the marine environment. A specific dilution factor of 38,681 was provided, which is significantly higher than any of the dilution factors applied in respect of other sites. Application of the default dilution factor of 10 gives a PEC:PNEC ratio of 1.34. However, for the purposes of the risk characterisation, the higher dilution factor of 38,681 has been

applied, on the basis that a detailed study of the estuarine environment where the site is located was provided which supported the specific dilution factor cited. In this case C_{water} is 0, and PEC_{water} is equivalent to PECregional (2.81 µg/l), giving a PEC:PNEC ratio of 0.17, as shown in Appendix A.2.

A number of other sites discharge to the sea or to an estuary. Many of these companies, e.g. 3, 8, JJ, KK, GGG, provided information indicating that effluents are immediately diluted by very large factors, without providing specific information on the degree of dilution. In each case however, specific data were provided on emissions to the environment that resulted in PEC:PNEC ratios below 1.0, despite application of the default dilution factor of 10 to these sites. The other sites discharging to the sea or estuaries, namely AA, FF, GG, AAA provided dilution factors of 1,400, 7,213, 573, 1,250, which were accepted as valid on the basis of the data provided. Finally, in relation to site 5, this company initially provided a site-specific dilution factor of >> 489 based on river flow into the estuary of location alone. The company has also carried out a detailed modelling exercise of the estuary, which suggested an annual average dilution of effluent of 16,800. Application of the model developed by Germany in the risk assessment of 4,4'-methylene dianiline for seawater dilution provided a dilution factor of 84 for salt-water dilution. This risk assessment concludes that the dilution factor of 500 used in Appendix A.2 represents a worst-case scenario, and indicates that this site is of low concern

The PECregional:PNEC ratio is below 1 (2.81 µg/l divided by 17 µg/l), indicating no concern.

Result

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

This conclusion applies to the aquatic compartment for production of acrylonitrile and further processing to fibres and other plastics, with the exception of processing to acrylic fibres at one site only.

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

This conclusion applies to the aquatic compartment for the production of acrylic fibres at one site only.

Acrylonitrile is toxic to aquatic organisms and is not readily biodegradable. Release into the aquatic environment could therefore possibly present some risk to aquatic species in the vicinity of plants producing or further processing acrylonitrile. Information from simulation tests and on the performance of wastewater biotreatment plants in a number of companies indicates that greater than 90% biodegradation is achieved in acclimated WWTPs. Of the 43 companies producing or processing acrylonitrile in the European Union, 33 have dedicated industrial WWTPs and a further 2 discharge to municipal WWTP. The PEC:PNEC ratio for all but 1 of these 43 companies is below 1.0, indicating that the risk is controlled by the reduction measures in place. It should be noted, however, that this conclusion applies only at a particular point in time to 42 out of the total of 43 European sites existing at that time which provided aquatic release data relating to the period 1994-1996, and cannot be extrapolated generally for the aquatic environment. The specific risk reduction measures (e.g. wastewater treatment) or particular characteristics of the assessed sites (e.g. high dilution factors due to effluent emissions into very large rivers or estuaries) cannot be extrapolated to sites not covered by this risk

assessment, for example new sites starting up after the data for this assessment were gathered, or sites located outside the European Union.

In the case of one site, site E, located on a large marine estuary, application of a specific dilution factor of 701 derived from modelling of the dilution due to seawater results in a PEC:PNEC ratio of 3.1. It is concluded that there are concerns for effects on the local aquatic environmental sphere as a consequence of exposure arising from production of acrylic fibres at this site.

3.3.1.2 Microorganisms in wastewater treatment plants

In relation to the risk assessment for microorganisms in wastewater treatment plants, a PNEC of 5 mg/l is estimated for sites with acclimated WWTPs, while for the *ab initio* situation a PNEC of 100 µg/l is assumed (Section 3.2.1.3). Section 3.1.4.1.3 identifies likely PEC's for WWTP. For sites with WWTP which produced measured data for effluent, concentrations ranged from 0 to 5.8 mg/l. Appendices A.1 and A.2 show that the PEC:PNEC ratios for sites having WWTP are all below 1.0. It can be concluded that there is little or no risk for microorganisms in industrial WWTP, although some risk may exist in non-acclimated WWTP. In practice the companies providing data for this report have indicated that, in the main, their WWTP operate continually and are fully acclimated to acrylonitrile waste.

Result

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

3.3.1.3 Sediment

A PNEC for sediment of 0.0126 mg/kg (based on PNEC (aquatic) of 17 µg/l) was derived using the equilibrium partition method as outlined in Section 3.2.1.4. PECs of between 0.002-0.044 mg/kg for sediment have been calculated (Appendices A.1 and A.2). Reflecting the parallel between the PEC:PNEC ratio for aquatic organisms and for sediment-dwelling organisms, the results of risk characterisation for sediment are similar to that for water. The PEC:PNEC ratio for sediment is above 1 for site E (3.46), indicating that there could be some risk to sediment-dwelling organisms in the vicinity of this plant.

Result

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

This conclusion applies for production of acrylonitrile and further processing to fibres and other plastics, with the exception of processing to acrylic fibres at one site only.

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

This conclusion applies for the production of acrylic fibres at one site only. The justification for this conclusion is as for water above. Site E has a PEC:PNEC ratio for sediment of 3.46, and it is

concluded that there are concerns for effects on the local aquatic environmental sphere as a consequence of exposure arising from production of acrylic fibres at this site.

3.3.2 Atmosphere

Although the atmospheric compartment is the major compartment of distribution of acrylonitrile, there is rapid photodegradation ($t_{1/2}$ approximately 5 days). All 43 production and further processing companies provided data on atmospheric emissions. These were generally low, being reduced by scrubbing of gaseous and volatile wastes before discharge to the atmosphere. As shown in **Tables 3.17, 3.18, 3.19, 3.20** and **3.21** the calculated $PEClocal_{air}$ for sites providing specific emission data lay between 0 and 0.240 mg/m^3 or 1-240 µg/l. Derivation of PEC:PNEC ratios for the atmospheric environment provided values of below 1.0 for all sites. Results of monitoring at the perimeter of acrylonitrile plants (**Table 3.22**) showed that levels were generally below 1 µg/m^3.

Little information is available on the effects of atmospheric acrylonitrile, other than a very limited study, reported in abstract form only, on its effects on pea stem sections *in vitro*, which indicated no effect on growth at a concentration of 9 mg/l. An investigation of the fumigant effect of acrylonitrile on a number of insect species known to infest food (Section 3.2.2.1.2) showed an LC_{50} of 0.5 mg/l in the most sensitive species, and therefore a PNEC of 0.5 µg/l. The quality of these studies is questionable, however, and taking into account also the measured levels in the vicinity of plants it was considered that there is little risk associated with exposure of ecosystems to atmospheric environmental levels of acrylonitrile. In addition, information provided regarding a catastrophic event which happened outside the EU some years ago and during which the contents of a large storage tank containing acrylonitrile were released very rapidly, showed damage to vegetation observed within a 100 m zone of the spill. This damage had disappeared within 3-4 months. No damage to vegetation was observed greater than 100 m from the spill where acrylonitrile concentrations of up to 20 ppm were measured, a concentration far greater than the expected fence-line value (0.46 ppb).

<u>Result</u>

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

This conclusion applies for atmosphere for the production and further processing of acrylonitrile. All 43 production and further processing companies provided data on atmospheric emissions. These showed that emissions were generally low, being reduced by scrubbing of gaseous and volatile wastes before discharge to the atmosphere. Derivation of PEC:PNEC ratios for the atmospheric environment provided values of below 1.0 for all sites. Acrylonitrile is also rapidly photodegraded.

3.3.3 Terrestrial compartment

A PNEC for soil of 0.00268 mg/kg (based on PNEC (aquatic) of 17 µg/l) can be derived. Calculation of $PEClocal_{soil}$ in accordance with Section 2.3.8.5 of the TGD (see Section 3.1.6.1 of this report) provided a figure of 0.3 mg/kg for PEC_{local} in soil pore water (agricultural soil) for production sites. This would result in a PEC:PNEC ratio of over 100 and processing sites would also exceed 1. It should be noted, however, that this is a worst-case exposure scenario and

appears unrealistic, given the information from industry that little industrial sludge from acrylonitrile production and processing facilities is spread on land in Europe. The majority of companies providing information on this aspect indicated that contaminated sludge is incinerated together with other wastes. Risk characterisation for the terrestrial compartment has therefore excluded the possibility of sludge application, and has been based on the values obtained from EUSES for $PECregional_{soil.}$

A value of $1.36 \cdot 10^{-4}$ mg/l has been derived from EUSES for $PECregional_{soil}$ (pore water, agricultural soil), indicating a PEC:PNEC ratio of 0.008. While direct comparison of the PNEC derived from the equilibrium partition method with the $PECregional_{soil}$ (pore water, agricultural soil) may not be strictly valid, the result indicates that there is little risk for the soil compartment. This conclusion is however based on the assumption that sludge from the WWTP is not applied to soil, an assumption which is supported for the European Union, based on the data supplied. It cannot be extrapolated to sites not covered by this risk assessment.

The estimate of $PECregional_{soil}$ reflects primarily point source emissions from production or further processing, and diffuse emissions from car exhausts etc. have not been taken into account in the EUSES input. However, even with a significant contribution to $PECregional_{soil}$ from such sources, the PEC:PNEC ratio will still be well below 1, again indicating little concern for this compartment. The PNEC of 9 µg/l derived from the pea straight growth test (Section 3.2.2) and the PNEC of 100 µg/kg derived for soil microorganisms would support this conclusion.

Result

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

This conclusion applies for soil for production and all uses of acrylonitrile.

3.3.4 Secondary poisoning

Exposure of species relevant for the food chain to low levels of acrylonitrile in the environment is theoretically possible. Section 3.1.3.2.4 indicates that acrylonitrile is unlikely to bioaccumulate in exposed biota, and this is supported by the low octanol:water partition coefficient. EUSES calculates a BCF for fish of 1.41, and an experimentally derived BCF of 48 in fish was probably in part attributable to binding of acrylonitrile to tissue macromolecules, as also demonstrated in rodents, rather than to true bioaccumulation. Toxicity studies in mammalian species (Section 4) provide little evidence of cumulative toxicity in a range of species.

Concentrations of acrylonitrile in biota are therefore expected to be very low, and there are no reports in the literature of detectable levels of acrylonitrile in aquatic biota. Bioaccumulation or biomagnification is not therefore anticipated. Values for acrylonitrile levels in exposed biota and for daily human intake from the environment have been derived via EUSES, and indicate a low level of concern. It is concluded therefore that the potential for secondary poisoning is very small.

Result

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

4 HUMAN HEALTH

4.1 HUMAN EXPOSURE (TOXICITY)

4.1.1 Exposure Assessment

4.1.1.1 General discussion

The most important routes of exposure to acrylonitrile are inhalation and dermal, for both production and processing to polymers and other products. Oral exposure during production should only occur by accident or poor work practice. While acrylonitrile is a liquid, the use of high temperatures during various stages of synthesis/reaction and the high volatility of acrylonitrile indicates that the major exposure route of concern to workers is inhalation. However all of the initial production and processing of acrylonitrile involves closed systems.

Acrylonitrile is released to air and wastewater during production and processing. The main human exposure is via air for occupationally exposed workers and to a minimal extent the population living close to production and processing plants. Dermal exposure to workers must be considered should direct handling/contact occur e.g. during processing or at fibre production. As acrylonitrile is produced and almost totally processed in a closed system, the main concern relating to exposure arises should ignition or explosion occur by accidental failure or breaches in the closed system.

The major potential source for indirect or consumer exposure is via the use or wearing of materials, textiles, furnishings etc. which may contain a very small percentage of unreacted acrylonitrile monomer, or via food which is packaged in containers made from acrylonitrile plastics, e.g. margarine tubs, fruit juice containers, vegetable oil bottles etc. Exposure via the air or in drinking water is also theoretically possible (see Section 4.1.1.4).

Subsequent exposure via the food chain is negligibly low as acrylonitrile will be extensively degraded following a short acclimation period if emitted to WWTP from industrial sources, either primary production or secondary processing plants, although available data indicate that it does not meet the criteria for ready biodegradability. Exposure to increasing concentrations of acrylonitrile over a period of several days to several weeks results in enhanced biodegradation of acrylonitrile, and 100% degradation of concentrations greater than 100 mg/l have been reported using acclimated microbial populations. Also, the measured log octanol/water partition coefficient is smaller than or equal to 0.3 and as bioaccumulation is therefore unlikely, the possibility of secondary poisoning is low.

4.1.1.2 Occupational exposure

The occupational exposure limit (OEL) for acrylonitrile in a number of EU countries and also in the US and Australia (1990) is 4.5 mg/m^3 or 2 ppm, and includes a notation referring to skin and carcinogenic effects/potential. This “skin” notation refers to the potential contribution to the overall exposure by the dermal route, by contact with acrylonitrile. Set out below is a list of these occupational exposure limits:

Germany (TRK-8hr, 1989)	3 ppm (7 mg/m^3)
Austria	2 ppm (4.5 mg/m^3)
France (VME-8hr)	2 ppm (4.5 mg/m^3)
France (VLE-15 min)	5 ppm (11.25 mg/m^3)
Netherlands	2 ppm (4.5 mg/m^3)
Spain	2 ppm (4.5 mg/m^3)
UK (MEL)	2 ppm (4.5 mg/m^3)
Ireland (OEL)	2 ppm (4.5 mg/^{3})
Sweden (OEL-8hr)	2 ppm (4.5 mg/m^3)
Sweden (OEL-15 min)	6 ppm (13.5 mg/m^3)
Hungary	0.23 ppm (0.5 mg/m^3)
USA (TLV)	2 ppm (4.5 mg/m^3)
Australia	2 ppm (4.5 mg/m^3)
Japan	20.36 ppm (45 mg/m^3)
USSR (STEL-15 min.,1995)	0.23 ppm (0.5 mg/m^3)

The Occupational Safety and Health Administration (OSHA) ceiling limit is 10 ppm in a 15-minute time period. OSHA further states that an employer shall assure that no employee is exposed to skin or eye contact with liquid acrylonitrile. The US National Institute for Occupational Safety and Health (NIOSH) established a OEL-TWA of 1 ppm with skin notation, a 15-minute ceiling of 10 ppm and placed the Immediately Dangerous to Life and Health (IDLH) value for acrylonitrile exposure at 85 ppm (30 min).

Currently acrylonitrile is classified for its carcinogenic effects as follows:

IARC: Group 2B, (Note: IARC in February, 1998, agreed to revise down their categorisation of acrylonitrile to Group 2B on the basis of recent epidemiological evidence).

MAK: Group 2A, carcinogenic in animal experimentation only.

NIOSH: Carcinogen, with no further categorisation.

ACGIH/TLV: A2, suspected human carcinogen.
(Documentation of the Threshold Limit Values and Biological Exposure Indices, 1996, ACGIH, US).

NOHSC: Category 2.
(Exposure Standards for Atmospheric Contaminants in the Occupational Environment. Canberra ACT, Australian Government Publishing Service).

EU: Category 2 carcinogen, may cause cancer.

4.1.1.2.1 Occupational exposure during acrylonitrile production and polymer production and processing

The processes used in acrylonitrile production and further processing have potential for exposure of workers and also the wider environment (the latter via wastes). These processes are therefore described in some detail below in relation to the potential for workplace exposure. Based on correspondence received from industry and information accumulated by the lead contact company

for the purposes of this risk assessment report, reflecting the hazard profile of acrylonitrile and the possibility of exposure to acrylonitrile during production/processing, the following actions are taken (personal correspondence, February, 1998):

- use of strictly closed systems;
- enclosing of special parts of plant equipment;
- use of the highest technically available safety valves and glands;
- use of rotary mechanical double acting seals with fluid package;
- use of technical exhaust systems (engineering controls including local exhaust ventilation);
- use of vent gas systems with connection to incineration;
- waste gas streams undergo cleaning/washing procedures;
- raw polymer is transported e.g. in closed systems under reduced pressure, so that the possibility of contact with the product cannot occur;
- before any maintenance work the systems are cleaned by rinsing with steam, air, nitrogen, etc. and sufficient latency periods are observed.

Acrylonitrile production

Acrylonitrile is produced by conversion of propylene, ammonia and air in the gaseous phase on appropriate fluidized bed catalysts (SOHIO process). With an almost complete propylene conversion the selectivity of the reaction to form acrylonitrile amounts to about 70%. Apart from acetonitrile and hydrogen cyanide (HCN) as by-products, low amounts of polymers, acetone, propionitrile and acrolein are produced. Operating conditions vary from 20-200 kPa gauge pressures (2.9-20 psig) and 400-500°C temperatures. Owing to the high conversion of propylene to acrylonitrile, a once-through operation with a residence time of <10s under slight excess pressure (1.3 to 2.5 bar) is employed. The reaction heat is utilised for the generation of high-pressure steam. After cooling down the reaction gases by indirect heat exchange, the temperature is further lowered by quenching with water; at the same time polymers and the discharged catalyst dust are scrubbed.

In the next step the residual ammonia is removed by saturation crystallisation with sulphuric acid. In a water-operated absorber, acrylonitrile and by-products are washed out and sent to crude nitrile distillation where crude acrylonitrile composed of about 80% acrylonitrile, 10% HCN, 5% water and other by-products is recovered overhead. The likewise formed acetonitrile can be recovered from a sidestream of the crude distillation or fed to the absorber waste gas. The separation of HCN as well as the water removal and pure distillation occur in the three downstream distillation columns. The whole processing unit of an acrylonitrile plant is operated under atmospheric pressure. Water removal and pure distillation are carried out under reduced pressure for purposes of product care. During start-up and shut-down of the plant, any reaction gas which does not meet specifications must be flared for safety reasons.

During the production of acrylonitrile the reactions take place in closed systems, so there are no defined emissions and the risk of exposure to acrylonitrile by workers will not occur. The waste gas produced at the separation of the reaction products consists mainly of nitrogen, carbon dioxide, carbon monoxide, propane, propylene, acetonitrile, HCN and low amounts of acrylonitrile. These wastes are typically sent to a combustion plant. The thermal combustion, which requires a combustion temperature of at least 800°C, permits an almost complete conversion of all combustible carbon compounds. A supporting fire is necessary because of the low calorific value of the waste gas of the acrylonitrile plant. During start-up and shut-down of an acrylonitrile plant, additional waste gases are produced. Prior to attaining the oxygen content, which is necessary for safety reasons, the waste gas has to be flared for short periods of time.

The waste gas from distillation (purification of the reaction products) and the tanks is sent through scrubbers.

Acrylonitrile is usually stabilised at normal temperatures and normal pressure and stored in nitrogen-superimposed tanks. For long-distance transportation acrylonitrile is preferentially carried in tank wagons or barges. Acrylonitrile-containing waste gases arise during filling and emptying operations and possibly during the purging of the tanks. The transfer of acrylonitrile from stationary to mobile tanks and vice versa is carried out via articulated arms or pressure-resistant hose-pipes with gas displacement devices or in combination with waste gas purification, where scrubbers are used for that purpose.

Production and processing of acrylonitrile polymers to fibres

The process is fully continuous, with precipitation polymerisation occurring in a single stage. Polymerisation is an exothermic reaction process and is preferentially carried out without pressure in a temperature range of about 40 to 70°C. In special cases it is also possible to work under pressure. Normal conversion levels of the polymerisation reaction under process conditions amount to about 80-90%. The average chain length of the macromolecules is adjusted through catalyst concentration, the reaction time, the temperature and possibly polymerisation modifiers.

In order to obtain the desired fibre properties, it is necessary to copolymerise comonomers. Such comonomers are for instance acrylic acid methyl ester, vinyl acetate, methacrylic acid methyl ester and sulphonate group-containing olefins, providing the dyeing affinity vis-à-vis cationic dyestuffs. Halogen-containing monomers are used in special cases to reduce flammability of the fibres.

The quality of the polymer types as starting products for textile fibres has to meet certain requirements e.g. the polymer has to dissolve uniformly in the relevant solvent. The formation of gel particles is for instance disadvantageous with regard to spinning. The spinning solution must not have any viscosity fluctuations, i.e. a constant molecular mass of the polymer is necessary. Furthermore, a constant whiteness and a constant dye affinity are essential.

Polymerisation

Polymerisation is usually carried out as a continuously operating precipitation with subsequent filtration and drying, although polymer may also be produced by solution polymerisation. Precipitation polymerisation is carried out in a stirred tank reactor into which the liquid acrylonitrile, comonomers, catalysts and auxiliary products as well as water are continuously fed prior to polymerisation It may be necessary to destabilise the acrylonitrile by distillation after stabilisation for storage purposes with polymerisation inhibitors. The polymer suspension is continuously withdrawn from the reactor. It has a solids content of about 15 to 35%. The polymerisation heat is removed by jacket cooling.

The output from this stage of the fibre manufacture is a very viscous solution (dope). In plant observations (confirmed by industry), even following accidents involving spillage of dope, air concentrations of acrylonitrile near the spillage have been low (taken within the context of permitted 8-hour TWA exposures). The reason for these low levels relates to the low monomer content of the dope, its high viscosity and the “skinning” effect where the surface coagulates.

After polymerisation the suspension is collected in a stirred tank reactor. If necessary, the polymerisation may be stopped by appropriate agents. Depending on the process, the suspension can be subject to intensive degasification whereby the quality requirements to be met by the

polymer have to be taken into account. In the subsequent continuously operated washing filter the solids content is separated, washed and sent to a dryer with a moisture of about 45 to 70%. Depending on the process, filtration can be single or double-staged. The filtrate from the washing filter has a residual monomer content of about 1 to 3% and is processed in an acrylonitrile recovery plant. Appropriate dryers are for instance pneumatic conveyor dryers or belt dryers. The polyacrylonitrile powder obtained is subsequently conveyed to intermediate storage in silos by screw conveyor or pneumatically with inert gas as the conveying agent.

The waste gases from vent pipes of the stirred-tank reactor and the suspension collector as well as the waste gases of the washing filter and intensive degassification, are sent for waste gas scrubbing or adsorption. The main part of the acrylonitrile trapped in the waste gases can be recovered as aqueous solution from acrylonitrile-rich partial streams by previous cooling. The acrylonitrile-containing waste air of the dryers is emitted. The acrylonitrile content in these large volumes of air is relatively low. The reactors are opened only in case of repair and breakdown, so that emissions during these operations are negligible.

The acrylonitrile/water mixtures contain the residual acrylonitrile, which was not converted during polymerisation. The effluent of the purification and cooling plants have different acrylonitrile contents varying with the process. They are either recycled into the process or sent to a stripping column together with the filtrate of the washing filter. The acrylonitrile recovered here is also returned to the polymerisation process. The vent pipes of the stripping columns are connected to the central disposal system.

Spinning of polymers to fibres

In the case of fibre production, a homogeneous solution is produced from the polymer and a solvent, e.g. dimethylformamide (DMF) or dimethylacetamide (DMAC). This solution is spun in hot inert gas in the case of dry spinning or in coagulation baths of DMF-water or DMAC-water in the case of wet spinning. Aqueous sodium thiocyanate (NaSCN) may also be used as a spinning solvent. The acrylonitrile polymers still contain residual amounts of acrylonitrile monomer, dependent on polymer type. They are mainly washed out (wet spinning) or released (dry spinning) in the spinning machines.

The viscous solution is extruded through multi-holed jets and thin streams of dope coagulate to form gelatinous filaments. Depending on the technology used, extrusion is into long baths of a suitable aqueous liquid or into vertical towers where the filaments are formed in air. The filaments are collected together and taken as “tows” through a succession of stages including partial stretch in a preheat bath, further stretching in a steam stretch tube, washing out residual impurities.

The enclosed spinning machines are vented if using DMF as a solvent, resulting in large volumes of air with a relatively low acrylonitrile content. The DMF-containing waste gases, which may also contain acrylonitrile vapour, are liberated from the DMF by scrubbers and emitted. In the case of spinning operations using DMAC spinning solutions in coagulation baths, the bath vapours contain low amounts of both solvents and acrylonitrile which are extracted and emitted.

Production of ABS plastics

ABS plastic consists of a homogeneous physical mixture of a butadiene-acrylonitrile-styrene graft polymer and a styrene-acrylonitrile copolymer. ABS is placed on the market as plastic granulate which is ready for processing. The technical production of ABS may be carried out via different processes e.g. pure emulsion polymerisation or combined solution/emulsion polymerisation.

Production of ABS plastics by emulsion polymerisation

This process consists of four steps:

1. Butadiene Polymerisation

Polybutadiene is obtained on a graft basis by emulsion polymerisation. It is produced as latex with a defined particle size distribution.

2. Graft polymerisation

In the presence of the polybutadiene latex, a styrene acrylonitrile monomer mixture is graft-polymerised by means of anionic emulsifiers. Production takes place without pressure in stirred tank reactors. Water, polybutadiene latex and the initiator are introduced and heated to temperatures in the range of 50 to 80°C. The emulsifier, acrylonitrile and styrene are added to the aqueous phase while the polymerisation temperature is controlled with a heating/cooling cycle. At the end of the feeding, the mixture is polymerised at a higher temperature.

After polymerisation the latex is stored until further processing. The intermediate storage without pressure is to compensate for the different production rhythms of polymerisation and processing. At the internal wall of the reactors polymer deposits develop which necessitate cleaning operations from time to time. Since polymer deposits can also develop during intermediate storage, these storage tanks must likewise be cleaned at certain intervals.

During the polymerisation, part of the acrylonitrile-containing gaseous phase is continuously withdrawn (if necessary, together with acrylonitrile-containing air) from the stirred-tank reactors which were emptied for cleaning purposes. The stored latex still contains low amounts of unreacted volatile acrylonitrile, styrene and butadiene. The acrylonitrile withdrawn during polymerisation and during reactor cleaning is passed to a waste gas combustion plant. The acrylonitrile, styrene and butadiene-containing displacement air of stored latex is connected to the same disposal system. Here the acrylonitrile is combusted with greater than 99% efficiency.

3. Resin polymerisation

The resin component is produced via emulsion polymerisation of styrene or α-methyl styrene and acrylonitrile. Resin polymerisation is carried out in stirred-tank reactors in a temperature range of 50 to 80°C without pressure, while water, emulsifier and auxiliary products are fed into the reactor and the monomer mixture is added under stirring. The polymerisation heat is removed via a heating/cooling cycle. The resin polymer latex is pumped off into storage tanks.

Polymer deposits at the internal wall which, after prolonged operating times, impair heat removal necessitate reactor cleaning from time to time. Prior to processing, the resin polymer latex is kept at intermediate storage in the same way as the graft latex.

Emissions of acrylonitrile, butadiene and styrene or α-methyl styrene occur at the vent pipes of the reactors and intermediate storage tanks. The acrylonitrile-containing waste gases are sucked

off prior to the opening of the stirred-tank reactors for repair or cleaning purposes. They are sent to the vent pipes also. The polymer generated during wet reactor cleaning releases volatile acrylonitrile practically completely to the aqueous phase. The wastes from the vent pipes of the reactors and from the intermediate storage tanks are sent to a waste gas combustion plant via a central waste gas pipe.

4. ABS Powder production

During the first processing stage the graft polymer latex is mixed with the resin polymer latex in predetermined proportions, and stabilisers are added. In other stages the mixed latex is coagulated, the solids are separated from water and the wet ABS is dried and granulated to powder at 100 to 180°C.

ACN-containing waste gases arise when the mixing tanks are filled, in the precipitation and washing processes as well as in the drying process. Slightly polluted waste air is produced during powder conveying, compounding/granulating and granulate conveying. While the tank vent pipes and the suction pipes of the washing stage permit an integration into the waste air combustion system due to comparatively high acrylonitrile loads, the dryer waste air is emitted due to the high volume flows involved.

Production of ABS polymers

The production of ABS polymers by combined solution/emulsion polymerisation involves three steps as follows:

1. SAN polymer production

The starting materials acrylonitrile and styrene or α-methyl styrene are continuously fed to the reactor with a circulation solvent (e.g. ethyl benzene). The reaction is conducted in a temperature range of between 90 and 170°C at pressures of up to 6 bars. The reaction heat can be removed from the pressurised reaction mixture via evaporative coolers. The polymer solution is continuously discharged from the reactor. In the subsequent solvent recovery plant, the solvent and the non-converted monomers are withdrawn by heat increase and pressure reduction. After condensation they are recycled into the polymerisation process. The degassed polymer melt is sent to the mixing unit. Acrylonitrile-containing inert gas streams escape from the solvent recovery and are sent for combustion.

2. Latex production

Latex production takes place in two stages. In the first stage, a base dispersion is polymerised in the reactor without involvement of acrylonitrile. In the second polymerisation stage, acrylonitrile and styrene are fed and grafted onto the base dispersion in another reactor. The polymerisation takes place at this stage at temperatures of between 50 and 80°C without pressure. The resulting aqueous dispersion with solids contents of between 30 and 50% (graft dispersion) is sent to an intermediate storage tank for further processing. The reactors must be cleaned at certain intervals.

During polymerisation the monomer-laden inert gases are removed. Suction systems at the openings for reactor filling, sampling and cleaning convey among others acrylonitrile-containing waste gases, which are emitted. In order to avoid emissions as a result of non-converted monomers (butadiene/styrene with a volume content of 0.05 to 5.0% acrylonitrile), the reactors and intermediate storage tanks are connected by gas displacement ducts to shift the displacement of air. Any excessive displacement air is sent for combustion.

3. Latex processing

Incorporation into the SAN melt.

The aqueous dispersion is sent via an intermediate storage tank to the precipitation and dewatering plant. The resulting product together with the SAN melt is continuously fed into the mixing unit and homogenised. The crude ABS is granulated. Near the discharge device of the mixing unit and during granulation, acrylonitrile-containing waste gases arise and are emitted. The acrylonitrile-containing waste gases from the precipitation and dewatering plants and from the mixing unit are sent for combustion.

Production of NB rubber by emulsion polymerisation of acrylonitrile

Nitrile butadiene (NB) rubber is a copolymer of acrylonitrile and butadiene. The exothermal reaction of the monomers is carried out in aqueous emulsifier solutions by means of initiators. There is no depolymerisation by heat exposure under the production conditions and the common technical processing conditions. When producing NB rubber in a continuous polymerisation process 5 steps are involved as follows:

1. Latex polymerisation

The continuous production of NBR precursor latex is done at temperatures below 35°C and at a pressure of up to 10 bars in reactors installed in cascades. All chemicals required for the reaction e.g. monomers, activators, emulsifier solutions and modifiers are fed into the cascades via dosing devices. In order to preserve certain polymer properties, the polymerisation is stopped at a conversion of about 70%. The reaction heat is removed in evaporators with liquid ammonia. In case of a breakdown of the cooling system, the temperature and the pressure rise very slowly, so that the release of monomers through safety valves or bursting discs can be safely prevented by chemical emergency shutdown. At the internal walls of the reactors polymer deposits gradually develop and necessitate a cleaning of the tank from time to time. No waste gas arises during polymerisation since it takes place in a closed system. In case of type changes and prior to repair and cleaning, the polymerisation tanks are emptied with pressurised nitrogen; the monomer-containing inert gas is subsequently expanded in the monomer separation unit. The tanks are filled with water thus the acrylonitrile residues are largely removed from the tanks; the displaced gas is also sent to the separation unit.

2. Latex degassing

The monomer-containing latex is continuously withdrawn from the polymerisation tank via pressuriser valves; it is introduced into the degassing tanks, which operate at reduced pressure, and are degassed with steam. A mixture of acrylonitrile, butadiene and steam is withdrawn during degassing and sent for recovery to the separation unit. The steam displaced from the buffer tank is also sent to the separation unit. The displacement air of the latex tanks is sent to a combustion unit.

3. Monomer recovery

In the separation unit, first acrylonitrile and, after compression, liquid butadiene are separated in several steps from the volatile degassing mixture. The aqueous acrylonitrile is concentrated by distillation, butadiene is distilled. Both monomers are returned to polymerisation. The intermediate storage tanks required for the aqueous acrylonitrile release their displacement air into the separation unit. The buffer containers for recovered acrylonitrile are connected to a scrubber in which the

displacement air is purified. The scrubbing water is circulated after stripping of the acrylonitrile. The waste gas from this scrubbing has a low-acrylonitrile content and is emitted.

4. Intermediate storage of latex

The intermediate storage with its selective latex mixing provides special product qualities or general homogenising; it guarantees continuous supplies of material to the precipitation units. Despite degassing, the latex contains residual monomers which might escape during storage, which lasts from one to three days. The displacement air and the released monomer vapours are sent to a thermal combustion unit.

5. Solid rubber processing

The processing steps are precipitation, washing, screw-dewatering, drying and assembly. The coagulation of the NBR latex with electrolyte solutions takes place in a closed system. Polymerisation auxiliaries and precipitation agents have to be washed from the precipitated rubber. The main water content is pressed off in screws to a large extent, and the rubber is then mechanically reduced to pellet size and dried in hot air streams to a residual moisture of less than 0.5%. The NB rubber is then pressed into bales or assembled as pellets or powder.

No acrylonitrile-containing waste gas will escape during coagulation, with the exception of fugitive emissions. During drying the residual volatile acrylonitrile is stripped. This waste air is emitted because of the large volumes of air involved. During the washing of the coagulate the acrylonitrile-containing waste gases are sucked off the headspace of the enclosed washing tanks and sent to a combustion unit. The efficiency of acrylonitrile combustion at temperatures of more than 800°C amounts to greater than 99%. The acrylonitrile-laden precipitation and washing waters are discharged into the sewage plant.

Dispersion production

Dispersions are mainly produced discontinuously by emulsion polymerisation of acrylonitrile. The production comprises the steps of premixing, polymerising and degassing. Furthermore, these steps include one or several filtration stages as well as storing, mixing and filling of the finished dispersion. The monomer mixtures and the aqueous emulsifier solution as well as auxiliary product and initiator solutions may be prepared separately in mixing tanks and either be fed into the reactor at once (batch process) or with continuous supply over several hours (feed process). The process of emulsion feeding is also applied, whereby monomers and the emulsifier solution are premixed in the same receiving tank. The polymerisation is carried out in stirred-tank reactors at a temperature range between 20 and 130°C. Depending on the components, it may be effected either without pressure or under pressure (up to 15 bars). The polymerisation heat is reduced by reactor cooling or, if necessary, by additional evaporative cooling.

The reaction is stopped at conversion levels of 80 to 99%. It results in aqueous polymer dispersions (lattices with solid contents of between 35 and 70%). The dispersion is possibly prefiltered and conveyed to an intermediate storage tank where it can be liberated from non-converted acrylonitrile and comonomers by stripping in a vacuum either directly or in a downstream unit. The filtered dispersion, which is also conditioned, if applicable, is finally stored in storage tanks before being filled into small containers or road tankers.

The feeding of the monomer-receiving vessels involves acrylonitrile-laden waste air. During polymerisation, acrylonitrile-containing inert gas may be withdrawn from the evaporative cooler. At the end of the polymerisation the autoclaves are emptied into the intermediate storage tanks and expanded if necessary. This involves the formation of acrylonitrile-containing inert gas or

displacement air, respectively. The degassing process gives rise to a monomer-containing vapour mixture which is liberated to a large extent from acrylonitrile by condensation of the steam portion. The aqueous phase may be discharged into a sewage plant. The tank air is sucked off during wet cleaning of the polymerisation and intermediate storage tanks as well as in case of open filtration and degassing devices. Large volumes of air are emitted with a very low residual acrylonitrile content. The rinsing water is sent to the sewage plant, the waste gas flows from the monomer-receiving vessel, reactors, intermediate storage tanks, condensers of the degassing unit and the latex storage tanks are sent to thermal combustion or scrubbing. There is 99% elimination of acrylonitrile in the waste gas, during combustion.

Conditions for measurement for emissions of acrylonitrile

Measurements should be taken when operations and workload of the plants are normal. In the case of discontinuous or intermittent emissions, the measurement period is fixed in such a way as to ensure the occurrence of emissions. If the measurement values reach a level which is atypical of normal plant operation, or unexplained losses are indicated as a result of calculation acrylonitrile balance, the underlying causes must be detected. If defects in the emitting plant or in the measurement system are found to be the cause, they must be eliminated according to the state of the art, and the measurements must be repeated.

At the planning stage of plant, sampling apertures have to be provided in such a way that a sampling probe may be used and that for instance the flow velocity can be measured with a Pilot tube. In general, lockable apertures with a diameter of 20 to 50 mm will be sufficient, preferably on the pressure side. The measurement point should be arranged as close as possible to the point where the waste gas is released into the atmosphere, in as much as this is possible with an acceptable technical expenditure. Measurement points and the related facilities must be accessible at any time without risk.

Sampling

Half an hour is the appropriate time basis for sampling in plants with continuous emissions. The results are considered equivalent to whether the half hour mean value was obtained merely with the method of integrating measurement (accumulative sampling) or whether it was obtained by application of an adequate evaluation method. When the sampling period is shorter than the time basis due to the applied measurement method, a sufficient number of samples must be taken during that period. Five samples are sufficient for continuous emissions; in the case of short intermittent emissions, the number of instantaneous samples which must be taken at regular intervals has to be increased according to the circumstances.

(SOURCES: (Guideline 2447: Emission Control: Acrylonitrile, VDI, June, 1996), European Industry Correspondence, and American Industry).

Analytical methods

1. OSHA Method 37 (OSHA, 1990)

This method is developed for a target concentration of 2 ppm (4.5 mg/m^3). Air is sampled by a personal sampling pump and drawn through a 7 cm long coconut shell charcoal absorbent tube containing two sections of charcoal (a 100 mg and a 50 mg section). Both sections are placed in separate vials, and 1 ml of desorbing solvent, acetone containing 0.1 µl/ml propionitrile, is added to each vial. Samples are analysed by gas chromatography using a nitrogen/phosphorous detector. The recommended air volume for this method is 20 l at a sampling rate of 0.2 l/min.

The detection limit of the analytical procedure is reported to be 0.36 ng per injection, and of the overall procedure to be 0.51 µg per sample (0.01 ppm (0.026 mg/m^3)) at the recommended sampling conditions. Pooled coefficients of variation obtained from replicate determinations of analytical standards at 0.5, 1 and 2 times the target concentration (2 ppm) was 0.0051. The desorption efficiencies at these concentrations were constant and averaged 89%. High relative humidities (above 80%) and high temperatures (above 25°C) may cause a decrease in the capacity of charcoal to adsorb and retain acrylonitrile.

2. NIOSH Method 1604 (Eller, 1994)

A known volume of air (between 4 and 20 l) is drawn through a 7 cm long glass tube, containing two sections of activated (600°C) coconut shell charcoal separated by a 2 mm urethane foam plug. Front and back sorbent sections are placed in separate vials, and 1.0 ml eluent (2% acetone (v/v) in carbon disulphide) is added to each vial. After a 30-minute stand, a sample aliquot of each vial is injected into a gas chromatograph equipped with a flame ionisation detector. No interferences are known. The working range is 1 to 67 mg/m^3 (0.5 to 31 ppm) for a 15 l sample. The estimated limit of detection is 0.05 mg/m^3 (0.02 ppm). The precision (variation coefficient) of the analytical measurement (excluding the air sampling) was 0.06.

3. EN 689 (DIN) and EN 482 (DIN)

Exposure measurements in workplace atmospheres are made in compliance with both the requirements on measurement strategy as laid down in (DIN) EN 689 and the requirements on measurement methods as laid down in (DIN) EN 482. For this purpose a defined volume of air is drawn through a silica gel tube by means of a sampling pump with tube holder. After extraction with diethyl ether, the quantitative determination is carried out gas-chromatographically using a flame ionisation detector (FID). The analytical detection limit for a two-hour sampling period is to 0.05 mg/m^3.

4. HSE Method MDHS 2/85 (HSE, 1985). Acrylonitrile in air - Laboratory method using porus polymer adsorption tubes and thermal desorption with gas chromatographic analysis.

This method is suitable for the determination of the concentration of acrylonitrile in factory and environmental atmospheres. The upper limit of the useful range is set by the adsorptive capacity of the porus polymer used and by the linear range of the gas chromatograph electrometer. A maximal sample volume of five litres is recommended for 0.5 g Porapak N for acrylonitrile concentrations up to 10 ppm (22 mg/m^3) and relative humidity up to 90% at 20°C. At higher concentrations (up to 100 ppm) five litres remains the maximal safe sampling volume, but the volume sampled may need to be reduced to avoid electrometer overload. A known volume of air is drawn through a porus polymer tube to trap the organic vapours present. The tube is transferred to a compatible thermal desorption apparatus. The organic vapours on the adsorbent tube are thermally desorbed under inert carrier gas into a gas chromatograph. The area of the resultant peak is determined and compared with areas obtained for standards.

5. HSE Method MDHS 1/86 (HSE, 1986 a). Acrylonitrile in air - Laboratory method using charcoal pump absorption tubes and gas chromatography.

This method is suitable for the determination of concentrations of acrylonitrile vapours in the range of 1 ppm to 50 ppm (2.2 to 109 mg/m^3) for samples of 20 litres at normal humidity. High humidity severely reduces the breakthrough volume and under these conditions sampling should not exceed 10 litres. A known volume of air is drawn through a charcoal tube to trap the organic vapours present using a calibrated sampling pump. The charcoal tube is transferred to a stoppered sample container and the analyte desorbed with carbon disulphide. An aliquot of the

desorbed sample is injected into the gas chromatograph. The area of the resulting peak is determined and compare with the areas obtained for standards.

6. HSE Method MHDS 55/86 (HSE, 1986 b) Acrylonitrile in air - Laboratory method using porus polymer diffusion samplers, thermal desorption and gas chromatography.

The method described is for the determination of the time-weighted average concentrations of acrylonitrile vapour in workplace atmospheres. It is suitable for the measurement of airborne acrylonitrile vapour in the concentration range 2 to 20 mg/m^3 (about 1 to 10 ppm, v/v) for exposure times between 30 minutes and 8 hours. This range may be extended to 0.1 mg/m^3 or lower for 8-hour sampling periods. The diffusive sampler is exposed to air for a measured time period. The rate of sampling is determined by prior calibration in a standard atmosphere. The acrylonitrile vapour migrates down the tube by diffusion and is collected on the porus polymer adsorbent. The collected vapour is desorbed by heat and is transferred under inert carrier gas into a gas chromatograph equipped with a flame ionisation director, where it is analysed. This is not a "reference" method in the strict analytical sense of the word.

Sampling methods

Currently (1997) the majority of large users and producers of acrylonitrile use personal sampling methods. These methods use a tube filled with absorbent (carbon) which is attached to the worker's clothing so as to be within the breathing zone of that worker. The tube samples the air breathed by the worker either by diffusion or in pumped mode, in which case the worker wears a battery powered metering pump. The acrylonitrile is eluted from the carbon with carbon disulphide and quantified by means of gas chromatography. Regarding air monitoring levels at workplaces these levels will vary depending on the location and process stage from which these samples are taken.

The sampling devices may be worn for the duration of a short task or could be worn for up to a whole shift. Most companies operate on a continuous basis involving various shifts rotas. Some sampling strategies cover only day shifts whilst others cover overnight and weekend shifts as well, when process activities can be significantly different from daytime operations. At the end of a sampling period the tube is analysed. In the simplest method, the adsorbent in the tube undergoes a colour change as a result of reaction with acrylonitrile. The length of the stain change indicates the cumulative exposure (dose = concentration · time). In other methods, the tube is packed with an adsorbent from which the acrylonitrile must be desorbed, generally into a gas chromatograph for quantitative and qualitative analysis.

In general, the colour stain tube methods have poorer accuracy and precision. The diffusive methods also have limited accuracy, first because of the relatively small total sample at low concentrations and second because the linearity predicted by Fick's Law of diffusion breaks down at very high concentrations. The pumped methods can be affected by variations in pump flow rate.

Potential for occupational exposure

Monomer handling

Bulk acrylonitrile is delivered by pipeline, railcar, road tanker, or ship into storage tanks. There is potential for personnel exposure when making and breaking connections for acrylonitrile transfer. Personal Protective Equipment (PPE) is used during such operations. There may also be release of vapour into the workplace from displacement from the storage tank as it is filled. This operation is however generally conducted in the open air ensuring good ventilation.

Filtration

It is necessary to remove lumps from the dope (polymer solutions) which otherwise could block the holes of the spinning jet. In the workplace the filters operate in rotation and are taken out of line (operation) to be shut down for cleaning infrequently. The process of opening up the filters and cleaning has traditionally been a manual process involving some potential for exposure to the residual levels of monomer in the dope. On this basis PPE must be worn, which typically includes impermeable suit, gloves and respiratory equipment.

Regarding ABS/SAN polymerisation at a Dutch site of production the handling activities of operators with potential for direct skin contact were monitored as follows:

1. Connecting and disconnecting hose from the road tanker to the storage tank of acrylonitrile. This procedure takes no more than five minutes. The operator wears natural rubber gloves during this manual handling procedure. The individual operator performs this manual handling task on average once in every two days.

2. Opening the bottom cover from the polymerisation tank. At the time of this operation the polymerisation tank does contain residues of water and polymerisation liquid. The acrylonitrile content is always less than 0.5% (w/w). Opening of the bottom tank cover requires no more than 2 minutes. During this activity the operator wears natural rubber gloves and normally his gloves do not come in contact with the liquid. The gloves are disposed of immediately after use. Cleaning of the reactor by high water pressure occurs in a closed system and so no exposure can occur. No aerosol is released from the reactor during cleaning activities. The individual operator does this handling on average once every two days.

The breakthrough time of acrylonitrile through natural rubber for gloves (USA “Best” gloves) was measured and was estimated to be approximately 48 minutes, when it was brought in contact with 100% acrylonitrile. Considering the short handling times at truck unloading and at opening bottom covers of polymerisation tanks (2-5 mins together with the disposal of gloves after use), dermal exposure is regarded as negligible.

During production the potential for dermal exposure to acrylonitrile cannot occur under normal working conditions as production occurs in a closed system. As described, methods for sampling and taking measurement are devised in such a way that exposure by this route should not occur. For processing again the risk or potential for dermal exposure to acrylonitrile is low to negligible based on confirmed good occupational hygiene practice and the methods used in processing are partially closed. In addition local exhaust ventilation and the strictly monitored use of personal protective equipment is applied.

The concentration of acrylonitrile in end use products is low. Therefore, airborne exposure during the handling of such products will be minimal, with most exposures below the limit of detection.

Personal Protective Equipment (PPE)

As outlined in the CEFIC Guidelines for the distribution of acrylonitrile, Rev. 2, 1995, when selecting personal protective equipment, two groups can be distinguished regarding protective clothing i.e. preventive and repressive clothing. The main difference between these two groups results from the difference in emphasis placed on the comfort and chemical resistance respectively of the materials concerned.

Preventive protection is only resistant to acrylonitrile for a short time and is used in activities where none of the substance is normally released, but the risk of this happening cannot be completely ruled out altogether. If acrylonitrile is released during work, the activities should be stopped immediately, following which the clothing should first be rinsed with plenty of water and then removed. An overall with a close-fitting hood and elastic fitting around the wrists and ankles is used for body protection. Disposable gloves are used to protect the hands. The material should first and foremost be gas- and liquid tight.

Repressive protective clothing is highly resistant to acrylonitrile for longer periods. This clothing is used for work in places where acrylonitrile is released and the workers may come in contact with it, e.g. during disaster control. In such instances an attempt must first be made to halt the release of the product from a distance. Repressive protection clothing for acrylonitrile consists of a gas-tight suit, e.g. of butyl rubber. The wearing of this repressive clothing can give rise to a high level of physical and mental stress and such suits must not be worn for longer than 20 minutes.

Breathing protection

For work purposes, air masks should be equipped with a clear view face piece, speaking diaphragm and air demand regulator. The air will be supplied from a 30-45 minutes capacity air cylinder, or medical air supply hose from air tanks or long duration cylinders. The use of the mask in the positive pressure mode affords greater protection and is preferred. Masks with carbon filter cartridges may be worn in emergencies, but only for a very limited period of time, especially if the concentrations of acrylonitrile are fairly high. Escape masks should only be used for escape purposes and during short exposure time.

Eye protection

This is incorporated into the full-face mask as mentioned above. A pair of close-fitting goggles may be used in emergencies. However attention should be paid to the fact that acrylonitrile can attack the skin, eyes and unprotected parts of the face.

Hand protection

For preventive clothing purposes, disposable gloves, made of high quality butyl rubber or neoprene, must be worn, while for repressive clothing requirement the hand protection element is incorporated into the need to wear a complete butyl-rubber gas-tight suit with independent air supply. (See **Table 4.1** for data on impervious material providing protection against contact with acrylonitrile).

Foot protection

Neoprene rubber boots.

Body protection

For preventive clothing purposes a PVC overall with a close-fitting hood and an elastic fitting around wrists and ankles should be worn, while for repressive clothing purposes a butyl rubber gas-tight suit with an independent air supply must be worn.

Circumstances Requiring Respirator Use

(BP Chemicals, personal correspondence, 1995)

Respiratory protection should be used in the following circumstances:

- during the installation of engineering controls,
- during maintenance and repair activities,
- during reactor cleaning where use of engineering controls is not feasible,
- when available engineering controls are not sufficient to reduce exposure below permissible exposure limits,
- during emergencies,
- in any situation where monitoring finds acrylonitrile vapour concentrations above 2 ppm.

Table 4.1 Data on impervious materials for acrylonitrile protection

Material	Thickness (mm)	Brealthrough (min)
Butyl rubber	0.70	> 480
Butyl rubber	0.31	180
Chlorinated polyethylene (CPE)	0.50	17
Neoprene	0.48	20
Polyethylene (PE) / DuPont Tyvek	n.a.	5
Polyvinyl alcohol (PVA)	0.76	42
Saranex-23 / DuPont	n.a.	23
Teflon	0.50	54

n.a. = Data not available

Industry has confirmed that PPE is worn as specified above. Although acrylonitrile is a known skin and respiratory system irritant and a severe eye irritant in animals, as well as a skin sensitiser, there is little evidence of these effects in workers. This information further confirms that PPE is worn by workers when there is a possibility that exposure to acrylonitrile may occur at the workplace.

4.1.1.2.2 Measured levels in the workplace

Exposure during production and further processing of acrylonitrile

Occupational exposure may occur during production and further processing of acrylonitrile. Considering the toxicological long-term effects of acrylonitrile, mean levels of exposure are considered to be more relevant for risk assessment purposes than peak values. Generally producers and processors of acrylonitrile comply with 95% confidence to the Occupational Exposure Limits of 2 ppm (4.5 mg/m^3) for an 8-hour TWA. In practice this means that the mean values are considerably lower than 2 ppm as indicated in **Table 4.2**, which shows mean values for the 8-hr TWA derived from production and various end uses of acrylonitrile.

Table 4.2 Summary table of mean exposure data from European-based industry

European Industrial Plants (1993)	Mean Level 8-hr TWA (ppm)
Production	≤ 0.45
Fibre	≤ 1.01
Latex	≤ 0.10
ABS – polymer	≤ 0.40
Acrylamide	≤ 0.20

(Source: Representative summary data from European producers and processors, supplied via Dutch Industry. Personal correspondence 16/3/'95)

As shown in **Table 4.3** for six of the European producers of acrylonitrile, personal average monitoring levels at the workplace varied from <0.12-0.49 ppm, with the range being between <0.1-2.21 ppm and the maximum recorded level was 5.5 ppm. Regarding users (for production of acrylonitrile fibres) the average personal monitoring levels recorded were from 0.21-0.43 ppm with a range of 0.009-2.56 ppm and a maximum recorded value of 3.6 ppm. For production of ABS polymers the average levels for personal monitoring were 0.08-0.16, with a range of between 0.05 to 2.0 ppm and a maximum recorded value of 8.6 ppm. In general the levels recorded were slightly higher for acrylonitrile users than for the producers of acrylonitrile, possibly reflecting the fact that acrylonitrile is initially produced in a closed system while manufacture of e.g. ABS polymers is carried out in a partially closed system with local exhaust ventilation and emission.

One German ABS/SAN producer submitted data showing that the mean value for all measurements since 1979 (approx. 1,500 PAS measurements) was 0.4 ppm and the mean value of approx. 800 continuous measurements was 0.2 ppm. A German acrylonitrile fibre production plant confirmed that "permanent safe" compliance was conserved at the workplace whereby mean values achieved through monitoring were 1/4 of the TRK i.e. < 0.75 ppm.

Table 4.3 Occupational exposure data for acrylonitrile production and further processing plants

Producer / user	Number of samples	Mean (ppm)	95% Confidence limit (ppm)	% Probability of conformance within 2 ppm 8 hrs	Range (ppm)
Producer 1 1993	97	0.49	-	98.46	<0.1 to 2.21
Producer 2 1993	37	-	-	-	max. 2.2
Producer 3 1994	25	<0.07	-	-	<0.04 to 0.56
Producer 4 1992-1994	10	0.08	< 0.77	-	0.001 to 1.6
Producer 5 1989-mid 1994	1,010	0.06	0.08	-	max. 5.5
Producer 6 1991-1992	74	<0.12	-	-	max. 0.3
User 1993 (Adiponitrile)	113	1.01	-	96.25	<0.1 to 6.7
User 1994 (acrylonitrile fibres)	1,116	0.31	-	99.84	<0.1 to 2.5
User 1994 (acrylonitrile fibres)	17	0.43	-	-	0.009 to 2.56
User 1989-1994 (acrylonitrile fibres)	-	<0.26	-	-	max. 3.6
User 1990-1993 (ABS)	66	0.16	0.64	-	0.05 to 2.0
User 1989-1994 (ABS)	638	0.08	0.09	-	max. 8.6
User 1990-1994 (ABS)	-	0.046	0.113	-	-
User 1990-1994 (ABS)	-	0.3	-	-	-
User 1989-1994 (ABS)	-	<0.26	-	-	max. 0.87

Source: Exposure data supplied from six EU producers and a variety of users, forwarded via BASF (personal correspondence, 1995)
Note: Some of the data include measurements taken when workers were wearing respiratory protection, where there was a potential to exceed 2 ppm (4.5 mg/m^3). This data reflects the different sources and ways of reporting the information, whereby some consider "the probability of conforming within 2 ppm over 8 hours", while others refer to "a 95% confidence limit" relating to exposures measured.

Specific exposure data were provided for 1994 from one European fibre processing plant. These data were provided for plant operators and associated maintenance personnel, as shown in **Table 4.4** below.

Table 4.4 Specific exposure data from one European fibre processing plant (1994)

No.of samples	Lowest result ppm	Highest result ppm	Mean result ppm	95% Probability of conformance with 2 ppm 8 hr	Total "Exposed" population size
270	0.1	5.0	0.2	0.3	100

The manufacturing process for acrylic fibres comprises 4 principal steps:

- Momomer receipt into bulk storage,
- Polymerisation (to dope),
- Spinning,
- Finishing including drying and baling.

For the various work areas and groups of workers, average personal exposures are less than 1 ppm 8-hour TWA. Set out below in **Table 4.5** are typical levels (during the 1990's) for selected tasks within the manufacturing of acrylic fibres.

Table 4.5 Personal exposures relating to the manufacture of acrylic fibre in Europe (1990's)

Work area	Exposure
Polymerisation operators	0.4 ppm
Spinners	0.5 ppm
Jet room operators *	0.4 ppm
Finish	0.1 ppm
Maintenance	0.2 ppm

* = Jet room is where spinning jets are cleaned and maintained and specific local extraction engineering is used to remove the emissions from this process.

Exposures within the spinning function were consistently the highest. A recent example of monitoring data from a fibre plant indicated that in a workforce of approximately 400 the mean exposure from about 1,800 samples was 0.2 ppm for 8-hour TWA (1995 data). There was a 99.9% probability of compliance with the 2 ppm 8-hour TWA exposure limit.

In a French fibre plant, short-term measurements (< 2 hour) were as follows (Cicolella et al., 1981):

Grinding	13 ppm	2-28 ppm (range)
Drying	3.4 ppm	1-7 ppm (range)
Wringing stations	15.8 ppm	3-46 ppm (range)

However the exposure levels measured at this French facility cannot be taken as representative of fibre plants in general. The values measured are for short-term exposures and do not relate to normal 8-hr TWA measurements, thus allowing for peak/short term measurements to be considered. Also while these three stations at the French plant had local exhaust ventilation, it was identified as being insufficient and subsequently improved. In addition the particular tasks outlined at which these measurements occurred are considered to be ones with potential for higher exposure than other non-specified tasks involving acrylonitrile fibre processing. Finally the data referred to for these plants date from before 1981, while other measurement data received from European industry relating to early and mid-1990's monitoring indicate that exposure measurements across Europe in fibre plants are generally less than 1 ppm.

The information on the occupational exposure scenario for Australia (Worksafe Australia, personal communication) correlates well with the above data. Australia imports approximately 2,500 tonnes per year, with the major use being the polymerisation of acrylonitrile butadiene styrene (ABS) and styrene acrylonitrile (SAN) solid (thermoplastic) polymers. The remainder is used in the manufacture of latex polymers (polymers dispersed in water) for adhesive and coating applications. Manufacturing of SAN polymers is carried out in a closed system and results of personal monitoring data indicate that atmospheric exposure levels (TWA) are <0.1 ppm (the limit of detection). ABS manufacture is carried in a partially closed system with local exhaust ventilation and emission control. Results of personal monitoring are generally <1 ppm.

In Canadian fibre plants personal 8-hour TWA levels were less than 1 ppm for unloading, reactors, wet spinning, maintenance and cleaning and processing in 1980. These measurements were identified as personal TWAs by the authors. In Canadian nitrile rubber plants the exposure levels averaged 2 ppm at the reactors and for maintenance and cleaning operations, 1.6 ppm at the coagulation and drying area and 1 ppm during sample taking (Guirguis et al., 1984).

Regarding monomer production in the US, surveys have been carried out with respect to full-shift personal exposures in four US acrylonitrile production plants (Zey, 1989b; Zey and McCammon, 1990; Zey et al., 1990a; 1990b). The monomer production operators had 8-hour time-weighted average (TWAs) personal exposures of 1 ppm or less from about 1978 to 1986, with some TWAs levels greater than 10 ppm. The highest average level recorded was 2.57 ppm. In three of these plants, maintenance employees averaged below 0.5 ppm, but in one plant the TWAs for these workers were about 1 ppm. Typical exposures to loaders of acrylonitrile into tank trucks, rail cars or barges varied from about 0.4 to about 0.6 ppm. Respirator use was noted for some of the higher measurements for production and maintenance workers and loaders in these plants. Laboratory technicians in these plants averaged about 0.25 ppm (n = 176; 0.01-2.0 ppm), except for one plant where the average was 1 ppm (n = 57; 0.1-9.4 ppm). Although measurement data were provided by year and several changes were made in these plants to reduce exposure levels, no trends over the years were observed.

Three US fibre plants had data for full-shift personal samples between the years 1977 and 1986. The average typical exposures for the operators at the polymerisation reactor were 0.9-1.6 ppm, based on more than 450 samples in each plant. The dope (viscous pre fibre solution) and spinning operators had exposure averaging below 1 ppm. The lower exposure occurred in the plant that dried the polymer before spinning operation resulting in a lower monomer content in the polymer. The other plant had a continuous wet operation without the drying stage. Exposure of maintenance workers averaged 0.2-0.7 ppm. Tank-farm operators, who are likely also to unload acrylonitrile monomer from trucks, rail cars or barges had homogeneous exposure levels (0.6-0.7 ppm) across plants, as did the laboratory technicians (0.1-0.4 ppm).

At a US facility making acrylonitrile-butadiene resin, the average exposure of the resin operators was about 1 ppm (Zey et al., 1990), while in another US resin plant the resin operators exposure averaged 0.3 ppm and compunders had lower levels (0.1 ppm). The average for maintenance workers in this plant was 0.3 ppm and tank-farm (unloading) was 0.2 ppm.

Full-shift personal samples taken at a US adiponitrile production plant measured an average of 0.5 ppm (218 samples), with a maximum value of 6.1 ppm.

Workplace personal monitoring results in Japan indicated levels of 0.1 ppm (0.21 mg/m^3) - 4.2 ppm (8.74 mg/m^3) for anticipated low and high exposure groups of workers, respectively. Urine levels measured in these workers indicated levels of acrylonitrile to be 3.9 µg/l - 359.6 µg/l for the low and high groups respectively. Thus urinary acrylonitrile values appear to show marked differences according to the different levels of exposure, even at environmental acrylonitrile concentrations below or equal to the current ACGIH TLV.

In the study performed by Sakurai et al. (1978), acrylonitrile concentration in air was measured in spot samples in six acrylic fibre factories in Japan on two consecutive days. On average 101 samples (where worker exposure exceeded 5 years) were taken per factory with the median concentration for the highly exposed population of workers being 5 ppm (11 mg/m^3). It should be noted however that in a later report by Sakurai, it was stated that the “exposure levels were not reliably reported”. These workers experienced irritation of the conjunctiva and upper respiratory tract following exposure to acrylonitrile. However reappraisal of this Sakurai et al.

study indicated that levels less than 10 ppm did not cause notable irritancy and the effects recorded were related to higher than 10 ppm exposure levels measured over years prior to this study being undertaken. Wilson (1948), identified upper respiratory symptoms, nasal irritation, nausea etc., in workers at a synthetic rubber manufacturing plant following exposure to “mild” concentrations of acrylonitrile, while Zeller et al. (1969), observed similar symptoms in workers exposed to acute inhalation of acrylonitrile fumes. Sartorelli (1966) recorded these symptoms also in an individual worker who was exposed to acrylonitrile vapours when a leakage occurred in a distillation apparatus. (See Section 4.1.2.6.5).

Improvements in Workplace Exposure Control, 1980-1998

Industry has confirmed that since 1980 major exposure control improvements have been carried out in workplaces using acrylonitrile. These improvements include:

- Delivery of raw material acrylonitrile by pipeline, eliminating the hazards associated with road transport in general and more particularly the risk of exposure when off-loading road tankers;
- Redirection of vents coming from storage facilities away from areas occupied by personnel;
- Improved and continuous air monitoring in areas of greater risk, enabling early identification and resolution of potential exposure problems;
- Refinements to the de-monomerisation process to reduce residual monomer levels in tow and finished fibres;
- Improvement in the engineering controls to pump seals so as to reduce the leakage potential;
- Improvement in ventilation including extract ventilation at critical locations in the process.

Exposure during use of acrylonitrile polymers

Based on measured occupational exposure levels of acrylonitrile in Germany between 1991 and 1995, 91% of all of the exposures were located below the analytical detection limit i.e. 0.05 mg/m^3 (0.01 ppm). 35% of the measurement data originated from the area relating to the production of plastic, 13% from the chemical industry, 9% from the electrical industry and 7% from paper and board production, with approximately 75% of the sampling performed stationary. Exposures above the analytical detection limit occurred in individual cases during the production of plastic (reaction vessels, mixers, extruders) in work areas without exhaust ventilation equipment. The measured data are presented in **Table 4.6** below. In carrying out these measurements, there was further differentiation according to whether or not technical measures for exposure reduction (ventilation) were taken at the workplaces.

Table 4.6 Measurement results (8-hr time-weighted averages for acrylonitrile) [a)]

Type of company/work area	No. of measurements	No. of companies	95%- value [mg/m^3]
Extruders for plastics, injection moulding	69	43	<0.05 *
- without technical measures (ventilation)	53	34	<0.05 *
- with technical measures (ventilation)	14	10	<0.05 *
Surface coating (spraying, brush application, roller application, filling, gluing)	20	16	<0.05 *
Paper and board production	16	6	<0.05 *
- without technical measures (ventilation)			
- with technical measures (ventilation)			

* Note: <0.05 mg/m^3 (0.1 ppm) is less than the analytical limit of detection

a) Source: German Industry (1991-1995)

4.1.1.2.3 Biological indicators of exposure to acrylonitrile

The main routes of absorption of acrylonitrile in occupational situations are the respiratory system and the skin. The effects may be local due to contact with the skin and mucosa or systemic following exposure via inhalation or skin routes. Acrylonitrile is eliminated in the urine, in part unchanged and in part after biotransformation to thiocyanate and mercapturic acids.

Thiocyanate is a metabolic product normally present in human urine, consequently it is present in the urine of non-occupationally exposed subjects. Diet and smoking are non-occupational sources. Sakurai et al. (1978) found increasing levels of urinary thiocyanate with increasing atmospheric concentrations of acrylonitrile in an exposed worker population smoking an average of 15 cigarettes/day. In the three sub-groups of workers studied, for mean environmental acrylonitrile concentrations of 0.1, 0.5, and 4.2 ppm (detected with personal samplers), the corresponding mean urinary thiocyanate values were 4.5, 5.78 and 11.41 mg/l. These values show a rather limited range compared to the urinary acrylonitrile values found by the authors at the same environmental acrylonitrile levels, indicating that the urinary thiocyanate has less discriminating power than urinary acrylonitrile in subjects with different degrees of exposure. However, it would appear that the test can still distinguish between subjects with exposure to levels lower or equal to the TLV proposed by the ACGIH (1991) and non-exposed subjects.

Determination of acrylonitrile in urine seems at present to be the most suitable indicator that will ensure distinction between exposed and non-exposed subjects and between groups of subjects with varying degrees of occupational exposure. The highest values are found at the end of the work shift. Evaluation of urinary thiocyanate is less discriminating in exposed than in non-exposed subjects and between different degrees of exposures. This however may well be due to the influence of factors other than exposure (i.e. diet, smoking).

4.1.1.2.4 Modelling of exposure

With regard to whether or not skin absorption of airborne acrylonitrile is an important route of exposure, Rogaczewska (1975) observed that the uptake of acrylonitrile vapour in rabbits via the dermal route was 1% of the uptake via the inhalation route. Rogaczweska and Piotrowski (1968) observed a dermal permeation rate in volunteers of 0.6 mg/cm^2/hour and van Hooidonk (1986) found a dermal permeation rate for human skin *in vitro* of 3.6 mg/cm^2/hour in the case of skin contact with pure acrylonitrile.

It is possible to derive the permeation coefficient by dividing the permeation rate by the water solubility of acrylonitrile (Wilschut et al., 1995). This results in a permeation coefficient between 0.008 and 0.05 cm/hour for aqueous solutions of acrylonitrile. This may be converted into the permeation coefficient in air by multiplying with the water/air partition coefficient of acrylonitrile (= 275 reciprocal dimensionless Henry coefficient). This results in an estimated permeation coefficient in air between 2.2 and 13.8 cm/hour. This is relatively small compared to the diffusive transfer of 400 cm/hour in air, so the permeation through the skin is the controlling factor. Using the permeation coefficient in air the dermal uptake from air may be compared to the uptake by inhalation. A rabbit inhales 0.015 m^3 of air per kg body weight per hour and has a dermal surface area of 725 cm^2 per kg body weight. At a concentration of 1,000 mg/m^3 in air the following absorption can be estimated:

- 15.5 mg via inhalation. In the case of 50 % retention this results in an actual uptake of 7.75 mg per kg body weight.

- between 1.6 and 10 mg per kg body weight via dermal uptake (= $0.001 \cdot 725 \cdot$ permeation coefficient [2.2 and 13.8, respectively]). This is 20 % to 129 % of the absorption by inhalation.

The estimated dermal uptake via air, derived from the permeation coefficient of pure acrylonitrile (or saturated acrylonitrile in water) in contact with skin is much higher than that experimentally observed by Rogaczewska (1975). The explanation for this finding may be that following direct contact of liquid acrylonitrile with the skin a reaction possibly occurs with skin proteins (Van Hooidonk, 1986), which increases the permeability. This is supported by the relatively long lag time (reaction time with skin proteins) of 20 to 30 minutes, whilst most compounds in this class (low molecular weight and octanol/water partition coefficient) have a lag time between 5 and 10 minutes.

Modelling using the SKINPERM Programme (ten Berge, personal correspondence, 1996, see Appendix D), predicts absorption by vapour more appropriately, because the vapour of acrylonitrile will not result in a concentration in the stratum corneum sufficiently high so as to react with the tissue macromolecules. However modelling by SKINPERM does not take into account the reaction with tissue macromolecules and so should not be used in estimating permeation of skin in contact with pure acrylonitrile. It is recommended that experimental observations should be used in preference when such data are available. An explanation of the SKINPERM Model used for the estimation of permeation of vapours through the skin is presented in Appendix D.

In SKINPERM it is assumed, that a worker inhales 1 m^3 of air per hour and that 100% of inhaled material is retained. In the case of 50% retention, the ratio (skin permeation/lung retention) becomes about 3%. Therefore should occupational exposure levels exceed 30 times the threshold limit value, in fact, respiratory protection would not be deemed sufficient for the protection of the worker against such a high exposure.

Modelling with EASE

The Estimation and Assessment of Substance Exposure (EASE) model developed by the EU has been used to predict occupational exposure to acrylonitrile. The main routes of concern regarding exposure would appear to be most importantly via inhalation and to a lesser extent via dermal. Regarding the dermal route of exposure, based on the information provided by industry and recent measurements, as well as the knowledge regarding engineering controls, use of

personal protective equipment and the application of good occupational hygiene practice, the risk of exposure via this route is low.

Four kinds of use scenarios are considered: production of acrylonitrile, polymerisation of acrylonitrile including processing stages for fibre production, plastics production including resin and ABS powder production and NB rubber production.

The vapour pressure of acrylonitrile at normal temperature i.e. 133.3 hPa @ 22.8°C (Verschueren, 1983) indicates that it is highly volatile. Inhalation is therefore the primary route of concern with respect to occupational exposure.

Regarding the potential for aerosol production this is not a concern in either the production and or processing of acrylonitrile or acrylonitrile products (confirmed by industry) and so has not been considered as a possibility when running the EASE model programme. Regarding the specified scenarios, possible breaching (for sampling, drumming purposes, cleaning etc.) has been considered but does not reflect the real situation. This reflects the fact that these occurrences occur only a maximum of once every 2 days for loading and even less frequently for purging of reactors, cleaning of reactors or intermediate storage tanks and loading onto barges or into tanks. Also when this type of work is being undertaken, where there is a possibility of exposure, personal protective equipment and/or LEV is always used. Based on measured data the levels of exposure are still low or below the level of detection.

Production of acrylonitrile (closed system)

For this operation the temperature range is between 400 and 500°C. The process involves a closed system and batch production methods. The exposure prediction for dermal exposure is very low and for vapour exposure is low (between 0 and 0.1 ppm) if the pattern of control is full containment. Some higher vapour exposures are likely to occur when breaching of the system is considered for sampling, loading, cleaning etc i.e. 100 to 200 ppm. However these are unrealistically high predictions for reasons already discussed. Regarding the sampling process the apertures used are placed and designed in such a way that a probe can be inserted efficiently so reducing the risk of exposure during this operation.

Polymerisation of acrylonitrile including processing of fibres (closed/partially closed system)

The temperatures for these processes including polymerisation (closed), spinning (partially closed), washing (closed) and drying (closed) range between 40 and 70°C. The pattern of use is non-dispersive for the partially closed system. The pattern of control for the closed systems is full containment and for the partially closed system local exhaust ventilation is used. The predicted dermal exposure is very low and for vapour exposure is 0 to 0.1 ppm for the closed systems. For the partially closed system the predicted dermal exposure is still low and for vapour exposure is between 10-50 ppm and 100-200 ppm. If breaching (sampling etc.) is considered the predicted vapour exposure is between 100 and 200 ppm.

Plastics production including resin processing and ABS powder production (closed system)

These processes involve non-dispersive use and the pattern of control is a closed system with full containment. For plastics production the processing temperature is 80°C and for ABS powder production the temperature is 180°C. For the closed system therefore the predicted exposure for dermal is very low and for vapour exposure is 0 to 0.1 ppm. When considering a breach in the system e.g. for sampling, the dermal exposure prediction remains as very low, while the vapour exposure becomes 100-200 ppm.

NB Rubber production (closed system)

The temperature for this process is 35°C. For this closed system the pattern of control is full containment. The predicted exposure for dermal is very low and for vapour exposure is between 0 and 0.1 ppm. When a breach in the system is considered, while the dermal exposure remains low the vapour exposure predicted is 10-50 ppm.

The dermal exposure is considered to be low for production and non-dispersive use in processing. Based on the predictions of the EASE model this was shown to be the case. EASE also indicated that the major route of concern for occupational exposure is via inhalation, reflecting the highly volatile nature of acrylonitrile. The results obtained using this model are useful as general indicators but do not reflect the practical day to day production, processing and use of acrylonitrile in the workplace. The model deals with continuous exposure and does not adequately accommodate on-site controls, work practices or systems. The measured values bear little resemblance to the predicted exposure values from EASE other than those estimated for production in fully closed systems.

4.1.1.2.5 Summary of occupational exposure

The main route of occupational exposure to acrylonitrile is by inhalation of the vapour of this volatile substance. There is also potential for exposure via the dermal route. However this route is considered to be of less importance, particularly if good occupational hygiene practice is assumed.

Workers are potentially exposed to acrylonitrile during production of the monomer and use of the monomer to produce acrylonitrile polymers. Although minor differences in exposure could potentially exist between these two scenarios, reflecting the extent of enclosure of the process, in practice this is not borne out by recent exposure data provided by industry for both production and further processing facilities in a number of European countries. These data indicate that maximum exposure levels lie well below the Occupational Exposure Limit of 2 ppm. In fact the value of 0.1 ppm provided for processing would appear to be representative, based on 1995 European data and measurement, and for acrylonitrile production the representative value measured throughout Europe is 0.45 ppm.

The higher levels of 5 ppm recorded by Sakurai et al. (1978) reflect the levels from approximately 20 years earlier and specifically relate to highly exposed workers in a user rather than a producer scenario. Engineering controls, use of PPE and improved monitoring have led to improved and much reduced levels being achieved in workplaces. For this risk assessment therefore the current situation in industry is considered to represent the relevant data and indicates that in general the actual average personal monitoring occupational exposure levels occurring are <1 ppm and in the majority of situations the levels are below the level of detection i.e. 0.05 mg/m^3 (0.1 ppm). When specific tasks such as purging, cleaning maintenance and sampling occurs, where there exists the potential for higher exposure levels, it is confirmed that engineering controls, use of personal protection equipment, strict adherence to good occupational hygiene practice is ensured. Even during these specific tasks the measured levels are well below 1.0 ppm.

However for the purposes of the risk characterisation a reasonable worst-case exposure level of 2 ppm has been chosen and carried forward, reflecting both production and further processing, and allowing a built in safety margin and degree of conservatism with respect to performance of risk characterisation.

During production there is no potential for dermal exposure to acrylonitrile under normal working conditions as production occurs in closed systems. As described, methods for sampling and taking measurements are devised in such a way that exposure via this route should not occur. For processing again the risk or potential for dermal exposure to acrylonitrile is low to negligible to low based on confirmed good occupational hygiene practice and the methods used in processing are partially closed. In addition local exhaust ventilation and the strictly monitored use of PPE are applied. However for the purposes of this report and in particular with regard to the area of risk characterisation a worst-case scenario for dermal deposition is assumed i.e. between 0.0 and 0.1 mg/cm^2/day, and is carried forward for the risk characterisation.

The concentration of acrylonitrile in end use products is negligible. Therefore, airborne exposure during the handling of such products will be negligible, with exposures below the level of detection.

4.1.1.3 Consumer exposure

Acrylonitrile is not sold to the general public alone or as part of a preparation. However should acrylonitrile occur as part of a preparation it is then regulated for under Directive 76/769/EEC, relating to restrictions on the marketing and use of certain dangerous substances and preparations. Under this legislation acrylonitrile would be regulated to ≤0.1% in substances and preparations placed on the market for sale to the general public, due to its carcinogenic properties. The potential for possible exposure of the consumer relates to products such as textiles or food packaging etc. containing a percentage of acrylonitrile, which may migrate or come in contact with the consumer/end users of these products. A specific acrylonitrile limit has been laid down in Commission Directive 90/128/EEC, relating to plastic materials and articles intended to come into contact with foodstuffs. This Directive requires that the specific migration limit from ABS into food or in food stimulant should be 0.02 mg/kg, based on the detection limit (DL) of the method analysis. It should be noted that this limit applies to food content, and not to the residual monomer in plastic.

Article manufacturers have to ensure no acrylonitrile migrates from the article into the food at levels exceeding the Specific Migration Limit (SML). Evidence can be provided by performing migration tests using simulants (water, 3% acetic acid, 15% ethanol and olive oil), time and temperature conditions equivalent to the intended and foreseeable usages. Such migration and food stimulant criteria are defined in EEC Directive 82/711 and EEC 93/8 (first amendment to directive 82/711) on the basic rules necessary for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs.

Based on the knowledge and identified uses of acrylonitrile in products, the main routes of potential consumer exposure are as follows:

- via dermal contact/absorption through the skin, due to slow release of acrylonitrile from the acrylic fibres from clothes to the skin;
- via consumption of foods packaged in acrylonitrile derived plastics due to migration of residual acrylonitrile monomer from the food packaging into food.

The most significant consumer applications in terms of volume of acrylonitrile consumed are acrylic fibres and ABS/SAN resins. Global growth in the demand for the derivatives of acrylonitrile is greatest in the developing regions of the Far East (including China). ABS/SAN resins represent the fastest growing acrylonitrile derivatives. Approximately 60% of acrylonitrile manufactured is used in the production of acrylic and modacrylic textile fibres. Fabrics account

for the largest percentage of products with acrylic-based fibres acting as a popular substitute for cotton and wool, going to make such items as carpeting, blankets and clothing in general (e.g. socks, shirts, sweaters etc.).

Approximately 20% of acrylonitrile manufacture is used to produce ABS and SAN resins. These resins result in such products as telephones, computer and TV housings, sports equipment and moulded automotive parts, pipe-fittings and products likely to come into contact with food e.g. blister packs, food wrappings, plastic food containers/tubs etc. The benefit of these resins relate to their rugged, durable plastic quality. These resins are impermeable to gases and ideal for shatter-proof bottles that hold chemicals and cosmetics, blister packs that keep meat fresh and medical supplies sterile.

Acrylonitrile can also be copolymerised with butadiene to produce nitrile rubbers and elastomers. Oil-resistant nitrile rubber, made from acrylonitrile, is used for hoses at gasoline service stations and in automobiles, trucks and buses. They are also used to make personal protective equipment, reflecting their properties of low permeability and resistance.

Acrylonitrile was formerly used as a fumigant/pesticide and in flour-milling. Industry have confirmed that on a world-wide basis acrylonitrile is no longer used in this manner or for this purpose and so this specific use is not considered regarding potential exposure to consumers.

4.1.1.3.1 Skin contact with fibres

Release of acrylonitrile monomer is expected to occur only at elevated temperatures of greater than 130°C. The unreacted acrylonitrile in polymers is tightly bound and diffuses slowly even at elevated temperatures. The levels of residual acrylonitrile in acrylic and modacrylic fibres are currently well below 1 ppm and since these fibres are always dyed using wet processes at elevated temperatures and used in conjunction with other acrylics, the resulting garments contain well below 1 ppm residual acrylonitrile.

A survey carried out by the US Consumer Product Safety Commission in 1978, on the potential for monomer migration or extraction etc., from consumer products containing acrylonitrile, indicated that levels of residual monomer in acrylonitrile fibres were extremely low, as summarised in **Table 4.7**. Furthermore, the survey indicated that the release of acrylonitrile in consumer products will not occur under normal conditions of use and this has been confirmed by the major fibre manufacturers (industry personal correspondence).

Table 4.7 Residual levels of acrylonitrile monomer in PAN fibres

Year and source	Company	Levels (mg/kg)
1978 (US Consumer Product Safety Commission)		
	American Cyanamid	0.2 - 0.9
	Dow Badische Co.	< 1.0
	DuPont	< 1.0
	Eastman Kodak	1.0 - 3.0
	Monsanto	0.2
1979	IARC Mono. No. 19	< 1.0
1994	BUA Report	< 1.0
	Bayer	< 0.1

Note: 1.0 mg/kg is equivalent to 1.0 ppm

Regarding potential extraction and migration of acrylonitrile, one company attempted to simulate the effect of human perspiration using 1% saline solution at 120°C for 1 month, and showed no transmission of acrylonitrile from the fabric to the solution. Another company performed a range of extraction tests, in which no transmission of acrylonitrile was identified (the detection limit was 1 ppm). Tests were also carried out by industry, which indicated that unreacted acrylonitrile is tightly bound and diffuses slowly, even at elevated temperatures. Fibres were heated in water at 60°C (140°F) for one hour, to simulate washing with hot water in a washing machine. Generally no acrylonitrile was detected in the extracts (detection limit was 0.04 ppm based on fibre weight). Therefore under normal wearing conditions, monomer migration from consumer products would be less than that encountered in this hot water test.

According to the IARC Monograph (No 19, 1979) residual acrylonitrile has been reported in a limited number of commercial polymeric materials derived from acrylonitrile for fibres the levels were generally less than 1 mg/kg (1 ppm).

Release process of acrylonitrile from fibres

Fibres of polyacrylonitrile have a size of 1.5 denier, which translates into a weight of 166.5 mg per 1 km of acrylic fibre. The density of polyacrylonitrile is 1,170 kg per m^3. The radius of the cylindrically shaped fibre was estimated from these data to be $6.73 \cdot 10^{-4}$ cm. The diffusion coefficient in the fibre material was estimated with the AMEM programme (OECD, 1984). The greatest diffusion coefficient of acrylonitrile was estimated to be $1.03 \cdot 10^{-5}$ cm^2/sec (silicone rubber) and the smallest diffusion coefficient $8.6 \cdot 10^{-13}$ cm^2/sec (PVC). From diffusion equations the half-life time for radial diffusion out of a very long cylinder of solid homogenous material, containing the volatile substance can be estimated:

$$T_{half} = \frac{R^2}{4D}$$

where, R = radius fibre (cm)
D = diffusion coefficient (cm^2/sec)
T = second

From this simple equation the time for release of 50% from the fibre may be estimated for the greatest diffusion coefficient (0.01 seconds) in the case of silicone rubber and the smallest diffusion coefficient (1.5 days) in case of PVC-like materials. For the purposes of this risk assessment it is assumed that the diffusion behaviour of acrylonitrile in polyacrylonitrile resembles that of PVC rather than that of silicone rubber. A half-life time for diffusion of 1.5 days means that every 5 days the concentration in polyacrylonitrile is reduced by a factor of 10. Generally a long period will elapse between manufacturing polyacrylonitrile fibre and final processing into clothing. Therefore the final concentration will be very low in the fibre after a period of months.

Fate of acrylonitrile released from fibres in clothing

A layer of clothes may be compared to a layer of stagnant air of about 3 cm, through which the volatile compound has to pass to the outside air via diffusion. The other diffusion barrier is the stratum corneum. The diffusion resistance via these pathways controls which part of the volatile chemical is released to air or is absorbed by the stratum corneum. Regarding dermal absorption of acrylonitrile released from acrylic fibres in clothes, the skin permeability coefficient of acrylonitrile vapour in air is estimated to be 1.14 cm/hr. The permeability of acrylonitrile through a layer of clothes is estimated to be 141 cm/hr (Lotens and Wammes, 1993). In practice therefore the pathway through clothes to the air is favoured over the pathway through the skin by a ratio of 141/1.14 = 124.

An estimation of the transfer coefficient is made according to ECETOC Document No. 35 (1997) and correlates well with the values estimated above.

$$D_{air} = 360 \cdot \sqrt{\frac{76}{Mw}} \qquad Kp_{air} = \frac{D_{air}}{\delta}$$

D_{air}	=	diffusivity in air (cm^2/h)
Mw	=	molecular weight
Kp_{air}	=	mass transfer coefficient from stagnant air layer to ambient turbulent air (cm/h)
δ	=	depth of layer with stagnant air (clothes) in cm

$Kp_{air\text{-}skin}$	=	$Kp_{water\text{-}skin} \cdot Kwa$
$Kp_{air\text{-}skin}$	=	permeation coefficient from air to skin (cm/h)
$Kp_{water\text{-}skin}$	=	permeation coefficient from aqueous solution to skin (cm/h)

$$K_{wa} = \frac{R \cdot T \cdot Wsb}{Vp \cdot Mw} \quad R = 8.314\, J/Mol/°K \quad T = 298°K$$

K_{wa}	=	water/air partition coefficient
R	=	gas constant (J/Mol/°K)
T	=	temperature (°K)
Wsb	=	water solubility (g/m^3)
Vp	=	vapour pressure (Pa)
Mw	=	molecular weight

The $Kp_{water-skin}$ is estimated according to Wilschut et al. (1995 a; 1995b) with the following equations:

$$Kp_{water-skin} = \frac{1}{\frac{1}{K_{lip} + K_{pol}} + \frac{1}{K_{aq}}}$$

$$\log K_{lip} = -1.326 + 0.6097 \cdot \log Kow - 0.1786 \cdot Mw^{0.5}$$

$$K_{pol} = \frac{0.0001519}{\sqrt{Mw}} \qquad K_{aq} = \frac{2.5}{\sqrt{Mw}}$$

K_{lip} = permeation coefficient through lipid part of stratum corneum
K_{pol} = permeation coefficient through protein part of stratum corneum
K_{aq} = permeation coefficient through water layer below stratum corneum
K_{ow} = octanol/water partition coefficient
Mw = molecular weight

$$Kp_{air-skin} = Kp_{water-skin} * K_{wa}$$

Substitution of the data of acrylonitrile in the equations above provide the following results:

$Kp_{air\ layer\text{-}\ \text{-->ambient air}}$ = 143 cm/hour
$Kp_{air\ layer\text{-}\ \text{--> skin}}$ = 0.78 cm/hour

where, for acrylonitrile:

Molecular weight	=	53
Vapour pressure	=	13,330 (Pa, 25°C)
Solubility in water	=	75,000 (25°C, mg/l)
Log[octanol/water part.]	=	0.16 (25°C)

The transfer from clothing via the air layer to ambient air proceeds 260 times faster than permeation from clothing via air through the skin. This means that no more than a half percent of acrylonitrile released from acrylic fibres will be absorbed via the skin (Wilschut et al., 1995a; 1995b).

Consumers wearing acrylic textiles

It is assumed that a consumer wears 1 kg of acrylic fibre containing 1 mg of acrylonitrile or 1 ppm in clothing during a period of 30 days. During these 30 days 1 mg is assumed to be fully released from the fibre. Per day 33 µg acrylonitrile is released. About 0.4% of this will be absorbed by the skin, contributing to an average daily load of 0.13 µg or 1.8 nanogram per kg

body weight per day. This dose is $>10^5$ times lower than the No Observed Effect Level in chronic rat studies.

It should be noted however that this is a worst-case analysis. The 1 ppm residual acrylonitrile level used in the calculation is considerably in excess of the levels detectable in even freshly spun fibre, and a figure of < 0.1 ppm is more realistic (industry, personal communication). Any residual acrylonitrile on fibre is likely to be greatly reduced before it reaches the consumer because of the subsequent processing of the fibre into a textile and then into a garment. This process includes wet dying and washing stages at elevated temperatures. The calculation also assumes that a garment is worn continuously for 30 days, and that all residual acrylonitrile is released within those 30 days.

Fate of acrylonitrile released from fibres in acrylic carpets

For the manufacture of such floor coverings i.e. acrylic carpets 6.7-17 dtex acrylic fibres are normally used. Often these fibres are blended with other synthetic or natural fibres. For the purposes of the following exposure assessment it is assumed that there is an acrylic fibre content of more than 90% bulk weight and that the carpets contain 0.8-1.2 kg/m^2 of acrylic fibres. In developing a scenario to assess the potential for consumer exposure to residual acrylonitrile via inhalation, the following assumptions have been made:

- a 1:1 mixture of 6.7 and 17 dtex fibres has been used for the carpet,
- the residual acrylonitrile monomer content is ≤ 1 mg per kg fibres,
- the average weight of the carpet is estimated at 1 kg acrylic fibres/m^2.

The radius of fibres has been determined by assuming a cylindrical form of the homogenous acrylic fibre material with a density of 1.17 g/cm^3. Therefore for a 6.7 and 17 dtex fibre the radius (R) is $1.35 \cdot 10^{-3}$ cm and $2.15 \cdot 10^{-3}$ cm, respectively.

Estimation of the acrylonitrile diffusion coefficient in the carpet fibres

This was calculated by applying the AMEM programme (OECD, 1984) (see above for details). By this simple equation the time for release of 50% from the fibre may be estimated for the greatest diffusion coefficient (0.04 seconds) in the case of silicone rubber and the smallest diffusion coefficient (6.1 days) in the case of PVC-like materials for a fibre of 6.7 dtex. For a fibre of 17 dtex these half-times are between 0.11 seconds and 15.6 days. It is generally known that diffusion in silicone is relatively fast. Therefore it is assumed that the diffusion behaviour of acrylonitrile in polyacrylonitrile is realistically more closely related to diffusion in PVC. Based on this understanding it can be concluded that the concentration of residual acrylonitrile is decreased by evaporation by a factor of 10 in 20 days for a fibre of 6.7 dtex and in 52 days for a fibre of 17 dtex. In this average scenario of the 1:1 mixture of 6.7 and 17 dtex fibres the average half-time T_{half} can be calculated as (6.1 +15.6)/2 equalling 10.85 days. The turnover time (T_r) is calculated from the T_{half} as follows:

$$
\begin{aligned}
T_r &= T_{half}/\ln(2) && \rightarrow 15.6 \text{ days} \\
T_{90\%} &= \ln(10) \cdot T && \rightarrow 36 \text{ days} \\
T_{99\%} &= \ln(100) \cdot T && \rightarrow 72 \text{ days} \\
T_{99.99\%} &= \ln(10{,}000) && \rightarrow 144 \text{ days}
\end{aligned}
$$

The average time to reduce the level by a factor of 10 is estimated to be 36 days, by a factor of 100 about 72 days and by a factor of 10,000 about 144 days. The release rate K_{el} is the reciprocal

of the turnover time T_r. The unit of the release rate is dependent on the unit by which the turnover time is indicated.

$$K_{el} = 1/T_r \ (day^{-1}) = 0.0639 \ day^{-1}$$
$$K_{el} = 1/T_r \cdot 24 \ (hour^{-1}) = 0.00266 \ hour^{-1}$$

Estimated average concentration of acrylonitrile in a room with an acrylic fibre carpet

For the purposes of this estimation the following parameters are assumed:

- total weight of acrylic fibres per m^2 carpet is ca 1 kg/m^2
- content of acrylonitrile is ≤ 1 mg/kg
- mass of acrylonitrile per m^2 (=M in mg/m^2)
- the height of the room is 2.5 metres (=H)
- the area of the room is 1 m^2 (A)
- the room is ventilated at 0.2 times per hour (=V_r)

From the turnover time T_r the release rate K_{el} from the fibre is estimated to be 0.00266 per hour. This means that in the 1st day about 0.062 mg will be released per m^2 and on the 36th day 0.0062 mg per m^2 will be released.

The average concentration of acrylonitrile in the room on the first day is estimated as follows:

$$C = K_{el} \cdot M \cdot A / V_r \cdot H \cdot A, \quad \text{that is} \quad C = 5.32 \cdot 10^{-3} \ mg/m^3$$

On the basis of the turnover time, it can be estimated that every 36 days the acrylonitrile level is decreased by a factor of 10 and that after 144 days more than 99.99% of the original acrylonitrile content has been lost. The resultant final level can therefore be estimated to be $5.32 \cdot 10^{-7}$ mg/m^3 at day 144.

The average level in the room over a year is estimated as follows. There are 8,760 hours in a year. The room therefore is ventilated per m^2 over a year with $0.2 \cdot 2.5 \cdot 8{,}760 = 4{,}380$ m^3 of air. In this volume 1 mg per m^2 is released. This is an average level of 0.23 microgram per m^3. This value is independent of the release rate of acrylonitrile on condition that the residual acrylonitrile is completely released in one year.

Other fibre exposures

In 1993 Montefibre compiled information on potential exposure to fibres. Their findings are summarised as follows. Polyester and acrylic fibres often have prolonged contact with human skin: the sucking of children's toys may lead to ingestion. However, regarding consumer exposure finishing agents (applied to the fibres) were examined in bacterial mutation and rat acute oral toxicity tests. Also finished fibres were tested for acute toxicity and then for sensitising potential (on guinea pig). Human volunteer trials for skin irritancy and sensitisation followed. No adverse reactions were observed. Only when fibres were burnt was toxicity seen: smoke from acrylic fibre proved to be more toxic than that from polyester fibre (due principally to hydrogen cyanide release). Migration tests showed that little material leached out from the fibres: <1 mg/dm^2 surface area in saline; 0.4 mg/dm^2 from acrylic fibres in methanol; and 3.6 mg/dm^2 from polyester fibres in chloroform. Analysis showed only fibre polymer components and finish in the saline and methanol leachates (Robatto et al., 1993).

In general, based on the comprehensive work carried out by this major fibre producer, it can be concluded that current and foreseeable users of products containing these chemical fibres are at negligible risk from exposure to residual acrylonitrile content in the products made from acrylic fibres.

4.1.1.3.2 Food packaging

A specific limit has been laid down in Commission Directive 90/128/EEC, relating to plastic materials and articles intended to come into contact with foodstuffs. In this Directive the specific migration limit from ABS into food or in food simulant is 0.02 mg/kg, based on the detection limit (DL) of the method analysis. It should be noted that this limit applies to food content, and not to residual monomer in plastic.

Article manufacturers have to ensure that no acrylonitrile migrates from the article into the food at levels exceeding the Specific Migration Limit (SML). Evidence can be provided by performing migration tests using simulants (water, 3% acetic acid, 15% ethanol and olive oil), under time and temperature conditions equivalent to the intended and foreseeable usages. Such migration and food stimulant criteria are defined in EEC Directive 82/711 and EEC Directive 93/8 (first amendment to Directive 82/711) on the basic rules necessary for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs.

Certain foods such as margarine, cold-packed cheese, peanut butter and other spreads are packaged in acrylonitrile-based plastics. Early investigations on these viscous or semi-solid foods showed that higher concentrations of acrylonitrile existed in the samples next to/in contact with the container walls compared to those taken from the middle of the food i.e. migrant acrylonitrile is distributed inhomogenously with the contents. To overcome this factor therefore the foods must first be made homogenous i.e. blended prior to sampling for detection of acrylonitrile. IARC Monograph (No. 19, 1979) indicated that residual acrylonitrile levels in a limited number of samples could contain 30-50 mg/kg ABS resins and samples could contain up to 15 mg/kg of SAN resins. However later work on residual content in ABS-based plastic containers has provided measured data indicating much lower levels of residual acrylonitrile monomer in food packaging containers. The FDA determined that the migration of acrylonitrile monomer from containers to vegetable oil and margarine could be as high as 37 ppb (Flood, 1980).

As illustrated in **Table 4.8**, Page and Charbonneau (1983) detected acrylonitrile in concentrations ranging from 8.4 to 31.1 µg/kg in cheese, honey, butter and peanut butter packaged in ABS-plastic, where the tub and lid had a residual acrylonitrile monomer content of 33-99.2 mg/kg and 26-141 mg/kg, respectively. No acrylonitrile could be detected in spreading fats (butter and coconut), which were sold in ABS-packaging having an acrylonitrile monomer content of between 1.6 and 5.2 mg/kg (with a limit of detection of 2.5 µg/kg). However regarding these studies certain analytical deficiencies were identified in these studies relating to the loss of acrylonitrile during analysis whereby a decomposition or reaction of acrylonitrile resulted in it being removed from the system. Acrylonitrile reacts with proteinaceous material, as demonstrated by the fact that peanut butter and cheese, which have the higher protein content of the foods examined, also exhibited the greater loss of acrylonitrile.

Table 4.8 Acrylonitrile levels in food contained in ABS polymers [a)]

Food	Container Material	Sample	Tub µg/kg	Lid mg/kg	Food [b)] µg/kg
Honey butter (natural)	ABS	A B C	60 99.2 44.7	119 141 125	13.1 20.1 15.5
Honey butter (cinnamon)	ABS	A B C	44.6 80.7 42.3	92.3 26 c)	19.55 15.7 23.75
Cold-pack cheese	ABS	A B C	33.0 62.0 54.9		24.05 27.4 29.4
Peanut butter	ABS	A B C	63.8 64.3 63.0		35.0 11.9 12.0
Soft butter spread	ABS	A B C	2.2 1.7 1.7		ND [d)] ND ND
Creamed coconut	ABS	A B C	5.2 1.8 1.6		ND ND ND

a) Source: Page and Charbonneau
b) Average of duplicate determinations in food
c) Not analysed
d) ND= not detected; < 2.5 µg/kg

Page and Charbonneau developed improved analytical procedures, and in 1985 conducted a survey on the packaging of luncheon meats whereby 17 samples were examined under infrared to identify the constituent parts of the packages. 10 packages indicated the presence of a nitrile-based polymer and they were used in the survey. Meat slices next to the nitrile-based polymer were examined as were slices from the centre (blanks/controls). The result of the survey was that no acrylonitrile was detected from any slice which had been in contact with the nitrile-based polymer package (known to have an acrylonitrile residual content of up to 2.6 ppm). These 10 samples came from 5 different companies and a variety of different luncheon meats, giving a good representative sample overall.

Gilbert and Shepherd (1981), using headspace gas chromatography, analysed the acrylonitrile monomer content in ABS margarine tubs and in soft margarine contained in these tubs. The packaging material of these tubs contained 1.5 to 10 ppm of monomeric acrylonitrile and the margarine was shown to contain <0.01 to 0.04 ppm. In fact the majority of the 35 samples analysed (both tubs and margarine) gave results at the end of the range. Similar levels of acrylonitrile monomer were identified for concentrated cooking butter and lard, stored in ABS containers.

In a UK government-commissioned survey (1982), the average residual acrylonitrile content of margarine tubs and margarine product (purchased in 1979) was 6.2 and 0.015 mg/kg respectively. When considering that the approximate daily intake of soft margarine is 10 g/person/day, results from migration and storage time analyses indicated that the likely intake of acrylonitrile in soft margarine was a maximum of 0.3 µg/person/day. This UK survey showed that the monomeric acrylonitrile content of ABS margarine tubs had been greatly reduced over

the previous decade, going from a maximum content of 138 mg/kg in 1975 to a maximum content of 10 mg/kg in 1980.

The tolerable specific migration limit is 0.020 mg acrylonitrile/kg food for food products contained in ABS plastic, as laid down by Commission Directive 90/128/EEC. If it is assumed that only 5% of human food is packaged in ABS and an average man consumes 2 kg of food and beverages/day, human intake will be no more than 2 μg/day or 0.03 μg/kg/day in the case of a bodyweight of 70 kg.

In July 1981 the Committee on Carcinogenicity of Chemicals (COC) in Food, Consumer Products and the Environment considered all of the toxicological and epidemiological evidence available on acrylonitrile. In their deliberations they commented on the fact that the only foodstuff in which measurable contamination with acrylonitrile is likely to occur is soft margarine where acrylonitrile-containing polymers are used for packaging. "The levels of contamination in this product are very low and taking this into account the Committee considers that the general public are not at measurable risk from acrylonitrile in food". The Food Additives and Contamination Committee also considered the report and endorsed the comments of the COC above. They also acknowledged and welcomed the reduction in levels of acrylonitrile already achieved and hoped for continued efforts in this direction.

Other studies have been performed with respect to the migration of acrylonitrile from containers into water/solutions. In a Japanese study (Tatsuno et al., 1979) contamination of foodstuffs with acrylonitrile monomer after long-term storage in nitrile-based containers was examined. The measured concentration of residual acrylonitrile in 55 samples of ABS and AS plastic ranged from 1 to 1,373 mg/kg. Migration of acrylonitrile from these resins into water gave values of 5 to 250 μg/kg after 24 hours at room temperature. Vas (1983) found that bottles made of nitrile-based plastic contained between 2 to 5 mg/kg of monomeric acrylonitrile, while the acrylonitrile content in the drink contained approximately 2 to 3 μg/kg, but was greater than 9 μg/kg in two samples. Gawell (1979) also sampled beverage bottles and their contents for acrylonitrile. Levels in the packaging ranged from 2-5 mg/kg, but only trace amounts (< 5 μg/kg) of acrylonitrile were found in the carbonated soft drinks and beers sampled.

4.1.1.3.3 Other sources of contamination

Both IARC (1979) and WHO (1983) reported that fumigation of food products with acrylonitrile was another possible cause of food contamination. For example shelled walnuts were found to contain up to 8.5 mg/kg acrylonitrile as a result of fumigation with acrylonitrile 38 days prior to analysis. However industry has confirmed (in 1997) that on a worldwide basis acrylonitrile is no longer used in this manner or for this purpose and so this specific use is not considered regarding potential exposure to consumers.

4.1.1.3.4 Summary of consumer exposure

Based on the identified uses of acrylonitrile in products, the main routes of potential exposure for consumers are via dermal contact/absorption through the skin and via the oral router due to consumption of foods packaged in acrylonitrile-derived plastics. Exposure could occur due to slow release of acrylonitrile from the acrylic fibres from clothes to the skin or via migration of residual acrylonitrile monomer from the food packaging into food.

With regard to the UK survey (1982), it has been shown that the monomeric acrylonitrile content of ABS margarine tubs has been greatly reduced, going from a maximum content of 138 mg/kg in 1975 to a maximum content of 10 mg/kg in 1980.

A specific limit has been laid down in Commission Directive 90/128/EEC, relating to plastic materials and articles intended to come into contact with foodstuffs. This Directive requires that the specific migration limit from ABS into food or in food simulant should be 0.02 mg/kg, based on the detection limit (DL) of the method analysis. This limit applies to food content, and not to the residual monomer in plastic.

Article manufacturers have to ensure no acrylonitrile migrates from the article into the food at levels exceeding the Specific Migration Limit (SML). Evidence can be provided by performing migration tests using simulants (water, 3% acetic acid, 15% ethanol and olive oil), time and temperature conditions equivalent to the intended and foreseeable usages. Such migration and food simulant criteria are defined in Directive 82/711/EEC and Directive 93/8/EEC (first amendment to Directive 82/711/EEC) on the basic rules necessary for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs.

The available data and more recent studies performed suggest that the risk of exposure via either the oral or dermal route of exposure is very low. This conclusion is based on the amount of residual acrylonitrile actually present in the products and on the actual amount of this monomer that can be released to give exposure to the consumer.

4.1.1.4 Humans exposed via the environment

The exposure of the general public to acrylonitrile via the environment and the possibility for secondary poisoning may be addressed at two levels: (1) exposure to background levels on a regional or continental basis, (2) exposure to potentially higher levels which may exist near industrial production and processing sites. Exposure in the latter case is restricted to residents living in the immediate area. In both cases, compartments of concern are biota, drinking water and air.

In considering exposure scenario (1), models of environmental distribution of acrylonitrile in the compartments of concern predict that levels of exposure will be low. EUSES (Sections 3.1.4.1.5, 3.1.5.2 and 3.1.7) provides values of 2.81 µg/l for the regional concentration of acrylonitrile in water (assuming inherent rather than ready biodegradability), 0.071 $\mu g/m^3$ in air, 3.96 µg/kg in wet fish, $1.30 \cdot 10^{-4}$ µg/kg in meat, $1.66 \cdot 10^{-2}$ µg/kg in plant leaves and $1.30 \cdot 10^{-3}$ in milk. Results of modelling using the Mackay Level 3 model and the geophysical parameters for Germany predicted $1\text{-}4 \cdot 10^{-4}$ µg/kg in fish and vegetables, $2.37 \cdot 10^{-3}$ µg/l for drinking water, $5 \cdot 10^{-4}$ $\mu g/m^3$ for air. Actual monitoring data indicate that levels of acrylonitrile are below the limits of detection in both air and drinking water, while no data were found for levels of acrylonitrile in biota. A further indication of the low regional concentrations of acrylonitrile is provided by the estimated annual regional releases for inland European production and processing sites, as shown in **Table 3.6**. Using the 10% rule, an estimated 4.3 tonnes of acrylonitrile is released annually to water on a regional basis, and 90 tonnes to air (the latter figure representing releases from all sites, rather than inland only).

EUSES also provides values for daily human intake from the environment, as follows and as already shown in Section 3.1.7:

Daily intake through drinking water (mg/kg/day):	$8.01 \cdot 10^{-5}$
Daily intake through consumption of fish (mg/kg/day):	$6.51 \cdot 10^{-6}$
Daily intake through consumption of leaf crops (mg/kg/day):	$2.84 \cdot 10^{-7}$
Daily intake through consumption of root crops (mg/kg/day):	$7.12 \cdot 10^{-7}$
Daily intake through consumption of meat (mg/kg/day):	$5.61 \cdot 10^{-10}$
Daily intake through consumption of milk (mg/kg/day):	$1.05 \cdot 10^{-8}$
Daily intake through intake of air (mg/kg/day):	$1.52 \cdot 10^{-5}$
Regional total daily intake for humans (mg/kg/day)	$1.03 \cdot 10^{-4}$

In general the possibility of exposure via the food chain is very low as acrylonitrile will be extensively degraded following a short acclimation period in water, is degradable in air and soil and the measured logPow (octanol/water partition coefficient) is smaller than or equal to 0.3. Exposure of humans via the food chain is therefore anticipated to be extremely small and is not considered further in this risk assessment.

In relation to exposure via air, the average level in the atmosphere (Mackay level 3 model applied to Germany) was estimated to be about $5 \cdot 10^{-4}$ µg/m^3. If the inhalation volume is 20 m^3 per day, if 50% of the inhaled acrylonitrile is retained and if a body weight of 70 kg is assumed, this would cause a daily uptake of $7 \cdot 10^{-5}$ µg/kg body weight. This uptake is negligible compared to the estimated uptake from food packaging and acrylic fibres.

It is concluded from these results that the potential for secondary poisoning following exposure on the regional scale is very small. As discussed in Section 3.1.5.2, relating to the derivation of $PECregional_{air}$ and $PECcontinental_{air}$, however, these results are based on the point source emissions from the various production and processing plants in Europe as the input into EUSES. Diffuse emission sources such as cigarette smoke, loss of monomer from plastics and fibres during use and, in particular, vehicle exhausts will contribute additionally to the above levels in various biota and to daily human intake from these biota. This is in part counterbalanced by the fact that the input into EUSES includes some default emission scenarios, and the values cited above can be regarded as a reasonable estimate of indirect exposure via the environment on a regional scale.

In relation to exposure scenario (2) consideration of the emissions data from the European industry presented in Section 3 of the report and in Appendices A.1, A.2, A.3, A.4 indicate that predicted local concentrations in water courses in the immediate vicinity of plants for inland European production and processing sites range from 0 µg/l (the background regional level from EUSES being 0.003 µg/l) to 6 µg/l. Higher levels were predicted in the vicinity of coastal sites without WWTP discharging directly into the sea, as discussed in Section 3.

In the US some acrylonitrile-containing wastes are disposed of by deep-well injection. This leads to a theoretical risk of contamination of drinking water supplies in the local area. Stochastic modelling of flow and transport processes was undertaken by Rhee et al. (1993) in order to predict underground waste movement. The conclusion from this analysis was that acrylonitrile concentrations in excess of drinking water criteria ($5.8 \cdot 10^{-2}$ µg/l) were unlikely to be detected beyond the (assumed) low permeability confining layers after simulation for 10,000 years. Disposal by deep-well injection is not practised in Europe, although the US model provides reassurances about likely low levels of acrylonitrile in the vicinity of a local emission source.

Given the continuing degradation of acrylonitrile which is predicted to occur following initial release to local surface water and the lack of bioaccumulation potential, it is anticipated that levels of acrylonitrile in local biota will be extremely low. An assumption is made that they will be similar to those predicted on a regional scale by EUSES, as reflected above.

As indicated above, the estimated annual regional releases for European production and processing sites are significantly higher to air (90 tonnes) than to water (4.3 tonnes). This reflects relatively high releases to air for a number of companies, as shown in **Table 3.1** to **3.4** and Appendices A.1, A.2, A.3, A.4. This would indicate that concentrations of acrylonitrile in air in the vicinity of production or processing sites may be relatively high and could present a risk to the local population. Predicted environmental concentrations of acrylonitrile in air ranged from 0 to 0.24 mg/m^3, based on the reported emissions (Appendices A.3 and A.4), with the majority lying below 0.03 mg/m^3. Several companies for which emission data are presented in this report provided the results of fenceline monitoring data. For one such, the measured average fenceline concentration in 1995 was 0.6 $\mu g/m^3$, 95% confidence limit 2.5 $\mu g/m^3$, which compares well with the predicted levels shown for a number of companies in Appendices A.3 and A.4. Another company has carried out a detailed fenceline and community monitoring survey over a number of years up to 1990. The 1990 survey involved 180 samples and covered 18 weeks of operation. Of the 180 samples, only 2 were above the limit of detection of 0.8 ppb, at 1.5 and 7.5 ppb. (1.9 ng/m^3 and 16.8 ng/m^3), and these two samples were taken within the site fenceline, close to the tank farm and jetty area.

VROM (1984) reported emission and concentration levels of acrylonitrile in the vicinity of 8 acrylonitrile producing or consuming plants in the USA. An average acrylonitrile concentration of 0.25 mg/m^3 (0.12 ppm) was found at a distance of 0.5 km from one ABS/SAN resin producing plant. At a distance of 0.7 km the concentration was 0.09 mg/m^3 (0.04 ppm), while at a distance of over 1 km air levels of acrylonitrile were less than 0.0005 mg/m^3 (0.0002 ppm). Air concentrations of acrylonitrile in the vicinity of other plants were in general less than 0.01 mg/m^3 (0.005 ppm).

It can be concluded that people living close to or in the surroundings of acrylonitrile production or processing plants are exposed to low to negligible levels of acrylonitrile in the air.

4.1.2 Effects assessment: hazard identification and dose (concentration) – response (effect) assessment

4.1.2.1 Toxicokinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

The toxicokinetic profile of acrylonitrile following inhalation or oral administration has been evaluated in the rat by a number of groups, notably Kedderis et al., Ahmed et al. and Pilon et al. Toxicokinetics have additionally been studied following intravenous and intraperitoneal administration. Results show that acrylonitrile is extensively absorbed and distributed after all routes of administration, metabolised via direct conjugation with glutathione or via cytochrome P450-mediated oxidation followed by urinary excretion of a range of metabolites including thiocyanate, cyanide and N-acetyl-S-(2-cyanoethyl)cysteine (2-cyanoethylmercapturic acid, CMA).

Toxicokinetics of acrylonitrile in the rat following oral administration

Absorption and distribution

Much of the information available on the toxicokinetics of acrylonitrile has been derived from oral gavage studies in the rat, with comparatively little information being available for other species. As indicated above, results show that acrylonitrile is extensively absorbed and widely distributed following oral administration. Kedderis et al. (1993a) demonstrated absorption of approximately 95-98% of an administered oral dose, while the work of Ahmed (1982) and others indicates that peak blood levels are achieved within 3 hours of oral administration (see also **Table 4.9** below). Gut et al. (1981) examined acrylonitrile concentrations in blood and liver after oral administration, and determined half-lifes of 61 and 70 mins, respectively. Higher levels in these tissues were achieved after i.v. and i.p. administration than after oral dosing, with a rapid decrease of initial high concentrations, giving a $t_{0.5}$ of 19 minutes for blood and a $t_{0.5}$ of 15 minutes for liver. However, the difference in the half-life findings between oral versus i.v. or i.p. administration appears to reflect slower absorption following oral administration rather than slow elimination.

Sandberg and Slanina (1980), using the oral route of administration, showed that acrylonitrile and/or its metabolites accumulated in the liver, kidney, intestinal mucosa, adrenal cortex, and blood. However most of the radioactivity was irreversibly bound to proteins (Peter and Bolt, 1981), making it difficult to determine whether the high levels of [^{14}C]-radioactivity in various tissues were due to free acrylonitrile, its metabolites or cyanoethylated proteins. Nerudova et al. (1981) suggested that free acrylonitrile is relatively uniformly distributed and that the higher concentrations of radioactivity seen in some organs and erythrocytes are related to reaction products of acrylonitrile with soluble and protein sulphydryls.

The tissue distribution, elimination and covalent binding of [1-^{14}C]-acrylonitrile have been investigated in a well-conducted study in the rat by Ahmed and co-workers (Ahmed et al., 1982; 1983a). Adult male Sprague Dawley rats (n = 3/group/time interval) given a single oral dose of 46.5 mg/kg of [1-^{14}C]-acrylonitrile and monitored over a 10-day period excreted 40% of the radiolabel in urine, 2% in faeces, 9% in expired air as $^{14}CO_2$, 0.5% as $H^{14}CN$ and 4.8% as unchanged acrylonitrile in 24 hours. Bile flow increased by 3 times after the administration of acrylonitrile and over a period of 6 hours. 27% of the ^{14}C was recovered in bile. Total excretion at the end of 10 days was approximately 75% of the initial dose, indicating a retention of approximately 25% of the dose.

Ahmed et al. showed that the highest initial concentration of radioactivity occurred in the stomach and intestines. High concentrations were found in liver, kidney and lung tissues up to 24 hours after administration. Heart, thymus, spleen, adrenals, brain and skin showed maximum concentrations between 3 and 6 hours, followed by a gradual decrease in radioactivity. Highest levels of [1-^{14}C]-acrylonitrile up to 72 hours were found in the G.I. tract, suggesting a possible resecretion process of acrylonitrile metabolites into the stomach or of binding of acrylonitrile, cyanide or other metabolites within the stomach mucosa. The levels of unbound radioactivity declined progressively with time in the various organs studied, although significant retention in red blood cells was noted for up to 10 days after the initial administration. In contrast covalent binding of radioactivity as a percentage of total radioactivity increased concomitantly, most of the covalently bound radiolabel being located in non-cytosolic cell fractions (nuclear, mitochondrial and microsomal fractions). The authors suggested that extensive interactions with gastric macromolecules could play a possible role in the development of tumours, ulcers and acrylonitrile-induced gastrointestinal bleeding reported by these and other authors, however no attempt was made to identify possible

DNA adducts. **Table 4.9** summarises tissue levels of radioactivity from [1-^{14}C]-acrylonitrile found by Ahmed et al. in key target organs of rats given a single oral dose of 46.5 mg/kg.

Table 4.9 Tissue levels of radioactivity in rats given a single oral dose of 46.5 mg/kg [1-^{14}C]-acrylonitrile

Tissue	[1 -^{14}C]-acrylonitrile, ng equivalent/mg protein [1]								
	1 hr	3 hr	6 hr	12 hr	24 hr	48 hr	72 hr	168 hr	240 hr
Expired air [2]	16.9+1.8	31.4+2.9	93.9+15	112.5+17	838.8+87	11.2+1.1	*	*	*
Blood [3]	79.5+8.0	90.0+9.1	71.7+7.2	63.0+7.0	46.6+5.4	28.7+4.0	28.7+7.0	21.5+ 27	17.9+1.3
Stomach	513.7+71	367.6+34	332.8+36	379.9+41	362.9+31	204.6+25	118.2+12	22.2+0.9	4.2+0.5
Liver	92.1+5.7	87.5+2.2	74.9+2.0	62.0+0.2	56.8+0.8	32.3+2.6	17.7+1.7	8.6+1.3	2.9+0.2
Kidney	74.2+2.4	76.5+1.3	72.0+4.2	63.3+0.5	48.1+0.4	26.2+3.4	14.1+2.8	6.85+0.5	3.95 +0.7
Lung	39.5+5.6	53.7+3.1	64.1+5.3	27.4+0.3	22.9+0.5	27.6+4.1	17.2+1.3	9.7+0.3	0.4+0.1
Heart	25.4+3.8	28.6+1.8	35.8+1.5	21.8+0.2	21.1+0.1	14.6+1.7	13.6+1.0	5.4+0.8	2.9+0.5
Thymus	17.9+2.9	25.6+2.0	27.7+2.5	20.8+0.1	21.1+0	14.0+0.8	7.5+2.2	3.6+0.5	1.8+0.4
Spleen	29.0+2.5	36.2+1.9	48.0+4.4	31.7+0.1	21.8+0.2	16.9+1.2	13.1+0.8	9.3+0.5	7.5+1.5
Adrenals	26.5+0.6	35.1+4.0	37.5+3.5	28.7+0.2	21.4+0	14.3+1.7	12.1+2.0	4.7+0.1	4.43+1.0
Brain	16.1+2.2	15.7+1.8	16.1+2.4	11.5+0.1	10.7+0.1	8.6+1.0	7.7+0.3	1.8+0	1.8+0.1
Skin	35.1+3.3	54.1+6.1	60.5+4.3	31.8+0.2	32.1+0.4	37.5+4.4	28.6+1.6	33.0+ 1.2	18.1+ 3.5

1) Values are means + S.E. of 3 animals
2) Expressed as µg equivalents in total ^{14}C CO_2 and ^{14}C HCN
3) µg equivalents/ml
* Values less than 0.05

Pilon et al. (1988a) observed that glutathione depletion resulted in greater uptake of a single oral dose of 4 mg/kg [2,3-^{14}C]-acrylonitrile into target organs (brain, stomach, liver, kidney and blood) of adult male Fischer 344 rats compared with non-GSH-depleted rats, monitored over a 24-hour period. Metabolism to 2-cyanoethylene oxide (CEO) and urinary excretion of thiocyanate (see “Metabolism”) were both increased by 300%. Glutathione depletion was achieved using a combined phorone/buthionine sulfoximine treatment (300 mg/kg and 2 mmol/kg, respectively) given 30 minutes prior to acrylonitrile exposure. In addition to increasing the uptake of radioactivity into these tissues, glutathione depletion caused an increase in covalently bound radioactivity between 6 and 24 hours after dosing. Pilon et al. suggested that glutathione could play a role in the extent of metabolism of acrylonitrile to the key metabolite 2-cyanoethylene oxide (CEO) by the cytochrome P450 system and hence the distribution of acrylonitrile-derived species to tissue macromolecules and nucleic acids.

Metabolism

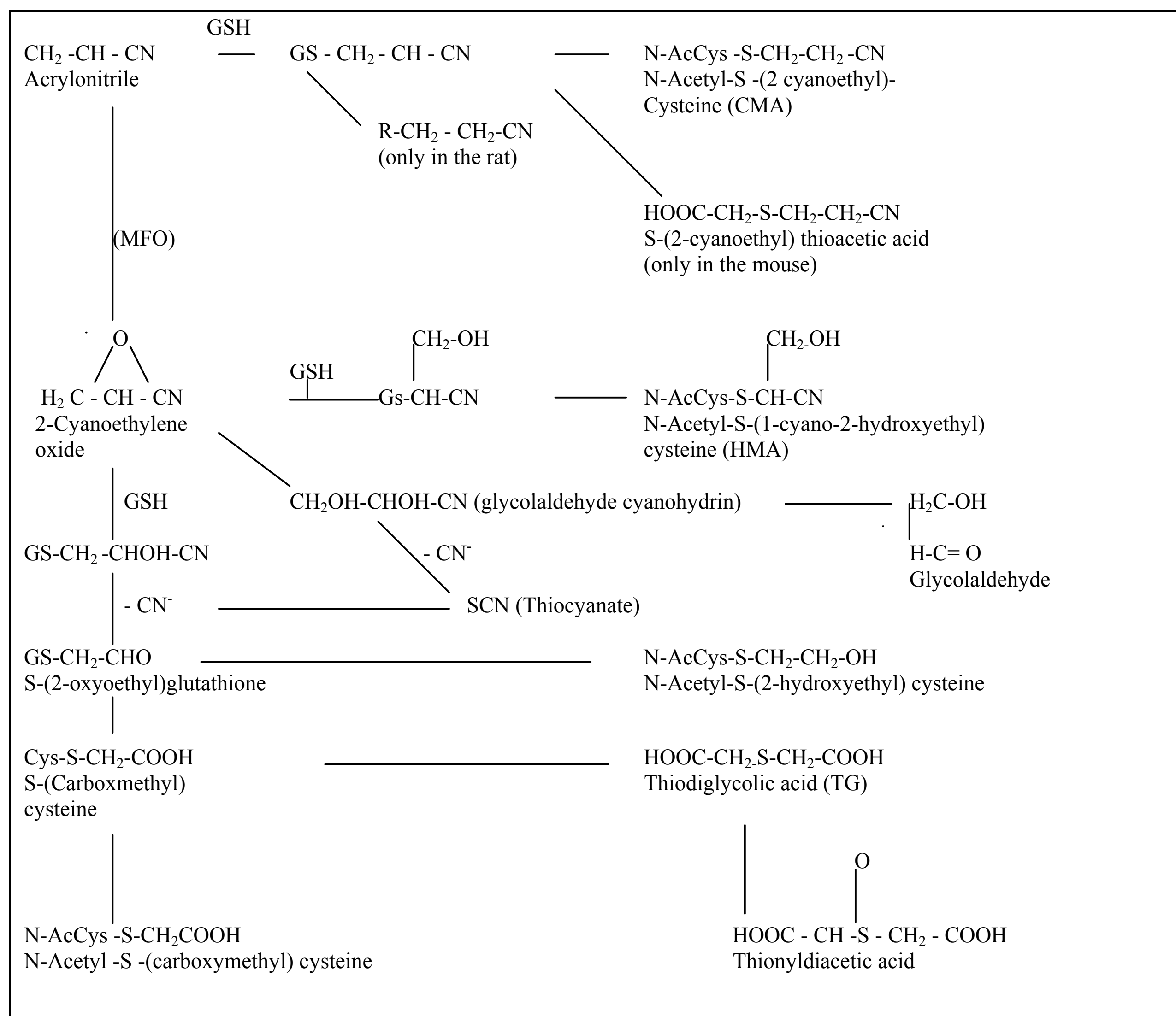
Following oral administration, a number of investigators (e.g. Dahl and Waruszewski, 1989; Fennell et al., 1991; Kedderis et al., 1993a; Burka et al. 1994; Gargas et al., 1995) have shown that acrylonitrile is metabolised by two pathways as follows:

1. direct conjugation with glutathione (GSH), either with or without catalysis by glutathione transferases and,
2. cytochrome P450-mediated oxidation to cyanoethylene oxide (CEO).

The oxidation of acrylonitrile to CEO can be considered an activation step, while conjugation of acrylonitrile or CEO with GSH can be considered as a detoxification step. The balance between these two processes may play a role in the carcinogenic susceptibility of different animal species. The predominant biotransformation pathway appears to be dependent on the systemic dose.

Figure 4.1 provides an overview of the various metabolic pathways for acrylonitrile.

Figure 4.1 Biotransformation of acrylonitrile (BUA, 1995)



GSH = Glutathione; MFO = Mixed -functional oxidases

In relation to pathway (1), conjugation of acrylonitrile with glutathione, it appears that this can occur non-enzymatically via a Michael reaction or via catalysis by GSH S-transferase. This reaction has been proposed to be responsible for the observed depletion of GSH from various tissues (brain, lung, liver, kidney, stomach, erythrocytes) after acrylonitrile administration (Cote et al., 1984; Gut et al., 1985). The binding of GSH and other protein sulphydryls by acrylonitrile and CEO is apparently responsible for inhibition of various -SH dependent enzymes following acrylonitrile administration. Prolonged failure to maintain adequate levels of intracellular glutathione results in impairment of redox processes in the cell and a increased binding of acrylonitrile and its epoxide to macromolecular structures such as cell proteins and nucleic acids,

as shown by the work of Pilon et al. (1988a). Exogenous thiols e.g. cysteine and N-acetylcysteine compete with GSH and endogenous thiols for acrylonitrile, giving some protection against the toxicity of acrylonitrile (Buchter et al., 1984; Benz et al., 1990).

A number of workers have shown that the major metabolite of acrylonitrile in the rat, rabbit and other animals is N-acetyl-S-(2-cyanoethyl)cysteine, following oral administration of acrylonitrile and conjugation with GSH (Dahm, 1977; Ahmed and Patel, 1979; Van Bladeren et al., 1981; Ghanayem and Ahmed, 1982). Langvardt et al. (1980) found a total of seven radioactive metabolites in rat urine, with 3 major metabolites (including thiocyanate and the N-acetyl-S-(2-cyanoethyl)cysteine, and possibly 4-acetyl-5-cyanotetrahydro-1,4-2H-thiazine-3-carboxylic acid). The chemical structures of the 4 remaining metabolites, representing one third of the total activity excreted, were not identified, but none contained the -CN group of acrylonitrile.

Fennell et al. (1991) and Kedderis et al. (1993a) determined the urinary metabolite profile of [2,3-^{14}C]-acrylonitrile over 72 hours following gavage administration of single doses ranging from 0,09 to 28.8 mg/kg to male F-344 rats and reported that metabolites arising from conjugation with GSH represent approximately 85% of all urinary metabolites. These workers identified 5 major components which accounted for 75-100% of urinary radioactivity. In relation to pathway (1) above, direct conjugation of acrylonitrile with GSH, Kedderis et al. observed that the excretion of N-acetyl-S-(2-cyanoethyl)cysteine and -S-(2-cyanoethyl)thioacetic acid, both derived from the GSH conjugate of acrylonitrile, increased non-linearly with dose suggesting the existence of a saturable pathway which competes with GSH for acrylonitrile. Kedderis et al. suggested that this was most likely to be the cytochrome P450-dependent pathway (2) above.

Similarly, a number of investigators have shown that in the case of a short-term peak dose following oral gavage (also following intravenous or intraperitoneal administration), resulting in saturation of the cytochrome P450-dependent pathway, metabolism to N-acetyl-S-(2-cyanoethyl)cysteine (pathway (1)) appears to predominate, however in the case of low oral doses (e.g. in dietary administration or administration in drinking water), or in the case of exposure to low levels of acrylonitrile by inhalation, pathway (2) via cyanoethylene oxide is more predominant (Müller et al., 1987; Kedderis et al., 1993a). Depletion of glutathione also results in a shift in the metabolic pathway from pathway (1) to pathway (2) (Pilon et al., 1988a).

The cytochrome P450-dependent pathway of acrylonitrile biotransformation includes a number of consecutive enzyme- catalysed or spontaneous reactions. Studies of the biotransformation of acrylonitrile point to an epoxidation of the vinylic double bond by the cytochrome P450 system to give the epoxide 2-cyanoethylene oxide (CEO). Both the parent molecule (acrylonitrile) and CEO are electrophilic and are reactive towards glutathione and other nucleophilic sites of tissue macromolecules. CEO is mutagenic (see Section 4.1.2.7) and has been hypothesised to be responsible for the carcinogenic activity of acrylonitrile since it reacts with DNA much more readily than the parent compound (Roberts et al., 1991). The liver is the major site of CEO formation and evidence suggests that CEO is then transported from the liver to target organs via the blood stream. However other organs such as the lung and the kidney are also metabolically capable/competent, giving rise to the possibility that CEO may also be formed in situ in organs other than the liver. Roberts et al. (1989) reported that rat lung cells were capable of metabolising acrylonitrile to the epoxide CEO. However the capacity to carry out this metabolism did not appear to be evenly distributed throughout the various lung cell types i.e. metabolic capacity appeared to be up to 7 times greater in the Clara cell-enriched fraction than in any other cell fraction.

CEO is in turn metabolised by conjugation with GSH at either the 2- or the 3- position of the molecule, as shown in **Figure 4.1**. The GS-2-CEO conjugate is further metabolised to the mercapturic acid N-acetyl-S-(1-cyano-2-hydoxyethyl)cysteine (Fennell et al., 1991), while the GS-3-CEO conjugate is metabolised to inorganic cyanide (CN) and ultimately excreted as thiocyanate following transformation by rhodanase. Oral administration of acrylonitrile to Wistar rats resulted in urinary excretion of 20 % of the dose of acrylonitrile as thiocyanate (Gut et al., 1975), while Lambotte-Vandepaer et al. (1985) observed an excretion of approximately 23% of the oral dose as thiocyanate. The relative proportion of the 2-GS and the 3-GS conjugate formed determines the amount of cyanide released, and differences in these pathways have been suggested to be responsible for differences in the acute toxicity of acrylonitrile in different species. Thus, Fennell et al. (1991) reported that more CEO was metabolised to cyanide in the mouse than in the rat, and that the acute toxicity of acrylonitrile was greater in the mouse than in the rat. Kedderis et al. (1993b) examined the tissue distribution of [2,3-^{14}C]-CEO in the tissues of F-344 rats and found wide distribution of radioactivity 2 hours after administration, with highest levels in the intestines, stomach and liver. By 24 hours, radioactivity had decreased by 70-90% in all tissues, with 53-64% of the total dose being excreted in urine.

Excretion

Despite its high volatility only about 5% of the total dose of acrylonitrile administered is estimated to be exhaled unchanged (Farooqui and Ahmed, 1983a). Ahmed et al. (1983a) showed that the net amount of unchanged acrylonitrile eliminated in expired air in Sprague Dawley rats (n=3) given a single oral dose of 46.5 mg/kg of [1-^{14}C]-acrylonitrile reached a peak at 30 minutes after dosing. Thereafter it rapidly decreased, not being detectable 2.5 hours after treatment. Although only 2.5% of radioactivity was exhaled as trapped $^{14}CO_2$ during the first 12 hours, a maximum of 9% of the dose was recovered as $^{14}CO_2$, 0.5% as $H^{14}CN$ and 4.8% as unchanged acrylonitrile in 24 hours.

Urinary excretion is the major excretory route for acrylonitrile, administered by the oral and other routes, with only about 3-8% of a given dose being excreted in the faeces (Ahmed et al, 1983a; Tardif et al, 1987; 1988; Kedderis et al, 1989; 1993a). The bulk of the urinary excretion takes place in 24 hours. Ahmed et al. showed that Sprague Dawley rats excreted 40% of the radiolabel in urine and 2% in faeces in 24 hours, in addition to the 14.3% excreted in expired air as described above. These workers also showed that the CO_2 excreted via respiratory air is mainly from the intermediate cyanide, by varying the position of the radiolabel within the molecule using 1-^{14}C- acrylonitrile and 2,3-^{14}C- acrylonitrile. The total excretion of radiolabel at the end of 10 days was approximately 75% of the initial dose administered, indicating a retention of about 25% of acrylonitrile either bound to macromolecules or in the form of non-excretable conjugates. Burka et al. (1994) administered 46 mg/kg of [2-^{14}C]-acrylonitrile by gavage to groups of 3 male F-344 rats and observed that after 24 hours 10. 7 + 0,8% had been excreted as CO_2, 2.0 + 0.4% as volatiles, 67.0 + 2.2% in the urine, 11.4 + 0.6% in faeces, with 9.8 + 0.2% remaining in blood and 4.1 + 0.02% in the tissues. The identity of the various urinary metabolites has been discussed in the paragraph “Metabolism” above.

Toxicokinetics of acrylonitrile in the rat following exposure by inhalation

Toxicokinetics data in the rat following exposure by inhalation overall support the picture seen after oral administration of acrylonitrile, although there are quantitative differences in the metabolic profile following inhalation versus oral administration.

Peter and Bolt (1984) exposed rats (no details of number and sex provided) to a concentration of 1,800 ppm over a 5-hour period and reported a biphasic exponential uptake of acrylonitrile from the exposure chamber. They calculated an almost quantitative retention of acrylonitrile (91.5%), with exhalation of only 8.5% of the inhaled dose.

Pilon et al. (1988b) also demonstrated biphasic uptake of [2,3-^{14}C] acrylonitrile in male F-344 rats, using exposure levels of 25-750 ppm, characterised by a dosage-independent rapid phase lasting for approximately 60 minutes and a subsequent slow phase which lasted from 60 minutes to the end of exposure. The rate of uptake for both phases was linearly related to the initial concentration of acrylonitrile in the chamber. Uptake was assessed by measurement of the concentration in the exposure chamber over a 240-minute period. Using the rate of the uptake curve for the rapid phase, Pilon et al. estimated a rate of 4.82 mg/kg/hr uptake at an exposure level of 100 ppm.

Pilon et al. showed that glutathione (GSH) depletion resulted in an increase in the rate of acrylonitrile uptake via inhalation, in both rapid and slow phases. Glutathione depletion was achieved using a combined phorone/buthionine sulfoximine treatment (300 mg/kg and 2 mmol/kg, respectively) given 30 minutes prior to acrylonitrile exposure. As with the oral study reported in the paragraph “Absorption and distribution” above, these workers also investigated the role of GSH in acrylonitrile metabolism and binding to macromolecules following exposure by inhalation of 100 ppm, equivalent to the 4 mg/kg oral dose. They showed that depletion of GSH resulted in a decrease in total radioactivity recovered in the brain, stomach, liver, kidney and blood and a concomitant decrease in the acrylonitrile-derived nondialysable radioactivity in these organs. In non-GSH-depleted rats, accumulation of radiolabel was greatest in brain RNA, but no radioactivity was detected in DNA of any organ examined. In GSH-depleted rats, the radiolabel concentration was higher in the brain RNA than in liver or stomach RNA, but was also 50% lower than that observed in brain RNA of non-GSH-depleted rats. Urinary excretion of thiocyanate (SCN^-), derived from the epoxide pathway of acrylonitrile metabolism, was doubled in GSH-depleted rats compared with non-depleted rats.

Young et al. (1977) exposed rats to ^{14}C acrylonitrile at levels of 5 or 100 ppm (11 or 220 mg/m^3) for 6 hours in a “nose only” chamber with a view to determining the recovery rate of acrylonitrile. During the first 9 days from the start of inhalation 82.2% and 68.5% was recovered in the urine for the high and lower doses, respectively, with 3-4% in the faeces and 6% and 2.6%, respectively as expired $^{14}CO_2$.

Exposure by inhalation resulted in urinary excretion of 16 % of the inhaled dose of acrylonitrile as thiocyanate (Gut et al., 1985). This was later confirmed by Tardif et al. (1987) who reported a urinary excretion of 15 % of the inhaled acrylonitrile dose as thiocyanate in Sprague-Dawley rats.

Tardif et al. (1987) examined the formation of the urinary metabolites N-acetyl-S-(2-cyanoethyl)cysteine (2-cyanoethylmercapturic acid, CMA), N-acetyl-S-(1-cyano-2-hydroxyethyl)cysteine (2-hydroxyethylmercapturic acid , HMA) and thiocyanate (SCN^-), in groups of 5 adult male Sprague Dawley rats exposed to 0, 4, 20 or 100 ppm acrylonitrile for 6 hours, and showed that thiocyanate was the major metabolite following inhalation exposure, with levels of excreted HMA being higher than levels of CMA. The latter represented only 8% of total urinary metabolites. As exposure levels of acrylontrile increased, excretion of thiocyanate became relatively more important, as shown by the ratio of excreted SCN^- to the sum of CMA and HMA, rising from 0.47 at 4 ppm to 0.89 at 20 ppm and 2.93 at 100 ppm. This finding paralleled the observations in the work of Kedderis et al. (1993a) of a non-linear increase in excretion of N-acetyl-S-(2-cyanoethyl)cysteine and -S-(2-cyanoethyl)thioacetic acid, both

derived from the GSH conjugate of acrylonitrile, with dose. Tardif et al. suggested however that the saturation of the cytochrome P-450 pathway occurred at much higher levels in Sprague-Dawley rats and that the pathway via cyanoethylene oxide was the major metabolic route for inhaled acrylonitrile up to 100 ppm.

Müller et al. (1987) investigated the quantitative urinary excretion of the acrylonitrile metabolites 2-cyanoethylmercapturic acid (CMA) and 2-hydroxyethylmercapturic acid (HMA) and also the metabolite S-carboxymethyl cysteine, its further metabolite thiodiglycolic acid, together with unchanged acrylonitrile in groups of 4 adult male Wistar rats exposed to 0, 1, 5, 10, 50 or 100 ppm acrylonitrile for 8 hours. These workers showed a dose-related increase in excretion of unchanged acrylonitrile in urine collected during the 8-hour exposure period, indicative of saturation of the metabolic processes for acrylonitrile. Mean levels at 100 ppm were 25 µmol/ml compared with 1 µmol/ml at 10 ppm. However in the subsequent 24-hour post-exposure period levels of unchanged acrylonitrile in urine fell to very low levels in all groups (mean 1.6 µmol/ml in the 100 ppm group).

2-cyanoethylmercapturic acid (CMA) was the predominant urinary metabolite during the 8-hour exposure period (mean 53.6 µmol/ml at 100 ppm), increasing with exposure level and with excretion reducing in the subsequent 24 hours (22.7 µmol/ml). In contrast more of the metabolite 2-hydroxyethylmercapturic acid (HMA) was excreted in the 24-hour post-exposure period than during the 8-hour exposure period (mean 4.7 µmol/ml at 100 ppm in the post exposure period compared with 2.7 µmole/ml during the exposure period). The authors detected lower levels of the metabolite S-carboxymethyl cysteine (2.43 µmol/ml at 100 ppm during the 8-hour exposure period, falling to 1.2 µmol/ml in the post exposure period, while levels of thiodiglycolic acid increased in the post exposure period (3.2 µmol/ml at 100 ppm, compared with 2.7 µmol/ml during the exposure period. They suggested that CMA was the most sensitive indicator metabolite of acrylonitrile exposure, noting from their previous work (Müller et al., 1980) that acrylonitrile is metabolised to a lesser extent in humans than in rodents and that HMA is only a minor metabolite in humans.

Toxicokinetics of acrylonitrile in the rat following exposure by other routes

Dermal

There are no specific toxicokinetics studies using dermal administration.

Intravenous or intraperitoneal

Silver et al. (1987) administered 100 mg/kg of [1-^{14}C] acrylonitrile to rats intravenously. All rats survived this dose but showed clinical cholinomimetic signs. Total radioactivity in tissues was determined at 15, 30, 60 or 90 minutes after dosing. Levels were found to be highest in the blood, liver, duodenum, kidney and adrenals 15-90 min post-exposure. Except for blood, the concentration of total radiolabel remained constant or declined with time over the 90-minute period post-injection. Total radiolabel increased in the blood during this period.

Tardif et al. (1987) examined formation of the urinary metabolites N-acetyl-S-(2-cyanoethyl)cysteine (2-cyanoethylmercapturic acid, CMA), N-acetyl-S-(1-cyano-2-hydoxyethyl)cysteine (2-hydroxyethylmercapturic acid, HMA) and thiocyanate (SCN^-) in groups of 5 adult male Sprague Dawley rats given a single dose of 0.6-15 mg/kg i.v. or i.p., and showed that CMA represented 74-78% of total urinary metabolites. In contrast levels of thiocyanate excreted were low. They suggested that conditions favouring rapid entry of substantial quantities of acrylonitrile

facilitate direct conjugation with glutathione to give rise to the mercapturic acid retaining the cyanide moiety (CMA) rather than resulting in direct release of cyanide.

Influence of route on the toxicokinetics of acrylonitrile

Although absorption and distribution of acrylonitrile is broadly similar following different routes of exposure, there are differences in the metabolic profile. These differences may be attributable to the presence of acrylonitrile-metabolising enzymes in the liver and the first pass effect following oral administration. Rats given an equivalent dose of acrylonitrile (4 mg/kg) by oral administration or inhalation excreted 16% and 27% of the dose as SCN (thiocyanate), respectively (Pilon, 1988 a; 1988b). CEO is an obligatory intermediate in the formation of SCN and therefore SCN excretion data suggest that a greater percentage of inhaled acrylonitrile relative to orally administered acrylonitrile is metabolised to CEO *in vivo*. In the studies of Pilon et al. (1988a; 1988b), using GSH-depleted rats (phorone/buthionine sulfoximine treatment) it was revealed that the depletion caused an increase (37 to 92%) in radiolabel irreversibly associated with macromolecules in all tissues after gavage but a decrease (30 to 53%) after inhalation exposure.

Following oral administration, Kedderis et al. (1993a) found a linear relationship between the excretion of mercapturic conjugates and the administered amount of acrylonitrile up to a dose level of 26.5 mg/kg of body weight. However at higher doses, the amount of mercapturic acids remained constant, which appeared to be a direct result of a depletion of available glutathione.

In the study of Tardif et al. (1987) in Sprague Dawley rats exposed to 0, 4, 20 or 100 ppm acrylonitrile for 6 hours or given a single dose of 0.6-15 mg/kg i.v. or i.p., 2-cyanoethylmercapturic acid (CMA), the end metabolite of the GSH conjugation pathway, represented 74-78% of the urinary metabolites when acrylonitrile was administered i.p. or i.v., but only 8% when given by inhalation. They suggested that conditions favouring rapid entry of substantial quantities of acrylonitrile facilitate direct conjugation with glutathione to give rise to the mercapturic acid retaining the cyanide moiety (CMA) rather than resulting in direct release of cyanide. However the data in the inhalation study were obtained from measurement of the metabolite in post exposure urine only, which may have resulted in a low estimate of CMA.

In contrast, Müller et al. (1987) estimated that up to 46% of an inhaled dose in rats may be CMA, excreted mainly during an 8-hour exposure period, although significant amounts were still excreted in the 24 hours after exposure (see Paragraph “Toxicokinetics of acrylonitrile in the rat following exposure by inhalation”).

Interspecies variation in toxicokinetics of acrylonitrile

Although the majority of the toxicokinetic data have been obtained in the rat, some studies have compared toxicokinetics in the mouse with those in the rat (Roberts et al., 1991; Kedderis et al., 1993a; 1995). The results have reflected a species difference with respect to the type and quantities of specific metabolites formed in the lung and liver. Evaluation of the kinetic parameters suggests that mouse lung and liver microsomes are more metabolically active than those of the rat. This difference is due partly to the fact that the mouse has more cytochrome P-450/mg of lung and liver tissue than the rat.

Species differences in the steady state level of cyanoethylene oxide may explain some species differences in the toxicity of acylonitrile. Roberts et al. (1991) studied the formation of cyanoethylene oxide (CEO) from acrylonitrile by subcellular liver fractions of mice, rats and

humans. Liver subcellular fractions from mice converted acrylonitrile to CEO 4 times faster than those of rat, and in turn liver fractions of rats were 1.5 times faster than those of humans.

Mice appear to excrete a higher percentage of a dose of acrylonitrile as thiocyanate compared with rats following all routes of administration. Kedderis et al. (1993b) reported a value of 0.67 for the ratio of acrylonitrile epoxidation to GSH conjugation in male B6C3F1 mice, compared with 0.5 in male F-344 rats, both species having been dosed orally by gavage with approximately 10 mg/kg [2,3-^{14}C]-acrylonitrile. Kedderis also reported that excretion of thidiglycolic acid (derived from the epoxidation pathway) was 10 times higher in the mouse than in the rat, although the toxicological significance of this finding is not known.

Kedderis et al. (1995) and Roberts et al. (1991), using microsomal fractions from rats, mice and humans, have also examined *in vitro* the extent of detoxification of acrylonitrile itself and the metabolite CEO, considering both GSH conjugation following hydrolysis by epoxide hydrolase and spontaneous hydrolysis. In microsomal liver fractions it was observed that subcellular liver preparations from mice detoxified acrylonitrile by GSH conjugation twice as fast as rat or human subcellular liver preparations. The latter two detoxified acrylonitrile at similar rates. Conjugation of CEO with GSH was more or less comparable in rat and mice and 1.5 times faster in human than in rat and mice. These results suggest that:

- mice detoxify acrylonitrile by conjugation with GSH twice as fast as rats and humans, and
- humans detoxify CEO by conjugation with GSH 1.5 times faster than rats and mice.

Kedderis et al. (1995) estimated the steady state level of CEO in mice, rats and humans, based on the integration of the formation rate of cyanoethylene oxide and the detoxification of acrylonitrile and CEO by conjugation with GSH. Results are shown in **Table 4.10**, in which the overall estimate of the steady state level of CEO and the other parameters are expressed as a ratio of the activity in rats or mice to that in humans.

Table 4.10 Comparison of the formation of CEO, the conjugation of acrylonitrile and CEO with glutathione and the steady state level of cyanoethylene oxide (CEO) in rats, mice and humans expressed as a ratio of the activity in mouse or rat to that in human

Reaction	Mouse	Rat	Human
Formation of CEO	x 6	x 1.5	1
Detoxification of acrylonitrile by conjugation with GSH	x 2	x 1	1
Detoxification of CEO by conjugation with GSH	x 0.67	x 0.67	1
Overall estimate of the steady state level of CEO	x 4.5	x 2.25	1

On the basis of the above reasoning, the CEO-level in mice is expected to be twice the level in rats. However Roberts et al. (1991) observed that following a single oral dose level of 4 mg/kg in B6C3F1 mice the blood level of CEO at 0.5, 1, 4 or 24 hours was consistently lower than in F 344 rat blood during the absorption, metabolism, and elimination of acrylonitrile. The magnitude of the difference was approximately 3 times.

The results of Roberts et al. (1991) and Kedderis et al. (1995) indicate that species differences do exist in the metabolism of acrylonitrile, which may explain some of the species differences in the toxicity and carcinogenicity. A definitive picture of the metabolism of acrylonitrile in rats and mice compared with humans cannot be established, given the different conclusions which can be

drawn from the *in vitro* data of Kedderis et al. and Roberts et al. (1991), using microsomal fractions from rats, mice and humans and the actual levels of CEO in blood provided by Roberts et al. It must be recognised, however, that the use of microsomal liver fractions may not be the most appropriate test system to compare the metabolic rate of activation and detoxification of acrylonitrile *in vivo. O*ne of the identified problems is the reactivity of acrylonitrile with tissue macromolecules, which is not fully considered in *in vitro* systems and the reversibility of the reaction with at least some tissue macromolecules which may result in the re-release of acrylonitrile into the blood.

In summary, in relation to the influence of inter-species variation in interpretation of the Margin of Safety (MOS) for repeated dose toxicity (Section 4.1.3), the above data indicate that mice excrete a higher percentage of administered acrylonitrile as thiocyanate (and hence metabolise more to cyanide, related in turn to a higher rate of formation of CEO than for rats or humans). The results of Kedderis et al. (1995), based on *in vitro* studies on rat, mouse and human microsomal fractions indicate that the rate of conjugation of either ACN or CEO with GSH is lower in humans than in either rats or mice, but that hydrolysis of CEO by epoxide hydrolase is very high, while this detoxification pathway is apparently absent in rodents. This indicates that CEO is detoxified by GSH in rats or mice, but predominantly by epoxide hydrolase in humans. The metabolite from this latter pathway, glycolaldehyde cyanohydrin, $CH(OH_2)$-CH(OH)-CN, is rapidly converted to hydoxyacetaldehyde and hydrogen cyanide, as shown in **Figure 4.1**.

Physiologically based dosimetry description for acrylonitrile in rats

The information developed on the metabolism and disposition, haemoglobin binding, and other macromolecular interactions of acrylonitrile has been used to develop a physiologically based dosimetry description for acrylonitrile in rats (Gargas et al., 1995). The oral absorption rate constant for acrylonitrile was determined from oral bolus pharmacokinetic studies (Kedderis et al., 1996). Sensitivity analysis of the dosimetry description indicated that the inhalation exposure route was much more sensitive to changes in metabolic and physiological parameters than either i.v. or oral bolus routes. Therefore inhalation pharmacokinetic data were obtained and compared to simulations of the dosimetry description. Rats were exposed to 186, 254 or 291 ppm acrylonitrile for 3 hours, and acrylonitrile and 2-cyanoethylene oxide concentrations were measured in blood, brain, and liver at selected post exposure time points. The dosimetry description accurately simulated the acrylonitrile inhalation pharmacokinetic data, providing verification of the parameter estimates (Kedderis et al, 1996). The verified rat dosimetry description for acrylonitrile and 2-cyanoethylene oxide could potentially be used as the basis for development of a dosimetry description for humans.

4.1.2.1.2 Studies in humans

Few data are available relating to the toxicokinetics of acrylonitrile in humans. The possibility of release of cyanide in humans following exposure to acrylonitrile is also recognised. Following an accident in which a man was fully covered with liquid acrylonitrile, (Vogel and Kirkendall, 1984), the victim had to be treated with cyanide antidotes over a period of 3 days, implying that, the metabolic pathway via cyanoethylene oxide exists in humans. Exposure in this accident involved the dermal route but the victim almost certainly also inhaled acrylonitrile and possibly even swallowed a quantity. This accident is discussed in further detail in Section 4.1.2.2.2.

The retention of acrylonitrile in the respiratory tract in 3 human volunteers exposed to a concentration of 20 mg/m^3 (9 ppm) for up to 4 hours was 46% and did not change throughout the

inhalation period (WHO, 1983). Jakubowski et al. (1987) exposed 5 male volunteers to a mean acrylonitrile concentration of 9.0 mg/m^3 (4.1 ppm) for a period of 8 hours. An average retention of 51.8% was found with no information being given about the activity during exposure.

In humans acrylonitrile is partly metabolised to thiocyanate. Blood thiocyanate levels in volunteers exposed to acrylonitrile concentrations below 45 mg/m^3 (22 ppm) for 30 minutes returned to normal within 24 hours, while elevated levels were still present 12 hours after exposure to 110 mg/m^3 (50 ppm) for 30 minutes (Wilson and McCormick, 1949). Urinary concentrations of CMA ranged from 50 to 200 ng/l in 13 workers exposed to airborne acrylonitrile concentrations between 3 and 10 ppm. Jakubowski et al. (1987) exposed 6 male volunteers to an acrylonitrile concentration of 5.0 ppm for 8 hours, (52 % retention). Five of them were also exposed to 2.6 ppm (the time period between these two exposures was not given). At the lowest level, 16.3% (range 12.8 to 20.8%) of the retained dose was excreted as CMA in the urine, while at the higher level this percentage was 26.4% (range 19.3 to 38.7%). Large inter-individual differences were observed as to the time period of maximum excretion. The half-life value for urinary CMA was approximately 9 hours. In a study by Sakurai et al. (1978), average urinary concentrations of acrylonitrile and thiocyanate ion of 360 µg/l and 11.4 µg/l respectively was measured (see also Section 4.1.2.2.2).

Roberts et al. (1991) and Kedderis et al. (1995) showed that hepatic microsomes or cytosols from male F-344 rats or B6C3F1 mice did not enhance the rate of hydrolysis of CEO, while human hepatic microsomes significantly increased the rate of hydrolysis of CEO but human hepatic cytosols did not. This difference was attributed to the presence of an inducible epoxide hydrolase in humans which plays a role in the hydrolysis of CEO, and suggests that humans possess an additional detoxification pathway for CEO which is absent in rodents.

Kedderis et al. (1996) confirmed high epoxide hydrolase activity and low glutathione transferase activity in humans compared in rodents. These authors concluded that species differences in 2-cyanoethylene oxide disposition pathways suggest that rodent data will not be useful for directly predicting the human disposition of this epoxide in humans. The active epoxide hydrolase pathway in humans should decrease the amount of CEO leaving the liver to the systemic circulation relative to rats, where this pathway is not operable.

4.1.2.1.3 Adduct production as a dosimeter of exposure to acrylonitrile

Adducts result from the reaction of the electrophilic molecule (acrylonitrile or its metabolites) with nucleophilic sites in DNA or other macromolecules. Measurements of these adducts provide an estimate of target tissue doses in both animals and humans, thereby eliminating some of the difficulties in high to low dose and species to species risk extrapolation. Both in a single exposure experiment and a drinking water experiment the relationship between adduct level and exposure concentration approaches linearity at low concentrations (below 35 ppm, acrylonitrile in drinking water) (Fennell et al., 1989b; 1991; Osterman-Golkar et al., 1994). However at higher concentrations, adduct levels increase more rapidly, indicating saturation of some metabolic process for elimination of acrylonitrile.

Acrylonitrile binds to haemoglobin both *in vitro* and *in vivo*, forming cyanoethyl adducts at a number of sites. In an experiment using rodents who were administered [2,3-^{14}C] acrylonitrile by single gavage, extensive binding was observed in haemoglobin (Fennell et al., 1989b). Characterisation of the bound residues indicated that the majority of the products were formed by direct reaction of acrylonitrile with cysteine residues. A non-linear response was observed,

suggesting that the internal dose of acrylonitrile increases in a non-linear manner with saturation of the oxidation of acrylonitrile to 2-cyanoethylene oxide. By measuring a rate constant for reaction of [2,3-^{14}C] acrylonitrile with haemoglobin *in vitro*, adduct measurements *in vivo* can be related to the internal dose of acrylonitrile in the blood and can provide information that can be compared with simulations of the physiologically based dosimetry description (Gargas et al., 1995).

While the majority of binding occurs at cysteine residues in the rat, other adducts are formed, including an adduct formed by reaction with the N-terminal valine residue (Fennell et al., 1991; Osterman-Golkar et al., 1994). CIIT have developed a method to measure this adduct, using a sensitive gas chromatography and mass spectrometric procedure. CEVal is a biomarker that appears to be specific for exposure to acrylonitrile, and the GC/MS method described can be used to assess exposure to acrylonitrile in humans. After measurement of the reaction rate constant for acrylonitrile with the N-terminal valine residue, comparisons of the dose calculated from total binding can be made with the dose calculated from the measurement of this specific adduct. This assay now enables the measurement of adducts in animals exposed to unlabelled acrylonitrile and in people exposed to acrylonitrile in the workplace or from other sources.

In an investigation of the adduct levels in rats administered acrylonitrile in drinking water for up to 112 days at concentrations ranging from 0 to 300 ppm, a non-linear response was observed, with higher levels of adducts formed at higher exposure concentrations (Osterman-Golkar et al., 1994). This response was similar to the non-linear response seen with gavage administration (Fennell et al., 1991). With an understanding of the kinetics of erythrocyte turnover and a stable adduct, the amount of adduct formed per day can be estimated (Fennell et al., 1992) and compared with the daily dose of acrylonitrile administered.

As part of a collaborative study Calleman et al. (1994), globin samples from workers in a Chinese factory which manufactures acrylamide from acrylonitrile were obtained. These samples were analysed for CEVal by GCMS. Elevated levels of CEVal were observed in these workers, with considerable variability among individuals. Low levels of adduct were observed in the control group, and slightly higher levels were observed in smokers. The considerable variation of adduct levels has since been attributed to dermal exposure, which could vary considerably among individuals.

Cigarette smoke contains acrylonitrile as a product of combustion. Baker et al. (1984) reported that acrylonitrile could be detected in the emissions from cigarette burning tests at levels of 13-17 µg per standard cigarette (70 mm in length and 25 mm in diameter, containing 1 g of tobacco. This may be a more significant source of exposure to acrylonitrile both for smokers themselves and for those in the vicinity of smokers than the sources of consumer exposure described in Section 4.1.1.3 or industrial emissions. Smokers have been shown to have elevated levels of CEVal. Fennell et al. (1995) conducted a study into the effects of smoking on adduct levels so as to derive information on the range of variation due to lifestyle and on the relationship between adduct formation and the approximate extent of exposure from the number of cigarettes smoked.

An approximate lethal dose of 50 mg/kg of acrylonitrile or a dose of 6 mg/kg of the metabolite cyanoethylene oxide by intraperitoneal injection to F 344 rats produced DNA-adducts in the liver to a small extent. Following acrylonitrile administration the 7-oxo-ethylguanine adduct was detected at a level of 108 µmol/mg DNA while following CEO the level was 48 µmol/mg DNA, but adducts were not found in the brain (Hogy and Guengerich, 1986). Although DNA adducts formed from CEO are assumed to be responsible for the carcinogenic effects of acrylonitrile in rats, very low levels of DNA adducts derived from acrylonitrile have been detected *in vivo*

(Guengerich et al., 1981; Hogy and Guengerich, 1986). This was confirmed in experiments where animals received chronic high doses of acrylonitrile, in which no detectable levels of DNA adducts were measured (Butterworth et al., 1992). Recent *in vitro* studies have shown that the initial cyanohydroxethyl addition products of the reaction of CEO with nucleotides and DNA are unstable (Yates et al., 1994), thus casting some doubt on the potential of using DNA adducts as dosimeters of acrylonitrile bioactivation. Additionally, the actual promutagenic DNA adducts derived from CEO or the critical target genes for mutations leading to tumour development are not known.

Existing evidence does not conclusively implicate DNA adduct formation as the mechanism of tumorigenicity, but raises the possibility that epigenetic effects might be involved. Although not completely studied, several lines of evidence suggest an epigenetic tumour-producing mechanism in the brain, possibly involving the formation of oxygen radicals. Increased lipid peroxidation has been demonstrated, which may be partially related to release of cyanide or some other mechanism. The formation of oxygen radicals could also lead to oxidative DNA damage. Studies by Whysner et al. (1996) noted that the acrylonitrile-induced tumours produced were via a mechanism involving the formation of 8-oxodeoxyguanosine, which these authors suggest may reflect oxidative damage.

4.1.2.1.4 Summary of toxicokinetics, metabolism and distribution

Acrylonitrile is rapidly absorbed and distributed after oral, dermal or inhalation uptake. The half-life in rat blood is approximately 1 hour. Biotransformation takes place through binding to glutathione, which mainly occurs non-enzymatically because of the high reactivity of acrylonitrile with nucleophilic centres. However acrylonitrile may also be metabolised by the cytochrome P450 system via an oxidative intermediate step i.e. CEO formation. Both acrylonitrile and CEO can alkylate macromolecular structures by means of a nucleophilic reaction.

Peter and Bolt (1984) and Pilon et al. (1988b) reported a biphasic exponential uptake of acrylonitrile following inhalation exposure, characterised by a dosage-independent rapid phase lasting for approximately 60 minutes and a subsequent slow phase lasting from 60 minutes to the end of exposure. Using the rate of the uptake curve for the rapid phase, Pilon et al. estimated a rate of 4.82 mg/kg/hour uptake at an exposure level of 100 ppm. Ahmed and co-workers (1982; 1983b) showed that rats given a single oral dose of acrylonitrile excreted 40% of the radiolabel in urine, 2% in faeces, 9% in expired air as $^{14}CO_2$, 0.5% as $H^{14}CN$ and 4.8% as unchanged acrylonitrile in 24 hours. Total excretion at the end of 10 days was approximately 75% of the initial dose, indicating a retention of approximately 25%. Highest levels of acrylonitrile were found in the G.I. tract up to 72 hours, suggesting a possible resecretion process of acrylonitrile metabolites into the stomach or binding of acrylonitrile, cyanide or other metabolites within the stomach mucosa. High concentrations were also found in liver, kidney and lung tissues up to 24 hours after administration.

Pilon et al. (1988a) observed that glutathione depletion resulted in greater uptake of a single oral dose of acrylonitrile into target organs (brain, stomach, liver, kidney and blood) of adult male Fischer 344 rats compared with non-GSH-depleted rats, monitored over a 24-hour period. Metabolism to 2-cyanoethylene oxide (CEO) and urinary excretion of thiocyanate were both increased. In contrast, following inhalation exposure, depletion of GSH resulted in a decrease in total radioactivity recovered in target organs and an increase in the rate of acrylonitrile uptake via inhalation in both rapid and slow phases. Urinary excretion of thiocyanate (SCN^-), derived from the epoxide pathway of acrylonitrile metabolism, was doubled in GSH-depleted rats exposed by inhalation, compared with non-depleted rats.

Acrylonitrile is metabolised by two pathways, (1) direct conjugation with glutathione (GSH), either with or without catalysis by glutathione transferases and (2) cytochrome P450-mediated oxidation to cyanoethylene oxide (CEO). The predominant biotransformation pathway appears to be dependent on the systemic dose. Fennell et al. (1991) and Kedderis et al. (1993a) identified 5 major components which accounted for 75-100% of urinary radioactivity. In relation to pathway (1), direct conjugation of acrylonitrile with GSH, the excretion of N-acetyl-S-(2-cyanoethyl)cysteine (CMA) and -S-(2-cyanoethyl)thioacetic acid, both derived from the GSH conjugate of acrylonitrile, increased non-linearly with dose suggesting the existence of a saturable pathway which competes with GSH for acrylonitrile, namely the cytochrome P450-dependent pathway (2).

In the case of a short-term peak dose following oral gavage (also following intravenous or intraperitoneal administration), resulting in saturation of the cytochrome P450-dependent pathway, metabolism to N-acetyl-S-(2-cyanoethyl)cysteine (pathway (1)) appears to predominate, however in the case of low oral doses (e.g. in dietary administration or administration in drinking water), or in the case of exposure to low levels of acrylonitrile by inhalation, pathway (2) via cyanoethylene oxide is more predominant (Müller et al., 1987; Kedderis et al., 1993a). Depletion of glutathione also results in a shift in the metabolic pathway from pathway (1) to pathway (2) (Pilon et al., 1988a).

Studies of the biotransformation of acrylonitrile by pathway (2) point to an epoxidation of the vinylic double bond by the cytochrome P450 system, giving the epoxide 2-cyanoethylene oxide (CEO). This is subsequently conjugated with GSH in the 2-position and then metabolised further to cyanide and then to thiocyanate, which is excreted in the urine. Conjugation of CEO in the 3-position and further metabolism ultimately results in the urinary metabolites N-acetyl-S-(1-cyano-2-hydoxyethyl)cysteine (2-hydroxyethylmercapturic acid, HMA) and thioglycolic acid.

Although absorption and distribution of acrylonitrile is broadly similar following different routes of exposure, there are differences in the metabolic profile. In the study of Tardif et al. (1987) in Sprague Dawley rats exposed by inhalation or dosed intravenously or intraperitoneally, 2-cyanoethylmercapturic acid (CMA), the end metabolite of the GSH conjugation pathway, represented 74-78% of the urinary metabolites when acrylonitrile was administered i.p. or i.v., but only 8% when given by inhalation. They suggested that conditions favouring rapid entry of substantial quantities of acrylonitrile facilitate direct conjugation with glutathione to give rise to the mercapturic acid retaining the cyanide moiety (CMA) rather than resulting in direct release of cyanide. However the data in the inhalation study were obtained from measurement of the metabolite in post exposure urine only, which may have resulted in a low estimate of CMA. Müller et al. (1987) estimated that up to 46% of an inhaled dose might be CMA, excreted mainly during exposure. Following oral administration, Kedderis et al. (1993a) found a linear relationship between the excretion of mercapturic conjugates and the administered amount of acrylonitrile up to a dose level of 26.5 mg/kg of body weight. However at higher doses, the amount of mercapturic acids remained constant, which appeared to be a direct result of a depletion of available glutathione.

Urinary excretion is the major excretory route for acrylonitrile, administered by the oral and other routes. From 60 to 100% of the administered dose is excreted in the urine, with only about 3-8% of a given dose being excreted in the faeces, and between 2.5 and 17% of labelled acrylonitrile has been recovered in exhaled breath, mainly as CO_2. The bulk of the urinary excretion takes place in 24 hours.

Limited data on toxicokinetics in humans indicate that the metabolic pathway via cyanoethylene oxide exists in humans. Thiocyanate has been detected in the urine of volunteers to 22 ppm

acrylonitrile for 30 minutes. Levels returned to normal within 24 hours, while elevated levels were still present 12 hours after exposure to 50 ppm for 30 minutes. Urinary excretion of N-acetyl-S-(2-cyanoethyl)cysteine (CMA), derived from the GSH conjugate of acrylonitrile has also been reported in workers exposed to airborne acrylonitrile concentrations between 3 and 10 ppm (Jakubowski et al., 1987). These data indicate that the metabolic pathways observed in experimental animals are also operative in humans. Additionally, Kedderis et al. (1996) confirmed high epoxide hydrolase activity and low glutathione transferase activity in humans compared with rodents suggesting that humans possess an additional detoxification pathway, epoxide hydrolase, for CEO. These authors concluded that species differences in 2-cyanoethylene oxide disposition pathways suggest that rodent data will not be useful for directly predicting the human disposition of this epoxide in humans. The active epoxide hydrolase pathway in humans should decrease the amount of CEO leaving the liver to the systemic circulation relative to rats, where this pathway is not operable.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

General

Acrylonitrile is a highly volatile liquid, with a wide range of uses that can result in exposure of humans. Acrylonitrile is acutely toxic to animals and humans. Based on animal studies and human evidence its acute effects can occur via inhalation, ingestion and dermal exposure. It is also a skin and eye irritant and has skin sensitising properties.

Clinical signs of toxicity

The clinical signs resulting from acute acrylonitrile administration have been examined in a number of different species and have been to found to vary very little between species. The developing clinical signs following acute exposure to acrylonitrile can be divided into four stages, as described by Nerland et al. (1989), based on the work of other workers. Immediately after administration the animal goes through an excitatory phase; the eyes water and the animal becomes agitated. A tranquil phase follows and cholinergic symptoms, such as salivation, lachrymation, urination and defecation occur. The latter signs may be a true cholinergic response. Atropine was reported to block the effect (Abreu and Ahmed, 1980), and application of 10-20 ng of acrylonitrile to an isolated guinea pig ileum caused vigorous contractions that were blocked by atropine. Next there is a convulsive phase in which the animal undergoes clonic seizures. The terminal stage preceding death is a paralytic phase in which the animal is immobile. These clinical signs indicate that the action of acrylonitrile is that of a typical nitrile, with toxic action probably due to a cleavage of the molecule to produce hydrogen cyanide, which is one of the key mediators of the toxicity. However, for any nitrile, there is a complex interplay of a number of factors that affect the outcome of acrylonitrile toxicity. These include the rate of cyanide liberation and detoxification, the dose of cyanogen, the route of administration, the species of animal and the presence of other bioreactive sites within the molecule (Nerland et al., 1989).

Lethality data

Reported oral LD_{50} values for various species lie in the range of 25 to 186 mg/kg body weight (BUA, 1995). Vernon et al., in a study carried out in 1969 but reported in the Journal of the American College of Toxicology in 1990, orally dosed four groups of 5 young adult male CF Nelson rats with 50, 100, 200 and 400 mg/kg and observed them for 14 days. All deaths occurred during the first 24 hours with no significant clinical signs being observed. The acute oral LD_{50} (in males) was 81 mg/kg, with 95% confidence limits of 62-107 mg/kg. The oral LD_{50} in mice was reported by Tullar (1947) to lie between 25-48 mg/kg, as summarised in WHO (1983).

Dermal LD_{50} values for various species were in the range of 148-693 mg/kg body weight, with the rat reacting most sensitively (BUA, 1995). In a study by Vernon et al. (1969) a single dose of 200 mg/kg was applied occlusively to the intact skin of 15 young adult male rabbits for an exposure period of 24 hours. This study resulted in death of all animals within the first 24 hours, with no clinical signs being noted. The acute dermal LD_{50} of acrylonitrile in this study was < 200 mg/kg. This indicates that acrylonitrile can readily penetrate the skin.

The LC_{50} values reported for a range of species following a 4-hour inhalation exposure lie in the concentration range of 300-1,210 mg/m^3. Dudley and Neal (1942) investigated the individual susceptibility of a range of species (rats, guinea pigs, rabbits, cats, dogs and monkeys) to a single 4-hour exposure to varying concentrations of acrylonitrile. The results indicated that rabbits were moderately susceptible to acrylonitrile, exposure to a level of 260 ppm (560 mg/m^3) for 4 hours causing 100% mortality in 4 to 5 hours, while a level of 135 ppm produced marked but transistory effects. Rats and cats were of about equal susceptibility, 100% mortality being observed in rats within 2–6 hours of exposure to 635 ppm (1,380 mg/m^3) acrylonitrile and in cats within 1.5 hours of exposure to 600 ppm (130 mg/m^3). Exposure of two monkeys to 90 ppm (196 mg/m^3) produced only slight transitory redness of the face, genitalia, etc. with full recovery in 12 hours.

Delayed mortality (25%) was observed in guinea pigs exposed to a level of 575 ppm (1,250 mg/m^3), mortality occurring as a result of lung oedema 3 to 5 days following exposure. In general guinea pigs appeared to be less sensitive to acrylonitrile than rats following inhalation exposure, but yet the lethality in both species after administration by other routes is comparable. This could be due to the lower respiratory volume per kg body weight of guinea pigs, i.e. 320 l/kg/8-hour compared to 150 l at rest for rats and guinea pigs respectively (Zielhuis and Van der Kreek, 1979).

In these acute experiments of Dudley and Neal (1942), the dog was shown to be the most sensitive species. Exposure to 110 ppm (240 mg/m^3) acrylonitrile was fatal in 2 out of 3 dogs exposed, while a 4-hour exposure to a level of 100 ppm resulted in convulsions followed by coma in 2 out of 3 dogs. One of these dogs recovered completely within 48 hours while the other showed partial paralysis of the hind legs for 3 days. The third dog exposed to 100 ppm showed severe salivation during the test but recovered fully within 24 hours. At an exposure level of 29 ppm (63 mg/m^3) for 4 hours, signs of toxicity in dogs were confined to slight salivation at the end of the test.

Dudley and Neal (1942) investigated the protective effects of sodium nitrite as one of three known antidotes to cyanide poisoning, the others investigated being thiosulfate and methylene blue. They showed that sodium nitrite was the most effective of the three in delaying the onset of symptoms of acrylonitrile toxicity and reducing the severity of the effects in rats and rabbits, although it had no such protective effect in guinea pigs. They suggested that it was likely that acrylonitrile was metabolised to hydrogen cyanide, as had been postulated for other nitriles.

The same authors also investigated the effect of increasing exposure levels of acrylonitrile and increasing duration of exposure, from 0.5 to 8 hours, in rats. Their results are summarised in **Table 4.11** below.

Table 4.11 Acute toxicity of acrylonitrile in the rat following inhalation exposure (Dudley and Neal, 1942)

mg/m^3 Acrylonitrile	Exposure period (minutes)	No. of rats exposed	No. of deaths
5,300	30	16	0
3,230	30	16	0
2,750	30	16	0
1,440	30	16	0
5,300	60	16	12
3,230	60	16	4
2,750	60	16	0
1,440	60	16	0
2,730	120	16	16
1,440	120	16	1
660	120	16	0
1,380	240	16	16
680	240	16	5
280	240	16	0
690	480	16	15
590	480	16	7
460	480	16	1
290	480	16	0
200	480	16	0

Appel et al. (1981) administered lethal doses of acrylonitrile to male Wistar rats by different routes of application (i.p. gavage and inhalation) in order to observe the effect of potential antidotes on the acute toxicity of acrylonitrile. Inhalation exposure for 30 minutes to 3,000 ppm (6,490 mg/m^3) of acrylonitrile proved to be lethal in all 6 rats examined, as shown in **Table 4.12**.

Table 4.12 Acute toxicity of acrylonitrile in the rat following inhalation exposure (Appel et al., 1981)

mg/m^3 Acrylonitrile	Exposure period (minutes)	No. of rats exposed	No. of deaths
1,406 (650)	180	3	1
2,055 (950)	120	3	1
2,380 (1,100)	120	3	3
3,461 (1,600)	30	3	0
5,192 (2,400)	10	3	0
5,624 (2,600)	30	3	1
6,490 (3,000)	30	6	6

The results from the Dudley and Neal (1942) and Appel et al. (1981) experiments above have been used to establish LC_{50} values for the rat, using the method of Probit Analysis (Finney, 1971). Results are shown in **Table 4.13**. The LC_{50} value for inhalation of acrylonitrile obviously decreases with increasing exposure time, the LC_{50} values after 4 hours of exposure being in the concentration range 1,030–1,210 mg/m^3, as shown by these two independent studies. The consistency between the results for the two studies should be noted, particularly considering the time gap of approximately 40 years between them.

Table 4.13 LC_{50} values for acrylonitrile in the rat, derived from the data of Dudley and Neal (1942) and Appel et al. (1981)

Exposure (minutes)	LC_{50} mg/m^3 (Dudley et al., 1942)	LC_{50} mg/m^3 (Appel et al., 1981)
30	7,880	5,740
60	4,000	3,410
120	2,030	2,030
240	1,030	1,210
360	690	890

Vernon et al., in a study carried out in 1985 and reported in the Journal of the American College of Toxicology in 1990, exposed a group of 10 young adult Sprague-Dawley rats (5 male and 5 female) to an acrylonitrile concentration of 1,008 ppm (2,240 mg/m^3) for one hour. There was no mortality. Clinical signs noted included shallow and rapid breathing, decreased activity, nasal discharge, salivation, lacrimation and coma (3 of 10 animals). Based on these observed clinical signs the CNS is indicated to be a target organ. The extremities of all animals appeared red after 37 minutes of exposure. However animals recovered fully within 5 minutes when exposed to fresh air. The acute inhalation (rat) LC_{50} was calculated to be $>$ 1,008 ppm (2,240 mg/m^3).

In respect of lethality in other species following inhalation exposure, the LC_{50} for dogs following a 4-hour exposure has been estimated from the data of Dudley and Neal (1942) to be 200 mg/m^3 (90 ppm), while exposure to 580-670 mg/m^3 (267-309 ppm) was fatal for three rabbits within 2-3 hours. **Table 4.14** summarises the lethality data for acrylonitrile in a range of species following different routes of administration.

Based on the information in **Table 4.14** the oral LD_{50} values for the various species range from 28 to 186 mg/kg body-weight. The sensitivity decreases in the order mouse, guinea pig, rabbit, and rat. The LD_{50} values for i.v., i.p. or s.c. administration are similar to those for oral administration. Dermal LD_{50} values ranged from 148 to 690 mg/kg bw for rat, guinea pig and rabbit, the rat being the most sensitive species. The LC_{50} values after a 4-hour exposure lie in the concentration range of 300 to 1,210 mg acrylonitrile/m^3. The sensitivity decreases in the order: mouse, guinea pig and rat.

Table 4.14 Acute toxicity of acrylonitrile by different routes of administration in a range of species [data obtained from WHO (1983) unless otherwise stated]

Species	Route	Toxicity
Mouse	inhalation	LC_{50} 300 mg/m^3 /4 hour
Rat	inhalation	LC_{50} 470 mg/m^3/4 hour
Rat	inhalation	LC_{50} 1,030 mg/m^3/4 hour [a)]
Rat	inhalation	LC_{50} 1,210 mg/m^3/4 hour [b)]
Guinea pig	inhalation	LC_{50} 990 mg/m^3/4 hour
Mouse	oral	LD_{50} 28-48 mg/kg
Guinea pig	oral	LD_{50} 50-85 mg/kg
Rat	oral	LD_{50} 72-186 mg/kg
Rabbit	oral	LD_{50} 93 mg/kg
Mouse	intraperitoneal	LD_{50} 47-5-0 mg/kg
Rat	intraperitoneal	LD_{50} 65-100 mg/kg
Mouse	subcutaneous	LD_{50} 25-50 mg/kg
Mouse	subcutaneous	LD_{50} 35 mg/kg [c)]
Hamster	subcutaneous	LD_{50} 60 mg/kg [c)]
Rat	subcutaneous	LD_{50} 80-96 mgkg [c)]
Rat	subcutaneous	LD_{50} 100 mg/kg
Guinea pig	subcutaneous	LD_{50} 130 mg/kg
Guinea pig	percutaneous(dermal)	LD_{50} 260-690 mg/kg
Rat	percutaneous(dermal)	LD_{50} 148-282 mg/kg
Rabbit	percutaneous(dermal)	LD_{50} 226 mg/kg
Rabbit	intravenous	LD_{50} 69 mg/kg
Guinea pig	intravenous	LD_{50} 72 mg/kg

a) Dudley and Neal (1942)
b) Appel et al. (1981)
c) Cote et al. (1984)

Target organ toxicity following acute exposure to acrylonitrile

Target organs of acute oral toxicity include the endocrine system, lung, brain, stomach and duodenum (Szabo et al., 1976; 1980; 1982a; 1982b; Jaeger et al., 1982). In the early studies of Dudley and Neal (1942) exposure of rats, guinea pigs, rabbits, cats dogs and monkeys to high

(acute) levels of acrylonitrile indicated marked flushing and reddening of the skin, particularly in rats and rabbits. This was considered to be due to either a dilatation of the blood capillaries or to some change in the respiratory cycle, which rendered the blood more highly oxygenated. Gross pathological examination showed marked lung congestion in all species except guinea pigs, in which the lungs were pale in colour and gave a frothy exudate on sectioning. Subsequent work in guinea pigs (Jedlicka et al., 1958) showed that lethal doses of acrylonitrile (50 or 100 mg/kg) caused dilatation of the right ventricle, congestion of the coronary blood vessels, hepatic and splenic congestion and inflammation of the intestinal mucosa.

The adrenal has been reported by Szabo and co-workers to be a primary target organ following acute administration of acrylonitrile. These workers showed rapidly developing adrenocortical haemorrhagic necrosis ("apoplexy") following either i.v. or oral administration (Szabo et al., 1976; 1980), and suggested that the effects seen could be due to peroxidative damage induced by acrylonitrile in the adrenal (Silver and Szabo, 1982).

Szabo and co-workers (1982a; 1982b; 1983) also reported that acute administration of acrylonitrile to the rat by the oral route produced pathological findings in the gastrointestinal tract. They reported a duodenal ulcerogenic effect that was markedly enhanced by pretreatment of the rats with the mixed function oxidase inducers polychlorinated biphenyl (PCB), Arochlor 1254 or phenobarbital. Ghanayem et al. (1985) also reported gastrointestinal haemorrhage. The severity of the gastrointestinal bleeding was independent of the route and was dose and time dependent. The gastric lesions were associated with a decrease in glutathione content of the stomach.

Other organs affected by acute administration of acrylonitrile have included liver, kidney and blood. Focal superficial necrosis of the liver in association with haemorrhagic gastritis was reported by Silver et al. (1982), following administration of acrylonitrile at 150 mg/kg in drinking water. As with acrylonitrile-induced adrenal necrosis, Silver and Szabo (1982) suggested that this could be due to peroxidative damage induced by acrylonitrile, while Ivanov et al. (1989) also reported increased peroxidation in the liver. Intraperitoneal administration of 20 mg/kg (and higher) of acrylonitrile produced nephrotoxic effects including glucosuria (Rouisse et al., 1986). Electron microscopic examination revealed a slight increase in dense bodies and moderate vesiculation of endoplasmic reticulum membranes in proximal tubular epithelium at dose levels of 40 mg/kg and higher. Rouisse and co-workers (1986) also identified altered haematological and clinical-chemical parameters which supported the observation of dose-dependent kidney damage, while Farooqui and Ahmed (1983a) and Gut et al. (1984) observed an effect on blood count and glucose metabolism. Although the blood is not believed to be the primary target organ in acrylonitrile toxicity, some haematological effects were observed in rats and guinea pigs administered lethal doses of acrylonitrile (WHO, 1983; VROM, 1984). Glutathione depletion has been reported in liver, kidney and lung (Szabo et al., 1977).

Neurotoxic effects of acrylonitrile

The central nervous system has been identified as a target organ in various animal species by a number of authors (Buchter and Peter, 1984; Ghanayem et al., 1991; Burhan et al., 1991), clinical symptomology being indicative of an effect on cholinergic transmission at dose levels in excess of 20, 40, and 80 mg/kg in rat, (Burhan et al., 1991), although no clinical symptoms of neurotoxicity were identified in a mouse study performed by Tanii et al. (1989) in which a single oral dose of acrylonitrile was administered to 4 animals at 4 dose levels of between 23 and 78 mg/kg (0.44 and 1.48 mmol). Of the 16 animals used in this study only 7 survived.

Neurotoxic effects reported following exposure to acrylonitrile may be mediated by cyanide, liberated *in vivo* as a result of metabolism (see Section 4.1.2.1.1, “Metabolism”). Hashimoto and Kanai (1965) suggested however that the toxicity of acrylonitrile was due not only to the liberated hydrogen cyanide but to acrylonitrile itself. This conclusion was based on the observation that a reduction of the acrylonitrile concentration in the blood by L-cysteine (which may have been caused by cyanoethylation of acrylonitrile with L-cysteine) was very effective in protecting animals from acrylonitrile poisoning. Benesh and Cerna (1959) and Hashimoto and Kanai (1965) applied lethal doses of acrylonitrile to rats and mice, pretreated with an intravenous dose of 0.5 to 1 gram thiosulphate. During these experiments the cyanide blood level remained far below the level of specific cyanide symptoms, and yet the animals still died. These results indicate that in addition to release of cyanide another mode of action plays a role in the acute toxicity of acrylonitrile.

The complexity of the neurotoxicity resulting from acute exposure to acrylonitrile is reflected in the evidence that classic cyanide antidotes are effective in preventing the acute toxicity in some animal models but are totally ineffective in others (Nerland et al., 1989a). The acute toxicity of nitriles in general apparently depends on the complex interplay of a number of factors such as the rate of cyanide liberation and detoxification, the dose of cyanogen, the route of administration, the species, and the presence of other bioreactive sites within the nitrile molecule. While acrylonitrile is cyanogenic, it is also metabolised to a reactive epoxide, 2-cyanoethylene oxide, and the parent molecule is also capable of non-enzymatically cyanoethylating essential functional groups in the body. All these factors contribute to the overall toxicity of acrylonitrile (as summarised by Nerland et al., 1989a; 1989b, based on studies from Dudley and Neal, 1942; Dudley et al., 1942; Abreu and Ahmed, 1980; Szabo et al., 1984; Tanii et al., 1986; Gut et al., 1985).

Effects on cholinergic transmission in the rat may result from an inactivation of acetylcholinesterase by cyanoethylation of the hydroxyl group of one serine residue (Buchter et al., 1984) or may be due to damage to acetylcholine muscarinic receptors by acrylonitrile or its metabolites (Ghanayem and Ahmed, 1986). These effects are particularly marked in glutathione-depleted animals.

Experimental evidence also suggests the involvement of acetylcholine muscarinic receptors in acrylonitrile-induced toxicity. Acrylonitrile produces “cholinomimetic” actions, such as salivation, diarrhoea and increased acidic gastric secretions, which are prevented by prior treatment with atropine (Ahmed et al., 1986). Burhan et al. (1991) demonstrated that acrylonitrile causes acute gastric haemorrhage and mucosal erosions. A possible mechanism of this acrylonitrile-induced GI bleeding may involve the interaction of acrylonitrile with critical sulfhydryl groups which in turn causes alteration of acetylcholine muscarinic receptors and leads to gastric haemorrhagic lesions. Pre-treatment of rats with atropine sulphate (1 mg/kg) 30 minutes before administration of 40 mg/kg of acrylonitrile significantly protected animals against the acrylonitrile-induced neurotoxicity seen in animals dosed at the same level (40 mg/kg) without any atropine. In addition, treatment of rats with the same dose of atropine sulphate after the first appearance of neurotoxic signs, prevented further progress of toxicity (Burhan et al., 1991).

Studies by Burhan et al. (1991) were designed to quantitatively characterise the acute phase of acrylonitrile-induced cholinomimetic neurotoxicity, and to determine the effects of dose, route of administration and atropine on such toxicity. Groups of 3 to 4 male Sprague-Dawley rats were administered doses of 20, 40 or 80 mg/kg acrylonitrile in distilled water by gavage, or in sterile saline subcutaneously. Control groups received vehicle alone. Two distinctive phases of acute neurotoxic effects were observed in the treated animals. Early after treatment with acrylonitrile,

rats exhibited salivation, lacrimation, miosis, diarrhoea, polyuria and peripheral vasodilation, reaching a maximum within 60 minutes of dosing. Other signs of toxicity observed but not quantified included flushing of the face, ears and extremities. This early phase was followed by a delayed phase (> 4 hours), which included central nervous system abnormalities such as respiratory depression, convulsions and, at high doses, death. The neurotoxic signs observed were dose-dependent regardless of the route of administration. The intensity of the early clinical symptomology, which reached a maximum in about 0.5-1 hour, was relatively similar after subcutaneous or oral administration, although oral administration produced more severe salivation and gastric secretion.

Brain slices prepared from male guinea pigs and frogs were used to study the potential neurotoxicological effects of acrylonitrile. The oxygen consumption of normal or potassium-stimulated slices was measured using a Warburg manometer in the presence or absence of acrylonitrile, cyanide or a range of narcotic agents. The results of these studies indicated that acrylonitrile caused inhibition of the respiration of brain slices, the effect being similar to that produced by the narcotic agents also tested in the system. The inhibitory effect was modulated by sodium thiosulphate, which completely suppressed the inhibition caused by cyanide. Acrylamide and acrylic acid were also tested and had no effect on brain respiration. These results suggest that the effects of acrylonitrile on the brain could be attributable to acrylonitrile itself rather than its metabolites. Acrylonitrile has also been reported to have a strong blocking effect on peripheral nerves similar to the effects of various local anaesthetics and general narcotics, although the active concentration *in vitro* was much higher than that estimated to be possible *in vivo*.

From these observations it seems likely that acrylonitrile acts both on the central and peripheral nervous system. The symptoms of acute acrylonitrile poisoning, such as general convulsions and paralysis of the lower limbs, seem to support this conclusion.

4.1.2.2.2 Studies in humans

Inhalation exposure

WHO (1983) and VROM (1984) summarised several cases of acrylonitrile poisoning whereby workers exposed to low acrylonitrile concentrations suffered from local effects such as irritation of the eyes, nose, throat and respiratory tract, headaches, vertigo and limb weakness at > 5ppm (11 mg/m^3). Slight liver enlargement and jaundice have also been reported. Workers in a synthetic rubber manufacturing plant exposed to acrylonitrile vapour at levels of between 16 (35 mg/m^3) and 100 ppm (219 mg/m^3) acrylonitrile for 20 to 45 minutes experienced mucous membrane irritation, headaches, nausea, feelings of apprehension and nervous irritability (Wilson et al., 1948). Low-grade anaemia, leucocytosis, kidney irritation and mild jaundice were also apparent; these effects subsided with exposure cessation. Zeller et al. (1969) observed that in 16 cases of acute inhalation of acrylonitrile fumes by workers, nausea, vomiting, headache and vertigo appeared within 5-15 minutes; none of the workers required hospitalisation.

More serious exposures have resulted in tremors, convulsions, unconsciousness, respiratory and cardiac arrest and even death (Buchter and Peter, 1984). One reported fatal case involved a 3-year old girl who slept overnight in a room recently sprayed with an acrylonitrile-based fumigant. Respiratory malfunction, lip cyanosis, and tachycardia were among the symptoms described prior to death (WHO, 1983). However five adults who spent the night and much of the day in a room fumigated with an acrylonitrile-based product complained only of eye irritation

and in general showed no signs of acrylonitrile poisoning (Grunske, 1949). The concentration of acrylonitrile in the air was not given. Several other cases of death in children were reported, but not described in detail, while adults only suffered mild irritation (Grunske, 1949).

Human volunteers exposed acutely (8 hours) to acrylonitrile at concentrations of 2.4-5.0 ppm (5.4-10.9 mg/m^3) exhibited no deleterious effects, indicating that acrylonitrile is not very irritating to the respiratory tract at these concentration levels (Jakubowski et al., 1987).

Dermal exposure

Dermal absorption of acrylonitrile may lead to systemic poisoning. A 10-year-old girl died after her scalp had been treated for lice with an insecticide formulation containing acrylonitrile (Ventox) (Lorz, 1950). The girl had impetigo and resultant widespread scratches on the skin of her scalp, which could have led to increased absorption of acrylonitrile. This case is reported in German and only a summary is presented in WHO (1983).

A number of serious effects have been reported following acute exposure of humans to acrylonitrile as a liquid or vapour, including local effects such as blisters on the skin and irritation of the mucous membranes of the nose, eyes and upper respiratory tract. Vogel and Kirkendall (1984) reported a case of a 24-year-old man whose face, eyes and body was sprayed by acrylonitrile when a valve burst when he was unloading the chemical from a ship. Dizziness, flushing, nausea and vomiting occurred within 30 minutes of exposure, followed by generalised erythema together with a mild conjunctivitis. The victim subsequently suffered hallucinations and convulsions. He received 15 antidotal treatments against cyanide poisoning, giving some indication of the severity of the systemic poisoning via dermal exposure. The recurrent clinical signs of cyanide poisoning over a 72-hour period suggest that the acrylonitrile or one of its metabolites was stored in tissues or was slowly absorbed from the skin (or possibly the gastrointestinal tract, given the nature of the incident). However since the patient was thoroughly bathed on three occasions, the likelihood that deposits on the skin played a role in his recurrent bouts was remote and indicated dermal absorption as the possible primary route of entry (Vogel and Kirkendall, 1984).

4.1.2.2.3 Summary of acute toxicity

Only limited data exist in respect of the acute effects of acrylonitrile in humans, based mainly on reports of specific incidents or accidents. The findings and approximate dose levels thought to be involved in these human experiences are consistent with the information obtained from animal studies. They indicate that acrylonitrile is toxic by the oral and inhalation routes, via contact with skin, and causes neurotoxic effects (which relate both to the acrylonitrile itself and also to the release of cyanide).

With regard to the acute lethality of acrylonitrile in animals, dogs appeared to be the most sensitive species following exposure via inhalation. However as outlined previously the acute toxicity of acrylonitrile is for the greater part caused by the release of cyanide, to which dogs are much more sensitive. Dogs are more susceptible to the toxicity of cyanide because they have lower levels of the detoxifying enzyme rhodanase in the liver than other mammals. Inhalation studies provided an approximate LC_{50} of 200 mg/m^3/4 hr in the dog, 300 mg/m^3/4 hr in the mouse and 990 mg/m^3/4 hr in the guinea pig. In rats the data of Dudley et al. (1942) and those of Appel et al. (1981) provided a figure of between 1,030 and 1,210 mg/m^3/4 hr, although a lower value of 470 mg/m^3/4 hr was reported by Knobloch et al. (1971).

Following oral dosing the mouse appeared to be the most sensitive species, with an oral LD_{50} ranging from 28 to 48 mg/kg body weight. The reported range in the guinea pig was 50-85 mg/kg, an oral LD_{50} of 93 mg/kg was reported in the rabbit, while in the rat the range was 72-186 mg/kg. No oral toxicity data exist for the dog.

The reported dermal LD_{50} for the rat lay between 148 and 282 mg/kg bodyweight, the dermal LD_{50} in the rabbit was 226 mg/kg and that in the guinea pig was between 260-690 mg/kg. The percutaneous LD_{50} in the rabbit was only 3 times higher than the intravenous LD_{50}, and was approximately 3 to 10 times higher in guinea pigs (see **Table 4.14** above), indicating that acrylonitrile can readily penetrate the skin. Acute administration of acrylonitrile produced pathological findings in the gastrointestinal tract, gastrointestinal bleeding apparently being independent of the route of administration since it was reported after oral or subcutaneous dosing, and changes have also been reported in the kidney, the liver and in haematological and clinical chemistry parameters. Acrylonitrile has been shown to induce dose- and time dependent cholinomimetic neurotoxicity in rats, regardless of the route of administration.

On the basis of the available animal data and using a weight of evidence approach, classification of acrylonitrile as toxic by inhalation, in contact with skin and if swallowed is appropriate (for classification, see Section 1.4).

4.1.2.3 Irritation

4.1.2.3.1 Studies in animals

<u>Skin</u>

Vernon et al. (1969) as reported in the Journal of the American College of Toxicologists (1990) applied 0.5 ml of acrylonitrile occlusively to the shaved skin of 6 young adult New Zealand White rabbits for a period of 24 hours. Evaluation of skin irritancy was made at 24 and at 72 hours, when the study was terminated. The scores at 24 and 72 hours after administration were averaged to produce 6 individual animal scores of 0 to 4 for both erythema and oedema. The 0-72 hour mean of the 6 individual animal scores for both erythema and oedema was 3.6, with slightly higher scores being obtained for abraded skin. This study indicates that acrylonitrile is strongly irritating to the skin and requires classification as an irritant (for classification, see Section 1.4).

Zeller et al. (1969) applied liquid acrylonitrile on a cotton pad for 15 minutes or 20 hours to shaved rabbit skin ($2.5 \cdot 2.5$ cm). The skin exposed for 15 minutes was then washed with concentrated polyethylene glycol and water. Skin exposed for 20 hours remained unwashed. The skin exposed for 15 minutes showed oedema only, however increasing the duration of exposure to 20 hours produced clear necrosis of the tissue. No further details are available.

<u>Eye</u>

In a BASF study (1963) approximately 0.05 ml of acrylonitrile was applied undiluted to the left eye of two rabbits. The right (control) eye was treated with NaCl solution. No findings were observed in the control eyes. The treated eyes at 1 hour both exhibited slight conjunctival redness (score of 1), diffuse corneal opacity, oedema (severe in one animal, score of 3) and miosis and secretion occurred in one animal. At 24 hours conjunctival redness (score of 2) and

corneal opacity remained with oedema in one animal (score of 2) and ciliary injection. At 48 hours conjunctival redness had reduced with some corneal opacity still remaining in one animal. At 72 hours the eye of one animal was clear of all effects, while the second animal still had conjunctival redness and petechiae, and diffuse milky corneal opacity. After 7 days the eye of the second animal had returned to normal.

Vernon et al. (1969) also carried out an eye irritancy study in rabbits. A dose of approximately 0.1 ml of acrylonitrile was instilled in one eye, the other eye serving as control. The eyes were examined and the grade of ocular reaction recorded at 24, 48 and 72 hours, when the study was terminated. The scoring system used was the original system proposed by Draize, and results from this study were presented in accordance with the Draize scoring system, taking into account both the intensity and the area of involvement. Acrylonitrile gave a maximum Draize score of 35 out of 110 at 24 hours, falling to 31 at 48 hours and 22 at 72 hours. It has not been possible to deduce accurately the scores for intensity alone, as required for the EU scoring system for classification, other than for iritis, where a mean score of 1.0 was obtained over 24-72 hours. Scores for corneal opacity are estimated to have been in the range of 1-2, with little reversibility of the damage over the period of the study. Scores for conjunctival redness and chemosis appear to have been in the range of 2-3, with some reversibility shown over the 3 days of the test.

In a study by Zeller et al. (1969) oedema and slight necrosis of the conjunctiva after 8 days were observed in rabbits. No further details are available.

In another low-volume study, following application of 0.05 ml of undiluted acrylonitrile, mild irritation was observed in the eye of the test animal (rabbit), and after one hour mild conjunctivitis had developed. By 24 hours however the eye had returned to normal (McOmie, 1949). Other investigators found a severe burn of the cornea after application of 0.02 ml of undiluted acrylonitrile in the rabbit eye (VROM, 1984).

In a study performed by Haskell Laboratories, DuPont in 1975, 0.1 ml of undiluted acrylonitrile was placed in the right conjunctival sac of each of two albino rabbits. After 20 seconds, the treated eye of one rabbit was washed with tap water for one-minute. The treated eye of the other rabbit was not washed. Observations of the cornea, iris and conjunctiva were made with a hand-slit lamp at one and four hours, and at 1, 2, 7, 14 and 21 days. Fluorescein stain and a biomicroscope were used at examinations after the day of treatment. In this study acrylonitrile produced moderate corneal opacity, moderate iritis and severe conjunctival irritation in the unwashed treated eye. The eye treated with acrylonitrile and promptly washed showed slight temporary corneal opacity, transient, moderate iritic congestion and moderate conjunctival irritation. The washed eye was normal within 3 days. The unwashed eye showed signs of healing by day 3 with partial circumcorneal vascularization. By days 14 and 21 mild opacity remained with traces of vascularization. Both rabbits showed possible systemic effects with pupil dilation at four hours. In conclusion therefore severe to moderate ocular effects occurred by treatment with acrylonitrile. These effects were not completely reversible in the unwashed eye, while by washing the eye the effects were considerably lessened as was the duration of these ocular effects. From the results of this study it was concluded that acrylonitrile should be regarded as a serious eye irritant.

On consideration of the above data, the classification as Xi; R41 (risk of serious damage to eyes) is appropriate.

Respiratory tract irritancy

No specific animal studies of respiratory irritancy such as the Alarie test have been carried out but both long-term and short-term toxicity studies in a range of species have indicated that acrylonitrile has irritant effects on the upper respiratory tract. Effects have included rhinitis, nasal discharge and hyperplastic changes in the nasal mucosa. The irritation of the throat and the respiratory tract is a delayed effect and provides no warning of exposure to acrylonitrile in the first period of exposure.

Classification as R37 is considered appropriate on the basis of these effects.

4.1.2.3.2 Studies in humans

Little information exists regarding specific human studies involving skin or eye contact. A male laboratory worker spilled "small quantities" of liquid acrylonitrile on his hands, resulting in diffuse erythema on both hands and wrists after 24 hours, followed by blisters on the fingertips on the third day. His hands were slightly swollen, erythematous, itchy and painful and the finger remained dry and scaly on the 10th day (Dudley and Neal, 1942). Wilson et al. (1948) noted that direct skin contact resulted in irritation and erythema and scab formation, with slow healing.

Skin contact has resulted in local irritation, erythema, swelling, blistering and burns. In one case report (Hashimoto and Kobayashi, 1961), lesions spread rapidly to parts of the body which had not been exposed and this was considered to be an allergic reaction. A producer of acrylonitrile reported 10 cases of skin complaints in employees (Bakker et al., 1991). Of these, 5 had irritant dermatitis while the other 5 proved to have an allergy to acrylonitrile on patch testing. Paresthesia was reported in one patient.

With regard to human experiences of acute exposure to acrylonitrile as a liquid or vapour a wide range of effects have been observed, including irritation of the mucous membranes of the nose, eyes and upper respiratory tract. Lachrymation, burning in the throat, coughing, sneezing, nausea, vomiting dizziness, visual disturbance, headache, coma, seizures and dermatitis have been described in some of the non-fatal cases (Davis et al., 1973). The seriousness of some of these effects however reflect very high exposure levels following for example accidental release of a large quantity of acrylonitrile.

Jakubowski et al. (1987) exposed human volunteers to acrylonitrile for 8 hours to concentrations of 2.4-5.0 ppm (5.4-10.9 mg/m^3). Volunteers exhibited no deleterious effects and acrylonitrile did not appear to cause irritancy to the respiratory tract at these exposure levels.

Vogel and Kirkendall (1984) reported a case of a 24-year-old man whose face, eyes and body were sprayed by acrylonitrile when a valve burst while he was unloading the chemical from a ship. Within 30 minutes the subject developed dizziness, flushing, nausea and vomiting. He showed generalised erythema, but no skin rash was observed. There was mild conjunctivitis but no corneal clouding; and funduscopic examination was normal.

Grahl (1970) refers to one volunteer who exposed himself to acrylonitrile for 70 seconds at levels of 370-460 ppm (800-1,000 mg/m^3) without experiencing an intolerable reaction, possibly indicating that acrylonitrile has little warning action even for acute high levels.

4.1.2.3.3 Summary of irritation

Based on the available animal data and limited human experience, the latter resulting mainly from accidental exposures, acrylonitrile is considered to be both a skin irritant and a severe eye irritant, and it also has irritating effects on the respiratory tract. In humans, the irritation of the throat and the respiratory tract appears to have a delayed action, with no sensation of irritation being felt in the initial period following exposure.

The data indicate that appropriate classification of acrylonitrile for this end point is Xi, R37/38-41 (irritant, irritating to respiratory system and skin, risk of serious damage to eyes). For classification, see Section 1.4.

4.1.2.4 Corrosivity

Isolated reports of a corrosive effect of acrylonitrile exist, as indicated above, relating in the main to exposure of humans in an accident situation. However, overall the available studies on both skin and eye irritation in animals and more recent human experience indicate that while acrylonitrile is irritant to skin, eye and respiratory tract it should not be considered as corrosive.

Classification of acrylonitrile as corrosive is not appropriate.

4.1.2.5 Sensitisation

4.1.2.5.1 Skin

Studies in animals

Koopmans and Daamen (1989) carried out a Guinea Pig Maximisation in compliance with EC and OECD guidelines. Sensitisation was induced by an intradermal injection of 2.5% acrylonitrile and an epidermal application of 2% acrylonitrile 7 days later. Animals challenged with acrylonitrile concentrations of 0.5% and 1.0% acrylonitrile showed a 95% positive sensitisation rate. Exposure to 0.2% on challenge caused an 80% sensitisation rate. It can be concluded that acrylonitrile has marked sensitising properties and should be classified as sensitising, using the EU criteria.

Studies in humans

In a case reported by Hashimoto and Kobayashi (1961) skin lesions were first observed at the site of contact with liquid acrylonitrile, which then spread rapidly to other neighbouring regions. Several days after contact the lesions spread to other parts of the body that had not been in contact with the liquid. It was concluded that these later skin lesions were indicative of an allergic type response to the initial exposure to acrylonitrile liquid.

A positive patch test for acrylonitrile was determined in 5 employees of an acrylonitrile processing and production plant who had contact dermatitis. The 8 control individuals did not show any allergic reaction to acrylonitrile (Bakker et al., 1991).

4.1.2.5.2 Respiratory tract

There are no data available.

4.1.2.5.3 Summary of sensitisation

Animal data provide clear evidence of skin sensitisation following exposure to acrylonitrile. There is also limited evidence of skin sensitisation in humans following skin contact with acrylonitrile. It should be noted however that only a handful of reported cases exists, in reports from industry rather than in scientific papers, among the many thousands of workers who have been exposed to acrylonitrile. Regarding respiratory sensitisation there are no data available relating to this end point for either animals or humans.

The appropriate classification for acrylonitrile for this end point is Xi; R43 (sensitising, may cause sensitisation by skin contact). For classification, see Section 1.4.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Inhalation studies in animals

Short-term inhalation studies in rats, rabbits, guinea pigs, dogs, cats and monkeys

Dudley et al. (1942) investigated the effects of daily inhalation exposure to acrylonitrile in a number of animal species using a range of exposure levels and durations. The sex of the animals used in these studies was not specified and control animals were not included in the experiments. These older studies also did not include quantitative data or statistical analyses.

Study 1

2 dogs and 4 rhesus monkeys were exposed for 4 hours daily, 5 days a week for 4 weeks, to an average concentration of 56 ppm (126 mg/m^3) of acrylonitrile in air. The 4 rhesus monkeys survived and showed no evidence of toxicity during the 4-week exposure period. After the first 4-hour exposure, one dog died in convulsions while the second dog developed a transitory weakness, showing a paralysis of the hind legs after the 5th, 13th and 14th exposures. Subsequent exposures were well tolerated. These experiments indicated that dogs are more susceptible to acrylonitrile than monkeys and that repeated exposure to concentrations of 56 ppm (126 mg/m^3) of acrylonitrile in air produced no signs of cumulative action in monkeys.

Study 2

16 rats, 16 guinea pigs, 3 rabbits and 4 cats were exposed for 4 hours daily, 5 days a week for 8 weeks, to an average concentration of 100 ppm (225 /m^3) of acrylonitrile in air. Exposure to this level was tolerated well by rats, the only sign of toxicity being a slight lethargy during exposure. Three of the 7 females gave birth to and raised normal litters. Guinea pigs gained weight moderately and showed a slight lethargy during the exposure but otherwise revealed no toxic effects. Rabbits survived for the 8 weeks but were drowsy and listless during the exposure, and showed no weight gain. Cats showed occasional vomiting, were lethargic and lost weight, with one animal developing transitory weakness of the hind legs after the 3rd exposure and dying

after the 11th exposure. The remaining three cats survived the entire exposure period with few untoward effects.

These experiments showed that rats, guinea pigs, and rabbits tolerate repeated exposures to 100 ppm (225 mg/m^3) of acrylonitrile in air over a period of 8 weeks. Cats were shown to be more sensitive to acrylonitrile than rodents. In these experiments there was no evidence of cumulative action of acrylonitrile.

Study 3

Sixteen rats (8 adult and 8 young animals), 16 guinea pigs, 4 rabbits, 4 cats and 2 rhesus monkeys, were exposed for 4 hours daily, 5 days a week, for 8 weeks to an average concentration of 153 ppm (344 mg/m^3) of acrylonitrile in air. Rats lost weight, their coats became rough, and their general physical condition was poor. 50% of the animals died during the third and fourth week of exposure. The 8 young rats showed definite impairment of growth and marked irritation of the eyes and nose. One of these 8 died during the third week of exposure. All of the 8 adult rats showed irritation of the eyes and nose. Four died by the end of the fifth week of exposure. Guinea pigs showed irritation of the eyes and nose and salivation during the first week of exposure. Three of the 16 animals died during the fifth week of exposure. The remainder of the animals gained weight slightly and were in fair condition at the end of the study. Rabbits showed moderate irritation of the eyes and nose. One of the 4 animals died during the fifth week of exposure. Cats were severely affected. All showed severe distress with each exposure and were frequently in collapse at the end of the exposure period. They suffered from marked nasal and conjunctival irritation and they all developed transitory weakness of the hind legs. One animal died after the second exposure. Monkeys showed sleepiness and weakness, loss of appetite, and frequently suffered from salivation and vomiting. One animal died after 6 weeks of exposure and the second animal was in complete collapse after each exposure, during the last 2 weeks of the study.

These experiments showed that repeated exposures to 153 ppm (344 mg/m^3) of acrylonitrile in air were definitely toxic to guinea pigs, rats, and rabbits, and were much more toxic to monkeys and cats. These exposures produced irritation of eyes and nose, loss of appetite, gastro-intestinal disturbances and an incapacitating weakness of hind legs from which the animals recovered relatively rapidly. Even with exposure to such high concentrations, no definite evidence of cumulative action was observed.

Histopathological findings

Paraffin sections were made from the spleen, kidneys, liver, lung, heart, pancreas, lymph nodes, stomach, duodenum, jejunum, ileum, and large intestine, from a representative number of animals in the above studies. Sections from all of the spleens and a representative number of livers were treated with acidulated ferro-cyanide to demonstrate the presence of or absence of iron-bearing pigment. A total of 680 sections from 18 rats, 6 rabbits, 6 cats, 16 guinea pigs, and 1 monkey were examined. A slight amount of hemosiderosis indicative of blood destruction was seen in the spleens of rats. This increased in degree and occurred in a greater number of animals with the higher concentrations. Negligible amounts were noted in the spleens of cats, guinea pigs, and rabbits.

Evidence of renal irritation was noted in most animals exposed. Hyaline casts were present in the straight collecting tubules of all of the animals exposed in studies 2 and 3, except for one cat and one rabbit receiving 100 ppm (225 mg/m^3), and the monkey exposed to 153 ppm (344 mg/m^3) of

acrylonitrile in air. Subacute interstitial nephritis, characterised by focal collections of lymphocytes, a few polymorphonuclear leukocytes and sometimes accompanied by small areas of fibrosis with occasional distension of the tubules, was found in a significant number of animals, although it was never extensive. The monkey and all the rats exposed to 100 ppm (225 mg/m^3) failed to show these changes. The species difference in relation to kidney involvement was otherwise not significant, although the guinea pigs and rabbits appeared to be the most affected. As these animals were symptomatically the least susceptible, it may be assumed that the greater renal damage indicates either more active excretion or a difference in species metabolism.

Subacute bronchopneumonia, characterised by congestion and oedema of the alveolar walls, extravasation of red cells and serum into the alveoli, and focal collections of lymphocytes and polymorphonuclear leukocytes, was present in all but one guinea pig and rabbit respectively, in the monkey, and about one-third of the rats. No pneumonic changes occurred in cats. Liver damage was observed only in cats. Weekly blood counts including red blood count, white blood count, haemoglobin, and differential counts were made on four rats and four rabbits during their 8-week exposure to 153 ppm (344 mg/m^3). The red and white blood counts and haemoglobin determinations remained within normal limits. The differential counts revealed an increase in eosinophils in both rats and rabbits, ranging from no eosinophils at the end of the first week to a maximum of 35, 42, 36, and 25% in the rabbits, and from 1% to a maximum of 21% in the rats. The cause of this marked increase in eosinophils is not known.

Target organs identified in the Dudley et al. studies, were the nervous system (transitory limb weakness and/or paralysis in dogs and cats), the kidney (histopathological changes in rats and rabbits), the upper respiratory tract (nasal irritation in all species studied) and the lung (bronchopneumonia, again in all species except cats).

Short-term inhalation study in rats, effects on intermediary metabolism

Gut et al. (1985) studied the effect on intermediary metabolism in rats following repeated exposure to acrylonitrile via inhalation. Male Wistar rats were exposed to acrylonitrile at a concentration of 130 ppm (280 mg/m^3) for 8 hours/day for 5 days. The body weight gradually decreased over the 5 days of exposure, and inspection of the abdominal cavity revealed a marked decrease of intra-abdominal fat. The weight of the liver decreased, while the weight of the brain did not. There were no changes in the absolute nor relative weights of the kidneys, lungs and adrenals. The relative weight of the liver significantly decreased ($P < 0.05$), but that of the brain increased by ($P < 0.05$) due to the body weight decrease.

Clinical chemistry and biochemical measurements showed a significantly decreased ($P < 0.05$) serum concentration of cholesterol and triglycerides, but the liver concentrations of phospholipids and esterified fatty acids were unchanged. The liver microsomal protein and cytochrome P-450 content decreased significantly ($P < 0.001$), while the levels of glucose, lactate and pyruvate in the blood and brain increased significantly (up to 250% compared to controls). Microscopic examination of the lungs, liver, kidneys and adrenals did not show histopathological changes and the numbers and enzyme activities of alveolar macrophages were also unaffected. This study in rats indicated that blood glucose could be a good marker as an indicator of exposure to acrylonitrile, but whether this indicator is sufficiently sensitive in humans needs to be researched further.

Short-term inhalation study in rats, effects on procoagulant activity of the lung

Following the observation of Sitrin et al. (1983) that there was a close association between acute lung injury and abnormalities of the coagulant system, Bhooma et al. (1992) studied the effect of acrylonitrile on the procoagulant activity (PCA) of rat lung. In this study 6 rats were exposed to acrylonitrile levels of 100 ppm (225 mg/m^3) for 5 hours/day for 5 days. The lungs (together with other organs) were removed. The lungs were lavaged 6 times with 5 ml cold, sterile, isotonic saline, with collection of the lavage effluent. Cells were counted in a haemocytometer chamber and viability was determined. Levels of PCA in macrophages and bronchoalveolar lavage (BAL) fluid were measured on day 1, 3, 5, 7, 14 and 28 days after acrylonitrile exposure. An increased coagulation capability of alveolar macrophages of the lung detected in this study was indicative of a lung-damaging effect. The elevation of alveolar macrophage procoagulant activity occurred from day 1 to 14 post-exposure. On the 28th day the levels returned to normal. BAL-PCA increased at this time, possibly due to the release of macrophagic PCA into BAL facilitated by fibrin degradation products.

Hopper et al. (1981) observed that the formation of fibrin networks on the surfaces of stimulated peritoneal macrophages impaired their mobility and that macrophage-associated PCA appeared to promote the formation of these fibrin networks. The Bhooma study also demonstrated fibrin network formation in the lung following exposure to acrylonitrile. The elevated macrophage PCA level up to day 14 and the decrease on day 28 illustrates the dynamic interplay between procoagulant activity and fibrinolytic factors. However it should be noted that the exposure level of acrylonitrile in the Bhooma experiment high (100 ppm) and the exposure regime was 5 hours/day for 5 continuous days. Since acrylonitrile is a known irritant it could be anticipated that a degree of lung irritation would occur at such a level, associated with an elevation of the macrophage PCA level.

90-day inhalation studies in rats, mice and dogs

90 day subacute inhalation studies (Brewer, 1976) were carried out in groups of 6 beagle dogs (3 male and 3 female) exposed to mean atmospheric concentrations of 0, 24 (54 mg/m^3) and 54 ppm (121.5 mg/m^3) acrylonitrile, and in 40 albino rats and 30 albino CD-1 mice exposed to 0, 24 (54 mg/m^3), 54 ppm (121.5 mg/m^3 and 108 (243 mg/m^3). The exposure regime was 6 hours per day, 5 days per week for a total of 57 exposures.

In the dog study at 54 ppm (121.5 mg/m^3), 1/3 males and 2/3 females died. All animals showed some decrease in weight gain. Clinical symptoms included rhinitis, ataxia and increased diuresis. Organ weights of the liver, kidney, spleen, adrenal gland, lungs, gonads, thyroid gland, heart and brain were similar to those of the control animals. The haematological and clinical chemistry findings were also similar to those in controls, other than a slight increase in serum alkaline phosphatase. Histopathological examination revealed focal macrophage infiltration, focal fibrosis and multifocal bronchopneumonia in 1/3 males and 3/3 females. Dogs exposed to 24 ppm (54 mg/m^3) acrylonitrile showed no mortality but signs of lung irritation were observed even at this low-dose level, comprising focal alveolar macrophage infiltration and multifocal bronchopneumonia (2/3) in female dogs only. Serum alkaline phosphatase was also slightly increased. The NO(A)EL for in dogs is thus below 24 ppm (54 mg/m^3).

In the rodent study, 5/40 control rats, 5/40 at 24 ppm (54 mg/m^3), 5/40 at 54 ppm (121.5 mg/m^3) and 18/40 at 108 ppm (243 mg/m^3) died during the study, while the comparable figures in mice were 23/30 controls, 21/30 at 24 ppm (54 mg/m^3), 15/30 at 54 ppm (121.5 mg/m^3) and 27/30 at 108 ppm (243 mg/m^3). Mortality in mice and rats was therefore unaffected by exposure to 24 and

54 ppm (54 mg/m^3 and 121.5 mg/m^3) acrylonitrile. However an increased lethality was seen in rats exposed to a level of 108 ppm (243 mg/m^3), with some increase in deaths also being seen in mice at this level. As in dogs, clinical symptoms included body weight retardation, rhinitis, ataxia and increased diuresis. Organ weights, haematological, clinical chemistry findings were similar to those in control animals.

Microscopic examinations of brain (cerebrum, cerebellum and pons), bronchi, small intestine, gonads, gall bladder (dogs only), heart, kidney, liver, lungs, lymph nodes, spleen, trachea and thyroid were carried out in untreated controls (dog) and dogs at 24 and 54 ppm (54 mg/m^3 and 121.5 mg/m^3), untreated controls (rats and mice) and rats and mice at 108 ppm (243 mg/m^3). Histopathological examination of tissues in dogs revealed treatment-related changes in the lung of some dogs at dose levels of 24 and 54 ppm (54 mg/m^3 and 121.5 mg/m^3). These changes were exposure-related with regard to incidence and relative severity. The changes consisted of focal to multifocal suppurative bronchopneumonia, and focal aggregates of alveolar macrophages in the alveolar lumina. These lesions were indicative of mild irritation. There were three mortalities at the 54 ppm (121.5 mg/m^3) dose level. The focal haemorrhages described in the lung of sacrificed animals were agonal lesions related to the method of sacrifice. Any other changes seen in other tissues were lesions of naturally occurring diseases and they were present among both control and test animals. The histopathological examination of the tissues in rats resulted in findings confined to the lung, only in rats exposed to 108 ppm (243 mg/m$^{3)}$), which were indicative of irritation. These changes consisted of a slight to mild increase in number of alveolar macrophages in the lumina of alveoli and a suppurative bronchopneumonia. No histopathological alterations were noted among the test mice.

Asphyxiation secondary to the sub-acute bronchopneumonia in affected animals was the major cause of death. Chronic respiratory disease was present in the lung and trachea of all the control and most of the test animals.

The quality of the Brewer studies is questionable. The presence of chronic respiratory disease in the rodents is noted, as is the high mortality in all exposure groups. Their value as pivotal studies for risk assessment purposes is therefore limited. The results suggest that the main effect of acrylonitrile inhalation in dogs was irritation of the lungs, to which the females seemed to be more sensitive than males. As with acute exposure, dogs were more sensitive to acrylonitrile than rats or mice, while the two rodent species seemed to be equally sensitive. The results indicate that the subchronic no observed adverse effect level (N(A)OEL) is less than 24 ppm (54 mg/m^3) in the most sensitive species, the dog, as lung irritation was still seen at this low-dose level. For the purposes of risk assessment (while recognising the limitations of the studies) this exposure level may be considered to be an LO(A)EL.

Two-year inhalation study in rats, Quast study (1980a)

The long-term inhalation study of Quast et al. (1980a) is one of the most important repeated dose studies for risk assessment purposes. Although it is a comparatively old study, it appears to have been well-conducted and valid for the risk assessment. Sprague-Dawley (Spartan sub-strain) rats (100/sex/concentration) were exposed for 6 hours per day, 5 days per week, during 2 years, to concentrations of 0, 20 and 80 ppm acrylonitrile (0, 45 and 180 mg /m^3). The control group was exposed to air alone. Additional animals were included for interim sacrifices at 6 months (n=7 /sex/dose) and 12 months (n=13 /sex/dose).

Clinical observations detected a variety of toxic effects characterised by decreases in body weight, early mortality, unthrifty clinical appearance, earlier onset of tumours and more

frequently observed palpable tumours. These observations were most apparent and occurred earliest in the high-dose group (80 ppm; 180 mg/m^3). A significant decrease in mean body weight was observed in rats exposed to 80 ppm (180 mg/m^3) acrylonitrile. Less significant, but similar weight decreases, were noted in the 20 ppm (45 mg/m^3) females after approximately a 1 month exposure. A treatment-related effect on mean body weight was not observed in males at this dose level (20 ppm; 45 mg/m^3). During the first 6 months of the study the exposed rats drank more water and appeared to excrete lower specific gravity urine than control animals.

Mean fasted body weights, mean organ weights, and organ to body weight ratios were examined within this study. In the 80 ppm (180 mg/m^3) group of male rats a significantly increased relative organ to body weight ratio ($p < 0.05$) was observed for the brain, heart, and testes. However, since body weight of fasted animals was significantly decreased ($p < 0.05$) in this group, the relative increase in these organs to body weight ratios were considered to be a reflection of the effect on body weight. In addition, the absolute kidney weight in the 80 ppm (180 mg/m^3) group of males was significantly decreased ($p < 0.05$). This observation was consistent with the decreased body weight and a decrease in the severity of chronic renal disease which was observed grossly and microscopically in these rats. The increased relative organ weights were a manifestation of the decreased body weight gain and do not indicate a specific target organ toxic effect. In the few surviving females there was a significantly increased ($p < 0.05$) liver to body weight ratio in the 20 ppm (45 mg/m^3) group. The increased liver to body weight ratio and the slight increase in the absolute liver weight in these rats, as well as in the single surviving rat in the 80 ppm (180 mg/m^3) group, were interpreted to be the result of increased extramedullary haematopoiesis in the liver. This was a result of the greater number of bleeding tumours in these rats and was not interpreted to be indicative of a primary hepatoxic effect due to acrylonitrile exposure.

During the course of the study haematology, urinalysis, and clinical chemistry determinations were performed at periodic intervals. The results showed that acrylonitrile exposure did not have a primary adverse effect on bone marrow, kidney, or liver function in either male or female rats. Occasional significant reduction of the packed cell volume (PVC), haemoglobin and in the RBC, and WBC counts were noted. However these were interpreted as being secondary changes associated with decreased growth and tumour induction and haemorrhage, generalised stress, and inflammatory reactions resulting from exposure to acrylonitrile.

A statistically significant increase in mortality ($p < 0.05$) was observed within the first year in both male and female rats administered 80 ppm (180 mg/m^3) acrylonitrile and in the females of the 20 ppm (45 mg/m^3) group during the last 10 weeks of the study. The apparent increase in the reported mortality for the 20 ppm (45 mg/m^3) females was principally due to early sacrifice of rats with large, benign, mammary gland tumours (Quast, 1980). In Sprague-Dawley the tumours are known to occur spontaneously at a high rate, but in this experiment the tumours were observed earlier and more frequently, and became larger in exposed animals. The neoplastic changes seen in this study are described in greater detail in Section 4.1.2.8.2. **Table 4.15** shows the cumulative mortality data in both male and female animals at the three exposure levels (i.e. 0, 20 and 80 ppm; 0, 45 and 180 mg/m^3). Statistically significant early mortality is indicated in both males and females at 80 ppm (180 mg/m^3). However the onset of early mortality begins much earlier into the study for male rats i.e. days 211-240, compared to females in which a significant increase in mortality was only seen at days 361-390.

Table 4.15 Cumulative mortality data for rats exposed to acrylonitrile via inhalation for 2 years (Quast et al., 1980a)

Days on test	Sex	0 ppm	20 ppm (45 mg/m³)	80 ppm (180 mg/m³)	Days on test	Sex	0 ppm	20 ppm (45 mg/m³)	80 ppm (180 mg/m³)
0-30	M	0	1	0	0-30	F	1	0	0
31-60	M	0	1	0	31-60	F	1	0	0
61-90	M	0	1	1	61-90	F	1	0	0
91-120	M	0	2	1	91-120	F	1	0	0
121-150	M	1	2	1	121-150	F	1	0	0
151-180	M	2	3	2	151-180	F	1	0	0
181-210	M	2	3	6	181-210	F	1	1	1
211-240	M	2	4	12 [a)]	211-240	F	1	1	2
241-270	M	2	5	13 [a)]	241-270	F	3	1	4
271-300	M	2	5	14 [a)]	271-300	F	5	1	6
301-330	M	3	6	16 [a)]	301-330	F	5	2	9
331-360	M	3	6	18 [a)]	331-360	F	7	2	11
361-390	M	4	8	19 [a)]	361-390	F	9	3	19 [a)]
391-420	M	6	9	22 [a)]	391-420	F	11	5	27 [a)]
421-450	M	11	12	24 [a)]	421-450	F	14	10	33 [a)]
451-480	M	14	15	28 [a)]	451-480	F	14	14	41 [a)]
481-510	M	19	26	39 [a)]	481-510	F	19	22	57 [a)]
511-540	M	23	34	47 [a)]	511-540	F	26	31	71 [a)]
541-570	M	27	38	56 [a)]	541-570	F	34	36	80 [a)]
571-600	M	35	47	63 [a)]	571-600	F	36	43	88 [a)]
601-630	M	43	59 [a]	76 [a)]	601-630	F	50	54	94 [a)]
631-660	M	62	68	83 [a)]	631-660	F	63	70	98 [a)]
661-690	M	71	72	85 [a)]	661-690	F	66	81 [a)]	98 [a)]
691-720	M	78	81	94 [a)]	691-720	F	71	88 [a)]	99 [a)]
721-735	M	82	86	96 [a)]	721-735	F	78	91 [a)]	99 [a)]
TOTAL No. Rats		100	100	100			100	100	100

a) significantly different from controls $p < 0.05$

Note: Data listed as number dead which is equal to percent dead

Complete histopathological examinations were done on 40 organs of the rats in the control and 80 ppm (180 mg/m³) groups. At least 23 selected organs were examined in 80% of the rats in the 20 ppm (45 mg/m³) group. Histopathological examination revealed increased pathological changes in the heart and lungs of male rats of both treatment groups. The authors indicated however that the changes seen were identical to effects seen in the control animals and were usually associated with chronic renal disease. Microscopic examination of the kidneys indicated a slight, non-statistically significant increase in the incidence of spontaneously occurring advanced chronic renal disease. However, this slight increase could have been due to increased

demand on the kidneys, resulting from increased water consumption seen earlier in the study (first months).

A treatment-related increase in extramedullary haemopoiesis in the liver and the spleen and an increase in focal liver cell necrosis was observed primarily at the 13-18 month and the 19-24 month intervals, with those in treated rats generally being observed at the earlier time intervals when compared with the controls. The finding of extramedullary haemopoiesis was considered (Quast, 2001, personal communication) to be secondary to the presence of large, benign mammary tumours in the animals, which occurred earlier in treated animals than controls. The presence of these tumours was frequently associated with haemorrhage and tissue damage or pressure necrosis due to contact with the wire mesh cage, the haemorrhage and blood loss in turn resulting in compensatory extramedullary haemopoiesis. The development of large, frequently ulcerated, necrotic and haemorrhagic ear canal (Zymbal gland) tumours in acrylonitrile treated rats contributed to this compensatory response. The presence of increased focal hepatic necrosis in these rats was also considered (Quast, 2001, personal communication) to be a secondary effect due to repeated episodes of blood loss and associated anaemia and hypoxia. It was concluded, therefore, that these findings were not indicative of a primary hepatotoxic effect of acrylonitrile. This is supported by the fact that the 6- and 12-month interim pathology data did not indicate any primary haemopoietic or liver toxicity attributable to acrylonitrile exposure, nor was any such effect demonstrated in other chronic toxicity studies in rats and dogs exposed to acrylonitrile by different routes (Quast, 2001, personal communication).

A treatment-related effect was observed in the nasal turbinate mucosa of all rats examined in the 80 ppm (180 mg/m^3) group as well as in some of the rats in the 20 ppm (45 mg/m^3) group. At the 6- and 12-month interim pathology evaluations, a grading system was used to demonstrate the concentration-related effect in the nasal turbinates. In general the changes were confined to the respiratory epithelium, were considered minimal in degree and reflected the known irritant effects of acrylonitrile. The changes in both exposure groups were qualitatively similar but much less severe in the 20 ppm (45 mg/m^3) group than in the 80 ppm (180 mg/m^3) group. In the chronic phase of the study (months 12-24) more pronounced changes were observed in males exposed to 80 ppm (180 mg/m^3), at month 19-24 of the study. In this latter part of the study, a grading system was not used, as it was considered that incidence data, as presented in **Table 4.16**, maximised the observed effect in each exposure group. The changes observed comprised suppurative rhinitis, hyperplasia in the region of the nasal turbinate mucosa lined by the respiratory epithelium, focal erosion of mucosa lining the respiratory epithelium, squamous metaplasia of the respiratory epithelium, etc., (incidence findings given in **Table 4.16** above). Similar occurrences (though fewer in number) were noted for the 20 ppm (45 mg/m^3) males only at the terminal kill. A similar pattern was noted for female test animals. Effects on the nasal turbinate mucosa were first observed at month 19 for the 80 ppm (180 mg/m^3) group, with similar though less frequent effects only being observed at terminal kill in the 20 ppm (45 mg/m^3) group. Some of these changes were noted in the female control animals either at 19 months or at the terminal kill. No tissues from the male control group were examined for these specific effects. These changes were confined to the turbinate region extending from the external nares into the region lined by respiratory epithelium. These changes were considered to be a result of irritation due to acrylonitrile exposure.

In addition, in two of the 80 ppm (180 mg/m^3) female rats there was a microscopic metaplastic proliferation of the respiratory epithelium. Although the incidence of this lesion was not statistically significantly increased, it was considered treatment-related, in view of its location in the same region of the nasal mucosa showing the degenerative and inflammatory changes and

because of the historically low spontaneous incidence of this change. **Table 4.16** summarises the changes in the nasal passages considered to be attributable to acrylonitrile exposure in this 2-year study in rats.

Table 4.16 Treatment-related histopathological changes in the nasal passages of rats exposed to acrylonitrile by inhalation for up to 2 years

Histopathological findings	Exposure level of acrylonitrile (ppm)		
	0	20	80
Hyperplasia of respiratory epithelium in the nasal turbinate mucosa			
Males	0/11	4/12	10/10
Females	0/11	2/10	5/10
Squamous metaplasia of respiratory epithelium in the nasal turbinate mucosa			
Males	0/11	1/12	7/12
Females	0/11	2/10	5/10
Hyperplasia of mucous secreting cells			
Males	0/11	7/12	8/10
Females	0/11	2/10	8/10

Note: the figures indicate no. showing effect/no. of animals in which the nasal passages were examined microscopically
(Quast et al., 1980a)

In addition to the changes observed in the nasal passages, treatment-related non-neoplastic lesions were also detected in the brain, characterised by focal perivascular cuffing and gliosis. In males at 20 and 80 ppm (45 and 180 mg/m^3) the incidence was 2/99[7] and 7/99[5] ($p < 0.05$, one-sided), respectively and for females the incidence was 2/100 and 8/100 ($p < 0.05$, one-sided), respectively.

In summary, Quast et al. (1980a) demonstrated treatment-related non-neoplastic changes in Sprague-Dawley rats exposed to 20 ppm or 80 ppm (45 or 180 mg/m^3) acrylonitrile for 6 hours/day, 5 days/week, for 104 weeks, consisting of effects on bodyweight and early mortality in both sexes in the 80 ppm (180 mg/m^3) group and in females at 20 ppm (45 mg/m^3). As a result of irritation due to acrylonitrile exposure, inflammatory and degenerative changes (hyperplasia and metaplasia of the respiratory epithelium) were present in the nasal turbinates of both the 20 and 80 ppm group (45 and 180 mg/m^3). A significantly increased number of rats in the 80 ppm (180 mg/m^3) exposure group also showed focal gliosis and perivascular cuffing in the brain.

This study is considered to be a pivotal study for risk assessment purposes. The key toxicological findings due to acrylonitrile exposure were local irritant effects in the nasal epithelium comprising suppurative rhinitis, hyperplasia, focal erosions, and squamous metaplasia of the respiratory epithelium, with hyperplasia of the mucous secreting cells. Effects were seen at the lowest effect level of 20 ppm (45 mg/m^3) used in the study, and this represents therefore a LO(A)EL. Application of a safety factor of 5 to the level of 20 ppm (45 mg/m^3) to give a suggested No Adverse Effect Level (NAEL) of 4 ppm (9 mg/m^3) is considered justifiable because of the

[7] One male animal in each of these groups died in the first 3 months of the study and group size is therefore described as 99 rather than 100 (See Table 4.15).

nature of the effect (local irritancy) and the conclusion that other systemic, non-neoplastic findings in acrylonitrile-treated rats were secondary to the tumorigenic effects of acrylonitrile, rather than due to direct systemic toxicity. The suggested NAEL is supported by the evidence from the study of Sakurai et al. (1978) that levels below 10 ppm (22.5 mg/m^3) did not cause notable irritancy in humans (see Section 4.1.2.6.5).

Inhalation carcinogenicity study in rats (Maltoni study)

Maltoni et al. (1977) studied the effects of inhalation exposure to 5, 10, 20, and 40 ppm of acrylonitrile, 4 hours daily, 5 days weekly, for a 12 month period on groups of 30 male and 30 female rats. One group of untreated rats acted as a control group for the study. After this 12-month exposure period the animals were kept under observation until spontaneous death with no further exposure to acrylonitrile. Slight increases in tumour incidences were observed in the mammary gland (males and females), forestomach (males) and skin (females), but none of these were statistically significant. The results were considered by the authors to indicate a "borderline carcinogenic effect".

No excess in mortality related to acrylonitrile treatment was observed in any of the animals. A slightly lower survival rate, though not statistically significant was noted in the control male rats. A statistically significant ($p < 0.01$) increase in malignant and total number of tumours occurred only in females at 5 ppm.

This study focused on neoplastic changes and provides little useful information for the assessment of chronic toxicity of acrylonitrile. No effect on body weight was observed. While this study is not suited to establishing or even estimating an NO(A)EL, there was no excess in mortality nor body weight changes and the borderline effects were seen in females only at 5 ppm. This study could be used as an indicator that an (NO(A)EL) lay between 5 and 10 ppm.

4.1.2.6.2 Oral studies in animals

Subacute toxicity drinking water study in rats

Humiston and Frauson (1975) showed treatment-related effects in adult Sprague-Dawley rats receiving acrylonitrile in drinking water up to a dose equivalent of 42 mg/kg/day weight for 90 days. Reduced water consumption was observed at dose levels above 10 mg/kg/day, while growth retardation occurred at levels of about 22 mg/kg/day and higher in female rats and at 42 mg/kg/day in males. Mean weekly food consumption was reduced for the first 7 weeks of the study at a dose level of 38 mg/kg, while at a dose level of 17 mg/kg/day it was reduced in the first 2 weeks. Increased relative liver weight was observed at acrylonitrile levels of 10 mg/kg/day and higher.

Subacute toxicity drinking water and gavage studies in rats with emphasis on effects of acrylonitrile on the gastrointestinal tract and adrenals

Szabo et al. (1984) carried out a number of studies in order to elucidate the sub-acute and chronic actions of acrylonitrile on the adrenals, stomach and duodenum by correlation of biochemical, functional and morphological findings, and to gain insight into the mechanisms of action of acrylonitrile. In total four studies were carried out with the aim of investigating dose and time dependency, age dependency and route of administration.

Study 1

Acrylonitrile at levels of 0.2% (2,000 ppm), 0.05% (500 ppm), 0.01% (100 ppm) and 0.0% (0 ppm) was given to adult female rats (3 to 5 animals per group including controls) in drinking water over a period of 2 weeks. Water and food intake were monitored continuously and body weights were recorded every 4 days. A group of pair-fed rats (with food restriction) was also included, with the aim of keeping body weights parallel with the 2,000 ppm dose group. A further group of rats was given 100 mg/kg of acrylonitrile by gavage twice daily, this dose being stated to be equivalent to the daily intake of the 2,000 ppm in drinking water group.

Study 2

Acrylonitrile was given to rats in drinking water at concentrations lower than those in study 1, namely 0, 1, 20, 100 and 500 ppm over a period of 60 days. Food and water intake and body weight were monitored as above. Additional groups were administered acrylonitrile by gavage once daily at levels corresponding to the intake of the drinking water animals, namely 0, 0.2 (1 ppm), 4.0 (20 ppm), 20 (100 ppm) or 60 (500 ppm) mg/kg body weight in distilled water.

Study 3

This study was undertaken following the observation that there could be an age-dependency with respect to the sensitivity of rats to effects of acrylonitrile on the adrenals. Weanling rats with an initial body weight of 35-40 g. and adult rats of 200-210 g. were given water containing 0, 20, 100 or 500 ppm acrylonitrile or given the corresponding amount of acrylonitrile by gavage daily for 21 days. This dose was 40 or 8 mg/kg/day for adult rats and 60 or 12 mg/kg/day for weanling rats.

Study 4

This last study was designed to assess the ability of the adrenals to recover from toxic insult, including production of steroid hormones, and to characterise further the adrenal ultrastructural changes noted in the previous experiments. Groups of young female rats were placed on drinking water containing 0, 100 or 500 ppm of acrylonitrile. One week later one control and two treated groups (i.e. one 100 ppm and one 500 ppm group) were unilaterally adrenalectomised. Three weeks later the adrenalectomised and certain control and acrylonitrile-treated groups were killed. The adrenals were rapidly removed, weighed and processed for electron microscopy. The remaining (non- adrenalectomised) control and experimental groups were kept for 60 days, when the animals were given ACTH. Four hours later these were killed and trunk blood was collected for plasma corticosterone determination.

Results

In general no overt signs of intoxification from acrylonitrile exposure was noted and mortality only occurred in the 2,000 ppm (study 1) dose group, in which 2/18 rats died from severe bilateral adrenal haemorrhage and necrosis. Decreased water and food intake was observed in both the 2,000 and the 500 ppm drinking water groups and following 100 mg/kg twice daily by gavage.

Adrenal weights were decreased in 7, 14 and 21 day studies in rats receiving 500 and 2,000 ppm acrylonitrile in drinking water, accompanied in the 2,000 ppm group by polyuria. Pair-fed controls to the 2,000 ppm group also showed a decreased relative adrenal weight, but urinary output was normal. However animals given the equivalent of 2,000 ppm (100 mg/kg twice daily) by gavage showed an enlargement of the adrenals, again accompanied by polyuria. Following

60 days administration of acrylonitrile in drinking water (study 2), there was also a significant increase in adrenal weight, which was particularly prominent in the group given 60 mg/kg/day (equivalent to 500 ppm) daily.

Histological examination of the adrenals from rats administered 500 and 2,000 ppm in drinking water for 7, 14 or 21 days revealed atrophy in the adrenal cortex, especially the zona fasciculata. In contrast, cellular hyperplasia with normal size or slightly shrunken cells was seen in the adrenals from rats given equivalent amounts of acrylonitrile by gavage and in animals administered 500 ppm in drinking water for 60 days. The results suggest that the effects of acrylonitrile on the adrenals were in part attributable to inherent toxicity and consequences of decreased food and, in particular, water intake, probably due to the unpalatability of acrylonitrile in drinking water, even at levels of 20 ppm. When this confounding factor was removed, by gavage dosing, the adrenals responded with hypertrophy and hyperplasia of the cortex.

Plasma levels of corticosterone showed a dose-dependent decrease in rats administered 100, 500 or 2,000 ppm acrylonitrile in drinking water, with larger decreases being seen when acrylonitrile was administered by gavage. The decrease noted in the 2,000 ppm group (14 days administration) was however even more marked in pair-fed controls. Plasma aldosterone levels were less affected by administration of acrylonitrile, effects only being seen at high levels and prolonged exposure. A significant decrease was observed only after administration by gavage of 60 mg/kg/day for 60 days.

Other effects reported in these studies were increased liver weights following a 21-day administration period, with a decrease being reported after 60 days. Kidneys were enlarged in the 100 ppm group after 60 days of administration and in the 500 ppm group after 21 days. Regional hyperplasia was observed in the gastric mucosa of rats receiving 100 and 500 ppm acrylonitrile in drinking water for at least 21 days.

While these studies were not designed with a view to calculating a NO(A)EL the information may be used to assess the effects produced by increasing doses of acrylonitrile, administered both via the drinking water and by gavage. Treatment-related effects occurred consistently at the 100 ppm level via drinking water, with 20 ppm representing a NO(A)EL. This was reported by the author to be equivalent to an intake of 4 mg/kg/day.

Subacute toxicity oral gavage studies in rats

Barnes (1970) administered 15 daily oral doses of 30 mg/kg to groups of 6 rats, followed by 7 doses of 50 mg/kg/day and then 13 doses of 75 mg/kg/day over a period of 7 weeks. No effects on body weight and no neurotoxic effects (gait, hindlimb activity) were observed.

Both these studies are reported as summarised in WHO (1983), VROM (1984), and DECOS (1994).

Subacute toxicity in rats using other routes of administration

Daily subcutaneous administration of 40 mg/kg body weight over 4 weeks, or daily intraperitoneal injections of 20 mg/kg for 6 weeks were not fatal to rats (Krysiak and Knobloch, 1971). Animals receiving acrylonitrile at either 40 or 20 mg/kg via s.c. or i.p. injection for 4 and 6 weeks respectively showed a significant lengthening of the time to perform correctly in a conditioned food reflex test and a significant decrease in the number of correct reactions achieved, compared to the controls or pre-treatment observations. Performance improved when the treatment was discontinued, thus indicating a treatment-related response with respect to exposure to acrylonitrile and its resultant effect on the nervous system of rats.

Heart and liver weights were significantly increased in adult rats receiving daily intraperitoneal injections of 50 mg/kg of acrylonitrile for a 3-week duration, and relative spleen and kidneys weights were also increased. Liver and kidney parenchymal degeneration and vacuolation of neuronal cells of the brain cortex was observed (Knobloch et al., 1971).

90-day gavage study in mice

A study by the Serota et al. (1996) using B6C3F1 mice has been carried out to determine the toxicity of acrylonitrile by oral gavage for a test period of 13 weeks with a view to setting dose levels for a subsequent carcinogenicity study. Doses of 1.2, 2.4, 4.8, 9.6, and 12.0 mg/kg/day were administered. A vehicle control, which received only distilled water, was run concurrently. Parameters used to determine toxicity included survival, clinical observations, body weights, clinical pathology, sperm morphology and vaginal cytology, gross pathology, and organ weights. 10 male and 10 female animals were assigned to each of the six groups (including the controls). Each mouse received an oral gavage dose of vehicle or acrylonitrile formulation for 5 days/week for 13 weeks. Additional animals (special study) were included in each group (41 to 71 male mice/group) for the collection of blood and tissue samples to examine acrylonitrile-associated cellular proliferation, apoptosis, haemoglobin adduct formation, and production of cyanoethylene oxide.

All core animals survived the 13 weeks of treatment. With the exception of a single mouse in the group dosed at 4.8 mg/kg/day (sacrificed in a moribund condition), all special study males survived through all scheduled sacrifices. Regarding clinical observations no treatment-related findings were noted. Sporadic occurrence of alopecia seen in several mice is a common background finding and was not considered to be related to treatment. Normal body weight gains were achieved except on one occasion due to a lack of supply of water overnight, which was detected the next day.

Biologically significant alterations were not detected in any of the haematological parameters evaluated in mice of either sex. Statistically significant declines in WBC values evident in the 2.4 and 9.6 mg/kg/day male treatment groups were not believed to be biologically significant as a dose-related trend was not identified. Again statistically significant elevations in WBC values occurring in the 4.8 and 12.0 mg/kg/day female treatment groups were not dose-related and were within the normal range for historical controls. A statistically significant increase in the mean HCT value in the 9.6 mg/kg/day female treatment group lay within a normal range. Statistically significant declines in lymphocyte counts present in the 1.2, 2.4, and 9.6 mg/kg/day male treatment groups were not believed to be of biological significance as a dose-related pattern was not identified and the values reported were within normal range. Similarly, significant elevations in lymphocyte counts evident in the 4.8 and 12.0 mg/kg/day female treatment groups and an elevation in neutrophils in the 12.0 mg/kg/day female group were believed to be within the normal biological variation range.

No treatment-related gross lesions were noted. The isolated findings in males of preputial gland cysts and enlarged inguinal lymph nodes, and the isolated findings in females of ovarian cysts and foci of ovarian tissue were considered to be unrelated to the treatment. These incidental lesions are normal findings in mice of this strain and age. A single tumour was observed in this study. The ovarian tumour was diagnosed as an ovarian choriocarcinoma, a germ cell tumour with trophoblastic differentiation. Although these tumours are rare, several have been reported in $B_6C_3F_1$ mice in studies conducted by the NCI and National Toxicology Programme (NTP) (Alison et al., 1987). Since this tumour occurred in a control mouse, it was not considered to be compound related.

Histopathological findings in tissues also indicated no treatment-related effects in this 90-day study. In investigations of sperm morphology and vaginal cytology the overall conclusion was that for male mice, only epididymal sperm motility was significantly decreased at both the 1.2 (lowest) and 12.0 (highest) mg/kg/day dose levels. This change was statistically significant compared to the control animals. However the effect occurred at only the highest and lowest dose levels and not at the intervening dose levels of 2.4, 4.8, and 9.6 mg/kg/day, indicating no clear dose-response relationship. No other male or female mouse parameters were significantly affected at any dose level.

In conclusion therefore no treatment-related effects on survival, clinical observations, body weights, clinical pathology, sperm morphology and vaginal cytology, gross or microscopic pathology, or organ weights were observed. Isolated statistically significant findings in several toxicological parameters were noted but considered to be unrelated to treatment. Based upon the conditions and findings of this study, male and female B6C3F1 mice administered acrylonitrile by gavage, 5 days/week for 13 weeks at dose levels up to 12.0 mg/kg/day exhibited no signs of toxicity. The NOEL for this study was therefore determined to be greater than 12.0 mg/kg/day for mice.

Short-term (180-day) drinking water study in dogs

Quast et al. (1975), administered acrylonitrile in drinking water at concentrations of 100, 200 or 300 mg/l to groups of 4 male and 4 female beagle dogs for 180 days (6 months). Average intakes of acrylonitrile were the following for males (with figure for females in parentheses): 10 (8) mg/kg body weight at 100 mg/l; 16 (17) mg/kg at 200 mg/l; and 17 (18) mg/kg at 300 mg/l. At 100 mg/l (10(8) mg/kg), in addition to a slight decrease in water and food intake, a slight increase in relative kidney weight was observed. Five dogs died, or were killed because of debilitation, in each of the two higher dosage groups. In the dogs receiving acrylonitrile at 100-300 mg/l in the drinking water, early signs of toxicity included roughening of the coat and, later, retching and vomiting. Terminal signs of lethargy, weakness, emaciation, and respiratory distress were noted (as summarised in WHO, 1983 and VROM, 1984).

Two-year drinking water study in rats (1)

The most informative drinking water study was performed by Biodynamics (1980b). Although it is a comparatively old study, it appears to have been well-conducted and valid for the risk assessment. Acrylonitrile was administered orally via drinking water to groups of 100 male and 100 female Fisher 344 rats at dose levels of 1, 3, 10, 30, and 100 ppm. These dose levels are estimated to be equivalent to average daily doses of 0.08, 0.25, 0.84, 2.49 and 8.36 mg/kg/day in males and 0.12, 0.36, 1.25, 3.65 and 10.89 mg/kg/day in females respectively. The control group comprised 200 male and 200 female animals. 30 males and 30 females/test group were used for interim kills (60 in controls). Interim necropsies were performed at 6, 12, and 18 months (10/sex/exposed group and 20/sex/control group). The study was terminated early because of the low survival rate.

While this study was performed as a long-term carcinogenicity study on acrylonitrile and is also reported in more detail in Section 4.1.2.8, the results of the study are relevant to the examination of the chronic toxicity of acrylonitrile, in that effects on survival and body weight were seen at relatively low doses and a NO(A)EL for such effects was established. The study has therefore been taken into account in the assessment of chronic toxicity for the purposes of this risk assessment. Other carcinogenicity studies reported in Section 4.1.2.8 provided little information on non-neoplastic changes and/or were conducted at higher dose levels and did not permit the identification of a NO(A)EL.

In this study, mortality in the males and females receiving 100 ppm was markedly greater than controls, while mortality in the males receiving 10 ppm and the females receiving 3 and 30 ppm was also somewhat greater than controls. Due to the low survival in the females at 100 ppm, all surviving females were sacrificed at 23 months. The males, however, continued on the test until month 26, when survival in the group receiving 100 ppm reached low levels; all surviving males were then sacrificed (via exsanguination under ether anaesthesia). During this study animals were observed twice daily for mortality and gross signs of toxicological or pharmacological effects. The general physical observations noted throughout the study were variable in incidence and did not occur in a pattern suggestive of an adverse effect due to treatment. The mortality data from this study are summarised in **Table 4.17** and **Table 4.18** below. It should be noted that because this study used 200 control animals/sex i.e. double the amount of test animals the mortality data for the controls must be halved for comparison purposes.

Table 4.17 Summary of mortality data in rats given acrylonitrile in drinking water over a 2-year period (Biodynamics, 1980b)

Level of acrylonitrile (ppm)	Mortality at the end of the 2-year dosing period [a]	
	Males	Females
0	48/140	29/140
1	18/70	20/70
3	24/70	24/70
10	33/70	20/70
30	26/70	29/70
100	56/71	54/69

a) Males terminated during month 26; females terminated during month 24·
In this Biodynamics study 30 animals were taken out for interim kills, and the actual incidence can therefore be related to at least a total population of 70 for treatment groups and 140 for controls.

Body weights for the males and females receiving 100 ppm were consistently lower ($p < 0.01$) than the controls, while body weights for the males only receiving 30 ppm were significantly lower ($p < 0.01$) than the controls. The body weights for the animals in the other treatment groups were generally comparable to controls throughout the study.

Food consumption for the females at 100 ppm was consistently slightly lower than controls on a grams/week basis, while this pattern was notable for the males of this group only following the first year of the study. On a grams/kg/day basis, however, food consumption for both males and females at 100 ppm was considered generally comparable to or slightly greater than controls as a result of the lower body weights for these animals. Differences from controls in food consumption for the other groups were sporadic and not indicative of a relationship to treatment. Water consumption for the males and females at 100 ppm was generally lower ($p < 0.01$) than controls on a ml/3 days basis; however, on a ml/kg/day basis, differences from the controls were less marked for the females and comparable to or greater than controls for the males. Sporadic differences from controls noted for the other groups were not considered to be treatment related.

Table 4.18 Cumulative mortality data in rats given acrylonitrile in drinking water over a 2-year period (Biodynamics, 1980b)

No. dying spontaneously, accidentally or killed in a moribund condition												
Month	**M 0**	**M 1**	**M 3**	**M 10**	**M 30**	**M 100**	**F 0**	**F 1**	**F 3**	**F 10**	**F 30**	**F 100**
1	0	0	0	0	0	0	0	0	1 *	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	1	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	1	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	1
8	0	0	0	0	0	0	1	0	0	0	0	0
9	0	0	0	0	0	1	0	0	0	1	0	0
10	0	0	1	0	0	0	0	0	0	0	0	1
11	0	0	0	0	0	1	0	0	0	0	0	1
12	0	0	0	0	0	1	0	0	0	1	0	1
13	0	0	0	0	0	2	1	0	0	1	0	0
14	1	0	0	0	0	0	0	0	0	0	0	3
15	0	0	1	1	0	1	1	0	0	0	0	4
16	0	0	1	1	0	4	0	1	3	1	2	6
17	2	0	0	1	1	2	0	0	3	0	2	3
18	1	0	1	0	1	7	3	1	3	1	4	7
19	0	1	1	2	0	5	1	0	2	0	8	4
20	2	1	1	3	2	3	3	5	3	5	5	5
21	4	2	2	0	5	7	5	9	3	1	1	9
22	4	2	0	3	1	7	7	0	2	6	1	5
23	8	3	1	5	4	3	6	4	4	2	5	4
24	11	5	3	6	4	1	0	0	0	1	1	0
25	11	2	5	5	5	4	-	-	-	-	-	-
26	4	2	6	6	3	7	-	-	-	-	-	-
Total	48	18	24	33	26	56	29	20	24	20	29	54

* Female animal dying spontaneously on Day 15 was replaced

Note: Males terminated during month 26; Females terminated during month 24
200 animals/control; 100 animals/test groups

There was an increased incidence of masses in the area of the ear in male and female rats at 30 and 100 ppm which died or were sacrificed after 12 months on the study. These masses were characterised as subcutaneous and narcotising or purulent, and were associated with the ear canals. Other gross lesions occurred in control and treated groups; they were however considered to be incidental findings and not uncommon in rats of similar age and strain. The number of malignant tumour-bearing rats was increased in the male and female rats at 10, 30 and 100 ppm when compared to control. This was due to an increased incidence of astrocytomas of the central nervous system (brain and/or spinal cord) and squamous cell carcinomas of the ear canal, as well as mammary gland carcinomas in the females at 100 ppm. The increases in the incidence of the aforementioned neoplasms were noted predominantly in animals dying, killed in a moribund state or sacrificed at scheduled intervals after the first year of the study. The incidence of

neoplasms in the rats at 1 and 3 ppm was considered comparable to controls. Other neoplastic and non-neoplastic lesions occurred sporadically in various tissues and organs but were not considered attributable to treatment.

Consistent, but not always statistically significant, elevations in the mean relative (to body weight) liver and kidney weights were noted ($p < 0.01$) for animals receiving 100 ppm at most necropsy intervals, while the mean absolute weights for these organs were generally comparable to the controls or slightly elevated. The mean relative heart weights were also elevated ($p < 0.05$) for this group at 18 months and termination. The increases in the mean relative weights of these organs at most necropsy intervals in animals receiving 100 ppm were considered treatment-related effects. In addition, at the terminal sacrifice the mean absolute and relative liver and heart weights were elevated ($p < 0.05$) for females at 30 ppm, while their mean body weight was comparable to controls. Other organ weight differences were noted, but were considered attributable to body weight differences or else they did not occur in a pattern suggestive of a relationship to treatment. Elevated ($p < 0.05$) mean organ/brain weight ratios were noted for heart and liver in the females receiving 30 ppm at termination. Other differences were sporadic and not treatment-related. For 100 ppm animals the mean absolute weights of the liver, kidney and heart as well as the brain, were not markedly different from the control animals.

Small but generally consistent reductions in haemoglobin (occasionally achieving statistical significance of $p < 0.05$), haematocrit and erythrocyte counts were noted for the females receiving 100 ppm throughout the study. These parameters were considered comparable to controls for males at this dose level. Alkaline phosphatase was slightly elevated ($p < 0.05$) in females receiving 100 ppm from 12 months onwards (to termination), while values for the males in this group were elevated ($p < 0.01$) at 18 months onwards (to termination). Slight elevations ($p < 0.05$) in alkaline phosphatase activity were also noted in females receiving 10 and 30 ppm, at termination only. Increased specific gravity of the urine was noted in males receiving 100 ppm at 18 months and termination.

In conclusion the clinical observations noted throughout this study were variable in incidence and did not occur in a pattern suggestive of a marked adverse effect due to treatment. There was an increased incidence of masses in the area of the ear and an increased incidence of astrocytomas of the nervous system (brain and spinal cord). These carcinogenic effects however will be discussed in detail later in this report (Section 4.1.2.8.1, "Oral carcinogenicity study in rats administered acrylonitrile in drinking water"). As stated above other neoplastic and non-neoplastic lesions occurred sporadically in various tissues and organs but were not considered attributable to treatment. Other than the increased number of malignant tumour-bearing animals in the groups receiving 10, 30 and 100 ppm histopathological evaluation revealed no treatment related changes.

The results of this study are considered to be valid for risk assessment purposes, and for derivation of a NO(A)EL. The majority of non-neoplastic treatment-related effects seen in this study occurred at 10 ppm and upwards. There was an increase in mortality in males at 10 ppm and in females an increase was observed at 3 and 30 ppm. Salsburg (1990) derived a dose-response relationship from the mortality data from this study. As it was shown that mortality in female rats at 3 ppm was somewhat different from controls, Salsburg proposed a dose of 1 ppm as the "no mean effect level" for this study, using multivariate statistical procedures. It should be noted however that in females the number of deaths at 10 ppm was lower than that at 3 ppm, and the statistical significance of the result at 3 ppm was furthermore due to the relatively low mortality in the female controls (see **Table 4.17**). Also the time of occurrence of the deaths in

the female test animals was not significantly different to that of the male test animals. In fact the first death occurred in the male group at 3 ppm during month 6, other than the one female animal who died spontaneously on Day 15 but was replaced. The beginning of a pattern relative to dose and duration begins with females during month 8 at 10 ppm. The deaths in females at the 3 ppm dose level began during month 16 of the study and considering the low mortality rate in the female control group the number of deaths occurring can be considered somewhat but not significantly greater. This increase is not reflected in the 10 ppm dose level and so indicates that a dose-response relationship has not been established for the 3 ppm level in females.

Due to the lack of a dose-related trend in females at the lower dose levels, it is concluded in this report that 3 ppm represented a NO(A)EL in the Biodynamics study, with this level in drinking water being equivalent to an average daily dose of 0.25 mg/kg/day in males and 0.36 mg/kg/day in females via the oral (drinking water) route of exposure in rats.

Two-year drinking water study in rats (2)

Biodynamics (1980a), administered acrylonitrile in the drinking water at doses of 0, 1, and 100 ppm to 100 Sprague-Dawley rats/sex/group. Interim necropsies were performed at 6, 12, and 18 months (10/sex/group). This study was terminated early because of low survival rates and no conclusions can be drawn from it regarding repeated dose toxicity.

Two-year drinking study in rats (3)

Quast et al. (1980b) conducted a 2-year study in male and female Sprague-Dawley rats (48 rats/sex and 80 controls/sex). Rats were exposed to nominal concentrations of acrylonitrile in drinking water at dose levels of 0, 35, 85, or 210 ppm for the first 21 days and thereafter, for the remaining duration of the study, to levels of 0, 35, 100, or 300 ppm. While this is a comparatively old study, it appears to have been well-conducted. It has not been used as the key study for risk assessment as the dose levels are quite high and another well-conducted study exists with lower dose levels (1, 3, 10, 30 and 100 ppm). The latter has been used in this risk assessment report as the key study for risk assessment purposes.

The equivalent mean dosages of acrylonitrile converted to mg/kg/day were estimated to be 3.4, 8.5 and 21.2 in male rats and 4.4, 10.8 and 25.0 in female rats. This is based on the assumption that a level of 10 ppm in drinking water is equivalent to 1 mg/kg, assuming a drinking water consumption of approximately 10% of body weight, with female rats drinking slightly more than males.

Cumulative mortality data for this study are presented in **Table 4.19**. The first death in this study occurred during the 4th month and by the end of the first year losses amounted to 33 (14 males and 19 females). The mortality of females in all treatment groups was considerably higher than their controls. The increased early mortality rate was directly correlated to increasing concentrations of acrylonitrile in the water. Early mortality was observed only in the 300 ppm group of males when compared to their respective controls. The total number of animals dead or removed from the study prior to the time of necropsy on day 746 was 206 males and 199 females (405 total = 90.4 %).

Table 4.19 Mortality of rats maintained for 2 years on drinking acrylonitrile (Quast et al., 1980b)

Dose Level (ppm)	Sex of animal	No. of animals	No. dead (% dead)
0	Male	80	73 (91.3)
35	Male	47 [a)]	42 (89.4)
100	Male	48	43 (89.6)
300	Male	48	48 (100) [b)]
0	Female	80	60 (75.0)
35	Female	48	44 (91.7)
100	Female	48	47 (97.9) [b)]
300	Female	48	48 (100) [b)]

a) In the 35 ppm male group there was one female rat that apparently was mis-sexed and placed on test in this group (She was removed from the study on day 56 and none of the data from this rat was reported in the study report)

b) Significantly increased ($p < 0.05$)

Two-year drinking water study in rats (4)

Gallagher et al. (1988) studied the carcinogenic effects in rats resulting from the ingestion of acrylonitrile in drinking water for a two-year period. Eighty male Sprague-Dawley derived CD rats were divided randomly into four experimental groups (20 rats/group) and were administered acrylonitrile in their drinking water at levels of 0, 20, 100 and 500 ppm. Animals receiving the highest concentration of acrylonitrile (0.05% or 500 ppm) had accelerated mortality, and the last rats from this group died just before the 2-year terminal killing. Survival in the control group and the remaining groups (20 and 100 ppm) was similar.

Animals were weighed at weekly intervals. The average body weight of the controls and the 20 ppm group was virtually identical throughout the course of this study. The animals receiving 100 ppm or 500 ppm of acrylonitrile showed a slower body weight gain than the controls in the first year of the study and a greater decrease in body weight gain than the controls during the second year. At intervals of one month, for periods of 1 week, food and water consumption was measured daily, with mean consumption calculated for each group of animals. No statistically significant differences in food and water intake were observed, but a trend towards decreased water consumption in animals ingesting 500 ppm of acrylonitrile was noted

There were no histopathological changes reported in this study which were indicative of chronic toxicity, as opposed to neoplastic effects of acrylonitrile.

Two-year drinking water study in rats (5) with emphasis on neurological and neuro-oncogenic effects

In this chronic lifetime study, Bigner et al. (1986) exposed 600 Fischer 344 rats to acrylonitrile in drinking water, the primary aim being to examine the neuro-oncogenic effects of acrylonitrile on the central nervous system. Other than for neurological and oncogenic effects the incidence and severity of effects is not presented quantitatively in the report of this study. Animals were 6 weeks old at the start of the study and were randomly assigned to four groups, as follows:

- Group I: This group contained 153 females and 147 males exposed to 500 ppm acrylonitrile. The animals from this group were used for studies of tumour morphology, biology and karyotype. Complete autopsies were performed on all animals that died spontaneously or were killed for tumour examination.
- Group II: Comparative survival and clinical symptomology studies were made on this group, which consisted of 50 females and 50 males exposed to 500 ppm acrylonitrile.
- Group III: As for group II, comparative survival and clinical symptomology studies were made on this group, which was exposed to 100 ppm acrylonitrile and consisted of 50 female and 50 male rats.
- Group IV: This control group received no acrylonitrile and consisted of 49 females and 51 males. As above the group was used in comparative survival and clinical symptomology studies.

Dose-related effects of acrylonitrile on weight gain and mortality were readily apparent in both sexes, with effects in weight gain appearing earlier in males, while deaths occurred earlier in females. It was not determined, however, whether these differential effects between the sexes were due to greater ingestion of acrylonitrile-containing water or to other sex-related factors. Within 2-3 weeks after the commencement of administration of acrylonitrile at 500 ppm to male rats, there was a significant decrease in mean weight. Females showed a similar pattern at 500 ppm but with a slightly longer period before the mean weight clearly diverged from that of the controls. Throughout chronic administration of acrylonitrile, this mean weight difference was observed in both sexes at the 500 ppm dose level. At 100 ppm the divergence of the mean weight curves from those of the controls began about 2 months after the start of administration in males but was not apparent in females until well into the second year of administration. A clear-cut dose-response effect in mortality was observed in both sexes. Females at both 500 and 100 ppm dose levels died slightly earlier than males, whereas only a few controls of either sex died during the first 18 months of the study.

Animals from all groups were observed daily, and in greater detail during weekly weighing, for neurological signs. The neurological effects frequently seen included paralysis, head tilt, circling and seizures. Other more non-specific signs, sometimes associated with brain tumours but also seen in their absence, included precipitate weight loss and huddling in a cage corner with decreased activity. The incidence of neurological signs (observed within 12-18 months) was closely related to acrylonitrile dose. The proportion of animals affected was 20/300 and 16/100 in the two groups dosed at 500 ppm acrylonitrile, compared to 4/100 in the 100 ppm dose level group and 0/100 in the controls.

From the rats exposed to 500 ppm acrylonitrile, 215 brains were examined. Most of the animals died or were killed for tumour donation between 12 and 18 months after the beginning of exposure. In these 215 rats, 49 primary brain tumours were found. The carcinogenic effects with respect to this study are discussed in more detail in Section 4.1.2.8.1, “Oral Carcinogenicity study in rats administered acrylonitrile in drinking water”.

Oral gavage study in rats (6)

Maltoni et al. (1977) conducted a study to evaluate the effects on adult Sprague-Dawley rats of acrylonitrile, administered by gavage in olive oil at a single daily dose of 5 mg/kg bodyweight 3 times weekly for 52 weeks. The study used 40 male and 40 female treated rats, and 75 male and female controls. The animals were examined weekly and weighed every 2 weeks during the

period of treatment and monthly after treatment was over, until spontaneous death. A complete autopsy was carried out on each animal. Histological examination of the Zymbal glands, interscapular brown fat, salivary glands, Tongue, lungs, liver, kidney, spleen, stomach, different segments of the intestine, bladder, brain, and any other organs with pathological lesions was performed.

Under these experimental conditions acrylonitrile administered by gavage did not show effects on the survival and body weight of the test animals. No treatment-related histological changes were observed in liver, kidneys and lung.

4.1.2.6.3 Dermal studies in animals

There are no available repeated dose animal dermal studies.

4.1.2.6.4 Summary of repeated dose toxicity in animals

Repeated exposure to acrylonitrile results in damage to the kidney, gastrointestinal tract, central nervous system and adrenal gland. The respiratory tract is also affected following inhalation of acrylonitrile. Repeated dose exposure by both the oral and inhalation route has been associated with lethality in a range of animal species. Dogs appear to be the most sensitive species to exposure to acrylonitrile by inhalation, with mortalities being seen at exposure levels causing no deaths in other species, however no long-term oral study has been carried out in the dog. In relation to target organ toxicity, the central nervous system appears to be a primary target organ, with neurofunctional changes being observed, although the evidence for frank neurotoxicity is limited. Nephrotoxicity is observed at high-dose levels, and the studies of Szabo et al. indicate an effect on adrenal hormones at relatively low levels, although the effects seen may be largely attributable to stress. Gastrointestinal lesions seen following oral dosing may in part be due to a local irritant effect. Increased liver and heart weights have been reported in several studies. In the case of the increases in liver weight, these do not appear to be adaptive in nature and parenchymal degeneration has been observed at high-dose levels.

Neurotoxicological effects can largely be explained on the basis of release of cyanide (see Section 4.1.2.1.1, “Metabolism”), which may also be the ultimate causative agent in relation to the repeat dose toxicity of acrylonitrile. Neurological disturbances appear to be the main effect of acrylonitrile at sublethal dose levels and indications are that these may be reversible. In the case of lethal dose levels there is also a direct effect on the central nervous system, which cannot be counteracted by cyanide antidotes. Irreversible damage occurs possibly by cyanoethylation of vital structures in the central nervous system. While acrylonitrile is cyanogenic, it is also metabolised to a reactive epoxide, 2-cyanoethylene oxide, and the parent molecule is also capable of nonenzymatically cyanoethylating essential functional groups in the body. All of these factors may contribute to the overall toxicity of acrylonitrile.

The most relevant study involving exposure via the oral route was performed by Biodynamics (1980b), in which acrylonitrile was administered orally via drinking water to 100 Fisher 344 rats/sex/group at dose levels of 1, 3, 10, 30, and 100 ppm and to a control group of 200/sex. While this study was performed as a long-term carcinogenicity study on acrylonitrile, details on non-neoplastic effects and dose levels at which such observations occurred are relevant to the examination of the chronic toxicity of acrylonitrile and are considered valid for risk assessment. Treatment-related non-neoplastic changes were seen at 10 ppm and upwards. Mortality was

increased in males at 10 ppm and in females an increase was observed at 3 and 30 ppm. However the increase at 3 ppm was small and overall there was not a dose-related trend in the range 0-10 ppm in females. In addition it is noted that in this study the female control animals had a low mortality rate which directly affects the comparison with mortality in the female test animals. The first true indication of a dose-response relationship for mortality in females began at the 10 ppm dose level. This study can be used to establish an NO(A)EL of 3 ppm (equivalent to an average daily dose of 0.25 mg/kg/day in males and 0.36 mg/kg/day in females for the oral (drinking water) route of exposure in rats. It should be noted that an assumed NO(A)EL of 3 ppm derived from this study is considerably lower than the apparent NO(A)ELs which can be derived from the other long-term oral studies in rats (20 ppm in Gallagher et al., 1988, or 5 mg/kg in Maltoni et al., 1977).

In the 13-week oral gavage study B6C3F1 mice conducted by the Serota et al. (1986), no treatment-related effects or dose-response effects were seen with respect to the parameters examined i.e. survival rate, clinical observation, body weights, clinical pathology, sperm morphology and vaginal cytology, gross pathology and organ weights. Any statistically significant effects seen in haematological parameters, for example, were generally within normal biological variation and did not reflect a dose-related pattern/trend. Based on the information in this study the NO(A)EL(oral gavage) for B6C3F1 mice was determined to be greater than 12.0 mg/kg/day.

In relation to inhalation exposure, the Quast et al. (1980a) study is considered to be a key study for risk assessment purposes. Non-neoplastic changes observed in Sprague-Dawley rats exposed to 20 ppm or 80 ppm acrylonitrile for 6 hours/day, 5 days/week, for 104 weeks, compromised growth retardation and early mortality in both sexes at 80 ppm group and in females at 20 ppm. As a result of irritation due to acrylonitrile exposure, inflammatory and degenerative changes (hyperplasia and metaplasia of the respiratory epithelium) were present in the nasal turbinates of both exposed groups (20 and 80 ppm), as already discussed. A significantly increased number of rats in the 80 ppm exposure group also showed focal gliosis and perivascular cuffing in the brain. Other non-neoplastic systemic effects in the 80 ppm and 20 ppm exposure groups, such as extramedullary haemopoiesis in the liver and spleen and focal necrosis in the liver, were considered to be secondary to the large mammary gland tumours and ear canal (Zymbal gland) tumours in these animals. It was concluded (Quast, 2001, personal communication) that they could not be regarded as a manifestation of primary hepatotoxicity or haemopoietic toxicity.

From the changes described in this study, it can be concluded that the NO(A)EL is less than 20 ppm (lowest dose administered), based on the nasal changes (local effect) which were evident at this concentration. This value of 20 ppm can be considered the LO(A)EL. Quast (2001, personal communication) has indicated that a No Observed Effect Level for the local effects of acrylonitrile on nasal respiratory epithelium in this rat inhalation study was likely to lie in the region of 10 ppm. This conclusion was based on experience in the execution of numerous acute to chronic inhalation studies and investigative studies of nasal irritation in the rat. Application of a safety factor of 5 to the level of 20 ppm to give a suggested No Adverse Effect Level (NAEL) of 4 ppm is considered justifiable because of the nature of the effect (local irritancy) and the conclusion that other systemic, non neoplastic findings in acrylonitrile-treated rats were secondary to its tumorigenic effects, rather than due to direct systemic toxicity.

A further study to be considered with regard to establishing a NO(A)EL is the Brewer (1976) 90-day study in dogs. In this study mild irritant responses were observed in the lung at 24 ppm (52 mg/m^3) acrylonitrile. The NO(A)EL for dogs, reported to be one of the most sensitive species regarding inhalation exposure to acrylonitrile, is thus below 24 ppm, and again

application of a safety factor of 5 would give a No Adverse Effect Level (NAEL) of 4-5 ppm. However, no direct comparison between the Brewer study in dogs and the Quast 2-year study in rats can be made, given the different duration of the studies and possible interspecies variation in response to both the systemic and the local irritant effects of acrylonitrile.

4.1.2.6.5 Studies in humans

The published information on repeated dose exposure to humans from acrylonitrile is limited to case reports of specific incidents at workplaces and epidemiological type reports which were mainly conducted retrospectively with a view to establishing data regarding specific end points especially in relation to carcinogenicity in humans. The problem with much of this information is that quantification of exposures and interactions with other chemicals at the workplace are often not considered.

In general according to older publications (as summarised by WHO, 1983), chronic acrylonitrile exposure in workers caused, among other symptoms, irritation to the skin and eye, nausea, vomiting, diarrhoea, gastritis, general weakness, heart and breast pain, dyspnoea, coughing, irritation, bronchitis and symptoms of neurasthenia. Clinical-chemical and haematological effects included changes of the blood count, reduced activity of T-lymphocytes, raised glutathione levels, increased cholinesterase activity as well as an increase of the concentration of acetylcholine. However exposure to other chemicals can be assumed for at least some of these workers and generally the exposures conditions are inadequately characterised for direct causation to be determined (BUA, 1995).

WHO (1983) also summarised workplace studies (Zotova, 1975; Delivanova, 1978; Enikeeva et al., 1976; Ivanov, 1983) indicating that effects such as reduced haemoglobin levels, erythrocyte counts and leucocyte counts occurred at 5 ppm (11 mg/m^3). Furthermore, symptoms of gastritis and colitis, as well as blepharoconjunctivitis and an immunosupressive effect were reported.

Wilson and McCormick (1949) identified upper respiratory symptoms, nasal irritation, nausea, vomiting, headache and vertigo, in workers at a synthetic rubber manufacturing plant following exposure to “mild” concentrations of acrylonitrile, while Zeller et al. (1969) observed similar symptoms in workers exposed to acute inhalation of acrylonitrile fumes. Sartorelli (1966) also recorded these symptoms in an individual worker who was exposed to acrylonitrile vapours when a leakage occurred in a distillation apparatus.

Ageeva (1970, as reported in WHO 1983) reported a significant decrease in an “epinephrine-like substance” and an increase in acetylcholine in acrylonitrile-exposed workers. Depression and lability of autonomic functions (lowered arterial pressure, labile pulse, diffuse dermographia, increased sweating, change in orthostatic reflex) were also observed in workers involved in acrylonitrile production.

Complaints of poor health, headache, decreased work capacity, poor sleep, irritability, chest pains, poor appetite, and skin irritation (during the first months of employment only) came from workers employed in the manufacture of acrylonitrile (Zotova, 1975). Babanov et al. (1959, as reported in WHO, 1983) reported that workers exposed to acrylonitrile concentrations at 0.6-6.0 mg/m^3 (0.3-3 ppm) for approximately 3 years suffered headaches, insomnia, pains in the heart region, general weakness, decreased working capacity, and increased irritability. The vocal cords were inflamed, and non-specific changes in the vestibular apparatus, pale mucous membranes and skin were seen. Blood pressure was reported to be reduced, and acrylonitrile was also reported to be an immunosuppressive.

VROM (1984) and WHO (1983) refer to the study of Sakurai and Kusumoto (1972), in which the health records of 576 workers from 5 acrylonitrile fibre plants, over a 10-year period, were examined. At exposure levels of 11 mg/m^3 (5 ppm) some subjective complaints such as headache, fatigue, nausea, nose bleeds, insomnia and some changes in liver function tests were reported. The effects were positively associated with the length of exposure, but not with the exposure level or age of the workers. A total of 4,439 examinations were made over the 10 years prior to 1970. The 576 workers were formed into 2 cohorts, one exposed to concentrations of acrylonitrile of below 11 mg/m^3 (5 ppm), the other to below 45 mg/m^3 (20 ppm). However, in a later report by the same author (Sakurai et al., 1978), it was stated that the "exposure levels were not reliably reported".

In this later study, performed by Sakurai et al. (1978), the authors investigated the health effects of exposure to acrylonitrile in 6 acrylic fibre factories, including the factories of the earlier (1972) study in Japan. Acrylonitrile concentrations in air were measured in spot samples in these 6 acrylic factories on 2 consecutive days. On average 102 samples (subjects with at least 5-year exposure to acrylonitrile were included) were taken in each factory. Workers from the same factories not exposed to acrylonitrile were used as controls. Medical examinations were performed on 102 acrylonitrile workers and 62 controls. The median concentration for the highly exposed population of workers was reported to be 5 ppm (11 mg/m^3). Medical histories of these workers showed that many of the workers initially experienced irritation of the conjunctiva and upper respiratory tract following exposure to acrylonitrile in the years preceding this survey. A typical complaint when exposed to high concentrations of acrylonitrile for a short duration was nasal discharge. Others experienced transient irritation of the scrotal skin when they had worked inside polymerisation tanks using respiratory protection. These acute symptoms of irritation appeared to decrease gradually with time and became infrequent at the time of this study. Clinical chemistry did not reveal any acrylonitrile-related differences between acrylonitrile workers and controls. Although some differences appeared to exist with respect to physical signs, none achieved statistical significance. The gradual lessening of reported symptoms have been attributed to improved measures to reduce exposure.

Exposure levels associated with these effects originate from the previous 5 years i.e. before improved hygiene measures were introduced, and subsequent appraisal of this study indicates that the symptoms of irritancy were associated with exposures well in excess of 5 ppm. This reappraisal indicated that levels less than 10 ppm did not cause notable irritancy. An average urinary concentration of acrylonitrile and thiocyanate ion of 0.36 mg/l and 0.011 mg/l respectively was measured. Sakurai et al. stated that their findings were not contradictory to those of Wilson et al. reflecting the older and less controlled workplace environment where levels could be up to 20 ppm.

In a more recent study (Kaneko and Omae, 1992), workers exposed to acrylonitrile at mean concentrations of 1.8 ppm (ca. 4 mg/m^3), 7.4 ppm (ca. 16 mg/m^3) and 14.1 ppm (ca. 31 mg/m^3), for a period of 5.6, 7 and 8.6 years respectively, were questioned about their subjective symptoms by means of a questionnaire. A medical examination was not performed. Compared with non-acrylonitrile workers, those questioned complained more often about irritation of the mucosa and respiratory tract, headaches and general weakness, no clear differences being observed between the 3 exposure groups.

Buchter and Peter (1984) described a case of a 57-year-old locksmith who was exposed to acrylonitrile for 14 years. The man had also been exposed to prussic acid, ammonia, phosphoric acid, propylene, hydrochloric acid and sulphuric acid. His complaints consisted of disturbance of memory, weakness, headache, dizziness, drowsiness, diminished vision and hearing, and low

blood pressure. The diagnosis after examination was cerebrovascular insufficiency due to disturbance of the circulatory function, aortic sclerosis, elevation of the erythrocyte sedimentation rate of unknown origin (no tumour), porphyrinuria, noise induced hearing loss. In an effort tolerance test the performance of the patient was only 10 to 20% of normal workers. However according to these authors, psychopathological development seemed to be more likely than chronic disease due to acrylonitrile, as the patient was strongly convinced of his own inability to work, in spite of normal cardiopulmonary work capacity and only slight disturbance of the circulatory function.

According to BUA (1995) a decrease of the testosterone level in serum occurred in workers in Rumanian factories who were exposed to acrylonitrile and other unspecified chemicals. The extent of exposure was not mentioned in the publication (Ivanescu et al., 1990).

Grigoreva (1990) reported a reduction in acid phosphatase, myeloperoxidase and succinate dehydrogenase activity in peripheral blood leucocytes of workers exposed for more than 10 years to acrylonitrile. Alkaline phosphatase activity was unchanged compared to controls, while the glycogen content was increased.

4.1.2.6.6 Summary of repeated dose toxicity in humans

Human evidence from case reports and workplace surveys are suggestive of neuropathological effects following exposure to acrylonitrile, the primary routes of exposure being inhalation and physical contact with the substance. It is evident that there is usually co-exposure with other chemicals, which makes it very difficult to interpret these epidemiological studies in production and processing plants.

WHO (1983) summarised workplace studies indicating that effects such as reduced haemoglobin levels, erythrocyte counts and leucocyte counts occurred at 5 ppm (11 mg/m^3). Furthermore, symptoms of gastritis and colitis, as well as blepharoconjunctivitis and an immunosupressive effect were reported.

Sakurai and Kusumoto (1972) reported that at exposure levels as low as 5 ppm (11 mg/m^3) some subjective complaints such as headache, fatigue, nausea, nose bleeds, insomnia and some changes in liver function tests. These effects were positively associated with the length of exposure, but not with the exposure level or the age of the workers. However it should be noted that in a later report by Sakurai et al. (1978) it was stated that the “exposure levels were not reliably reported” and reflected historical data where the actual exposure levels were greatly in excess of 5 ppm (11 mg/m^3). In fact the study of Sakurai et al. (1978) in acrylonitrile workers indicated levels in excess of 10 ppm did not cause notable irritancy. WHO (1983) cited the study of Babanoov et al. (1959), in which similar subjective complaints, together with inflammation of the vocal cords, were reported by workers exposed to approximately 3 years to airborne acrylonitrile levels of 0.6-6.0 mg/m^3 (0.3-3 ppm).

Overall, the human data are difficult to assess in relation to establishment of a dose-response relationship. However many of the findings in the animal repeat dose exposure studies, especially the neurological and irritation effects, reflect the reported findings in workers. The respiratory tract appears to be a key target organ following inhalation of acrylonitrile, both in humans and in experimental animals.

4.1.2.7 Mutagenicity

4.1.2.7.1 Mutagenicity studies *in vitro*

Bacterial mutagenicity studies using *Salmonella typhimurium*

A large number of bacterial mutagenicity studies have been carried out using a range of strains of *Salmonella typhimurium,* with and without metabolic activation. In general the methodology employed was in conformity with current Annex V methods. Results overall indicate that acrylonitrile is a bacterial mutagen (IARC, 1982; 1987; 1998), the mutagenicity in general being dependent on the presence of exogenous metabolising systems (rat, mouse or hamster liver S9) and more marked in strains sensitive to base-substitution mutagens.

Milvy and Wolff (1977) studied the mutagenic potential of acrylonitrile using *Salmonella typhimurium* strains TA 1535, TA 1978 and TA 1538. Exposure of the bacteria was achieved by spotting acrylonitrile liquid (5 -20 µl per plate) on a "lawn" of Salmonella, by a preincubation method and by exposing the bacteria to an atmosphere containing acrylonitrile (57-8,500 ppm). A positive response was observed in the presence of mouse liver S9 in all 3 strains, independent of method, indicative of a potential of acrylonitrile to produce both base substitution and frameshift mutations. Exposure to 57 ppm (vapour) for 4 hours produced a doubling of revertants compared with controls in TA 1535, while exposure to 137.5 ppm for 3 hours resulted in a quadrupling of revertants. In view of the high volatility of acrylonitrile, Milvy and Wolff suggested that the experimental condition most useful for quantitative studies was exposure of the bacteria to acrylonitrile in the vapour phase.

de Meester et al. (1978) carried out a series of experiments to study the mutagenic potential of acrylonitrile in *Salmonella typhimurium* strains TA 1530, TA 1535, TA 1537, TA 1538, TA 1950, TA 1978, TA 98 and TA 100, using the classical plate incorporation and fluctuation methods and also gaseous exposure of plates. Test concentrations in the plate incorporation method and fluctuation assays ranged from 2.5-200 µg/ml, while in the studies involving gaseous exposure plates were incubated for 1 hour in an atmosphere of 0.2% acrylonitrile. Higher exposure levels resulted in excessive cytotoxicity. Positive responses in some strains in the presence of metabolic activation (rat liver S_9) were seen in all three test systems. The activating capacity of S_9 for acrylonitrile was significantly influenced by pre-treatment of the animals with known modulators of liver metabolising enzymes, e.g. methyl-3-cholanthrene, butadiene and styrene. The mutagenic response effect was particularly pronounced in *Salmonella* strains sensitive to base-substitution mutagens e.g. TA 1530, TA 1535 and TA 1950, a less pronounced effect being seen in those strains which are reverted by frameshift mutagens e.g. TA 98, TA 100 and TA 1978.

Lijinsky and Andrews (1980) studied the mutagenicity potential of a number of vinyl compounds in *Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98 and TA 100. In this study, acrylonitrile was tested both with and without metabolic activation, using S_9 from Aroclor-induced hamsters and the plate incorporation method. These authors obtained a positive response in strain TA 1535 only, at dose concentrations of 100 (3-fold increase in revertants compared with controls), 250 (5-fold increase in revertants), 500 (5.5-fold increase in revertants), and 1,000 µg/plate (the maximum non-toxic dose used, 8.7-fold increase in revertants), only in the presence of metabolic activation.

A study by Zeiger and Haworth (1985) formed part of the IPCS collaborative study. The study used a preincubation method and *Salmonella* strains TA 97, TA 98, TA 100 and TA 1535 at dose levels of 100, 333, 1,000, 3,333, 6,666 and 10,000 µg/plate, with and without metabolic activation (rat and hamster S9). Acrylonitrile exhibited a clearly positive mutagenic effect in strains TA 1535 and TA 100. The magnitude of the response was, for example, a quadrupling of revertants compared with controls at a level of 6,666 µg/plate in the presence of 10% rat liver S9 and a 10-fold increase at this dose level in the presence of 10% hamster liver S9. Cytotoxicity was evident at a dose level of 10,000 µg/plate. These results confirmed the need for metabolic activation and the authors also demonstrated that that the mutagenic response achieved increased with an increase in the S_9 concentration.

Liber (1985), as part of the IPCS collaborative study, studied the potential for acrylonitrile (and other compounds) to induce 8-azaguanine-resistant mutations in *Salmonella typhimurium* in the presence or absence of a rat-liver-derived S9. The dose levels of acrylonitrile used were 50, 200 and 500 µg/ml, with each concentration being performed in duplicate, resulting in 4 independent determinations of mutation frequency for each treatment condition. In the first assay performed, a dose level of 500 µg/ml yielded a mutation frequency in the range of $33\text{-}41 \cdot 10^{-5}$ in the absence of S9, the result being above the 99% upper confidence limit of the background. No evidence of a positive response was obtained in the presence of S9. When the experiment was repeated, acrylonitrile failed to induce a significant response although the mutation frequency observed at 500 µg/ml was higher than the control. The results obtained are therefore equivocal, although they appear to indicate that acrylonitrile is a weak mutagen in this assay system.

Not all bacterial mutagenicity studies using *Salmonella typhimurium* have shown acrylonitrile to be mutagenic even when using metabolic activation. Rexroat and Probst (1985, IPCS collaborative study) tested ten suspected genotoxic compounds including acrylonitrile, using the plate incorporation method, tester strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100, at dose levels of 50, 100, 500, 1,000, and 5,000 µg/plate. The test was conducted with and without metabolic activation using a liver S9 fraction from Aroclor-1254-induced rats. In this study treatment with acrylonitrile induced no revertants or evidence of mutation. Some evidence of cytotoxicity was observed in TA 100 at dose levels of 100 µg/plate and above, but no cytotoxic effects were seen in the other strains.

A similar negative result was obtained by Matsushima et al. (1985, IPCS collaborative study), using a preincubation method, tester strains TA 98, TA 100, TA 97 and TA 102, with and without rat S9, and dose levels of 20, 50, 100, 200, 500, 1,000, 2,000, and 3,000 µg/plate. Brams et al. (1987) tested 50-750 µg/ml acrylonitrile in the *Salmonella* assay, using the plate incorporation method and strains TA 97, TA 98 and TA 100 in the presence of rat liver S9, and obtained a negative response. They also carried out the SOS chromotest procedure using 5.3 ng/ml-13.3 mg/ml acrylonitrile and again obtained a negative response.

Acrylonitrile was observed to be weakly mutagenic in a plate incorporation method study performed by Baker and Bonin (1985, IPCS collaborative study) using *Salmonella* strains TA 97, TA 98, TA 100 and TA 102, with and without metabolic activation and dose levels of 320, 1,000, 3,200, and 10,000 µg/plate. While acrylonitrile produced a weak though statistically significant increase in revertant colonies with tester strain TA 102, this effect was marginal. A dose-related increase occurred in only one assay, which is not considered adequate to confirm that a satisfactory dose-relationship had been established for acrylonitrile.

In a study by Jung (1986) acrylonitrile was tested for mutagenicity in the *Salmonella typhimurium* strain TA 102. The mutagenicity studies were conducted in the presence and

absence of a metabolising system derived from rat liver homogenate. A dose range of 6 different doses from 4 µg/plate to 5,000 µg/plate was used, with toxicity being observed at 500 µg/plate and above. Acrylonitrile proved to be non-mutagenic in this test system both with and without exogenous metabolic activation.

Lambotte-Vandepaer et al. (1980) demonstrated that urine collected from rats and mice treated with acrylonitrile was mutagenic in *Salmonella typhimurium* strain TA 1530, in the absence of metabolic activation. Pre-treatment of the animals with phenobarbital abolished the direct mutagenicity of urine in rats and reduced that from mice. The addition of beta-glucuronidase to the incubation mixtures enhanced the mutagenic activity of urine from acrylonitrile-treated animals. The authors of this study also indicated that animal urine might retain its mutagenic activity for as long as a week after collection. This study may demonstrate the potential for mutagenic metabolites to be generated from acrylonitrile *in vivo*, however the results are difficult to interpret given the effects of pre-treatment with enzyme inducers and the species differences observed, which do not correlate with the findings of other toxicological studies.

As already indicated, although some of the results obtained are equivocal or negative the overall interpretation of the studies indicates that acrylonitrile exhibits mutagenic potential in the Ames bacterial mutagenicity assay using *Salmonella typhimurium* in the presence of metabolic activation.

Bacterial mutagenicity studies using *Escherichia coli*

Acrylonitrile has been shown to be mutagenic in a study performed by Venitt et al. (1977), using the *E. coli* WP2, WP2 *uvrA*, WP2 *uvrA polA* and WP2 *lexA* series of bacteria. In the first series of experiments the authors used a plate incorporation assay method and dose levels of 75 or 150 µmol per plate (8,000 or 16,000 µg/plate), in the presence or absence of an exogenous metabolising system. Acrylonitrile caused a dose-related increase in the number of revertant colonies compared with untreated bacteria in three of the four strains. WP2 *lexA* was not detectably reverted by acrylonitrile, and a slight drop in revertant yield could be accounted for by cytotoxicity at both dose levels. Of the three strains showing a statistically significant response, WP2 was rather more sensitive to the mutagenic effect of acrylonitrile, giving a 4-fold increase over the spontaneous level compared with a 3-fold increase for WP2 *uvrA* and a 2-fold increase for WP2 *uvrApolA*. Doses above 150 µmol/plate caused a decline in mutagenic response, concomitant with cytotoxicity as demonstrated by a dose-related reduction in the density of the bacterial lawn. The addition of a mixed-function function metabolising system (0.5 ml/plate of S9 mix) prepared from the livers of Aroclor 1254-induced CB Hooded male rats, had no detectable effect on the mutagenic action of acrylonitrile. It can be concluded that this compound is a direct acting mutagen for these bacteria.

While the mutagenic effects of acrylonitrile in *E. coli* were highly reproducible and statistically significant, the effects seen were weak and reliably demonstrable only over a narrow range of doses when measured using this plate incorporation method. For this reason the authors (Venitt et al., 1977) sought confirmation by using the simplified fluctuation test as described by Green et al. (1976). The assay resulted in a significant ($p < 0.001$) dose-related increase in mutations in both WP2 and WP2 *uvrApolA* following acrylonitrile treatment, although WP2 *uvrApolA* was more sensitive than was WP2, showing a significant effect at concentrations of acrylonitrile of 0.1-0.4 mM where there was no significant increase in mutations in WP2. On the basis of one experiment WP2 *uvrA* was similar to WP2 in its response to acrylonitrile. WP2 *lexA* was not detectably mutated by any of the concentrations that mutated the other three strains.

The results of the fluctuation assay confirmed those of the plate incorporation tests, showing mutagenic activity at acrylonitrile concentrations 4-20 fold below levels used in the plate tests in the absence of cytotoxic effects. The differential response of the tester strains to the mutagenic action of acrylonitrile i.e. WP2 and WP2 *uvrA* equally mutable, WP2 *lexA* not detectably mutable etc., suggests that acrylonitrile causes non-excisable mis-repair DNA damage, thought to be associated with the generation of DNA strand breaks (Venitt et al., 1977).

Mutagenicity studies using yeast assay systems

A number of investigators have used various strains of *Saccharomyces cerevisiae* with the aim of establishing whether acrylonitrile has the potential to induce such effects as forward and reverse mutation, mitotic chromosome loss, mitotic recombination and other genetic effects

Using the yeast *Saccharomyces cerevisiae*, strains D61-M, D6 and D7, as part of the IPCS collaborative_study, Parry and Eckardt (1985a; 1986) achieved positive increases (criterion, at least a 2-fold increase in mutant colonies) in mitotic aneuploidy, induction of point mutation (reversion of the *adenine 2-40* locus or the *leucine 1-92* locus) and mitotic recombination following exposure to aqueous acrylonitrile at concentrations up to 5,000 µg/ml without metabolic activation. In a study by Zimmermann et al. (1985, IPCS collaborative study), acrylonitrile at dose levels of 0.27-0.99 µl/ml without exogenous metabolic activation induced a clear-cut dose-dependent increase in total cycloheximide-resistant colonies, approximately 10-fold at 0.99 µ/ml, and was considered to be genetically active in strain D61-M. However there was no indication of mitotic aneuploidy, and it was concluded that acrylonitrile could induce mitotic recombination and mutation but that the induction of mitotic chromosomal malsegregation is not necessarily associated with this effect.

Mehta and von Borstel (1985, IPCS collaborative study) demonstrated that acrylonitrile had mutagenic activity in 3 strains of *Saccharomyces cerevisiae* exposed to levels of 0.8, 8, 80 or 800 µg/ml in the presence or absence of S9 prepared from the livers of Aroclor 1254-induced rats. Mutational frequency at the highest exposure levels ranged from 10- to 20-fold of control in the three strains, cytotoxicity was very marked at 800 µg/ml.

Two further studies (Arni, 1985; Ferguson, 1985; IPCS collaborative study) confirmed the potential of acrylonitrile to induce mutations in *Saccharomyces* with or without metabolic activation. Arni (1985) demonstrated that following treatment with acrylonitrile at levels of 6.25-50 µg/ml there was a significant increase ($p < 0.01$) in the incidence of gene conversions (tryptophan-prototrophic colonies) in the yeast strain *Saccharomyces cerevisiae D7*, in the absence of metabolic activation at concentrations of 25 and 50 µg/ml, while a level of 100 µg/ml had an inhibitory effect on the growth of the yeast cells. Ferguson (1985) found that acrylonitrile at levels of 30 or 60 µg/ml produced a large increase (from a background level of approximately 5% to over 50 % at 60 µg/ml) in respiratory-deficient ("petite") mutations in this yeast strain under conditions optimising endogenous metabolic activation.

In the study performed by Inge-Vechtomov et al. (1985, IPCS collaborative study) acrylonitrile at a concentration level of 800 µg/ml in the presence of S9 prepared from the livers of Aroclor 1254-induced female Wistar rats caused an significant increase ($p < 0.001$) in illegitimate mating in *Saccharomyces cerevisiae*. Lower concentrations (0.8, 8, 80 µg/ml) had no effect. Mitotic recombination was also increased. This genetic activity was shown to require metabolic activation. Brooks et al. (1985, IPCS collaborative study) also demonstrated that acrylonitrile produced significant increases in the frequency of mitotic gene conversion (up to 10-fold increase in prototrophy at a level of 500 µg/ml) in the stationary- and log-phases of yeast culture

(*Saccharomyces cerevisiae JD1*), in the presence of Aroclor 1254-induced rat liver S9 and in the optimised yeast P-450 assay. The mitotic gene conversion observed occurred at the his_4 and trp_5 loci of the yeast strain.

In contrast Loprieno et al. (1985, IPCS collaborative study) examined the mutagenic activity of acrylonitrile in the forward-mutation system in *Schizosaccharomyces pombe* P1 and showed no dose-related increase in frequency of mutation. Experiments were carried out in duplicate with dose levels ranging from 16 to 250 µg/ml, in the absence and presence of phenobarbital/b-naphthoflavone-induced rat liver S9. However as a possible explanation for the discrepancy between these results and those of previous studies it should be noted that the sensitivity of the *S. pombe* P1 forward mutation system is limited by the numbers of cells screened (20,000-40,000) for mutants. Unlike reverse mutation systems, it is also limited by the fact that in forward-mutation systems a small increase in induced mutants of one specific type must compete for detection with spontaneous mutations of all kinds. The authors of this study suggest that the advantage of the forward-mutation system is not in the detection of weak mutagens such as acrylonitrile, since the resolution of the system is limited, but in the detection of point mutagens with diverse mechanisms.

Rizzi et al. (1984) used a forward-mutation assay in *Schizosaccharomyces pombe* in the growth phase, involving incorporation of acrylonitrile at dose levels of 0.2 to 250 µg/plate in the presence and absence of rat liver S9 (phenobarbital and Aroclor 1254-induced rats). The frequency of mutants was 3 times greater than that of the controls at doses of 0.2, 0.5 and 1.0 µg/plate (-S9), 3 times greater at doses of 0.2, 0.5, 1.0 and 10.0 µg/plate in the presence of S9 from phenobarbital-induced rats and 5 times greater at the same doses in the presence of S_9 from Arochlor-induced rats. This study also examined DNA repair in HeLa cells, discussed in Section 4.1.2.7.1, "*In vitro* studies to detect DNA damage and repair". The results suggested that in this system acrylonitrile is mutagenic at low doses.

Finally, Whittaker et al. (1990) reported that acrylonitrile at levels up to 1.36 mg/ml did not induce mitotic chromosome loss in Saccharomyces cerevisiae, although cell respiration was inhibited, indicating a possible effect on mitochondrial function.

Mutagenicity studies using *Aspergillus nidulans*

Carere et al. (1985a; 1985b, IPCS collaborative study) used two methodological approaches, the plate incorporation assay and a liquid test procedure to detect acrylonitrile-induced somatic segregation in *Aspergillus nidulans* diploid strain P1, using germinating conidia. This test system exploits the endogenous metabolic activity of the test organism. The results of these studies demonstrated a significant increase ($p < 0.01$) in mitotic crossovers in the plate-incorporation assay at an acrylonitrile concentration of 806 µg/ml, while at 2,015 µg/ml a non-statistically significant increase was associated with a marked decrease in survival (10% of control). In the liquid test procedure, acrylonitrile at concentrations of 0.8-4.0 mg/ml induced haploid and diploid non-disjunctional segregants (up to 10-fold increase on historical control values).

Mutagenicity studies using Mouse Lymphoma test systems

Garner and Campbell (1985, IPCS collaborative study) tested acrylonitrile for the ability to induce mutations to ouabain or 6-thioguanine resistance in mouse lymphoma L5178Y cells. Only agents inducing missense mutations will mutate at the ouabain (*oua*) locus while frame-shift, base-substitution and deletion mutagens are active at the 6-thioguanine locus. Cells were exposed for 2 hours to dose levels in the range 12.5-200 µg/ml (i.e. 5 doubling concentrations),

in the presence or absence of S9 from Aroclor 1254-induced rat liver, with a subsequent single expression time of 48 hours for ouabain and 7 days for 6-thioguanine.

Results indicated that the maximum mutation frequency at the *oua* locus was $0.19 \cdot 10^{-6}$ viable (surviving) cells in the presence of S9 and $0.8 \cdot 10^{-6}$ in the absence of S9, compared with 0 for the negative control and up to $42.1 \cdot 10^{-6}$ for the positive control benzo-a-pyrene. The mutation frequency at the 6-thioguanine locus was $32.3 \cdot 10^{-6}$ in the presence of S9 and $34.6 \cdot 10^{-6}$ in the absence of S9, compared with 19 and 18 respectively for the negative control and up to $569 \cdot 10^{-6}$ for the positive control benzo-a-pyrene. No information on cytotoxicity was provided in the paper although survival was determined in the experiment. The results for acrylonitrile-induced mutations at the 6-thioguanine locus were statistically significant.

A study in mouse lymphoma L5178Y cells conducted by Lee and Webber (1985, IPCS collaborative study) examined mutations at the thymidine kinase ($TK^{+/-}$) locus. Experimental design involved a treatment time of 2 hours and a subsequent expression time of 4 days, and dose levels were between 80 and 225 µg/ml in the presence and absence of exogenous metabolic activation (S9 from Aroclor 1254-induced rat liver). The results of one experiment indicated mutational frequencies ranging from $16.8 \cdot 10^{-6}$ viable cells at 125 µg/ml to $22.7 \cdot 10^{-6}$ at 177 µg/ml in the absence of S9, with cytotoxicity and poor survival being observed at a level of 210 µg/ml. In the presence of S9, the mutational frequencies were $6.1 \cdot 10^{-6}$ at 125 µg/ml and $28.0 \cdot 10^{-6}$ at 177 µg/ml. Mutational frequency in the solvent control (DMSO) was $6.4 \cdot 10^{-6}$ while figures for the positive control lay in the range $119\text{-}149 \cdot 10^{-6}$. Results of a second experiment were comparable, and the authors concluded that acrylonitrile was mutagenic in this test system, in the presence and the absence of metabolic activation.

Similar results were achieved in another study using the L5178Y/$TK^{+/-}$ assay system (Amacher and Turner, 1985, IPCS collaborative study) at dose levels of 5-69 µg/ml (+S9 from uninduced rat liver) and 22-43 µg/ml (-S9). Treatment was for 3 hours and expression time was 48 hours. Survival rates in the presence of S9 were 5-92% and in the absence of S9 were 28-73%. Mutational frequency rose to approximately $85 \cdot 10^{-6}$ at a dose level of 69 µg/ml in the presence of S9 and $45 \cdot 10^{-6}$ at a dose level of 43 µg/ml in the absence of S9. Mutational frequency in controls was $17 \pm 4 \cdot 10^{-6}$. The authors concluded that acrylonitrile was clearly mutagenic in this system, with and without metabolic activation.

Myhr et al. (1985, IPCS collaborative study) confirmed this result in the L5178Y/$TK^{+/-}$ assay, using dose levels of 12.5-30 nl/ml (10-24 µg/ml), an exposure period of 4 hours and an expression time of 48 hours. Acrylonitrile induced a positive dose-related mutagenic response in the absence of S9, mutational frequency increasing 5.7-13-fold over the dose range, while relative growth was 44-55% of control.

In contrast, Oberly et al. (1985, IPCS collaborative study) observed only a weakly positive response in the L5178Y/$TK^{+/-}$ assay, with or without S9 from Arochlor-induced male Fischer 344 rats. These authors used an exposure period of 4 hours, an expression time of 48 hours and acrylonitrile concentrations of 1-60 µg/ml. In the absence of S9 excessive cytotoxicity was apparent at 60 µg/mg (4% survival), with a 4-fold increase in mutational frequency over control being seen at this level. Survival at lower concentrations ranged from 100% at concentrations up to 10 µg/ml to 29% at 50 µg/ml, and mutational frequencies ranged from 0.24 to $0.69 \cdot 10^{-6}$, the latter result, at 50 µg/ml, representing only a 2.6 fold increase over mutational frequency in controls. Similar results were obtained in the presence of S9, although cytotoxicity was very marked at both 50 and 60 µg/ml, and survival was only 10-12% control at 40 or 30 µg/ml, these

dose levels showing a respective 3.5-fold and a 2.8-fold increase in mutational frequency over control.

Styles and Clay (1985, IPCS collaborative study) obtained a negative result for acrylonitrile in the L5178Y/TK$^{+/-}$ assay, using dose levels of 12.5-100 µg/ml, an exposure period of 2 hours, and an expression period of 48 hours, with or without S9 from Arochlor-induced Sprague Dawley rats. Mutational frequencies were similar to control at all dose levels (approximately $0.035 \cdot 10^{-6}$), and there was also no evidence of cytotoxicity. These authors also obtained a negative result with acrylonitrile at levels up to 100 µg/ml in relation to mutations at the *oua* locus, using a L5178Y/TK$^{+/+}$ cell line and similar experimental conditions to the thymidine kinase locus experiments. The reason for the apparently conflicting results of Styles compared with other authors is not clear, particularly as these authors did obtain positive results with a number of other known or suspected mutagens.

Anderson and Cross (1985) investigated the mutagenic potential of acrylonitrile in the mouse lymphoma P388F TK-/+ cell line in the presence or absence of S9, using logarithmic increasing concentrations up to 161 µg/ml. They demonstrated an increased mutation frequency in the presence of S_9 (over 20-fold increase over control at 161 µg/ml). This dose level gave approximately 40% survival, but showed no response without S9 at concentrations up to 80 µg/ml even though cell survival was reduced to approximately 50% at this dose level.

<u>*In vitro* mutagenicity studies using human lymphoblasts</u>

Crespi et al. (1985, IPCS collaborative study) investigated the mutagenic activity of acrylonitrile in human lymphoblasts (TK6, *TK* locus), using dose levels of 5-50 µg/ml, an exposure period of 3 hours in the presence of S9 and 20 hours in its absence, and an expression period of 72 hours. S9 metabolic mix was prepared from Arochlor-induced rat liver. Mutational frequency was increased 3.5-fold in the presence of S9 at both 40 and 50 µg/ml, relative survival at these exposure levels being 37% and 26% respectively. In the absence of S9 mutational frequency was increased 2-fold compared with control at 15 µg/ml but only 1.3-fold at 20 µg/ml, associated with marked cytotoxicity at this exposure level (18% survival). The authors concluded that acrylonitrile gave a positive response both in the presence and absence of metabolic activation. These authors also examined mutagenic activity in the metabolically competent AHH-1 cell line (hypoxanthine guanine phosphoribosyl transferase locus) using dose levels of 5-25 µg/ml, an exposure period of 28 hours and an expression period of 6 days. Acrylonitrile also gave a positive response in this cell line, with an approximate 4.5-fold increase in mutational frequency over control at 25 µg/ml, at 16% relative survival, a response similar to that for the positive control benzo-a-pyrene (3.1 µg/ml).

Recio and Skopek (1988) also used the TK human lymphoblast cell line, the heterozygous thymidine kinase (*tk*) locus being the genetic marker, to study the mutagenic potential of both acrylonitrile and its metabolite 2-cyanoethylene oxide (CEO) in the presence and absence of S9 from Arochlor-induced male Sprague Dawley rats. The exposure period was 2 hours and the expression period was 6-8 days. Acrylonitrile was not mutagenic in the absence of S9, producing less than a 2-fold increase in mutation frequency over a concentration range of 0.4-1.5 mM (21-80 µg/ml). In the presence of S9, a statistically significant mutagenic response (4-fold increase, $p < 0.05$) was seen at the highest exposure concentration assessed experimentally, 1.4 mM (74 µg/ml). Survival was reduced to approximately 10% at a concentration of 1.5 mM. 2-Cyanoethylene oxide induced a 17-fold increase in mutation frequency without metabolic activation at 100 µM. The results from these experiments confirm that acrylonitrile is weakly

mutagenic in mammalian cells, while the mutagenicity exhibited by CEO suggests that this metabolite may in fact be the ultimate mutagenic metabolite of acrylonitrile.

In vitro chromosomal aberration and sister chromatid exchange studies

The potential for acrylonitrile to induce sister chromatid exchange (SCE) and the induction of DNA single breaks in adult human bronchial epithelial cells obtained from autopsy specimens and used in the 3rd or 4th passage was investigated by Chang et al. (1990). Cultures were exposed for 20 hours to levels of 150, 300, 500 or 600 µg/ml acrylonitrile, before assessment of SCE and DNA strand breaks using standard methodology. Cytotoxicity, as measured by colony forming efficiency, was marked at the highest exposure level of 600 µg/ml, but the lower concentrations were not associated with toxicity. SCEs were significantly increased ($p < 0.01$) at dose levels of 150 and 300 µg/ml, the incidence of SCE per cell being 6.6 and 10.7, respectively, compared with 3.7 in unexposed control cultures, the incidence falling at 600 µg/ml due to toxicity. The extent of DNA single strand breaks appeared to be positively correlated with increasing levels of acrylonitrile in the culture. The authors suggested that the observed genotoxic effects on bronchial epithelial cells was of interest in relation to the higher incidence of lung cancer reported in acrylonitrile workers in epidemiological studies.

Gulati et al. (1985, IPCS collaborative study) investigated the potential of acrylonitrile to induce chromosome aberrations and SCE in cultured Chinese Hamster Ovary (CHO) cells. In the SCE assay, cells were exposed to levels of 0.16-30 µg/ml for 26 hours without metabolic activation or for 2 hours in the presence of S9 from Arochlor-induced male Sprague Dawley rats. In the chromosome aberration studies cells were exposed to 5-100 µg/ml for 8 hours without S9 or for 2 hours in the presence of S9. Acrylonitrile caused a 2-fold increase in SCE frequency over solvent control at a dose level of 30 µg/ml, the incidence of SCE per cell being 19.6 (-S9) and 17.0 (+S9) compared with compared with 8.1 in solvent controls. SCEs were also slightly increased at lower exposure levels. The effect was only seen in cultures harvested after a delay of 12 hours in addition to the standard harvest time of approximately 28 hours, dependent on experimental protocol, consistent with the observation that acrylonitrile caused a significant cell cycle delay. Chromosomal aberrations were also increased in CHO cells exposed to acrylonitrile, 11% of cells exposed to 100 µg/ml showing aberrations compared with 2% in controls. Aberrations were also weakly increased at lower exposure levels.

A similar study was carried out by Natarajan et al. (1985, IPCS collaborative study). CHO cells were exposed for 1 hour to 1, 2 or 4 mM acrylonitrile (53, 106 or 212 µg/ml) and fixed between 25 and 36 hours after treatment for evaluation of SCEs. Exposure to 2 mM acrylonitrile in the presence of S9 produced an increase in SCE to 19.3 per cell from the control value of 11.9, but no effect was seen at the lower dose level, or in the absence of S9, while cytotoxicity at 4 mM resulted in very limited cell survival. These authors also showed an increase in chromosomal aberrations in cells exposed to acrylonitrile at 4 mM for 1 hour and fixed at three different times 13 to 19 hours after treatment. The significance of this finding is questionable given the cytotoxicity seen at this exposure level, and there was no reliable evidence of aberrations at the lower exposure levels.

Danford (1985, IPCS collaborative study) also examined chromosome aberrations and aneuploidy in the Chinese hamster liver fibroblast cell line CH1-L exposed to acrylonitrile at levels of 2.5, 6.25, 12.5 or 25 µg/ml for 2 hours without S9, followed by fixation at 36 hours after treatment. 200 Cells per treatment group were analysed. Danford found acrylonitrile to be the most potent of 10 potential mutagens tested, with 14.5% of cells exposed to 25 µg/ml showing aberrations, compared with 1% in controls. Smaller but statistically significant

increases were also observed at levels of 2.5 and 12.5 µg/ml. No effect on chromosome number was observed, indicating that acrylonitrile did not induce aneuploidy. This was confirmed in a study by Parry (1985, IPCS collaborative study) in Chinese hamster liver cells (CH1-L) exposed to 2.5-25 µg/ml acrylonitrile. In contrast Sehgal et al. (1990) found that acrylonitrile at 5 and 50 mM inhibited microtubule assemby in microtubule preparations from *Drosophila melanogaster*, and from mouse brain, indicating a potential aneuploidy effect.

Ishadite and Sofuni (1985, IPCS collaborative study), using Chinese hamster lung fibroblast (CHL) cells, also demonstrated that acrylonitrile could induce chromosomal aberrations in the absence of S9. Cells were exposed for 24 or 48 hours to 3.13, 6.25 or 12.5 µg/l, higher levels being cytotoxic. At the highest dose level, the number of cells showing aberrations, excluding gaps, was 19% at 24 hours and 30.5% at 48 hours, compared with 0.3-0.5% in saline controls. The number of polyploid cells was also increased.

Chromosome aberrations, SCE and polyploidy were also investigated by Priston and Dean (1985, IPCS collaborative study) in a rat liver cell line (RL_4) having epithelial cell characteristics and intrinsic metabolic capability. The normal cell cycle for the cell line was 13 hours and harvesting of cells was carried out at 22 or 30 hours, dependent on an initial study of proportion of 2nd division metaphases. Cells were exposed to levels of 1.25, 2.5, 5 or 10 µg/ml, plating efficiency being reduced by approximately 50% at an exposure level of between 5-10 µg/ml. At these levels, the authors found no evidence of SCE induction or chromosomal aberrations. The authors noted cell cycle delay at 10 µg/ml, with an associated reduction in the number of 2nd division metaphases. Unlike Gulati et al., however, they did not compensate additionally for this cell cycle delay, and thus potential SCEs or chromosome aberrations induced by acrylonitrile may have been missed in this study.

Perocco et al. (1982) demonstrated that exposure of human lymphocytes to 0.5 µM acrylonitrile (26.5 µg/ml) resulted in a significant increase in SCE. Obe et al. (1985, IPCS collaborative study), exposed fresh human lymphocytes to acrylonitrile at concentrations of 1 and 10 µg/ml for 24 hours in the absence of S9 and for 1 hour in the presence of S9 from Arochlor-induced rat liver. Metaphases were prepared 24 hours after treatment in both cases. In contrast to the earlier results of Perocco, these authors were unable to demonstrate SCE-induction by acrylonitrile or any of the other nine compounds tested in the IPCS collaborative study.

Finally, a study of micronuclei induction *in vitro* in CHO cells was carried out by Douglas et al. (1985, IPCS collaborative study), a positive result being obtained both in the presence and absence of S9. These authors also examined induction of DNA strand breaks by acrylonitrile, using an alkaline sucrose gradient elution technique, and demonstrated increased DNA damage at very high concentrations (3.7 mg/ml and 53.1 mg/l), this result being of little toxicological significance.

In vitro studies to detect DNA damage and repair

Bradley (1985, IPCS collaborative study) demonstrated that exposure of freshly isolated rat hepatocytes to an exposure level of 65.8 µg/ml acrylonitrile caused an increase in single strand breaks in DNA, using the alkaline elution technique, with a 5.7-fold increase in the elution slope relative to control. In contrast Lakhanisky and Hendricks (1985, IPCS collaborative study) did not demonstrate DNA single strand breaks in CHO cells in culture, details of exposure levels and times were not provided.

As part of an overall study to determine the possible mutagenic and genotoxic activity of acrylonitrile, Rizzi et al. (1984) carried out a DNA repair assay using HeLa cells. In this study

incorporation of [^{3}H]TdR into DNA was measured in 4 groups, namely control and acrylonitrile-treated cells in the absence of hydroxyurea (-HU) and control and treated cells exposed to hydroxyurea (+HU). The results showed that the -HU/+HU relationship between treated and control cells and the value of +HU between treated and control cells were statistically significant at acrylonitrile dose levels of 0.18 and 0.036 mM ($p < 0.01$ and $p < 0.09$ respectively). These results suggest that in both systems acrylonitrile is a mutagenic and genotoxic agent at very low doses. In contrast, Martin and Campbell (1985, IPCS collaborative study) did not demonstrate unscheduled DNA repair in HeLa cells. Details of exposure levels and times were not provided in this study.

Glauert et al. (1985, IPCS collaborative study) demonstrated unscheduled DNA synthesis in primary rat hepatocyte cultures by quantifying the amount of tritiated thymidine incorporated into DNA in the presence of hydroxyurea following treatment with 1-10,000 µM acrylonitrile for 18 hours, after which the cells were immediately harvested for analysis. At an exposure level of 1,000 µM, UDS was increased to 129% of the control value.

Butterworth et al. (1992) also measured chemically induced DNA repair in primary rat hepatocyte cultures caused by a number of different chemicals including acrylonitrile and cyanoethylene oxide (CEO), using autoradiographic techniques. The primary hepatocyte cultures were prepared by collagenase perfusion and treated with the compounds in the presence of 10 µCi/ml ^{3}H-thymidine. The test chemicals were dissolved directly in the media at concentrations as follows; acrylonitrile 0.01, 0.1, 1.0, and 10 mM, CEO, 0.01, 0.1 and 1.0 mM. Concentrations of 10 mM for acrylonitrile and 1.0 mM for CEO proved to be toxic as judged by the morphological appearance of the cultures. In general 25 cells were scored per slide, 3 slides per treatment group from each of two tests, with primary cell cultures from a different animal being used in each test. Any individual cell with a net nuclear grain (NG) of > 5 was considered to be in repair. Statistical significance compared to controls was determined by the unpaired t-test for the equality of two means. A response was judged positive at $p < 0.05$ for an NG value greater than zero. Neither acrylonitrile nor CEO produced a DNA repair response in this *in vitro* hepatocyte assay.

A similar autoradiographical methodology approach was used by Probst and Hill (1985, IPCS collaborative study) and Williams et al. (1985, IPCS collaborative study), also using freshly isolated rat hepatocytes, exposed to 0.5-500 nmoles/ml acrylonitrile for 20 hours (Probst and Hill) or 0.1-100 µg/ml for 18-20 hours (Williams et al.). These authors found no evidence of induction of DNA repair by acrylonitrile.

A human mammary epithelial cell (HMEC) DNA repair assay was performed in secondary cultures of HMEC by Eldridge et al. (1992). In principle the methods used were analogous to those described for the hepatocyte DNA repair assay performed by Butterworth et al. (1992) described above. The secondary cultures of normal HMEC were derived from residual surgical material from mammoplasties of 5 healthy women. The cell line used has lost the ability to activate genotoxicants metabolically, but retains the capacity for DNA repair. Based on historical controls, any individual cell with greater than or equal to 6 NG was considered in repair. The population average NG for 25 to 80 cells was calculated for each slide, two slides per treatment group. The percentage of cells in repair was also calculated. The unpaired t-test for the equality of two means was used to compare NG between control and treated cultures. The Chi square test was used to test for significant differences in the percentage of cells in repair (IR) between control and treated cultures. A response was judged positive at $p < 0.05$ for NG and/or IR with an NG value greater than zero. Although CEO was cytotoxic to HMEC, it did produce a positive UDS response, confirming its genotoxic potential. In contrast no activity of acrylonitrile

was observed in the HMEC DNA repair assay i.e. a negative response, although it was very cytotoxic in this assay.

4.1.2.7.2 Mutagenicity studies *in vivo*

Studies in Drosophila melanogaster

Benesh and Shram (1969) carried out a study on eukaryotic gene mutation *in vivo,* in which the occurrence of sex-linked recessive lethal mutations in *Drosophila melanogaster* was examined following administration of 0.1% acrylonitrile by intra-abdominal injection. The result of this study proved negative. Experimental details of this study are limited, and the results are of limited value.

Vogel (1985, IPCS collaborative study) tested acrylonitrile for activity in a *Drosophila* mitotic recombination and somatic mutation (SRM) assay using *white* and *white-coral* as genetic markers. Female *Drosophila melanogaster* were given food containing 5-20 mM acrylonitrile during an egg-laying period of 4 days and developing offspring were cultured for 10-11 days. Hatching females were scored for the chosen genetic markers, a total of 1,528 eyes being scored. Acrylonitrile at a concentration of 5 mM in the food produced a 3.5-fold increase in eye mutations, the incidence of single spot mosaics rising to 1.24% compared with 0.33% in controls. Twin mosaic spots were also increased. Higher concentrations of 10 and 20 mM in food resulted in lethality and sterility. The authors suggested that this SRM-assay may be more sensitive than the traditional sex-linked recessive lethal method, particularly for chronic exposure of *Drosophila* larvae.

Wurgler et al. (1985, IPCS collaborative study) also used a *Drosophila* mitotic recombination and somatic mutation (SRM) assay to examine the mutagenic potential of acrylonitrile, the genetic markers chosen being wing cell spots. The *Drosophila* used in the study were of two types, DNA repair-proficient and excision-repair-deficient. The various treatments in this study included exposure of 48- or 72-hour-old *Drosophila melanogaster* larvae to gaseous acrylonitrile at a level of 1 µl in a 1,150 ml chamber (0.8 ppm) for 0.5 or 1 hours, acute feeding of 48 or 72-hour larvae with 15 or 80 mM acrylonitrile in food for 2 hours, and chronic feeding with 1.5 mM over a 96-hour period. The authors concluded that acrylonitrile was a weak mutagen (marginally positive) in the *Drosophila* wing spot test, on the basis of a positive response in both the DNA repair-proficient (51.9% wings with spots, compared with 28.7% in controls) and excision-repair-deficient larvae proficient (90% wings with spots, compared with 74% in controls) following chronic feeding with 1.5 mM over a 96-hour period. Results in the other exposure regimes were not consistent, some positive and some negative results being obtained.

Fujikawa et al. (1985, IPCS collaborative study) examined the potential of acrylonitrile to induce sex chromosome aneuploidy in the *Drosophila melanogaster* ZESTE system. Following exposure to *in vivo* mutagens, exceptional offspring (mutations) derived from segregation errors during female meiosis are recognised by characteristic eye colours: (1) *zeste*-eyed females, (2) *white*-eyed males. Developing *Drosophila* larvae were exposed to acrylonitrile for 4 days. Exposure was accomplished by addition of 1 ml of 1, 2, 4 or 8 mM acrylonitrile onto the surface (18 cm^2) of the culture vessel. The larvae were then allowed to develop and the eyes of adult males examined for colour mutations. These authors observed a significant increase in red spot eyes indicative of a somatic mutation, the frequency being 0.39% in the cultures exposed to 8 mM acrylonitrile, compared with 0.1% in control ($p = 0.018$). No increase was seen at lower dose levels.

Osgood et al. (1991) also used the *Drosophila melanogaster* ZESTE system. Groups of 20 adult female *Drosophila* aged 2-3 days were exposed to 2.7 ppm acrylonitrile vapour for 0, 10, 30, 50 or 70 minutes and were reunited with groups of 10 males and permitted to deposit eggs for 2 days. They were then transferred to a new incubation bottle for a second 2-day sampling, then discarded. F1 offspring were counted and scored for mutations on days 10 to 17 following establishment of a brood. Total numbers scored (two broods) ranged from 6,000-10,000. Acrylonitrile was found to be relatively non-toxic at the concentration tested, with 13% females being killed at this level after 70 minutes exposure.

In this study, there was some evidence of chromosome loss, as evidenced by the appearance of small numbers (3-4) of *white*-eyed males, following 50 or 70 minutes exposure to acrylonitrile, and a single observation of chromosome gain (*zeste*-eyed female), following 30 or 70 minutes exposure. The mutations occurred predominantly in Brood A, obtained from the first sampling period. Overall the incidence of mutations was statistically significant following 50 minutes exposure ($p < 0.009$) or 70 minutes exposure ($p < 0.005$) but was much less than that observed for two other nitriles, acetonitrile and proprionitrile tested at concentrations of 131 ppm and 51 ppm, respectively, in the same experiment.

The overall conclusion to be drawn from these *Drosophila* studies is that acrylonitrile is capable of producing mutations *in vivo* in this organism.

Unscheduled DNA synthesis *in vivo*

Hogy and Guengerich (1986) measured *in vivo* DNA repair by incorporation of ^{3}H-thymidine during unscheduled DNA synthesis (UDS) occurring in the presence of hydroxyurea suppression of replicative DNA synthesis. Two hours after an oral dose of 50 mg/kg to male F 344 rats, acrylonitrile produced a 3-fold increase in incorporation of ^{3}H-thymidine in liver, indicative of an effect on DNA repair, but there was no concomitant increase in the brain.

The preferred method of measuring DNA repair *in vivo* is by autoradiography of tissues of animals treated with the compound of interest, in this case acrylonitrile. Hurtt et al. (1987) and Butterworth et al. (1992) used autoradiography to determine unscheduled DNA synthesis in spermatocytes of rats exposed to acrylonitrile at a single oral dose of 75 mg/kg, or during 5 days to an oral dose of 60 mg/kg. Incorporation of thymidine incorporation into nuclear DNA was not significantly different between treated and control animals. The authors concluded that UDS was not induced by acrylonitrile.

A number of authors (Tardif et al., 1987; Ahmed et al., 1992) have examined unscheduled DNA synthesis or repair in lung tissue, reflecting the observation of a possible increase in incidence in lung cancer in acrylonitrile-exposed workers. These workers dosed young male Sprague-Dawley rats orally with a single dose of 46.5 mg/kg of unlabeled acrylonitrile and then measured the replicative DNA synthesis and UDS in lung DNA by the ratio of ^{3}H–thymidine incorporated following hydroxyurea suppression. They observed a 1.5-fold increase of thymidine incorporation into lung DNA 30 minutes after dosing, rising to a 3.3-fold increase after 24 hours. At all time points, a significant decrease in lung DNA synthesis was observed in acrylonitrile-treated animals compared to controls. The DNA replicative index, calculated as the amount of ^{3}H-thymidine incorporated in DNA of acrylonitrile-treated animal/control, was significantly lower than 1.0 at all time points, thus implying a significant decrease in lung DNA replication resulting from a single oral dose of acrylonitrile. As replicative DNA synthesis is blocked by hydroxyurea, the authors attributed the thymidine incorporation to DNA repair. Given the lack of a validated UDS assay using lung as a target tissue, the results of these studies must however be

interpreted with caution. The methodology employed, namely determination of radioactivity associated with the nucleic acid cell fraction by liquid scintillation counting, is also not regarded as the most reliable means of establishing evidence of DNA- repair, preference being given to autoradiographical techniques as used by Butterworth et al.

Ahmed et al. (1994) also studied acrylonitrile-induced gastric DNA damage and UDS in adult, male Sprague-Dawley rats in the presence and absence of the P450 inhibitor SKF 525-A, which slows acrylonitrile oxidation to CEO. UDS in animals exposed orally to 23 or 46 mg/kg was increased in a dose and time dependent manner, up to a maximum of 6-fold at 2 hours post exposure, with a slow exponential decrease to baseline at 24 hours. SKF treatment prior to acrylonitrile exposure decreased the UDS observed to 35% of that observed in SKF 525-A untreated animals, consistent with an important role for CEO in the induction of UDS in acrylonitrile-exposed animals.

Dominant lethal assay in rats

Working et al. (1987) tested acrylonitrile for its ability to induce dominant lethal mutations in male F344 rats, together with its structural analogue acrylamide, which is a germ cell mutagen in rodents, causing dominant lethal mutations. Three groups of 50 male rats were gavaged daily for 5 days with acrylonitrile (60 mg/kg in normal saline), while acrylamide (acting as a positive control) was administered at 30 mg/kg in normal saline and a negative control group were given vehicle only. After a 1-day recovery period, a single female was placed with each male. Cage pans were examined every day for evidence of mating (vaginal plugs) and females were replaced each week for 8 weeks. All females were necropsied 18 days after the first day of cohabitation, and the number of live, dead and resorbed foetuses and corpora lutea counted. Pre-implantation loss was calculated as the difference between corpora lutea number and the total number of implants; post-implantation loss was defined as the number of dead and resorbed foetuses.

Acrylamide produced increased post-implantation losses ($p < 0.05$) for 3 weeks after exposure (peaking at 3.0 losses/female at week 2) and also increased pre-implantation losses for 4 weeks post-exposure (up to 6.6 losses/female at week 2). In contrast acrylonitrile did not cause increases in either index during the 8 weeks study period. Neither compound reduced the mating rate, but acrylamide significantly reduced the pregnancy rate during week 2 (78% pregnant compared with 96% in the control). In contrast, acrylonitrile demonstrated no fertility effects and does not appear to be a dominant lethal mutagen in male rat germ cells *in vivo*. This study is considered to be valid for risk assessment purposes, although it must be noted that only one dose level was used in the study, as opposed to the three dose levels normally employed.

Mouse micronucleus studies

Leonard et al. (1981) investigated the clastogenicity of acrylonitrile in mouse bone marrow *in vivo*, following intraperitoneal administration of a single dose of 20 or 30 mg/kg acrylonitrile to NMRI mice. The results of the study indicated that neither the percentage of chromosomal aberrations nor micronuclei differed from controls. However a micronucleus study performed *in vitro* did induce a positive response at a dose level of 3,710 µg/plate both with and without metabolic activation (Douglas et al., 1985). The apparent conflict in the results obtained *in vivo* and *in vitro* may be due to detoxification of the epoxide metabolite CEO via glutathione conjugation *in vivo*.

Hachiya et al. (1984; 1986; 1987) found acrylonitrile to be negative in the bone marrow micronucleus test in mice after i.p. administration of 20 mg/kg. The sampling times and number

of erythrocytes counted are considered to be acceptable. After i.p. injection no single-strand breaks were detected in rat liver, but alkali-labile sites were produced at one order of magnitude lower at least than with ethylene dibromide or ENU. This paper was mainly in the Japanese language and so only the figures and tables could be evaluated.

Chromosomal aberration and sister chromatid exchange studies *in vivo*

Leonard et al. (1981) investigated the clastogenicity of acrylonitrile in mouse bone marrow *in vivo*, following intraperitoneal administration of a single dose of 20 or 30 mg/kg acrylonitrile to NMRI mice. The results of the study indicated that neither the percentage of chromosomal aberrations nor micronuclei differed from controls.

Sharief et al. (1986) examined SCE and chromosomal aberrations in bone marrow cells from male C57B1/6 mice (n = 4 per group) administered a single i.p. injection of 60-100 mg/kg acrylonitrile. Bromodeoxyuridine (BrdUrd) was administered as a subcutaneous implant 30 minutes before dosing with acylonitrile. Animals were killed 24 hours after dosing for SCE analysis and 16 hours after dosing for analysis of chromosome aberrations, a total of 30 metaphases being examined for each animal. A slight increase in SCEs was detected at a dose level of 30 mg/kg, to 4.7 $\pm$ 0.62 SCEs per cell, compared with 4.0 $\pm$ 0.79 in untreated animals and 3.5 $\pm$ 0.34 in saline controls. Statistical significance ($p < 0.05$) is cited for this result, but the justification for this claim is not clear, and overall the result is not indicative of induction of SCE. One animal per group survived at each of the higher dose levels of 45 and 60 mg/kg, an increase in SCE to 7.3 $\pm$ 4.00 being seen in the animal receiving 45 mg/kg while the animal receiving 60 mg/kg had a comparable incidence of SCE to control. Some decrease in mitotic index was apparent at these higher dose levels, but no effect was apparent at 30 mg/kg and below. Sharief et al. did not detect an increase in chromosome aberrations in the bone marrow cells of mice dosed with 60-100 mg/kg acrylonitrile, a positive response was however obtained with the positive control cyclophosphamide. Overall, given the limitations of this study, the result is of limited value.

4.1.2.7.3 Evidence of mutagenicity in humans

Thiess and Fleig (1978) examined chromosomal damage in peripheral lymphocytes of 18 workers exposed to acrylonitrile for an average of 15.4 years. Co-exposure to styrene, ethylbenzene, butadiene and butylacrylate existed. Average air concentrations of acrylonitrile of 5 ppm (11 mg/m^3) were measured, representative of normal operating conditions, although higher peak exposures will have been present due to faults and manual operation. The frequency of chromosomal aberrations in peripheral lymphocytes of acrylonitrile workers was not increased compared to the unexposed controls.

Borba et al. (1996) measured chromosomal aberrations and SCEs in 14 workers employed in the continuous polymerisation area and in 12 maintenance workers in an acrylic fibre plant. Twenty workers in administration in that plant served as a control group. The study provides no information on acrylonitrile exposure level or duration of exposure, nor is information provided on exposure to other substances. There was no difference in SCEs between the two exposed groups and the controls. Maintenance workers were reported to show a higher incidence of chromosome aberrations than either the polymerisation workers or the control group. Reflecting however the mixed exposures likely for this group and the absence of a similar finding in the polymerisation workers it is unlikely that this finding can be linked to acrylonitrile exposure.

4.1.2.7.4 Cell transformation studies

Cell transformation assays cannot be regarded as providing information on genotoxic potential, recent research indicating that their primary value is in the detection of non-genotoxic carcinogens. Nevertheless a number of groups used such assays in the detection of potential carcinogens in the initial phases of development of short-term tests for carcinogens. This assay system was included in the IPCS collaborative study co-ordinated by Ashby and de Serres.

Parent and Castro (1979) showed that exposure of primary Syrian golden hamster embryo cells (HEC) in culture to 12, 25, 50 or 100 µg/ml acrylonitrile for 18 hours produced small numbers of foci of morphologically transformed cells capable of growth in the focus assay previously developed by these authors. These foci were reported to be indistinguishable from the foci induced by known chemical carcinogens. Incidences of transformed foci were 3/9 dishes at 100 µg/ml at a relative survival rate to control of 6% and 2/6 dishes at 50 µg/ml, with a survival rate of 76%. In an alternative approach, Parent and Castro examined the transformation of HEC cells treated with acrylonitrile 5 hours after they had been inoculated with simian adenovirus (SA7). They demonstrated an 8.9- and 8.4-fold increase in transformed SA7 foci at 200 and 100 µg/ml acrylonitrile respectively compared to cultures treated only with SA7. Exposure of HEC before inoculation with SA7 resulted in only a slight increase (1.8-fold to 2.1-fold) in foci of transformed cells.

A number of other researchers have also examined the effects of acrylonitrile in the SHE assay. Sanner and Rivedal (1985, IPCS collaborative study) showed an increase in transformation frequency in three independent experiments at exposure concentrations ranging from 5 to 50 µg/ml respectively, although dose-response relationship was poor and this could not be explained by differences in cloning efficiency, which was reasonably consistent throughout the exposure levels. The highest transformation frequency observed was 2.5% at 50 µg/ml, compared with 0.25% in controls. Barrett and Lamb (1985, IPCS collaborative study) reported similar results, using exposure levels of 0.01 to 1 µg/ml, higher exposures being associated with excessive toxicity. As in the study of Sanner and Rivedal, the relationship of dose and response was poor in this study, the highest transformation frequency of 0.35% being seen at the lowest exposure level of 0.01 µg/ml.

Weakly positive results were achieved by Lawrence and McGregor (1985, IPCS collaborative study) in a study designed to test the potential of acrylonitrile to induce morphological transformation in mouse fibroblasts (C3H/10T1/2 in culture, both with and without metabolic activation. No transformed foci were observed at 10-40 µg/ml acrylonitrile in the absence of S9. However in the presence of S9 mix from Arochlor-induced male rats, one Type II transformed colony was identified at 8 µg/ml; two Type II foci were recorded for 16 µg/ml; and one Type II focus was observed at the 32 µg/ml concentration (type II = massive piling up of cells maintaining essentially the normal morphology of cell line).

Acrylonitrile produced morphological transformation in Balb/c-3T3 cells and also produced ouabain-resistant variants (Matthews et al., 1985, IPCS collaborative study), when co-cultured with primary rat liver cells isolated from Fischer 344 rats. A positive response was only seen at the lowest of three exposure levels used in the study, 8.8 µg/ml, which gave an incidence of 2.35 foci per vessel, compared with 1.09 in control. The incidence of foci at an exposure level of 16.7 µg/ml was comparable to control, with a survival rate of 22%, while at an exposure level of 25 µg/ml survival was zero. No effect was seen in this cell line cultured without primary rat liver cells.

Banerjee and Segal (1986) demonstrated that acrylonitrile in the dose range 3-200 µg/ml produced morphological transformations in C3H/10T1/2 mouse fibroblast cells, exhibiting a peak at 12.5 µg/ml, when 4.5 foci/culture were seen, compared with 0.13/culture in controls ($p < 0.01$). Transformations decreased with increasing doses and increasing cytotoxic effects. The same authors also noted that acrylonitrile produced dose-dependent transformation in N1H/3T3 cells at doses of 2.0-100 µg/ml. The incidence of transformed foci was higher than that seen in C3H/10T1/2 cells, an incidence of 14.1 foci/culture being seen at an exposure level of 100 µg/ml compared with 0.17 in controls.

The overall outcome of the various studies above would indicate that acrylonitrile has the ability to cause cell transformation.

4.1.2.7.5 Summary of the mutagenic profile of acrylonitrile

Acrylonitrile is weakly mutagenic in reverse mutation assays in *Salmonella typhimurium* and specific strains of *Escherichia coli*, the effect generally requiring the presence of metabolic activation, although a number of authors have reported negative results in the *Salmonella* assay. Positive results have also been obtained in mutagenicity assays using yeast and Aspergillus, and in mammalian cell lines including mouse lymphoma cells ($TK^{+/-}$ locus and *oua* locus) and the TK6 human lymphoblast cell line, again generally in the presence of metabolic activation only and frequently only at cytotoxic concentrations. Acrylonitrile induces sister chromatid exchanges and chromosomal aberrations in *in vitro* studies, however negative responses have generally been obtained in DNA repair assays using rat hepatocytes and human mammary epithelial cells *in vitro*.

A number of *in vitro* assays have included the metabolite epoxide cyanoethylene oxide, CEO. The responses of the metabolite in several of the test systems described above indicate that it is a direct acting mutagen. Coupled with the observation that acrylonitrile is mutagenic *in vitro* mainly in the presence of S9, indicating that metabolic activation is required to exert the mutagenic potential, it may be concluded that the DNA active compound is CEO and that acrylonitrile itself has relatively low DNA reactivity. The epoxide has been shown to bind to DNA with a much greater affinity than acrylonitrile.

In *in vivo* studies, acrylonitrile overall appeared to be negative in a dominant lethal assay in rats, and was also negative in two mouse micronucleus studies, although the lack of experimental detail available on these latter studies makes them of limited value for risk assessment purposes. Conflicting results have been obtained in studies of unscheduled DNA synthesis. Negative results have been obtained in studies using rat liver hepatocytes *ex vivo* and in rat spermatocytes using autoradiographical techniques, while UDS has been reported in rat lung and in the gastrointestinal tract *in vivo,* using the methodological approach of determination of radioactivity associated with the nucleic acid cell fraction by liquid scintillation counting, which is regarded as being less reliable than autoradiography. A number of studies in *Drosophila*, using a range of genetic markers, have given positive results.

In summary, acrylonitrile has been shown to be weakly mutagenic in *in vitro* systems, indicative of a genotoxic potential. However these findings are not reliably reflected in the *in vivo* situation, suggesting that acrylonitrile or its active metabolites do not reach target tissues *in vivo*, possibly due to the detoxification of the epoxide metabolite CEO via a glutathione conjugation pathway which may not exist in *in vitro* test systems. Nevertheless, the body of evidence presented above on the *in vitro* mutagenicity of acrylonitrile, together with the positive results in *Drosophila*,

leads to the conclusion that for the purposes of this risk assessment, acrylonitrile could be regarded as genotoxic or at least mutagenic, despite the recent publication of Whysner et al. (1998) which argues for a possible non-genotoxic mechanism for the tumour induction in experimental animals.

4.1.2.8 Carcinogenicity

A number of long-term studies have been carried in rats exposed to acrylonitrile orally via drinking water and by gavage and also by inhalation. The animals were shown to develop tumours of the central nervous system, forestomach, intestines, Zymbal gland (a sebaceous tissue associated with the ear duct of rodent species) and the mammary glands.

4.1.2.8.1 Oral carcinogenicity studies in rats

Oral carcinogenicity study in rats administered acrylonitrile in drinking water (1)

As already described in Section 4.1.2.6.2, “2-year drinking study in rats”, Quast et al. (1980b) conducted a two-year study in male and female Sprague-Dawley rats (48 rats/sex and 80 controls/sex). Rats were exposed to nominal concentrations of acrylonitrile in drinking water at dose levels of 0, 35, 85, or 210 ppm for the first 21 days and thereafter, for the remaining duration of the study, to levels of 0, 35, 100 or 300 ppm. While this is a comparatively old study, it appears to have been well conducted. It has not been used as the key study for risk assessment as the dose levels are quite high and another well-conducted study with lower dose levels (1, 3, 10, 30 and 100 ppm) exists. The latter study has been used in this risk assessment report as the key study valid for risk assessment purposes.

The equivalent mean dosages of acrylonitrile converted to mg/kg/day were estimated to be 3.4, 8.5 and 21.2 in male rats and 4.4, 10.8 and 25.0 in female rats. This is based on the assumption that a level of 10 ppm in drinking water is equivalent to 1 mg/kg, assuming a drinking water consumption of approximately 10% of body weight, with female rats drinking slightly more than males.

Cumulative mortality data for this study were presented in **Table 4.19 (**see Section 4.1.2.6.2). The first death in this study occurred during the 4th month and by the end of the first year losses amounted to 33 (14 males and 19 females). The mortality of females in all treatment groups was considerably higher than their controls. The increased early mortality rate was directly correlated to increasing concentrations of acrylonitrile in the water. Early mortality was observed only in the 300 ppm group of males when compared to their respective controls. The total number of animals dead or removed from the study prior to the time of necropsy on day 746 was 206 males and 199 females (405 total = 90.4 %).

During the course of this 2-year study, haematology, urinalysis, and clinical chemistry determinations were performed at periodic intervals. The results of these determinations indicated that ingestion of acrylonitrile did not have a primary adverse effect on bone marrow, kidney or liver function in either male or female rats. However the presence of acrylonitrile in drinking water resulted in a variety of toxic effects in both male and female rats. There was a dose-related decrease in water and food consumption, and reduced body weight gain in all treatment groups, with females being more severely affected than males. Clinical observations showed that acrylonitrile-treated animals were unthrifty, exhibited early mortality compared to

controls and had an earlier onset of tumours, many of which were detectable on external examination and palpation. While these observations were initially noted in the highest dose level rats, the same observations occurred at the lower doses as the study progressed.

Gross and microscopic examination of tissues revealed a variety of pathological findings in treated rats which occurred with statistically significant increased or decreased frequency compared to the respective control animals. Certain non-neoplastic age-related changes, for example chronic nephropathy, were less frequent in the treated animals compared to controls. This can be interpreted to be due to the early mortality and decreased food and water consumption in treated animals. An increased incidence of endocardial fibrosis was noted only in males at the 300 ppm level.

Both male and female acrylonitrile-treated rats exhibited a statistically significant increased incidence of various tumour types. A statistically significant increase was seen in the incidence of tumours of the CNS, ear canal (Zymbal) gland. The occurrence of the various tumour types (zymbal gland, forestomach, Tongue, small intestine, mammary gland and multifocal glial cell tumours (astrocytoma)), are summarised in **Table 4.20**. These effects were detected first at the highest dose level (300 ppm) and later in the lower dose groups (100-35 ppm). Tumours of the subcutaneous tissue, mammary region, and pinna of the ear were not significantly different in treated and control rats.

Table 4.20 Tumours observed in rats administered acrylonitrile in drinking water for up to 2 years (study 1) (Quast et al., 1980b)

Organs affected by tumour	Dose levels showing elevations in tumour incidence
Central nervous system	35, 100 and 300 * ppm (male & female)
Zymbal gland	35, 100 and 300 * ppm (female) 300 * ppm (male)
Stomach (non-glandular)	35, 100 * and 300 * ppm (male) 100 and 300 * ppm (female)
Tongue	300 * ppm (male and female)
Small intestine	35 and 300 * ppm (male) 100 * and 300 * ppm (female)
Mammary gland: Malignant Total no. of rats with mammary gland tumour, malignant and benign combined	 300 ppm (female) 35 and 300 ppm (female)

* Statistically significant compared to controls ($p < 0.05$)

Histopathological observations revealed that a significantly increased incidence of CNS tumours, characterised as astrocytomas, was observed in rats in all dose groups. In addition, a significantly increased incidence of a focal or multifocal glial cell proliferation suggestive of an early tumour of the same cell type was observed in the 35 and 300 ppm groups. In each category of the two identified proliferative changes in the CNS, it was observed most frequently in the cerebral cortex, followed by the brain stem in the region of the cerebellum, and less frequently in the cerebellum and the thoracic spinal cord. In general, the changes of a proliferative type in the cerebral cortex sections were most frequently observed in the section obtained from the middle of the cerebral hemisphere.

Histopathological examination of the tongue showed a statistically significant increase in incidence of squamous cell tumours and for the nonglandular portion of the stomach (forestomach) the increase in incidence was in squamous epithelial tumours. On gross examination there were many rats with multiple papillomas present in this region of the stomach. Upon microscopic examination of these stomach tumours some were found to be papillomas only, others were carcinomas only, and yet other rats had both a papilloma and a carcinoma present. The earliest tumours were papillomas, while later in the study carcinomas were also frequently observed. Stages of the lesion progressed from hyperplasia and hyperkeratosis, to papilloma, and ultimately carcinoma (papillary and ulcerating) formation, with some overlap in the sequence of lesion development. These observations were dose related in severity at the 100 and 300 ppm groups. There were greater numbers of rats with a carcinoma in the stomach at the highest dose level, and they also showed a decreased latency period compared to the lower dose groups. The carcinomas present in the nonglandular stomach were predominantly papillary in type, with only a small proportion of the rats with a carcinoma having the ulcerating type. Only a single ulcerating carcinoma of the nonglandular stomach invaded through the wall of the stomach and extended locally into the mesentery.

In general, rats ingesting the highest dose of acrylonitrile (300 ppm) showed the earliest onset and greatest number of tumours which infrequently metastasised. Female rats exhibited a slightly greater toxic and tumorigenic response than males, which was concluded to be the result of the higher dose of acrylonitrile (mg/kg/day) consumed by the females than males.

Oral carcinogenicity study in rats administered acrylonitrile in drinking water (2)

In a chronic lifetime study Bigner et al. (1986) exposed 600 Fischer 344 rats to acrylonitrile in drinking water, the primary aim being to examine the neuro-oncogenic effects of acrylonitrile on the central nervous system. Other than for neurological and oncogenic effects the incidence and severity of effects is not presented quantitatively in the study report. Animals were 6-weeks-old at the start of the study and were randomly assigned to four groups, as follows:

- Group I: This group contained 153 females and 147 males exposed to 500 ppm acrylonitrile. The animals from this group were used for studies of tumour morphology, biology and karyotype. Complete autopsies were performed on all animals that died spontaneously or were killed for tumour examination.
- Group II: Comparative survival and clinical symptomology studies were made on this group, which consisted of 50 females and 50 males exposed to 500 ppm acrylonitrile.
- Group III: As for group II comparative survival and clinical symptomology studies were made on this group, which was exposed to 100 ppm acrylonitrile and consisted of 50 female and 50 male rats.
- Group IV: This control group received no acrylonitrile and consisted of 49 females and 51 males. As above the group was used in comparative survival and clinical symptomology studies.

Results

Dose-related effects of acrylonitrile on weight gain and mortality were readily apparent in both sexes, with effects in weight gain appearing earlier in males (significantly decreased), while deaths occurred earlier in females. However it was not determined whether these differential effects between the sexes were due to greater ingestion of acrylonitrile-containing water or to other sex-related factors.

There were no reported histopathological changes due to chronic toxicity reported in this study. The incidences of Zymbal gland tumours, stomach and skin papillomas and of brain tumours were higher in acrylonitrile exposed animals than in controls. While the increased incidence of tumours, other than brain tumours is noted, this study specifically deals with the question of biological significance and histogenesis of neuro-oncogenic effects in rats chronically exposed to acrylonitrile.

- Effects on body weight and mortality

There was a significant decrease in mean body weight within 2-3 weeks after the commencement of administration of acrylonitrile at 500 ppm to male rats. Females showed a similar pattern at 500 ppm but with a slightly longer period before the mean weight clearly diverged from that of the controls. Throughout chronic administration of acrylonitrile, this mean weight difference was observed in both sexes at the 500 ppm dose level. At 100 ppm (Group III) the divergence of the mean weight curves from those of the controls began about 2 months after the start of administration in males but was not apparent in females until well into the second year of administration. A clear-cut dose-response effect in mortality was observed in both sexes. Females at both 500 and 100 ppm dose levels died slightly earlier than males, whereas only a few controls (Group IV) of either sex died during the first 18 months of the study.

- Neurological signs

Animals from all groups were observed daily, and in greater detail during weekly weighing, for neurological signs. The neurological effects frequently seen included paralysis, head tilt, circling and seizures. Other more non-specific signs, sometimes associated with brain tumours but also seen in their absence, included precipitate weight loss and huddling in a cage corner with decreased activity. The incidence of neurological signs (observed within 12-18 months) was closely related to acrylonitrile dose. The proportion of animals affected were 20/300 and 16/100 in the two groups (Group I and II) dosed at 500 ppm acrylonitrile, compared to 4/100 in the 100 ppm dose level group and 0/100 in the controls.

- Brain tumours

A total of 215 brains were examined from the rats (Group I) exposed to 500 ppm acrylonitrile. Most of these animals died or were killed for tumour examination between 12 and 18 months after the beginning of exposure. Out of these 215 rats, 49 primary brain tumours were found. Tumours were observed in the cortex (approximately 75%), brain stem and cerebellum. When the tumours were differentiated according to size, 10/49 (20%) were found to be larger than 5 mm in greatest diameter, 28/49 (58%) were between 1 and 5 mm in diameter and were detectable by visual examination of an H. & E.-stained slide without a microscope, leaving 11/49 (22%) that could only be detected microscopically.

Despite the variation in their size and regardless of their location in the brain, all 49 primary tumours were remarkably similar in their cellular and architectural features. The lesions were densely cellular in the centre with diffusely infiltrative margins. The cells were consistently uniform in size with round or oval nuclei and moderate amounts of pink or clear cytoplasm. Multinucleated giant cells were not seen. Very rarely, tumours contained focal necrosis surrounded by palisading nuclei, but endothelial proliferation was not present in any of the 49 brain tumours. Infiltrating cells at the periphery of lesions often accumulated around small blood vessels, forming perivascular cuffs. Neuronal stellitosis by tumour cells was also observed frequently. Tumour cells gathered in the subpial regions and invaded the ventricles and subarachnoid space in lesions where these spaces were accessible.

The tumours found proved to be similar to, and probably indistinguishable from, a subset of spontaneously occurring rat brain tumours that have been generally classified as astrocytomas or anaplastic astrocytomas by light microscopic evaluation of H. & E.-stained slides. Despite this superficial similarity to astrocytomas, karyotypic analysis did not provide definite evidence to identify any of the neoplastic cells as astrocytic. No glial fibrillary acidic protein (GFAP) was detectable in the tumour cells, despite prominent staining of reactive and normal astrocytes in the same section. Electron microscopy revealed no distinctive intermediate filaments or junctions, nor was there evidence of differentiation of the neoplastic cells. This is in conflict with the hypothesis that the neoplastic cells found in this study are astrocytic in origin.

Regardless of the classification of the primary brain tumours, the occurrence of neurological signs in a dose-related manner and the ability to detect most of the brain tumours macroscopically in the group I animals (500 ppm) in this study suggest that the lesions are biologically significant and are capable of causing death. With respect to brain tumour occurrence in long-term toxicity studies in rats, Koestner (1986) noted that some of these microscopic tumours are transplantable, and so it would be prudent to take them into account in an assessment of carcinogenic activity. However it was stressed that these lesions are difficult to distinguish from reactive gliosis.

Oral carcinogenicity study in rats administered acrylonitrile in drinking water (3)

Gallagher et al. (1988) studied the carcinogenic effects in rats resulting from the ingestion of acrylonitrile in drinking water for a two-year period. Eighty male Sprague-Dawley derived CD rats were divided randomly into four experimental groups (20 rats/group) and were administered acrylonitrile in their drinking water at levels of 0, 20, 100 and 500 ppm. Animals receiving the highest concentration of acrylonitrile (0.05% or 500 ppm) had accelerated mortality, and the last rats from this group died just before the 2-year terminal killing. Survival in the control group and the remaining groups (20 and 100 ppm) was similar.

As already reported in Section 4.1.2.6.2 animals were weighed at weekly intervals. The average body weight of the controls and the 20 ppm group was virtually identical throughout the course of this study. The animals receiving 100 ppm or 500 ppm of acrylonitrile showed a slower body weight gain than the controls in the first year of the study and a greater decrease in body weight gain than the controls during the second year. At intervals of one month, for periods of 1 week, food and water consumption was measured daily, with mean consumption calculated for each group of animals. No statistically significant differences in food and water intake were observed, but a trend towards decreased water consumption in animals ingesting 500 ppm of acrylonitrile was noted. There were no histopathological changes reported in this study which were indicative of chronic toxicity, as opposed to the neoplastic effect, following exposure to acrylonitrile.

The necroscopy results revealed no tumours in the heart, brain, liver, lungs, kidneys, adrenals, or testes of experimental animals or controls, with the exception of a few primary or metastatic tumours as outlined in **Table 4.21**.

Table 4.21 Tumours observed in rats administered acrylonitrile in drinking water for up to 2 years (study 2) (Gallagher et al., 1988)

Site of tumour	No. of animals with tumours at increasing dose levels of acrylonitrile (ppm) [a]			
	0	20	100	500
Blood (lymphoproliferation)	1	0	0	0
Soft tissue	1	1	5	1
Forestomach	0	0	0	4
Zymbal gland	0	0	1	9
Pituitary	5	3	1	0
Pancreas	1	0	2	0
Kidney	0	0	0	1
Parathyroid	1	1	0	1
Skin	0	0	2	1

[a] 20 rats per group per concentration of acrylonitrile

The only conclusive dose-related lesions were those found in the forestomach, pituitary and Zymbal gland. Papillomatous proliferation of the squamous epithelium of the forestomach was observed in 4 animals receiving 500 ppm acrylonitrile. Although one of these pre-neoplastic/neoplastic lesions (from a single animal) showed cytological atypia, invasion of the submucosa by proliferating epithelium was not seen. Zymbal gland tumours were associated with acrylonitrile exposure in a dose-related manner in animals exposed to 100 or 500 ppm

acrylonitrile. All of these lesions were centred around the ear canal, and most were locally destructive and histologically poorly-differentiated squamous carcinomas with numerous abnormal mitotic figures. One metastatic lesion which morphologically resembled the primary tumour was observed in the lung. In some cases growth of the tumours restricted mouth opening and contributed to the death of the animal.

Pituitary adenomas were found in 5 of 18 control animals (28 %). The incidence decreased among the animals receiving increasing concentrations of acrylonitrile. These tumours expanded locally but were not noted to be invasive or metastatic; however, they were a major cause of mortality among the control and low-dose groups. Although cytological atypia was often pronounced, in the absence of other features of malignancy, it was clear that these lesions represented benign neoplasms. In some adenomas, multinucleated giant cells were seen. Immunocytological staining revealed the presence of prolactin in the cytoplasm of several scattered adenoma cells. This lower incidence of pituitary tumours in acrylonitrile-treated rats is interesting. Increased mortality among the high-dose animals possibly contributes to the apparent protective effect noted in that group by prematurely reducing the number of animals at risk. Most of the animals with pituitary adenomas in the other groups died between 16 and 22 months. This, however, together with the dose-response relationship, suggests that the effects observed were not simply due to attrition among the acrylonitrile rats. The high-dose rats dying during that period were examined microscopically for tumours, and none were found.

The results of this study revealed that in rats ingesting 0, 20, 100 or 500 ppm acrylonitrile in drinking water for 2 years, body weight gain was consistently retarded and mortality was slightly accelerated in the high-dose group (500 ppm). There was a dose-dependent increase in the incidence of tumours in the Zymbal gland. The occurrence of age-dependent pituitary adenomas was, on the other hand, dose-dependently suppressed. Tumours in other systems e.g. the central nervous system, respiratory tract, or urogenital tract, were not related to chronic exposure to acrylonitrile. Papillomas of the forestomach were increased, however, indicating a trend towards the development of forestomach papillomas following chronic exposure to acrylonitrile at the highest concentration (500 ppm), almost certainly due to the irritant action of acrylonitrile. The overall incidence of tumours in control and treated rats was not however statistically significant.

Oral carcinogenicity study in rats administered acrylonitrile in drinking water (4)

The Biodynamics (1980a) study administered acrylonitrile in the drinking water at doses of 0, 1, and 100 ppm to 100 Sprague-Dawley rats/sex/group. The actual intake was calculated to be 0.093 or 7.98 mg/kg/day in males, and 0.146 or 10.69 mg/kg/day in females. Interim necropsies were performed at 6, 12, and 18 months (10/sex/group). The study was continued for 19 months in females and 22 months in males. This study was then terminated early because of low survival rates in animals. There was increased incidence of astrocytomas of the brain and spinal cord, carcinomas and adenomas of the Zymbal gland, squamous cell carcinomas and papillomas of the forestomach in the high-dose group (100 ppm).

Oral carcinogenicity study in rats administered acrylonitrile in drinking water (5)

The most informative drinking water study, already described in some details in Section 4.1.2.6.2, was performed by Biodynamics (1980b). Acrylonitrile was administered orally via drinking water to groups of 100 male and 100 female Fisher 344 rats at dose levels of 1, 3, 10, 30, and 100 ppm. These dose levels are estimated to be equivalent to average daily doses of 0.08, 0.25, 0.84, 2.49 and 8.36 mg/kg/day in males and 0.12, 0.36, 1.25, 3.65 and 10.89 mg/kg/day in females respectively. The control group comprised 200 male and 200 female animals. Interim

necropsies were performed at 6, 12, and 18 months (10/sex/exposed group and 20/sex/control group). The study was originally designed to have a duration of 24 months, however, to ensure at least 10 animals/sex/group for histopathological evaluation at termination, the females were sacrificed early i.e. 23 months. The males were continued on the study until the 26th month when similar survival levels were reached at which time all remaining animals were sacrificed.

In this study, mortality in the males and females receiving 100 ppm was markedly greater than controls, while mortality in the males receiving 10 ppm and the females receiving 3 and 30 ppm was also significantly greater than control. The mortality data from this study were summarised in **Table 4.17** (see Section 4.1.2.6.2).

Body weights for the males and females receiving 100 ppm were consistently lower ($p < 0.01$) than the controls, while body weights for the males only receiving 30 ppm were significantly lower ($p < 0.01$) than the controls. The body weights for the animals in the other treatment groups were generally comparable to controls throughout the study.

Food consumption for the females at 100 ppm was consistently slightly lower than controls on a grams/week basis, while this pattern was notable for the males of this group only following the first year of the study. On a grams/kg/day basis, however, food consumption for both males and females at 100 ppm was considered generally comparable to or slightly greater than controls as a result of the lower body weights for these animals. Differences from controls in food consumption for the other groups were sporadic and not indicative of a relationship to treatment.

Water consumption for the males and females at 100 ppm was generally lower ($p < 0.01$) than controls on a ml/3 days basis; however, on a ml/kg/day basis, differences from the controls were less marked for the females and comparable to or greater than controls for the males. Sporadic differences from controls noted for the other groups were not considered to be treatment related.

Small but generally consistent reductions in haemoglobin (occasionally achieving statistical significance of $p < 0.05$), haematocrit and erythrocyte counts were noted for the females receiving 100 ppm throughout the study. These parameters were considered comparable to controls for males at this dose level. Slight increases in alkaline phosphatase activity ($p < 0.05$) were noted for the females receiving 100 ppm from 12 months onwards (to termination), while values for the males in this group were elevated ($p < 0.01$) at 18 months onwards (to termination). Slight elevations ($p < 0.05$) in the alkaline phosphatase activity were also noted in females receiving 10 and 30 ppm, at termination only. Urine specific gravity was increased in males receiving 100 ppm at 18 months and at termination.

Consistent, but not always statistically significant, elevations in the mean relative (to body weight) liver and kidney weights were noted ($p < 0.01$) for animals receiving 100 ppm at most necropsy intervals, while the mean absolute weights for these organs were generally comparable to the controls or slightly elevated. The mean relative heart weights were also elevated ($p < 0.05$) for this group at 18 months and termination. The increases in the mean relative weights of these organs at most necropsy intervals in animals receiving 100 ppm were considered treatment-related effects. In addition, at the terminal sacrifice the mean absolute and relative liver and heart weights were elevated ($p < 0.05$) for females at 30 ppm, while their mean body weight was comparable to controls. Other organ weight differences were noted, but were considered attributable to body weight differences or else they did not occur in a pattern suggestive of a relationship to treatment. Elevated ($p < 0.05$) mean organ/brain weight ratios were noted for heart and liver in the females receiving 30 ppm at termination. Other differences were sporadic and not treatment-related. For 100 ppm animals the mean absolute weights of the liver, kidney and heart as well as the brain, were not markedly different from the control animals.

A dose-related increased incidence of palpable masses on the head, i.e. in the area about the ears and eyes and in the cervical region, was noted in the males and females receiving 30 and 100 ppm which died or were sacrificed after 12 months. The masses observed in the area of the ear were characterised as subcutaneous and necrotising or purulent, and were associated with the ear canals. An increased incidence of masses in the mammary region was also noted in females receiving 100 ppm and in males receiving 3 and 10 ppm.

Histopathology showed that the number of malignant tumour-bearing rats was increased in the male and female rats at 10, 30 and 100 ppm, when compared to controls. This was due to an increased incidence of astrocytomas of the central nervous system (brain and/or spinal cord) and squamous cell carcinomas of the Zymbal gland, as well as mammary gland carcinomas in the female at 100 ppm. The increases in the incidences of these neoplasms were noted predominantly in animals dying, killed in a moribund condition or sacrificed at scheduled intervals after the first year of the study. The incidence of neoplasms in the rats at 1 and 3 ppm was considered comparable to controls. Other neoplastic and non-neoplastic lesions occurred sporadically in various tissues and organs but were not considered attributable to treatment. The number of rats with specific tumours per dose level are summarised in **Table 4.22**.

Table 4.22 Incidence of tumours observed in male rats administered acrylonitrile in drinking water for up to 2 years

Tumour site and type	Male rats, dose Level in mg/kg/day					
	0	0.08	0.25	0.84	2.49	8.36
Brain/spinal cord, astrocytoma	3/200	2/100	1/100	2/100	10/99	21/99
Zymbal gland, carcinoma	1/189	0/97	0/93	2/88	5/94	8/93
Tumour site and type	**Female rats, dose level in mg/kg/day**					
	0	0.12	0.36	1.25	3.65	10.89
Brain/spinal cord, astrocytoma	199	1/100	2/100	4/100	6/99	24/99
Zymbal gland, carcinoma	93	0/94	1/92	2/90	2/94	7/86

Note: It should be noted that the table above reflects the no. of animals as per study design. However in this Biodynamics study 30 animals were taken out for interim kills, and the actual incidence can therefore be related to at least a total population of 70 for treatment groups and 140 for controls.

In general the physical observations noted throughout this study were variable in incidence and did not occur in a pattern suggestive of adverse effects or toxicity, other than those relating to neoplastic effects, following long-term exposure to acrylonitrile via drinking water. Non-neoplastic lesions occurred sporadically in various tissues and organs but not considered attributable to treatment Therefore other than the increased number of malignant tumour-bearing animals in the groups receiving 10, 30, and 100 ppm histopathological evaluation revealed no treatment related changes.

The main tumours observed in rats exposed to acrylonitrile are microscopic brain tumours and Zymbal gland tumours. This is a consistent finding in chronic oral and inhalation studies on exposure of rats to acrylonitrile. Therefore considering similar tumour pattern and metabolism, both the oral Biodynamics (Biodynamics, 1980a) study above and the Quast (1980b) inhalation study (discussed in the following section) have been used for the purposes of risk assessment.

Included as Appendix B are the calculations of T_{25} (Dybing et al., 1997) made by Sanner (1998, personal communication) for both oral and inhalation routes and the application of these data for risk characterisation based on the Biodynamics (1980b) and Quast et al. (1980a) studies.

Oral carcinogenicity study in rats administered acrylonitrile by gavage (6)

Maltoni et al. (1977) conducted a study to evaluate the effects on adult Sprague-Dawley rats of acrylonitrile, administered by gavage in olive oil at a single daily dose of 5 mg/kg bodyweight 3 times weekly for 52 weeks. The study used 40 male and 40 female treated rats, and 75 male and female controls. The animals were examined weekly and weighed every 2 weeks during the period of treatment and monthly after treatment was over, until spontaneous death. A complete autopsy was carried out on each animal. Histological examination of the Zymbal glands, interscapular brown fat, salivary glands, Tongue, lungs, liver, kidney, spleen, stomach, different segments of the intestine, bladder, brain, and any other organs with pathological lesions was performed. Under these experimental conditions acrylonitrile administered by ingestion did not show effects on the survival and body weight of the test animals. No treatment-related histological changes were observed in liver, kidneys and lung.

In this study, acrylonitrile did not affect the percentage of animals bearing benign and malignant tumours, the number of animals bearing malignant tumours only, the number of total malignant tumours per 100 animals or the incidence of Zymbal gland carcinomas, extrahepatic angiosarcomas, hepatomas and encephalic gliomas. The only increase in incidence of tumours was in the mammary gland and forestomach of female rats.

4.1.2.8.2 Inhalation carcinogenicity studies in rats

As with the oral route of administration a number of inhalation studies in rats have been performed in order to evaluate effects caused by long-term exposure to acrylonitrile, in particular any carcinogenic effects which may result from such exposure.

Study 1

Maltoni et al. (1977) studied the effects on groups of 30 male and 30 female rats of inhalation exposure to 5, 10, 20, and 40 ppm of acrylonitrile, 4 hours daily, 5 days weekly, for a 12-month period. One group of untreated rats acted as a control group for the study. The animals were kept under observation until spontaneous death. No effect on body weight was noted.

A statistically significant increase in the percentage of animals bearing benign and malignant tumours ($p < 0.01$), malignant tumours alone ($p < 0.01$) and in the number of total malignant tumours per 100 animals was found in several treated groups, although a strong dose-response relationship was not established. No increase in Zymbal gland tumours, extrahepatic angiosarcomas and hepatomas was observed. 3/60 and 2/60 encephalic gliomas were observed in animals exposed to the two highest concentrations of acrylonitrile. While this finding did not achieve statistical significance, it is biologically significant given that the brain was clearly shown to be the target organ in rats following oral administration.

Maltoni suggested that the carcinogenicity of acrylonitrile was influenced by the age of the animals at the start of treatment and was dependent on the concentration administered and duration of treatment (Maltoni et al., 1977).

Study 2

The long-term inhalation study of Quast et al. (1980a) is one of the most important studies for risk assessment purposes. Sprague-Dawley (Spartan substrain) rats (100/sex/concentration) were exposed, for 6 hours per day, 5 days per week, during 2 years, to concentrations of 0 (control), 20 (44 mg/m^3) and 80 (176 mg/m^3) ppm. The control group was only exposed to air. Additional animals were included for interim sacrifices at 6 months (n=7 /sex/dose) and 12 months (n=13 /sex/dose). During the course of this study, haematology, urinalysis, and clinical chemistry determinations were performed at periodic intervals.

In this study, clinical observations detected a variety of toxic effects characterised by decreases in body weight, early mortality, unthrifty clinical appearance, earlier onset of tumours and more frequently observed palpable tumours. These observations were most apparent and occurred earliest in the high-dose group (80 ppm).

Mortality

A statistically significant increase in mortality ($p < 0.05$) was observed within the first year in both male and female rats administered 80 ppm acrylonitrile and in the females of the 20 ppm group during the last 10 weeks of the study. The apparent increase in the reported mortality for the 20 ppm females was principally due to early sacrifice of rats with large, benign, mammary gland tumours (Quast, 1980a). In Sprague-Dawley the tumours are known to occur spontaneously at a high rate, but in this experiment the tumours were observed earlier and more frequently, and became larger in exposed animals. Cumulative mortality data were presented in **Table 4.15** (see Section 4.1.2.6.1).

Non-neoplastic lesions

As already reported in detail in Section 4.1.2.6.1, primary treatment-related effects were observed in the nasal turbinate mucosa of all rats examined in the 80 ppm group as well as in most of the rats in the 20 ppm group. The changes in both groups were qualitatively similar but much less severe in the 20 ppm group than in the 80 ppm group. These changes were confined to the turbinate region extending from the external nares into the region lined by respiratory epithelium. The inflammatory and degenerative changes present in the nasal turbinates were characterised by suppurative rhinitis, hyperplasia, focal erosions, and squamous metaplasia of the respiratory epithelium, with hyperplasia of the mucous secreting cells. These changes were interpreted to be a result of irritation due to acrylonitrile exposure.

In addition, in two of the 80 ppm female rats there was a microscopic metaplastic proliferation of the respiratory epithelium. Although the incidence of this lesion was not statistically significantly increased, it was considered treatment-related in view of its location in the same region of the nasal mucosa showing the degenerative and inflammatory changes and because of the historically low spontaneous incidence of this finding.

Focal perivascular cuffing and gliosis was reported in the brain. In males at 20 and 80 ppm the incidence was 2/99 and 7/99 ($p < 0.05$, one-sided) respectively and for females the incidence was 2/100 and 8/100 ($p < 0.05$, one-sided) respectively. A treatment-related increase in extramedullary haemopoiesis in the liver and the spleen and an increase in focal liver cell necrosis was observed primarily at the 13-18 month and the 19-24 month intervals, with findings in treated rats generally being observed at the earlier time intervals when compared with the controls. The finding of extramedullary haemopoiesis was considered (Quast, 2001, personal communication) to be secondary to the presence of large, benign mammary tumours and ear canal (Zymbal gland)

tumours in the animals, which occurred earlier in treated animals than controls. It was concluded, therefore, that these findings were not indicative of a primary hepatotoxic effect of acrylonitrile.

Neoplastic changes

An increased incidence of brain tumours was observed, although they were rarely the cause of death. Many of the tumours could not be detected by gross pathology, but were identified histopathologically as focal or multifocal glial cell tumours (astrocytomas). The incidence was significantly increased for both male and females at the 80 ppm exposure level compared to the controls. The incidence of proliferative glial cell lesions, suggestive of early tumours, was significantly increased in the 80 ppm males, but not in the females at any dose level. Collectively, proliferative changes in the glial cells i.e. tumours and early proliferation suggestive of tumours, were significantly increased in the 20 ppm and 80 ppm females but only in the 80 ppm males, compared to controls.

Recorded deaths were often attributable to severe ulceration of the Zymbal gland or mammary tissue tumours, and at the highest dose level (80 ppm) were also due to suppurative pneumonia due to the irritant effects of acrylonitrile on the lungs. The occurrence of Zymbal gland tumours was observed to be significantly increased in both male and female animals in the 80 ppm group (11/100 in both male and female, $p < 0.05$). For females the highest incidence occurred during the 13 to 18 month interval. An incidence of 3/100 was also seen in males exposed to 20 ppm, compared with 1/100 for control males, but no Zymbal gland tumours were seen in females at this exposure level. These tumours showed an increased incidence and a decreased latency period which was consistent with the life records of the palpable masses in this region. The type of tumour observed in both males and females was sebaceous squamous cell carcinoma of the external auditory canal gland, without metastasis.

Table 4.23 Brain tumours and pre-neoplastic changes in the brain in rats following inhalation exposure for up to 2 years

Lesion	Acrylonitrile exposure level (ppm)		
	0	20	80
(Multi) focal glial cell proliferation			
Males	0/97 [a)]	0/93 [a)]	7/83 [a)]
Females	0/99 [a)]	4/99 [a)]	4/99 [a)]
Astrocytoma			
Males	0/97 [a)]	4/93 [a)]	15/83 [a)]
Females	0/99 [a)]	4/99 [a)]	17/99 [a)]
Total:	0/97 [a)] (M) 0/99 [a)] (F)	4/93 [a)] (M) 4/99 [a)] (F)	22/83* [a)] (M) 21/99* [a)] (F)

* $p < 0.05$, one-sided

a) These figures from the Quast study have been adjusted for mortality

(Quast et al., 1980a)

The main tumours observed in rats exposed to acrylonitrile are microscopic brain tumours and Zymbal gland tumours. This is a consistent finding in chronic oral and inhalation studies on acrylonitrile in rats and is supported in particular by the key study above (Quast et al., 1980a). Considering similar tumour pattern and metabolism, both oral (Biodynamics, 1980b) and inhalation (Quast et al., 1980a) studies on acrylonitrile can be used for quantitative risk assessment. Appendix C contains details of such a quantitative risk estimate for acrylonitrile using these key studies.

For the purpose of this report an estimate of the T_{25} has been derived from the Quast data (using the approach described by Dybing et al., 1997), taking the incidence of the most common tumour type, malignant astrocytomas, as a basis for the calculation. The incidence in males, adjusted for mortality, was 0/97 at 0 ppm, 4/93 at 20 ppm (4.3%) and 15/83 at 80 ppm (18%), and in females was 0/99, 4/99 (4%) and 17/99 (17.2%). The incidence at 80 ppm was statistically significant in both sexes and was used to derive the T_{25}. The daily dose in animals exposed to 80 ppm can be derived as follows: 6 hours $\cdot$ inhalation volume $\cdot$ mg acrylonitrile/m^3 $\cdot$ (5/7) (average over 7 days a week) = 6 hr $\cdot$ 6 l/hr $\cdot$ 180 mg/m^3 $\cdot$ 1/1,000 $\cdot$ (5/7) = 4.63 mg/rat/day. Given a mean bodyweight of 400 g for males and 300 mg for females, the daily dose per kg body weight is therefore 11.6 mg for males and 15.4 mg for females. The T_{25} after 2 years is then estimated to be 25/18 $\cdot$ 11.6 mg/kg/day = 16.1 in males and 25/17.2 $\cdot$ 15.4 = 22.4 mg/kg/day for females. Alternatively, considering the tumour incidence data presented in **Table 4.21** (Gallagher et al. 1988), the T_{25} can be estimated to be approximately 125 ppm.

Included as Appendix B are the calculations of T_{25} made by Sanner (1998, personal communication) for both oral and inhalation routes and the application of these data as applied to the risk characterisation based on the Biodynamics (1980b) and Quast et al. (1980a) studies. In the calculation using the Quast et al. (1980a) study the calculated T_{25} value is 14.9 mg/kg/day, reflecting a consistency in results subject to whether the incidence value was adjusted for mortality or not i.e. 15/83 as per **Table 4.23** or 15/99 (unadjusted per Quast et al. study).

An alternative approach to estimation of the carcinogenicity risk is presented in Appendix C. This approach uses an extensive analysis of the microscopic brain tumour incidence in F344 rats, exposed to acrylonitirle via drinking water at 5 different concentrations (Biodynamics, 1980b).

Felter and Dollarhide (1997) re-evaluated the database to support an inhalation cancer risk assessment with respect to exposure to acrylonitrile. In doing so, they examined various animal inhalation studies but found many of the studies limited for use in quantitative risk assessment. However the study on which they proceeded was the Quast et al. (1980a) study, it being deemed as the only study suitable for quantitative risk assessment. Astrocytomas, Zymbal gland tumours, and tumours of the small intestine and tongue were statistically significantly increased. The astrocytomas and Zymbal gland tumours are the most clearly associated with acrylonitrile exposure as these tumour types were seen in each of the independently conducted bioassays. The relevance of Zymbal gland tumours for humans is however highly questionable, as there is no comparable target organ in humans. Therefore the Felter and Dollarhide analysis focused on modelling of the astrocytoma incidence data. Both benign and malignant tumours were included because there was a clear progression of this tumour type such that the benign tumours had the potential to become malignant.

The authors modelled the astrocytoma tumour incidence data using a polynomial model and a derivation of the human equivalent concentrations (0, 7.5, and 30 mg/m^3) relative to the dose levels used in rats (Quast et al., 1980a) of 0, 20 (42 mg/m^3), 80 (168 mg/m^3) ppm. In accordance with the EPA (1996) guidelines, both the ED_{10} and the LED_{10} were calculated. For male, rats the ED_{10} and LED_{10} were calculated to be 14.6 and 9.1 mg/m^3 respectively. For female rats, the ED_{10} and LED_{10} were calculated to be 12.2 and 9.1 mg/m^3 respectively. The cancer potency is then reported as the slopes of the lines drawn from the ED_{10} or the LED_{10} to the origin. The results of this assessment showed that male and female rats have similar susceptibilities. As the data from the female rats resulted in a slightly higher slope factor, these data were used by the authors to calculate the inhalation unit risk. Therefore using the methodology of the EPA's 1996 cancer risk assessment guidelines, they derived a risk estimate of between $8.2 \cdot 10^{-6}$ (based on the ED_{10}) to $1.1 \cdot 10^{-5}$ (based on the LED_{10}) associated with lifetime continuous exposure to 1 µg/m^3 acrylonitrile ($0.44 \cdot 10^{-3}$ ppm). These authors comment that these estimates are 6-8 fold lower than the US EPA's previous estimates of risk. This in turn supports the conclusion that the weight of evidence derived from the human studies does not indicate a causal association between exposure to acrylonitrile and lung, as discussed in detail and evidenced in Section 4.1.2.8.6 of this report.

With regard to deriving this inhalation cancer risk assessment for acrylonitrile certain assumptions (EPA defaults values etc) had to be made, which by their nature are health protective. For this reason therefore it is unlikely that the cancer potency in this scenario has been underestimated and indeed it is more possible that an overestimation of actual risk to humans had occurred. In addition to the assumptions mentioned, it should be noted that it is considered appropriate to calculate cancer risk at low levels of exposure based on an assumption of low-dose linearity. This does not take into account the protective or adaptive mechanisms that humans have for dealing with exposures to xenobiotics (e.g. detoxifying metabolic reactions, DNA repair etc.).

4.1.2.8.3 Dermal carcinogenicity studies in animals

There are no available carcinogenicity studies in animals using the dermal route of exposure.

4.1.2.8.4 Summary of carcinogenicity studies in animals

A number (8 in all) chronic bioassays have been conducted, both via the oral (drinking water or gavage) route and by inhalation. All of the studies to date have been performed using rat only, although different strains of rat were tested, and a broad range of dose levels has been used. The results of these studies have shown that acrylonitrile is carcinogenic to rats following either oral administration or via inhalation. Common target organs identified were the central nervous system (brain and spinal cord), Zymbal gland, gastrointestinal tract (tongue, non-glandular stomach and small intestine) and mammary gland. The most important finding arising from the studies is the general consistency of the findings of primary brain and Zymbal (ear canal) tumours, irrespective of route of administration. The results of the carcinogenicity studies carried out on acrylonitrile are summarised in **Table 4.24**.

Table 4.24 Summary of the findings of 8 carcinogenicity studies carried out with acrylonitrile in rats

Source	Dose / Concentration	Route of Exposure	Effects
Biodynamics (1980b)	0, 1, 3, 10, 30,100 ppm	Ingestion (drinking water)	Increase in mortality, decrease in water / food consumption and body weight gain. Elevation of heart weight. Astrocytomas of CNS. Carcinomas of ear and mammary gland.
Bigner et al. (1986)	0, 100, 500 ppm	Ingestion (drinking water)	Brain tumours
Quast et al. (1980b)	0, 35, 85, 210 ppm (21 days) 0, 35, 100, 300 ppm (remainder of study)	Ingestion (drinking water)	Increase in various tumour types (CNS, Zymbal gland, forestomach, Tongue, small intestine, mammary gland)
Gallagher et al. (1988)	0, 20, 100, 500 ppm	Ingestion (drinking water)	Tumours of Zymbal gland. Increase in forestomach papillomas.
Maltoni et al. (1977)	5 mg/kg/bw	Ingestion (gavage)	Increase in forestomach and mammary tumours.
Quast et al. (1980a)	0, 20, 80 ppm	Inhalation	Increased mortality. Non-neoplastic nasal lesions. Tumours of CNS, Zymbal gland, Tongue, small intestine, mammary gland.
Maltoni et al. (1977)	0, 5, 10, 20, 40, 60 ppm	Inhalation	Tumours of mammary and Zymbal glands, angiosarcomas, encephalic gliomas
Biodynamics (1980a)	0, 1, 100 ppm	Ingestion	Increase of astrocytomas of the brain and spinal cord, zymbal gland and forestomach tumours

In both the acrylonitrile inhalation (Quast et al., 1980a) and drinking water studies (Biodynamics, 1980b), a linear relationship was observed between the incidence of astrocytomas and the dose level of acrylonitrile administered. There was no increase in tumour incidence at 3 ppm (oral) and a small increase at the lowest dose of 20 ppm (inhalation). While there is no doubt that acrylonitrile is an animal carcinogen, its mechanism of action with respect to inducing carcinogenicity is still relatively unclear (see Section 4.1.2.8.5 below). Based on current information and with no definitive contrary evidence, acrylonitrile must be considered to be a carcinogen for which a threshold cannot be reliably identified for the purposes of risk assessment. As such, from the current regulatory position one cannot state or establish a NOEL for acrylonitrile for this particular end point, since no safe threshold can be established.

It should be noted that the long-term inhalation study of Quast et al. (1980a), has been used as the most pertinent long-term carcinogenicity study for the purposes of the health assessment, particularly when considering that the occupational exposure occurs predominantly via the inhalatory route of exposure.

4.1.2.8.5 Mechanism of carcinogenicity

DNA adducts formed from the metabolite CEO are postulated to be responsible for the carcinogenic effects seen in rats following exposure to acrylonitrile. In fact the measurement of DNA adducts is probably the most definitive technique to demonstrate DNA damage indicating a genotoxic mechanism of action. *In vitro* assays have indicated the presence of three different DNA adducts, with CEO appearing to be the reactive metabolite of acrylonitrile. However very few DNA adducts derived from acrylonitrile have been detected *in vivo*.

Following acute exposure to acrylonitrile in rats (50 mg/kg of acrylonitrile or 6 mg/kg of CEO via i.p. route to male, F344 rats), the 7-oxo-ethylguanine (7-OEG) adduct was not found in the brain, although it was detected in the liver (Hogy and Guengerich, 1986). Also, after chronic exposure (rats given 500 ppm in drinking for up to 15 months) no adducts were found either in the liver or brain (CIIT, 1991). More recent *in vitro* studies have shown that the initial cyanohydroxethyl addition products of the reaction of CEO with nucleotides and DNA are unstable (Yates et al., 1994), further complicating the potential of using DNA adducts as dosimeters for acrylonitrile activation. In fact adducts in the liver and the occurrence of unscheduled DNA synthesis of DNA in liver cells are the only clear evidence of *in vivo* adduct formation. No genotoxic effect (i.e. DNA binding) has been adequately documented in the brain to account for the carcinogenicity in this specific target organ. Existing evidence therefore does not conclusively implicate DNA adduct formation as the mechanism of tumourigenicity, but raises the possibility that epigenetic effects might be involved. This is an area that requires further detailed research before a definite conclusion can be reached.

Although not completely studied, several lines of evidence suggest an epigenetic tumour producing mechanism in the brain, possibly involving the formation of oxygen radicals. Increased lipid peroxidation has been demonstrated, which may be partially related to release of cyanide or some other mechanism. The formation of oxygen radicals could also lead to oxidative DNA damage. Whysner et al. (1996) postulated that the acrylonitrile-induced tumours produced were produced via a mechanism involving the formation of 8-oxodeoxyguanosine, suggesting oxidative damage. In considering the possibility of an epigenetic mechanism involving oxidative stress, the production of tumours may only occur after exceeding the capacity of glutathione and other antioxidants to prevent oxidative damage to either DNA or lipids. It appears that there are no simple answers available for the most basic mechanistic questions regarding acrylonitrile tumourigenicity in rodents. In the meantime from a risk assessment standpoint the evidence still indicates that *in vitro* and *in vivo* DNA adducts have been found, which could indicate that acrylonitrile is a carcinogen that acts by a gen otoxic mechanism.

Although acrylonitrile is accepted as a rodent carcinogen, relating this fact to the assessment for the human scenario is complex, particularly as the mechanism of carcinogenicity is not clear. The *in vitro* genotoxicity tests show some mixed results, both with and without metabolic activation, and the *in vivo* genotoxicity results also prove to be unclear but generally proving negative.

Overall acrylonitrile has demonstrated a unique and unusual pattern of tissue-specific mutagenic and carcinogenic activity. However the mechanisms responsibility for this specificity are not understood and further work in this area would prove valuable, especially regarding the induction of brain tumours. Understanding the mechanism of action is essential when endeavouring to assess the potential human risk from carcinogenicity following exposure to this agent. Should a genotoxic mechanism for tumour induction be positively identified, especially regarding the induction of brain tumours in rats, it would support the contention that acrylonitrile has the potential for human carcinogenicity (Williams, 1987). It should be noted that a mouse bioassay has been commissioned which may assist in establishing the mechanism of action by which tumours are produced and therefore aid in the assessment of potential risk to man following exposure to acrylonitrile.

4.1.2.8.6 Epidemiological studies

There have been a number of epidemiological studies of workers exposed to acrylonitrile. Only the largest of these approached an acceptable statistical power for detection of a significant elevation of cancer risk (12.5% with a power of 80%) associated with exposure. More seriously, accurate quantification of exposure to acrylonitrile was not possible for most of the cohorts studied, and in some cases it is likely that a proportion of the exposed cohort were not, in fact, exposed.

The second drawback of the published literature is that many of the cohorts were exposed to levels of acrylonitrile that can be presumed to be higher than current exposure. Thus their direct application to present day workforces is uncertain. To add to these problems, some studies report workers whose exposure was to multiple chemicals, including acrylonitrile, that are known or suspected carcinogens. In addition confounding factors such as cigarette smoking have not been considered in most of these studies. Finally, shortfalls in mortality for acrylonitrile workers, beyond levels explainable by the "healthy worker effect", suggest that in some studies case-findings were incomplete.

Notwithstanding the above-mentioned problems there is a large amount of epidemiological information relating to workers exposed to acrylonitrile. Both consistency and inconsistency across the studies can be used as a means for determining causal associations rather than using individual study size. Two meta-analyses exist (Rothman, 1994; Collins and Acquavella, 1998) which endeavour to overcome the power issue by combining consistent studies.

For the purpose of this risk assessment report the studies are presented generally in two sections i.e. "old" and "new". In August, 1997, the Acrylonitrile Industry Group hosted an epidemiological Conference (on occupational exposure to acrylonitrile) in Oxford, UK (Doll Ed., 1998). As an inherent part of this conference four new studies were presented and discussed in detail, as follows:

- UK study (Benn and Osborne, 1998) is an extension of an already existing study by Werner and Carter (1981);
- Wood et al. (1998) updated the O'Berg et al. (1980; 1985) and Chen et al. (1987) studies;
- Swaen et al. (1998) updated the original Swaen et al. (1992) study;
- Blair et al. (1998) updated the Collins et al. (1989) study and provided new information on 6 other plants not previously studied.

The information from these "new" studies is valuable since it reflects the most recent data available and so may to some extent explain contradictory findings or in fact eliminate previously perceived problems associated with exposure to acrylonitrile arising from the "older" studies.

However, for completeness of the report and for a fuller understanding of the problems of the epidemiological studies on acrylonitrile, it is necessary to follow through from the "old" to the "new" studies. It is not appropriate to discount the information acquired in place of the more current data and it is anticipated that each individual study will contribute to a final analysis regarding the potential risk to a workforce from exposure to acrylonitrile. Also while the "old" studies record higher average exposure levels compared to current levels, it is considered that no cancer excess occurred even at these higher levels, indicating that the current exposures are probably safe.

"Old Studies"

O'Berg et al. (1980) observed 25 cases of cancer, including 8 cases of respiratory cancer, in 1,345 male textile workers exposed to acrylonitrile with a follow-up period of 10 years. Estimated levels of exposure were between 5 and 20 ppm of acrylonitrile. No quantitative exposure was available but jobs were rated by a qualitative exposure assessment that included information from work history cards, interviews with supervisors, and, for salaried workers, a survey form. The analysis compared the incidence and death rate from cancer and respiratory cancer of the workers with overall incidence and mortality data for all DuPont employees. Although the analysis examined duration of exposure to acrylonitrile and job category, little detail was provided by category of outcome.

A total of 89 deaths were observed, compared with an expected number of 77.4 based on DuPont mortality rates, and 121.1, based on United States rates. A total of 25 cases of cancer were found, compared with 20.5 expected according to the DuPont rates. There were 8 cases of respiratory cancer, with 4.4 expected, and 3 cases of prostate cancer, with 0.9 expected. All of the cancer cases, except for one non-respiratory cancer, occurred among 1,128 workers with 6 or more months exposure (SIR = 126, SMR = 113). A trend of increased cancer incidence was seen with increased duration of exposure and increased length of follow-up time. The excess of respiratory cancer incidence was statistically significant and remained so upon evaluation of the contribution of smoking (5 observed as opposed to 1.6 expected, p-value = 0.02).

However regarding this 1980 study by O'Berg it should be noted that both cancer morbidity and cancer mortality were considered. The information above reflects the cancer morbidity scenario. Concerning cancer mortality however there was no evidence of any increase, based either on the DuPont company rates or on national statistics. As no national vital statistics were available for cancer morbidity, O'Berg used DuPont cancer morbidity rates. However these data were flawed in that consideration was not extended to cover DuPont company leavers. One major weakness in using this Registry approach is that credible evaluation of cancers with long latencies cannot be successfully performed as these cancers will occur after workers are retired. On the other hand "survivable" cancers which occur at younger ages can be evaluated using the company Registry data. It is suggested that smoking was considered as a confounder, whereas in fact it was only verified that all lung cancer cases except one were smokers. According to Doll (Acrylonitrile Epidemiology Conference, 1980) this 1980 O'Berg study raised some suspicion about the carcinogenicity of acrylonitrile in humans, but did not amount to decisive evidence.

Three other studies, Delzell and Monson (1982), Thiess et al. (1980) and Werner and Carter (1981) reported a statistically significant increased incidence of lung cancer following exposure to acrylonitrile. However each of these studies suffered from problems relating to their methodology e.g. exposure to other chemicals/carcinogens, no smoking history, exposure not quantified.

Delzell and Monson studied 327 male workers at a rubber manufacturing plant and reported a statistically significant increase in lung cancer among workers employed 5 or more years. However the increase in lung cancer mortality (9 observed versus 5.9 expected) was not statistically significant in this study.

Thiess et al. studied 1,469 workers employed 6 months or more in acrylonitrile processing. A statistically significant increase in lung cancer and a nearly significant increase of cancer of the lymphatic and haematopoietic system were observed. It should be noted that the workers involved in this study experienced a mixed exposure of polycyclic aromatic hydrocarbons, vinyl chloride, beta-naphthylamine etc., and all cancer cases were known smokers.

Werner and Carter studied 1,111 men employed at least one year in polymerisation of acrylonitrile and spinning of acrylic fibre. A statistically significant increase was seen for stomach cancer in all age groups and for pulmonary cancer in the 15-44 year age group. When considering the data and findings of this study however it should be noted that the cohort consisted of 40% Welshmen, while in the vital statistics of the UK only 4% Welshmen are represented. The incidence of stomach cancer was relatively higher than in the UK population, so the expected mortality for stomach cancer has to be corrected for the background stomach cancer mortality in Wales. One other study reported a statistically non-significant increase in deaths from cancer from exposure to acrylonitrile (Monson, 1978), but workers were also exposed to other carcinogens. Five additional studies, as detailed below, reported no evidence of increased risk, but all of these studies suffer to some degree from deficiencies in design and/or methodology.

Kiesselbach et al. (1979) examined the mortality rate, the cancer rate, and the type of cancer in relation to the period of exposure to acrylonitrile in 884 workers. The results revealed that the general mortality of the exposed group was markedly lower than that of the normal population (i.e. 58 cases as opposed to an expected 104), possibly related to the “healthy worker effect”. The mortality rate for malignant tumours, cardiovascular, brain, respiratory, and gastrointestinal diseases, suicide, and other causes of death however were not different from expected. No relationship was found between length of exposure and mortality from tumours.

Interpretation of the studies of Kiesselbach et al. (1979), Thiess et al. (1980), Werner and Carter (1981) and Delzell and Monson (1982) is limited however for reasons such as incomplete follow-up, relatively short-observation time for latency or a small-cohort size.

O’Berg et al. (1985) studied cancer morbidity and mortality in 1,345 workers (cohort relating to O’Berg et al, 1980), who started between 1950 and 1966 at a DuPont textile fibre plant, US. The follow-up period for the estimation of expected deaths started on 1st January 1956 and ended on 31st December 1981. It ended for the estimation of expected cancer incidence on 31st December 1983. Smoking habits were not considered. The cancer incidence findings were not very different from the cancer mortality findings. There were a total of 43 incident cases of cancer observed versus 36.7 expected according to company-wide rates. For lung cancer there were 10 cases observed versus 7.2 expected. There were fewer incident lung cancer cases than deaths because of the differing time periods for follow-up (the incidence data refer to the period 1956-1983, whereas the mortality data refer to 1950-1981). The respiratory cancer incidence was no longer significantly increased based on DuPont Company rate.

For prostate cancer 6 cases observed and 1.8 expected. All 6 cases of prostate cancer occurred among wage workers, for whom the expected number was 1.5. Prostate cancer incidence was significantly increased, but no significant relationship between incidence and cumulative exposure was found. There were 7 cases of lymphopoietic cancer versus 3.7 expected, and for the wage workers the respective numbers were 6 versus 2.9 expected. There was no significant increase in cancer mortality based on US rates.

The only analysis linking the amount of cumulative exposure or induction time to any effect was for lung cancer. This analysis showed 7 cases of lung cancer versus 3.5 expected among workers with more than 20 years since first exposure (the category of longest duration), 6 cases versus 2.8 expected for workers with the highest category of cumulative exposure. The highest ratio of observed to expected for lung cancer was for those in the combined category of long duration and greatest cumulative exposure, in which there were 4 cases versus 1.8 expected. The primary difference between this study and the earlier 0'Berg study (1980) is the finding on prostate cancer. The lung cancer excess, while modest overall, appears to be related to the amount of exposure (Rothman, 1994).

Chen et al. (1987) studied cancer morbidity and mortality in 1,083 workers who started work between 1944 and 1970 at a different DuPont textile fibre plant, US. The observation period for latency covered the period from 1944 until 1981 for mortality based on US and DuPont rates and from 1944 until 1983 for morbidity based on DuPont rates. The follow-up period for the estimation of expected deaths covered only the period from 1957 until 1981. The mean observation time for latency was 21.3 years. Smoking habits were not considered.

There were 92 deaths observed in the cohort during the follow-up period, substantially fewer than the 124.0 expected on the basis of DuPont mortality rates and the 177.2 expected on the basis of rates for all white males in the United States. The deficit in deaths was more striking for salaried employees than for wage employees. Among the wage workers, there were 18 cancer deaths versus 20.4 expected from the DuPont rates and 24.1 expected from the United States rates. There were 7 deaths from lung cancer among the wage workers, compared with 7.9 expected on the basis of the DuPont rates.

No significant excess in cancer mortality was observed, based upon both US and DuPont rates. Respiratory cancer incidence was not increased. There were 37 cases of cancer identified during the period 1956-1983. However there was no excess seen for lung cancer (5 cases observed versus 6.9 expected, p-value = 0.82), but there was an excess for prostate cancer incidence, in that 5 prostate cancer morbidity cases were observed compared to the expected 1.9 (p-value = 0.04), based on DuPont rates.

Attention must be given to the interpretation of the increased incidence of prostate cancer morbidity as reported by O'Berg et al. (1985) and by Chen et al. (1987). In the O'Berg study no relationship was found between cumulative exposure and an increased incidence of prostate cancer. Chen et al stated that 3 of the prostate cancer morbidities had a latency time of more than 20 years. Both O'Berg and Chen used the internal DuPont Cancer Register for estimating the expected prostate cancer morbidity. A major drawback with using this Register as a reference is that workers leaving DuPont for other jobs etc. are no longer followed with respect to morbidity for the internal DuPont Cancer Register purposes of record keeping. In addition, in the DuPont studies smoking habits were not considered. Prostate cancer has been associated with smoking habits, exposure to cadmium and exposure to perfluoro-octanoic acid. Also even considering the cases of prostate cancer, mortality from prostate cancer was not increased.

Collins et al. (1989) studied cancer mortality in 1,774 workers exposed to acrylonitrile, who worked between 1951 and 1973 at the Fortier plant (LA) and between 1957 and 1973 at the Santa Rosa plant (FL) of American Cyanamid Company. The observation period for mortality follow-up ended on 31st December 1983. The mean observation time for latency was about 20 years. Exposure assessment included industrial hygiene measurements in 1977, which the authors indicated was representative of exposures going back to the beginning of plant operations. Their publication did not provide analysis of observed and expected mortalities by cumulative exposure, stratified by latency years. The authors did provide data for respiratory cancer mortality and for all cancer mortality.

There was no significant difference between observed and expected cases of cancer or between the sub-groups, stratified by cumulative exposure to acrylonitrile and by latency time. In addition smoking habits were taken into account but were available for only 58% of the cohort. The total number of deaths among exposed workers was 145, giving an overall SMR of 0.67. The relatively large number of deaths made this the largest study of acrylonitrile exposure. Despite the sizeable deficit relative to the expected number for total deaths, the number of cancer deaths (N=43) was about equal to the expected number (SMR 1.01). The 15 lung cancer deaths observed were also about the same as the number expected according to the general population comparison. There were 2 deaths from prostate cancer compared with 1.49 expected. The relative risk estimates reported for the four exposure categories were 1.1 for exposure under 0.01 ppm per year, 0.7 for exposure in the range of 0.01-0.7 ppm per year, 0.7 for exposure of 0.7-7.0 ppm per year and 1.2 for exposure greater than 7.0 ppm per year. No internal comparison was presented outside of the analysis for lung cancer.

The study by Swaen et al. (1992) is considered to be the best quality with respect to the “older” studies, due mainly to the extensive exposure assessment data and the useful Dutch system in operation regarding the registration of cause of death. The study used a cohort of 2,842 workers from 8 chemical plants situated within the Netherlands. These workers were exposed to acrylonitrile for at least 6 months between 1st January 1956 and 1st July 1979. By necessity the exposure assessment was a hybrid methodology consisting of measurements from some companies and indirect assessments based on interviews with key personnel. Jobs were classified into exposure level ranges. Use of respiratory protection was documented for various jobs, but it was not taken into account when the exposure scores were assigned. Therefore actual exposure was probably lower than assessed exposure in many cases, particularly for workers with high levels of peak exposure, for which respiratory protection was mandatory. Both average and peak exposure levels were taken into account when the jobs were classified. Cumulative doses were calculated for each worker according to the job exposure assessments and the time spent in specific jobs. The mean observation time for latency was about 17 years, and the observed cancer mortality in the exposed cohort was similar to the expected mortality. Specific analyses were carried out so as to investigate dose-response relationships and latency for total mortality and lung cancer mortality.

Overall, in the exposed cohort, there were 134 deaths observed, with 172.2 expected according to national mortality rates. Approximately the same ratio of observed to expected deaths was found for the unexposed cohort as for the exposed cohort. There were 42 cancer deaths in the observed cohort, with an expected number of 50.8, giving an SMR of 0.83, not very different from that for all deaths. For lung cancer, there were 16 deaths in the exposed cohort compared with 19.5 expected, which gives an SMR for lung cancer of 0.82. When lung cancer risk was examined according to three categories of dose, there was a marginal excess (8 deaths versus 7.2 expected) in the highest category and deficits for the lower dose categories. The authors attributed the rising SMR by dose category to a waning of the healthy worker effect for longer-

term employees. This interpretation was supported by the trend in the ratio of deaths to expected deaths by dose category; for low dose (<1 ppm-years), the ratio was 0.67; for moderate dose (1-10 ppm-years) it was 0.78; and for high dose (≥10 ppm-years) it was 0.83. There were 2 prostate cancer deaths in the exposed cohort, compared with 0.5 expected. These 2 deaths occurred in the group with the highest exposure and the longest "latency".

Overall, no indications were found of a carcinogenic effect in this cohort of workers exposed to acrylonitrile. The findings from this study are mostly reassuring with respect to the lack of an effect of acrylonitrile exposure on lung cancer risk. However the excess for prostate cancer, small in absolute terms but larger in relative terms, corroborates the findings of several other reports ("older" studies).

A review of all mentioned epidemiology ("old") studies on acrylonitrile occupational exposure was performed by Rothman (1994). He selected 8 studies for his meta-analysis on the basis of the quality of the studies. A summary of observed and expected numbers of cancer and respiratory cancer deaths in the studies selected for this meta-analysis is presented in **Table 4.25**.

As **Table 4.25** shows, the weighted total for the observed number of deaths was close to the total for the expected number of deaths for all cancers (SMR 1.03) and for respiratory cancers (SMR 1.07). These findings are a quantitative indication that in the aggregate these studies do not show a strong relation between work in an environment in which there is the possibility of exposure to acrylonitrile and subsequent death from cancer, respiratory cancer in particular.

Table 4.25 Meta-analysis of cancer mortality in workers exposed to acrylonitrile (Rothman, 1994)

Source	Cohort size	All cancers		Respiratory cancers	
		Observed deaths	Expected deaths	Observed deaths	Expected deaths
Kiesselbach et al. (1979)	884	20	20.4	6	6.9
Thiess et al. (1980)	1,469	27	20.5	11	5.7
Werner and Carter (1981)	1,111	21	18.6	9	7.6
Delzell and Monson (1982)	327	22	17.9	9	5.9
O'Berg et al. (1985)	1,345	31	27 (wage) *	12	10. 2(wage) *
Chen et al. (1987)	1,083	18	20.4 (wage)*	7.0	7.9 (wage) *
Collins et al. (1989)	1,774	43	42.6	15	15.7
Swaen et al (1992)	2,842	42	50.8	16	19.5
Total	10,835	224	218.2	85	79.4
Summary SMR		1.03		1.07	
90% Confidence Interval		0.92-1.15		0.89-1.28	

* Wage = relates to job and earning status, whereby exposure is expected to be higher for "wage" rather than salary earners (who are most usually office based)

Ward and Starr (1993) explored the discordance between laboratory animal and human study findings. According to the US EPA results, lifetime exposure to 1 $\mu g/m^3$ acrylonitrile translates into an increased risk of all cancer mortality of 1 in 6,700 people and into an increased risk of brain cancer mortality of 1 in 12,000 people. Ward and Starr assumed that workers were exposed to an average level of 2 to 5 ppm acrylonitrile. They determined the statistical power of the

epidemiological studies on acrylonitrile-exposed workers with respect to the detection of increased incidence of all cancer mortality or of brain cancer mortality, as predicted from animal studies by the US EPA (1983). The power of the studies used was high enough to detect the US EPA predicted increases of cancer due to occupational exposure to acrylonitrile. However, these predicted increases were not found in the epidemiological studies. The authors concluded that in fact the upper bound estimate of the acrylonitrile inhalation cancer potency as estimated by the US EPA was too high to be consistent with the human acrylonitrile exposure experience with regard to occupational exposure scenarios.

"New Studies"

Blair et al. (1998) performed a mortality study of industrial workers exposed to acrylonitrile. The study consisted of a cohort of 25,460 workers (18,079 white men, 4,293 white women, 2,191 non-white men and 897 non-white women) employed at 8 acrylonitrile producing or processing plants in the US. These workers, employed from the 1950's up to 1983, were followed up until 1989 so as to determine their vital status and cause of death. This large-scale investigation included a high quality, and well-documented procedure to develop qualitative estimates of historical exposures, which therefore provided the opportunity to evaluate exposure-response relationships. Smoking habits were considered as a confounding factor. Mortality rates for exposed workers were compared with unexposed workers in the cohort using Poisson regression to minimise the "healthy worker effect" problem. Set out below in **Table 4.26** are the exposure characteristics of the plants selected for the Blair et al. study.

Table 4.26 Exposure characteristics of selected plants (Blair et al., 1998)

Plant No. and acrylonitrile process	Year of first use of acrylo-nitrile	Other exposures	No. of personal samples taken for monitoring of acrylonitrile	Estimates of exposure for exposed Jobs (TWA 8-hr ppm) Median (mean)
1. Fibres	1958	Methylmethacrylate sodium thiocyanate dimethylformamide	1,100	0.10 (1.88)
2. Monomer	1965	Ammonia, propylene, hydrogen cyanide	2,300	0.39 (2.17)
3. Monomer, Resins, Acrylamide	1960	Ammonia, propylene, hydrogen cyanide, methyl acrylate, butadiene	400	3.46 (6.13)
4. Fibres	1958	Vinyl bromide, methyl acrylate, zinc chloride	2,300	0.34 (5.30)
5. Fibres, Adiponitrile	1952	Vinyl acetate, bromide, hesamethylene-diamine	1,400	0.42 (3.37)
6. Monomer, Acrylamide	1954	Ammonia, hydrogen cyanide, acetylene, propylene, sulphuric acid	500	0.54 (0.63)
7 Resins	1959	Butadiene, styrene	1,900	0.36 (1.34)
8. Monomer	1953	Ammonia, hydrogen cyanide, acetylene, propylene	2,100	1.90 (1.45)

In this study a procedure was created to develop quantitative estimates of exposure by job/department/time period. Personal monitoring of acrylonitrile exposures was performed in all 8 plants in 1986 by the study investigators using the recommended NIOSH method (1984). 7 of

the plants conducted their own monitoring simultaneously with the study monitoring. Company and study monitoring results were compared to identify any systematic differences that might exist between data from the various companies, but no major differences were found. 7 of the 8 plants had measurements dating back to 1977-78 and one plant started monitoring in 1960. Over 18,000 measurements were available from the companies between 1960 to 1989 and over 12,000 of these were personal samples. The estimated time-weighted average for an eight-hour period (TWA-8), covering a minimum time period of one year, served as the primary index of historical exposure developed for this study. In addition to TWA-8 estimates, other exposure assessments included:

- the frequency of peaks (defined as 15-minute exposures that averaged 20 ppm or greater),
- TWA-8 estimates taking into account respirator use,
- a dermal score to account for skin contact,
- the total mass inhaled (based on a semi-quantitative estimated level of physical activity associated with the job, the respiratory rate expected to be associated with that level of activity, the average tidal volume, and the estimated air concentration).

The entire cohort generated 545,369 person-years of follow-up. Of the total person-years of follow-up, 348,642 were assigned to workers after their first exposure to acrylonitrile and 196,727 person-years were associated with individuals never exposed, or with the time period prior to first exposure among workers who started employment in unexposed jobs. Over 44,000 person-years occurred among workers after their cumulative exposure exceeded 8.0 ppm-years. Exposure to chemicals other than acrylonitrile also occurred. Worker years of exposure totalled approximately 55,000 for benzene, 8,000 for butadiene, 50,000 for formaldehyde, 45,000 for styrene, 10,000 for sulphuric acid and 54,000 for vinyl chloride.

Analyses by various indicators of exposure including cumulative (ppm-years), average, peak, intensity, duration and lagged exposure revealed no elevated risk of cancers of the stomach, brain, breast, prostate or lymphatic and haematopoietic system. Mortality for lung cancer was elevated among the highest quintile of cumulative exposure[8], compared to unexposed group of workers. Relative risks (RR) and 95% confidence intervals from the lowest to the highest quintile of cumulative exposure were 1.1 (0.7-1.7), 1.3 (0.8-2.1, 1.2 (0.7-1.9), 1.0 (0.6-1.6) and 1.5 (0.9-2.4), respectively. When limiting data to 20 more years since first exposure the RRs by quintile become 1.1 (0.6-2.2), 1.0 (0.5-2.1), 1.2 (0.6-2.2), 1.2 (0.6-2.1) and 2.1 (1.2-3.8). To evaluate RRs at a wider range of cumulative exposure, analyses were also conducted for decile categories[9].

The RR did not continue to increase at higher levels and actually decreased at the ninth (RR=1.7) to the tenth decile (RR=1.3). Adjustments made in relation to possible confounding from tobacco use served to reduce the RR for lung cancer in the upper quintile slightly (from 1.5 to 1.4). Separate analyses for wage and salaried workers, long-term and short-term workers, fibre and non-fibre plants, and by individual plants revealed no clear exposure response patterns and tests for trend were not statistically significant.

This study provided no evidence to indicate that exposure to acrylonitrile at the levels experienced by these workers could be associated with any significant increased relative risk for most cancers of interest namely stomach, brain, breast, prostate, or lymphatic and

[8] For the purposes of analysis the population was divided into 5 groups or quintiles on the basis of estimated levels of exposure.

[9] Population divided into 10 groups, or deciles, on the basis of estimated levels of exposure.

haematopoietic system. The excess of lung cancer seen in the highest quintile, particularly when exposure was more than 20 years could be taken as providing evidence for a carcinogenic effect at the highest exposure. However, the analyses of exposure-response do not provide strong or consistent evidence for a causal association between acrylonitrile exposure and lung cancer. No dose-response effect was identified and the risk of lung cancer did not increase with increasing exposure, within this highest category.

In the second of these "new" studies Benn and Osborne (1998) studied the mortality of UK acrylonitrile workers. This study is an extended and updated study of the original Werner and Carter (1981) study. The cohort consisted of 2,763 male workers, employed between 1950 and 1978, for at least one year, at 6 factories involved in the polymerisation of acrylonitrile and the spinning of acrylic fibres. The follow-up period lasted until December 1991. The original study (Werner and Carter, 1981) looked at 1,111 male workers, employed as stated above, and found significant excesses of stomach cancer overall, in particular in those workers aged 55 to 64 years, and excesses of lung cancer in those aged 15 to 44 years. As the results of this study were inconclusive it was decided to extend the study by enlarging the population so as to include more recently exposed workers (i.e. those employed on polymerisation or as spinners at some time between 1969 and 1978), and also to extend the follow-up period. National death rates from England and Wales derived from mortality and population data provided by the Office of Population Census and Surveys (OPCS) were used to calculate expected deaths at all of the factories except one factory in Scotland, where Scottish rates were used. The standardised mortality ratios (SMRs) were calculated in the normal manner and 95% confidence intervals (95% CI) for the SMRs calculated under the assumption that the observed number of deaths followed a Poisson distribution. Results are described as "significant" when the calculated SMR value is outside the 95% CI for the national population.

The results showed that overall there was a deficit in mortality for the combined analysis population, reflecting a significant deficit in circulatory disease deaths, and deficits in most other causes. All cancers combined showed a deficit, and for most individual cancer sites (including lung and stomach) the observed numbers were close to the expected numbers. Set out below in **Table 4.27** is the analysis of mortality for total population by cause.

Table 4.27 Mortality of UK workers involved in the polymerisation of acrylonitrile and production of pan fibres (Benn and Osborne, 1998)

Cause of death	Obs.	Exp.	SMR
All Causes	409	485.5	84.2
All malignant neoplasms	121	137.1	88.2
-stomach	11	11.4	96.2
-large intestine-not rectum	11	8.8	125.7
-rectum	6	6.0	100.2
-trachea, bronchus and lung	53	51.5	102.8
-genito-urinary organs	12	14.9	80.8
-lymphatic and haematopoietic	5	10.0	49.9
Endocrine, nutritional and metabolic diseases	5	5.8	86.4
Circulatory disease	200	232.2	86.1
-Ischaemic heart disease	151	167.6	90.1
-cerebrovascular disease	27	33.9	79.1
Respiratory disease	31	41.4	74.8
-bronchitis	13	12.2	107.0
Disease of the digestive system	8	13.8	58.0
Suicides and violence	11	12.9	85.5

One particular factory involved in this study (Factory 5) influenced greatly the overall deficit in mortality observed in the total study population. **Table 4.28** outlines the comparison of mortality at Factory 5 and other factories in the study.

Table 4.28 Comparison of mortality of UK workers in polymerisation of acrylonitrile and production of pan fibres – Comparison of factory 5 with other factories

Cause of death	Factory 5			Other factories			
	Obs.	Exp.	SMR	Obs.	Exp.	SMR	p-values
All Causes	246	319.1	77.1 **	163	166.4	97.9	0.019 *
All malignant neoplasms	70	91.7	76.3 *	51	45.4	112.2	0.039 *
- stomach	4	7.5	53.4	7	4.0	177.2	0.050 *
- trachea, bronchus & lung	30	33.8	88.8	23	17.8	129.4	0.178
Circulatory disease	120	151.7	79.1 **	80	80.5	99.3	0.118
Respiratory disease	18	25.9	69.5	13	15.6	83.4	0.617

* significant at 5% level,
** significant at 1% level
(Note: p-value refers to the test for homogeneity of the data)

With respect to mortality by job category, workers were counted in a category if they worked in it for one year or more; workers who changed jobs may thus appear in more than one category. In the two "high-exposure" categories (polymer or control room worker and spinner) cancer SMRs were raised but not significantly so. In the "end of line"' workers (a category used exclusively in factory 5) the "all cancers" SMR was significantly reduced. On grouping the workers according to the level of acrylonitrile exposure categories that they had worked in (i.e. high, other, and little or no exposure), mortality from each of the examined causes other than respiratory disease was higher in the high-exposure group than in the other two groups. However, only stomach cancer showed a clear and statistically significant trend across the three groups. For lung cancer the SMR was lowest in the middle group. **Table 4.29** illustrates the findings for mortality related to level of exposure.

Again, the overall results were determined by the individual results achieved in one particular factory (Factory 5), whereas for the other factories combined the all cause mortality appeared close to the expected. For all cancers combined, there was a significant deficit at Factory 5 (SMR 76.3, 95% CI 59.5-96.4), compared with a non-significant excess at the other factories combined (SMR 112.2, 95% CI 83.5-147.5). These factories indicated non-significant excesses in lung cancer and stomach cancer, though it is difficult to interpret the figures at individual factories due to the smallness of the numbers involved.

Table 4.29 Mortality related to level of exposure (Benn and Osborne, 1998)

Cause of death	Obs.	Exp.	SMR
High acrylonitrile exposure			
All causes	170	181.2	93.8
All malignant neoplasms	58	50.1	115.8
- stomach	7	4.2	166.3
- trachea, bronchus and lung	27	19.1	141.1
Circulatory disease	81	86.9	93.2
Respiratory disease	11	15.7	70.2
Possible acrylonitrile exposure			
All causes	97	124.7	77.8 *
All malignant neoplasms	22	35.9	61.2 *
- stomach	3	2.9	102.7
- trachea, bronchus and lung	7	13.3	52.6
Circulatory disease	49	59.1	83.0
Respiratory disease	7	10.0	69.9
No/little acrylonitrile exposure			
All causes	142	179.6	79.1 **
All malignant neoplasms	41	51.12	80.2
- stomach	1	4.31	23.2
- trachea, bronchus and lung	19	19.1	99.5
Circulatory disease	70	86.25	81.2
Respiratory disease	13	15.76	82.5

* significant at 5% level,

** significant at 1% level

Regarding analysis by age, lung cancer mortality showed an increased SMR in the 15 to 44 and 45 to 54 age groups (SMR 284.4, 95% CI 104.4 - 618.9; SMR 148.7, 95% CI 83.2 - 245.2) and a deficit for the older age groups. However further analysis of these lung cancer excesses showed that the excess in the age group 45-54 was in Factory 5, while the excess in the 15-44 age group was in the other factories. While analysis by length of employment showed a tendency for increased all cause and circulatory disease mortality with time, the trend was less clear and not statistically significant. No clear trends were observed for cancer.

Finally, while there was no excess of cancer deaths above the expected, there were indications of excess cancer in those workers employed at jobs where there was highest acrylonitrile exposure, and in particular there was an excess of lung cancer in workers aged under 55 years. When considering the results of this experiment it should be noted that the study was hampered by certain limitations such as lack of exposure measurements and lack of information on smoking habits.

With regard to this study, no measured exposures were available for the earlier years covered. Werner and Carter (1981) attempted to estimate historical levels, but they judged that the only available methods of estimation were so subjective as to be of little value. Up to 1979 acrylonitrile exposure was subject to a threshold limit value of 20 ppm (8-hour time weighted average). In that year the Health and Safety Commission, in view of concern about possible carcinogenicity, issued a statement that in accordance with general policy on toxic substances, exposure should be reduced to a level as low as reasonably practicable. A stated objective was to work within a control limit of 2 ppm (8-hour TWA) by about 1981. The statement provided for a staged reduction of limits in the interim period, with a limit of 5 ppm immediately and 4 ppm from the first quarter of 1980. While it seems reasonable to assume a general downward trend in exposures, the lack of precise information and the fact that work histories were not updated after workers were recruited into the study meant that length of employment up to 1978 or 1980 was used as a proxy measure for exposure. Considering the shortcomings of this study, the overall findings indicate that United Kingdom acrylonitrile workers did not appear to have increased mortality risk.

The third “new” study (Swaen et al., 1998) is a further investigation and update of the original study performed by Swaen et al. (1992), whereby the specific mortality patterns among 2,842 workers exposed to acrylonitrile at the workplace were investigated. The total cohort (8 chemical companies participated) consisted of 6,803 workers of whom 2,842 had been exposed to acrylonitrile for at least 6 months prior to 1st July 1979. The “control” group, for comparison purposes, consisted of 3,961 workers at a nitrogen fixation plant, employed over the same time interval as the study cohort. Extensive industrial hygiene assessments were conducted to quantify past exposure to acrylonitrile, including the use of PPE (Personal Protective Equipment) and exposure to other potential carcinogenic agents. All 6,803 workers were followed-up for mortality until 1st January 1996. Causes of death could be obtained from the existing Dutch Central Bureau of Statistics.

Exposure assessment was carried out by an industrial hygienist who contacted the company industrial hygienist, thus ensuring a uniform approach for all companies. The first step was to make an inventory of the available measurements in each plant. These formed the basis for the exposure assessment, together with temporal information on changes in the production process, task rotation, industrial hygiene and total production. Information on the work environment and control measures was obtained through interviews with plant personnel. A job-exposure matrix was constructed for this study. In this matrix, the job history was described in detail, giving information on the job held in a specific period and in a specific workplace. Within each

department job classes were constructed which included all the job titles believed to have had a similar exposure profile based on the exposure assessments. The 8-hour time weighted average (TWA) exposure assessment results of all workers in an exposure class were grouped to determine the average exposure level of that job in that workplace for each calendar year. Based on this outcome it was decided in which exposure range each exposure job class was placed for that year. Ranges used were 0 to 0.5 ppm, 0.5 to 1 ppm, 1 to 2 ppm and 2 to 5 ppm. There were no exposures thought to be greater than 5 ppm TWA. For one company it was possible to carry out the exposure assessment on an individual worker level rather than job title, since exposure estimates had been recorded in the medical files of each worker.

The exposure assessment had some limitations. For instance, respirator use and the potential for skin exposure, which were not taken into account, may have resulted in a different exposure than assessed. Various other exposure characteristics were studied, such as exposure to peak concentrations, exposure to established carcinogens and respirator use. Peak exposures were defined as intervals with elevated exposure ranges 0-10, 10-20, 20-30 ppm occurring on a regular basis, at least once a week. An assessment of the occurrence of peak exposures could be made for all but one of the participating companies. In addition, an inventory was made of exposure to other agents considered to be potential human carcinogens by the International Agency for Research on Cancer.

Table 4.30 outlines information relating to the specific companies involved in this study. The follow-up was 99.6% complete and 99.3% of the deaths by cause could be ascertained (via the Central Bureau of Statistics, CBS). Adjustments for differences in age distribution, follow-up period and temporal changes in background mortality rates were made using Standard Mortality Ratios (SMR) for a range of separate causes of death. Cumulative dose-effect relationships were investigated after classifying the exposed workers into 3 exposure categories and 3 latency periods. The results show that although there are some small fluctuations in cancer mortality, there does not appear to be any cancer excess related to occupational exposure to acrylonitrile.

Table 4.30 Acrylonitrile use in the 8 companies in the Swaen et al. (1998) study and number of exposed workers employed

Plant	Type of ACN use	No. of workers	Start of exposure	Highest stel	Average exposure range
1	ACN and ABS production	594	1969	20	0.5
2	Acrylate plant	382	1959	20	1-2
3	Catalyst experimental plant*	30	1973	20	0.5-1
4	Acrylate plant	38	1973	10	0-1
5	ABS producers	715	1967	20	0.5
6	Fibre plant	645	1962	30	1-5
7	ABS plant	266	1966	30	0-1
8	Resin plant	210	1967	20	0-2

* During the determination of the exposure assessment, it was found that exposure to acrylonitrile in this plant only occurred 5% of the time worked. Therefore the workers from this plant were excluded from further analyses.

Set out below in **Table 4.31** and **Table 4.32**, are the values obtained relating to total mortality and lung cancer mortality, in workers exposed to acrylonitrile, grouped into the three exposure cumulative categories and latency periods, for the Swaen et al. (1998) study. Of the 6,803 study

subjects, 6,774 could be completely followed, being either until the end date of the follow-up, until the emigration date, or until death resulting in a completeness of follow-up of 99.6%. In the total study population 1,273 deaths were observed. Compared to the 706 deaths observed in the earlier study this is approximately a doubling of the observed number of deaths. The number of deaths in the exposed group increased from 134 to 290. In either group the observed mortality is still lower than the expected, an indication of the "healthy worker effect". For 9 (0.7%) deceased study subjects it was not possible to trace the actual cause of death, either because the person had died abroad or because it was not possible to link the record with the CBS cause of death file.

Table 4.31 Total mortality in workers exposed to acrylonitrile grouped into 3 exposure cumulative categories and latency periods

Exposure	**Total mortality**		
	Obs.	**SMR**	**95% CI**
Low (<1 ppm/year)			
<10 years' latency	7	43.1	17.3-88.8
10 to 20 years' latency	20	108.3	66.1-167.3
>=20 years' latency	12	128.2	66.2-224.0
Total	39	88.5	62.9-121.0
Moderate (1 - 10 ppm/year)			
<10 years' latency	35	71.7	50.0-99.8
10 to 20 years' latency	71	91.5	71.4-115.4
>=20 years' latency	42	87.2	62.8-117.9
Total	148	84.8	71.7-111.5
High (10 + ppm/year)			
<10 years' latency	35	119.8	83.5-166.7
10 to 20 years' latency	47	94.6	69.5-125.8
>=20 years' latency	21	76.8	47.5-117.4
Total	103	97.0	79.1-117.6

SMR Standard Mortality Ratio
CI Confidence Interval
Latency was defined as time since the particular exposure group was entered (Doll Ed., 1998)
(Swaen et al., 1998)

Table 4.32 Lung cancer mortality in workers exposed to acrylonitrile by 3 exposure cumulative categories and latency periods

Exposure	Lung Cancer Mortality		
	Obs.	SMR	95% CI
Low (<1 ppm/year)			
<10 years' latency	0	0.0	0.0-283.8
10 to 20 years' latency	3	120.6	24.2-352.5
>=20 years' latency	2	146.6	16.5-529.4
Total	5	97.3	31.4-227.1
Moderate (1 - 10 ppm/year)			
<10 years' latency	1	21.4	0.3-118.8
10 to 20 years' latency	16	148.2	84.6-240.6
>=20 years' latency	7	99.7	39.9-205.4
Total	24	106.6	68.3-158.7
High (10 + ppm/year)			
<10 years' latency	4	89.5	24.1-229.1
10 to 20 years' latency	11	150.2	74.9-268.8
>=20 years' latency	3	87.0	17.5-254.2
Total	18	118.1	69.9-186.6

SMR Standard Mortality ratio
CI Confidence interval
Latency was defined as time since the particular exposure group was entered (Doll Ed., 1998)
(Swaen et al., 1998)

The fourth and final "new" study presented at the 1997 Epidemiology Conference in Oxford, was the DuPont Study, performed by Wood et al. (1998), which updated the O'Berg et al. (1980; 1985) study and the Chen et al. (1987) study. This study assessed the risk of cancer mortality and incidence in a cohort of 2,559 male employees who were exposed to acrylonitrile during the production of Orlon, at two plants, during the period 1944 to 1991. Vital status follow-up was 99% complete. The production processes at these two facilities are identical and so a single protocol to estimate exposure for each work area/job was completed and the populations could be combined for health analysis purposes. Standard Mortality Ratios (SMRs) have been used to assess cancer mortality using the US population and the registry for all DuPont employees as a basis for comparison. Standard Incidence Ratios (SIRs) have been used to evaluate cancer incidence using the DuPont employee registry for comparative purposes. Indicators of exposure used in the analysis of mortality and morbidity of specific cancer sites of interest are as follows:

- latency (less than 20 years and 20 years),
- duration of exposure,
- highest level of exposure ever experienced,
- cumulative exposure (ppm years).

The average duration of exposure for workers in the cohort was 7.6 years with an average cumulative exposure (ppm-years) of 57.6 ppm-years.

A single exposure assessment procedure was developed with the objective of standardising exposure classifications across work areas and job titles in the 2 plants. For both plants, acrylonitrile monomer was received by tank car and stored in the recovery tank farm. It was then transferred to the polymer area where it was polymerised and the final product was made and packaged. The following data were used for the exposure assessment:

- a general history of the plant,
- process descriptions of where acrylonitrile was used and engineering and/or operating changes in the process which would impact on potential sources of exposure,
- a matrix of work area names and job titles held in the relevant production areas during the years of acrylonitrile use,
- documentation of personal protective equipment used,
- air sampling data, both area and personal,
- plant production records,
- details of work conditions and practices as described by long-term employees (including retirees) in panel meetings held to assist with exposure assessment.

After relevant job titles at each plant were standardised, a panel of long-term employees reviewed all job title/work area designations for their respective plants. The appropriateness of the assumptions made by the industrial hygienists were assessed and workplace conditions were further elaborated, such as whether or not the odour from acrylonitrile was detectable during normal operations (approx. 20 ppm) or workers experienced symptoms of exposure such as headaches and nausea (greater than 20 ppm). Area and personal air monitoring data were routinely collected beginning in 1975.

These data, with consideration of requirements for use of personal protective equipment, were the principal factors used to estimate the ppm levels of job assignments at the 2 plants after 1975. Changes in processes, engineering, and ventilation were confirmed to be reflected in the monitoring data. In order to estimate the exposure levels prior to 1975, information related to the above named changes as well as the panel of knowledgeable employees (and prior employees) who could detail working conditions, were the primary sources of information. An estimate of exposure was made in ppm acrylonitrile for a 40-hour work week for each potentially exposed job title/work area combination by time period. The exposure estimates were ranked into 4 groups low, moderate, high and very high based upon the distribution of all jobs at both plants. The arithmetic mean midpoints for the 4 groups are 0.11 ppm, 1.10 ppm, 11.0 ppm and 30.0 ppm, respectively (See **Table 4.33**).

Table 4.33 Estimated ranges for each acrylonitrile exposure level category (Wood et al., 1998)

Estimated Exposure Range (ppm)			
Exposure group	**Equal to or**	**less than**	**Mean**
Low	0.0001	0.20	0.11
Moderate	0.20	2.00	1.10
High	2.00	20.0	11.0
Very High	20.0	<100.0	30.0

The mean cumulative exposure in ppm-years was 61.4 at plant 1 and 52.1 at plant 2, while the mean cumulative exposure was 57.6 ppm-years for the total cohort. More than 50% of plant 1 cohort were exposed prior to 1956, while 23.2% of plant 2 cohort were exposed prior to 1956 i.e. before similar processing to plant 1 commenced at plant 2 in 1957 (See **Table 4.34**).

Table 4.34 Exposed workers by year of first exposure and plant

Year of first exposure	Plant 1		Plant 2		Both Plants	
	N	%	N	%	N	%
1947-1950	84	5.9	167	14.6	251	9.8
1951-1955	658	52.0	98	23.2	756	39.2
1956-1960	191	65.4	217	42.2	408	55.1
1961-1965	134	74.8	189	58.7	323	67.7
1966-1970	192	88.3	181	74.5	373	82.2
1971-1975	37	90.9	74	81.0	111	86.5
1976 and later	130	100.0	217	100.0	347	100.0
Total	1,426		1,143		2,559 *	

* Ten study subjects worked at both plants
(Wood et al., 1998)

Overall vital status was unknown for only 17 (1.2%) of the plant 1 cohort and 6 (0.5%) for plant 2 cohort. Approximately 18% of the combined cohort was deceased at the follow-up, giving a total of 454 deaths. Death certificates were obtained for all but 2 of the 454 identified decedents. The cohort for both mortality and morbidity analyses is restricted to males who were potentially exposed to acrylonitrile for at least 6 months (females are not included in the analysis because only 25 exposed female employees were identified). The mortality ratios for sites of interest for the combined cohort are compared using both the DuPont and US population referents (See **Table 4.35**).

Table 4.35 Standardised mortality ratios fot the acrylonitrile exposed workers with US and DuPont mortality rates as reference

Cause of Death (ICD 9th Rev.) *	Obs	US		DuPont **	
		SMR	95%CI	SMR	95%CI
All causes of death	454	69	62,75	91	84,99
All malignant neoplasms (140-209)	126	78	65,93	86	71,102
Buccal cavity & pharynx (140-149)	2	43	5,155	67	8,241
Digestive system (150-159)	27	69	45,100	72	48,105
Respiratory system (160-163)	47	74	55,99	89	65,181
Prostate (185)	11	129	64,230	106	53,189
Urinary system (188-189)	7	91	36,187	90	36,185
Lymphatic/haematopoietic (200-209)	9	57	26,109	55	25,105

* Code of the International Classification of Diseases (ICD), 9th Revision
** DuPont data includes brain & CNS cancer with other and unspecified sites
Obs = Observed no. of deaths; SMR = Standardised Mortality Ratio; 95%CI = 95% Confidence Interval for SMR
(Wood et al., 1998)

Overall mortality proved to be lower than expected compared to both the US population and the DuPont employee population (observed = 454, SMRs 69 and 91, respectively). All cancer death ratios with the exception of prostate cancer were lower than the US and DuPont population referents. The SMRs for specific sites including prostate were not significantly different from expected. Analyses of all cancers, and prostate, respiratory and digestive cancer mortality by indices of exposure did not show any significantly associated increases or a consistent pattern suggestive of a dose-response relationship. Similarly regarding cancer morbidity no significant patterns were identified.

Meta-analysis of studies of acrylonitrile workers (Collins and Acquavella, 1998)

In this meta-analysis, 25 epidemiology studies of workers exposed to acrylonitrile were analysed using meta-analysis techniques to assess the findings for 10 cancer sites. The predominant focus in the available studies was on worker mortality rates. All but 4 of the studies assessed were industrial cohorts studies, with the remaining 4 being two nested industrial case control studies and two general population case control studies (which were restricted to bladder cancer and astrocytic brain cancer) and were not specific for acrylonitrile exposure. The two nested case control studies were restricted to prostate cancer and lymphatic and haematopoietic cancers with acrylonitrile exposure. **Table 4.36** provides a summary description of most of these studies.

Table 4.36 Summary description of studies included in meta-analysis (Collins and Aquavella, 1998)

Author	Company location	Acrylonitrile use	Study design	Study period	No. of workers
Keissel-bach et al. (1977)	Bayer Germany	monomer production and resin	cohort mortality	1950-77	884
O'Berg (1980)	DuPont US	fibres	cohort mortality and incidence	1950-76 mortality and incidence	1,345
Thiess et al. (1980)	BASF Germany	resins	cohort mortality	1955-78	1,469
Ott (1980)	Dow US	styrene copolymers	cohort mortality	1950-75	100
Zack (unpublished, 1980)	Monsanto US	monomer production and fibres	cohort mortality	1952-77	352
Werner and Carter (1981)	8 plants UK	fibres and resins	cohort mortality	1950-78	1,111
Herman (unpublished, 1981)	Uniroyal US	nitrile rubbers and resins	cohort mortality	1951-77	Not reported
Gaffey and Strauss (unpublished, 1981)	Monsanto US	fibres	cohort mortality	1952-77	1,077
Delzell and Monson (1982)	Goodrich US	nitrile rubbers	cohort mortality	1940-78	327
Marsh (1983)	Monsanto US	styrene copolymers	nested case control	1949-76	13 cases, 52 control
O'Berg et al. (1980, updated 1985)	DuPont US	fibres	cohort mortality and incidence	1950-81 mortality, 1980 incidence	1,345
Burke (unpublished, 1985a)	DuPont US	monomer production	cohort mortality and incidence	1957-80 mortality, 1956-83 incidence	700
Burke (unpublished, 1985b)	DuPont US	monomer production	cohort mortality and incidence	1962-82 mortality 1962-83 incidence	472
Chen et al. (1987)	DuPont, US	fibres	cohort mortality and incidence	1957-81 mortality 1956-83 incidence	1,083
Zhou (1991)	Fushun Chemical China	fibres	cohort mortality	1971-88	1,811
Swaen et al. (1992)	8 plants NL	fibres and others	cohort mortality	1956-88	2,842
Mastrangelo (1993)	Enichem-fibre IT	fibres	cohort mortality	1959-90	671
Siemiatycki et al. (1994)	Population of Montreal Canada	tailors using acrylic fibres	case-control	1979-86	484 cases, 1,879 controls
Wood et al. (1998)	DuPont US	fibres	cohort mortality and incidence	1947-91mortality, 1956-91 incidence	2,559
Benn and Osborne (1998)	8 plants UK	fibres and resins	cohort mortality	1950-91	2,763
Swaen et al. (1992, updated 1998)	8 plants NL	fibres and others	cohort mortality	1956-96	2,842
Blair et al. (1998)	8 plants US	fibres and others	cohort mortality, with case cohort	1950-89	25,460

This meta-analysis focused on the evaluation of heterogeneity as an indicator of factors that need to be considered in making a proper causal inference about acrylonitrile and cancer. The absence of heterogeneity indicates consistency of results and is therefore important for the

generalisability of the meta Relative Risk (mRR). Heterogeneity was evaluated via graphical and statistical methods. The relative risk was calculated as an inverse variance weighted average of relative risks from the individual studies.

The predominant industries represented in the cohort studies were monomer production, fibres and resin manufacture. Of the 14 study cohorts, 8 were done in the US, 2 in Germany, and 1 each in the UK, the Netherlands, Italy and China. The average duration of follow-up for the study groups was 30.2 years for cohort mortality studies and 28.6 years for cohort incidence studies. The percentage lost in follow-up ranged from 0 to 12 percent in the cohort mortality studies with a mean of 4%. Loss of follow-up was not reported in the incidence studies. The percentage of death certificates not obtained in these studies ranged from 0 to 6% with a mean of 3%.

Based on the results from the 14 unique study groups, all cause mortality was about 20% less than general population rates and the results were heterogeneous ($p < 0.00001$). All specific causes of mortality were at or below expected levels with the single exception of bladder cancer (mRR = 1.4, 95% CI 0.9-2.0). All specific causes of death were homogeneous across studies with the single exception of colon cancer ($p = 0.0062$).

The cancer incidence studies gave similar results to the mortality studies. Most cancer incidence rates were at expected levels with the possible exception of prostate cancer (mRR = 1.4, 95% CI 0.8-2.6). The incidence rates from the three studies for all causes were homogeneous.

As an inherent part of this meta-analysis various parameters were examined, such as study design, country of study, acrylic fibre plants versus others, publication bias and other exposures present at the plants involved. Only publication bias, country of study, and other plant exposures showed substantive findings. The analysis presented specific results for total mortality, lung cancer, prostate cancer, and brain cancer, since these cancer sites were of particular interest given the results of human or animal studies. Bladder cancer was given special attention based on the findings recorded above.

There was considerable heterogeneity in the data, which was in part due to one obvious outlier study, that of Zhou and Wang (1991). This study showed a very high mortality rate. However, it is possible that local mortality variation or absence of the healthy worker effect was responsible for this result, or, more likely, there was a problem with vital status follow-up or non-comparability of the comparison group. There was no description of the method of follow-up in this study, and the authors stated that the death information might not be comparable to the national population. Even when this study is omitted from the analysis, there was still considerable heterogeneity in the results ($p = 0.00004$).

For lung cancer mortality, cumulative relative risk by date of the study before 1992 among acrylonitrile workers was slightly greater than 1.0. This could be a chance finding or reflect an early preference for publication of positive findings. However after 1992 the cumulative lung cancer rates are at expected levels. The early studies were smaller than the four recent (“new”) studies as evidenced by the wide confidence intervals. The 1998 studies of Blair et al., Wood et al., Swaen et al., and Benn and Osborne all have narrow confidence intervals and the SMRs are close to 1.0. The mRR for all studies is 0.9 (95% CI 0.9-1.1).

Several of the acrylonitrile studies used in this meta-analysis examined workers by level of exposure. It is important to separate workers with low and brief exposures from more highly exposed workers. However only 7 studies (Thiess et al., Delzell and Monson, Mastrangelo et al., Blair et al., Wood et al., Swaen et al., and Benn and Osborne) examined cancer risk by level of exposure and most of these evaluations are for lung cancer. These 7 studies, which present data

for highly exposed workers, have higher rates for lung cancer (mRR = 1.0, 95% CI 0.9-1.1) than those studies which did not specifically examine workers with higher exposure (mRR = 0.7, 95% CI 0.4-1.4). The highest exposed workers in the 7 studies produced an mRR of 1.2 (95% CI 1.0-1.5). None of the studies found a trend with exposure level. Three of the studies (Blair et al., Wood et al., and Swaen et al.) made semi-quantitative estimates of likely exposure which allowed for the examination of workers with comparable high exposures. These 3 studies which estimated the likely exposure had an mRR for lung cancer of 0.9 (95% CI 0.8-1.0) compared to an mRR of 1.1 (95% CI 0.9-1.4) for the studies which did not include an estimation of likely levels of exposure. On combining the +8 ppm-years category in the Blair et al. study, the 10-50, 50-100, and 100+ ppm-years categories in the Wood et al. study, and the 10+ ppm-years category in the Swaen et al. study, the mRR for these studies was 1.1 (95% CI 0.9-1.4).

Latency is the term often applied to the period between initial exposure and death from a disease. Most occupational carcinogens do not show increased risk for 15 or 20 years after first exposure. Eight studies consider latency periods of 15 years or longer. Studies which considered latency had an mRR of 1.0 (95% CI 0.9-1.1) compared to an mRR of 0.9 (0.7-1.1) for those studies which did not. Only the studies of Blair et al. (RR=1.3, 95% CI 1.0-1.63) and Delzell and Monson (SMR=1.7, 95%CI 0.7-3.5) give any indication of elevated rates in the longer latency category. The 6 other studies report SMRs equal to or less than 1.0 for this category. The mRR for this category is near one (1.2, 95% CI 1.0-1.4).

As with the previous meta-analysis, performed by Rothman (1994), no excess of all cancer or lung cancer among acrylonitrile workers was identified. The 1998 Blair et al. study has almost 5 times more person years of exposure than does the Wood et al. study, but the latter study has considerably more expected deaths from lung cancer than the Blair study in the highest exposure category (46.5 in Wood study versus 17.3 in the Blair study). It is possible that this difference in the highest exposure category was caused by different methods in the exposure assessment. Also the larger number of expected deaths in the Wood study in the higher exposure categories relative to the Blair study may have resulted from older workers with longer durations of exposure to higher levels of acrylonitrile. Therefore the Wood study may provide more information about higher cumulative exposure to acrylonitrile than the Blair study.

There was some indication of excess bladder cancer in three studies (Kiesselbach et al., 1972; Thiess et al., 1980; Delzell and Monson, 1982), a finding not reported previously. However, the excess seems to be restricted to plants with potential exposure to aromatic amines, and therefore is unlikely to be related to acrylonitrile exposure. The excess prostate cancer incidence reported by O'Berg et al. (1985), Chen et al. (1987) and confirmed by Wood et al. (1998) has raised concern that exposure to acrylonitrile may increase prostate cancer incidence risk. However, there was no increase in cancer rates with increasing exposure and this finding has not been seen in the mortality studies or in other incidence studies. Also, the excess of prostate cancer in the Wood et al. (1998) study was limited to a narrow reporting period (1978-1983), when improved diagnostic procedures were introduced. A deficit was observed (SIR-0.3, 95% CI 0.0-1.4) from 1983-1991. Accordingly, the evidence does not support an association between prostate cancer and acrylonitrile exposure.

There is little evidence that acrylonitrile workers have increased cancer rates even though exposures in some groups of workers were at levels which cause tumours in rats. All cancer sites examined in these workers show null or near null findings when studies are considered together. For lung cancer Collins and Acquavella, (1998) were able to evaluate consistency across studies, strength of the association, and some aspects of internal consistency within studies such as dose-response and latency. The excess risk of lung cancer from acrylonitrile exposure, if any, is small.

For less common cancers such as brain and prostate cancer the authors were only able to evaluate consistency across studies. They found a relatively imprecise estimate of risk for prostate and brain cancers in acrylonitrile workers, where acrylonitrile cannot be completely ruled out as the cause. In the authors' opinion, based on the available studies, a causal relationship between acrylonitrile exposure and cancer is not supported.

The studies did not have the power to detect a 50% increase in all cases of cancer and lung cancer mortality. The Relative Risk in **Table 4.37**, represents the upper bound of cancer risk that could be detected in the meta-analysis.

Table 4.37 Relative risk to be detected from the expected number of cases in the Collins et al. meta-analysis study (1988)

Cancer mortality	Mortalities observed	Expected	Relative Risk to be detected at a significance of 5% with 80% power
All cancer	783	922.8	1.083
Stomach	37	48.2	1.38
Colon	55	65.4	1.33
Liver	9	13.8	1.75
Lung	314	336.4	1.14
Prostate	33	32.9	1.47
Bladder	14	8.8	1.95
Brain	58	59.4	1.34
Lymphatic and Haematopoietic	52	68.6	1.32
Hodgkin's Disease	7	9.7	1.9
Leukaemia	23	32.6	1.46
Non-Hodgkin's Lymph.	22	26.3	1.53

For the purpose of this risk assessment report Collins reanalysed the data from his major meta-analysis above, excluding the studies of Kiesselbach et al. (1979), and Siemiatycki et al. (1994), as these were considered to be possible outliers. On completing this reanalysis, the only major difference noticed from the original findings of the meta-analyses was that the bladder cancer meta-relative risk (mRR) was reduced overall from 1.4 (95% CI 0.9-2.0) from the 10 studies reporting bladder cancer relative risk in the original report to 1.1 (95% CI 0.7-1.7) in the present analysis. Excluding the two studies also reduced the heterogeneity as evidenced by the change in P-values from 0.18 in the original analysis to 0.45 in this present analysis. This finding indicates that the studies of Kiesselbach et al. and Siemiatycki et al. had a significant influence on the overall mRR for bladder cancer in the original analysis and so indeed may be outliers.

In conclusion there is little evidence that acrylonitrile workers have increased cancer rates even though exposures in some groups of workers were at levels which have caused tumours in rats. The available and extensive epidemiological information available (including the four "new" studies) do not support a causal relationship between acrylonitrile exposure and cancer.

Specific sites of concern identified from the studies considered

Astrocytic Brain Tumours

The only data available are based on a case-referent study (Thomas et al., 1987), conducted on 300 brain tumour cases and 386 referents who had died from causes other than brain tumour, epilepsy, cerebrovascular disease, suicide or homicide. Case-referent studies offer an underused possibility of studying rare end points, such as occurrence of astrocytic brain tumours. No statistically significantly elevated odds ratios (OR = 0.9, 95% CI 0.5-1.6) were associated with employment in the chemical industry. However, despite the large number of cases, the study had a low power to detect an association of acrylonitrile with astrocytic tumours. In addition, exposure information was based on next-of-kin data and consequently highly unreliable. The overall indication is that no excess of astrocytic brain tumours occurred among these workers.

The studies of Keisselbach et al. (1979), Herman (unpublished, 1981), Burke (unpublished, 1985 (Memphis plant), Mastrangelo et al. (1993), Swaen et al. (1998) and Wood et al. (1998) report RRs for brain and central nervous system cancers in excess of 1.0. Thiess et al. (1980) Delzell and Monson (1982), Burke (unpublished, 1985 (Beaumont plant)), and Blair et al. (1998) report RRs less than 1.0. The mRR for brain cancer is 1.2 (95% CI 0.8-1.7). Only 2 studies report brain cancer rates by exposure level (Blair et al., 1998; Swaen et al., 1998). There is an increase in risk with level of exposure in either study. Although the estimates were imprecise, the RRs were higher in the unpublished studies (mRR = 1.1, 95% CI 0.6-9.3) than in the published studies (mRR = 1.1, 95% CI 0.7-1.5). There also was a tendency to not report SMR less than 1.0. Studies reporting expected deaths had an mRR of 2.2 (95% CI 0.7-6.4) compared to an mRR of 1.1 (95% CI 0.7-1.6) for studies not reporting expected deaths (Meta-analysis study, Collins and Acquavella, 1998). These authors concluded that based on the available studies (including the most recent studies) there is little support for a causal relationship between acrylonitrile exposure and brain cancers. Overall it should be noted that overall there was no excess of brain cancers in the cohort studies.

Lung Cancer

While some studies have reported small excesses of lung cancer mortality (Thiess et al., 1980; Werner and Carter 1981; Delzell and Monson, 1982; O'Berg et al., 1985) other studies have reported no excess (Collins et al., 1989) or small deficits (Kiesselbach et al., 1979; Swaen et al., 1998; Mastrangelo et al., 1993).

In general workers with less than 6 months-1 year exposure to acrylonitrile showed no excess of lung cancer. However in one of the studies (O'Berg et al., 1980), from 6 months onwards the risk of cancer at all sites (lung, colon, prostate, bladder, etc.) appeared to increase with increasing duration of exposure. Wage workers (maintenance mechanics), were generally more at risk by virtue of their greater potential for exposure than salary workers. O'Berg subsequently extended the original database (1985) and reported findings of increasing lung cancer with increasing cumulative exposure. However this trend of increasing lung cancer was not observed in the corresponding lung cancer mortality data. Some occupational cancer can develop 20 or more years after the initial exposure. Observed lung cancer mortality and incidence exceeded expected cases twenty years after first exposure in the O'Berg study. However 2 lung cancers cases were inadvertently excluded from the O'Berg study which may raise concern regarding completeness of cohort ascertainment. While Werner and Carter (1981) identified an excess in lung cancer in those aged 15-44 years, unlike O'Berg (who identified most of the lung cancers as smokers), smoking habits were not considered.

The Thiess et al. (1980) study reports lung cancer rates by duration of exposure. The standardised mortality ratios (SMR) are elevated for each duration of exposure category but showed no trend. The Delzell and Monson study (1982), which examined duration of employment and years since first employed showed no trend with duration of exposure but achieved an increased SMR after 15 years since starting employment. Collins et al. (1989) examined cumulative acrylonitrile exposure and found a modest U-shaped curve in the effect estimates for lung, but this did not achieve an overall linear trend.

Swaen et al. (1992) reported only a small (non-significant) increase in lung cancer mortality with increasing dose and latency for the highest exposure group i.e. 10+ ppm. However this observation may well reflect the fading out of the HWE (healthy worker effect) rather than an actual increased risk from lung cancer following exposure to acrylonitrile. Overall Swaen et al. could not identify a trend in lung cancer mortality data by cumulative exposure and no increased risk with latency. Mastrangelo et al. (1993) also found no trend with duration of exposure or increased risk.

Considering the evidence presented in the “new” studies and the known limitation of the “old” studies, the overall conclusion drawn is that there is no consistent trend in the data. It remains possible, nevertheless, that there is an small increased risk of lung cancer in workers exposed to acrylonitrile, but it is likely that it applies only at high levels of exposure and requires a lengthy period of exposure to manifest itself.

In the Blair et al. (1998) study there was no evidence to indicate that exposure to acrylonitrile at the levels experienced by these workers could be associated with any significant increased relative risk for most cancers. The excess of lung cancer seen in the highest exposure quintile, particularly when exposure was more than 20 years may indicate some risk at the highest exposure. However, no dose-response effect was identified and the risk of lung cancer did not increase with increasing exposure within this highest exposure category. In the Benn and Osborne study (1998), lung cancer mortality showed an increased SMR in the 15 to 44 and 45 to 54 age groups and a deficit for the older age groups. However when considering the results of this particular study it should be noted that the study was hampered by certain limitations e.g. lack of exposure/measured data and lack of information on smoking habits. Overall the results of the Swaen et al. (1998) study did not indicate any cancer excess related to occupational exposure to acrylonitrile. Similarly in the Wood et al. (1998) study, no significantly associated increases in cancer were identified, nor dose-response relationships established regarding cancer mortality or morbidity.

As with Rothman’s meta-analysis (1994), Collins and Acquavella (1998) found no excess of all cancer or lung cancer among acrylonitrile workers. In addition, these authors found that the conclusions of Rothman held even when considering the information recently available in the “new” epidemiological studies and when analyses are conducted with respect to exposure level and induction/latency periods. For lung cancer Collins and Acquavella, evaluated consistency across studies, strength of the association, and some aspects of internal consistency within the studies such as dose-response and latency. Overall their conclusion was that if any excess of lung cancer related to acrylonitrile exposure should exist it is small.

Prostate Cancer

An increased incidence of prostate cancer morbidity was reported by O’Berg et al. (1985), and by Chen et al. (1987). In the O’Berg et al. (1985) study a trend analysis did not reveal a significant relation between cumulative exposure to acrylonitrile and prostate cancer morbidity.

This can be seen from the overall SMR in **Table 4.38** below. All 6 cases occurred at least 20 years after first exposure to acrylonitrile in the workplace. Two cases were found in the lowest (< 2 cumulative exposure units) exposure group (2 versus 0.3 expected), 0 cases in the middle (2-12 cumulative exposure units) exposure group 0 versus 0.4 expected) and 4 in the high (> 12 cumulative exposure units) exposure group (4 versus 0.7) expected. The SMR for prostate cancer between the lowest and highest exposure group was not different despite the > 6 times difference in the exposure level. A dose dependent trend could not be observed and therefore a causal relationship between acrylonitrile and prostate cancer morbidity cannot be established based on the O'Berg et al. (1985) data.

Table 4.38 Observed and expected cases of prostate cancer, DuPont company rates (O'Berg et al., 1985)

	Cumulative exposure (years · level)							
	<2 exposure units		2–12 exposure units		13+ exposure units		Total	
Latency	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.
<2	0	0.1	0	0.1	0	0.2	0	0.4
20 +	2	0.2	0	0.3	4	0.6	6	1.1
Total	2	0.3	0	0.4	4	0.7	6	1.5
Overall SMR	6.7		0		5.7		4	

Chen et al. (1987) stated that 3 of the prostate cancer morbidities had a latency time of more than 20 years. Both O'Berg and Chen used the internal DuPont Cancer Register for estimating the expected prostate cancer morbidity. A major drawback with using this Register as a reference is that workers leaving DuPont for other jobs etc. are no longer followed with respect to morbidity and mortality for the internal DuPont Cancer Register purposes of record keeping. In addition, in the DuPont studies smoking habits were not considered. Prostate cancer has been associated with smoking habits, exposure to cadmium and exposure to perfluoro-octanoic acid.

Finally regarding the "new" studies, Blair et al. (1998), in a very large study of over 25,000 workers, found no excess of prostate cancer. Swaen (1992; updated 1998) failed to find any effects in a large, well designed Dutch study. Likewise, Wood et al. (1998) reporting on DuPont employees, found no significant cancer excess, and no suggestion of increased risk at long latencies or high exposure levels.

According to the meta-analysis of Collins and Aquavella (1998), the studies of Thiess et al., Delzell and Monson, and Burke (Memphis plant) report no prostate cancers but confidence intervals were wide. Keisselbach et al., and Wood et al. reported small excesses, and Burke (Beaumont plant) reported excesses based on a single case. The large studies of Blair et al. and Swaen et al. report slight deficits of prostate cancer. The mRR for the prostate cancer mortality is 1.0 (95%CI 07-1.5). Only the Blair et al., Swaen et al., and Wood et al. studies report exposure level analyses for prostate cancer risks, perhaps because most other studies had no more than 2 prostate cancer deaths. None of these studies however show an increasing risk with increasing exposure. The single nested case-control study of Marsh (1993), does report duration of exposure for the prostate cancer cases and controls. There were no cases in the highest category of 10 or more years of exposure. The two unpublished studies of Burke (Memphis plant) and Burke (Beaumont plant) which reported prostate cancer mortality reported 1 death from prostate cancer (mRRs = 3.9) compared to a mRR of 1.0 for the published studies.

Overall the mRR for prostate cancer incidence is 1.4 (95% CI 0.8-2.6). The Wood et al. study found 12 prostate cancer cases versus 7.6 expected. The other 2 studies that examine incidence found no cancer cases with 0.8 and 0.1 expected cases respectively. The Wood et al. update of the earlier DuPont studies (Chen et al. and O'Berg et al.) found only 1 new case versus 3.89 expected (SIR = 0.3, 95% CI 0.0-1.4) in the update period. Therefore, the cases of prostate cancer are limited in time. In addition the excess of prostate cancer in the Wood et al study was limited to a narrow reporting period (i.e. 1978-83), when improved diagnostic procedures were introduced. Also, no trend with exposure level was observed and there was no accompanying increase in prostate cancer deaths (Collins and Acquavella, 1998).

Bladder Cancer

Though Collins and Acquavella (1998) in their meta-analysis presented data that suggested that bladder cancer might be increased in acrylonitrile workers, this is mainly due to the inclusion of results from the Kiesselbach et al. (1977), and Siematycki et al. (1994) studies. Since completing meta-analysis, for the purpose of this risk assessment report Collins re-evaluated the data excluding the two studies mentioned. This resulted in the bladder cancer meta relative risk (mRR) being reduced overall from 1.4 (95% CI 0.9-2.0) from the 10 studies reporting bladder cancer relative risk in the original meta-analysis report to 1.1 (95% CI 0.7-1.7). Excluding these two studies also reduced the heterogeneity as evidenced by the change in P-values from 0.18 in the original analysis to 0.45. This information confirmed that both the Kiesselbach and Siemiatycki studies had significant influence on the overall mRR for bladder cancer in the original meta-analysis. It is considered that these two studies are outliers and the concern regarding the original analysis outcome for bladder cancer is reduced.

Blair et al. was the only study to report exposure levels for bladder cancer, and it was found that there was no increased risk with increasing exposure levels. It should also be noted that the most potent occupational cause of bladder cancer are aromatic amines and that three of the studies reported the presence of aromatic amines in the plant environment (Keisselbach et al., Thiess et al., and Delzell and Monson). Their data are consistent with the possibility of these substances being confounding factors.

Additional considerations when evaluating epidemiological studies (ten Berge, 1998, personal communication)

Cohort mortality studies are designed to show an increased risk from occupational exposure to chemicals. In evaluating the increased risk of dying from cancer by occupational exposure to acrylonitrile only the cohort mortality study of Blair et al. (1998) was analysed, this being the study with the largest cohort size. Also taking only one study instead of pooling data from more studies avoids the risk of adding up numbers of workers in overlapping cohorts. The study of Blair et al. involved more than 16,000 workers in the USA with occupational exposure to acrylonitrile. The expected causes of death in the Blair et al. study set out in **Table 4.39** below were derived from the general USA death statistics.

Table 4.39 Expected causes of death in the Blair et al. (1998) study

Cause of death	Observed	Expected	Probability RR * > 1
All causes	1,217	1,738	$4.5 \cdot 10^{-4}$
All cancer	326	407	$1.9 \cdot 10^{-5}$
Colon	19	32	$9.3 \cdot 10^{-3}$
Lung	134	149	0.12
Prostate	16	17.8	0.39
Bladder	6	7.5	0.38
CNS	12	17	0.14
All Leukemia	27	45	$2.7 \cdot 10^{-3}$

* RR = Relative Risk

In the far right column the probability is presented that the relative risk (RR) for different tumour end points is > 1. This probability is based on the assumption that the expected mortalities follow a Poisson distribution. It is obvious from this table, that the probability of an increased specific cancer mortality > 1 is always smaller than 0.40. The probability of an increased all cancer mortality is even very much lower ($1.9 \cdot 10^{-5}$).

In the Wood et al. study (1998) the exposure was on average 10 times higher than in the Blair et al. study. On the basis of the Wood et al. study the occurrence of 126 cancer deaths versus 161.5 expected makes it highly improbable (probability for RR > 1 is $2.2 \cdot 10^{-3}$) that acrylonitrile is a versatile carcinogen in man. For lung cancer 47 deaths were observed versus 63.5 expected (probability for RR > 1 is $1.87 \cdot 10^{-2}$). The observed/expected ratios were always smaller than 1 for all cancer mortalities except for prostate cancer. However this increase (observed 11, expected 8.53) is significant. In addition, a trend of an increased prostate cancer mortality or morbidity with increased cumulative exposure could not be demonstrated.

Much more sensitive than Poisson probability evaluations is the analysis for trend. In the Blair et al. study (1998) the relation between cumulative exposure between 0.01 and more than 8 ppm years and specific cancer mortality was extensively studied. No consistent significant trend with cumulative exposure could be detected in comparison with the cancer mortality data from the general USA vital statistics.

In the Wood et al. study (1998) this relationship was studied both for cancer morbidity and cancer mortality (prostate, lung, intestines) for much higher cumulative exposure regimes, between 0.1 and more than 100 ppm years. Wood et al. used as reference for cancer mortality both the general USA vital statistics and the internal DuPont company rates and for cancer morbidity there was no alternative but to use the internal DuPont Company rates. A consistent significant trend with cumulative exposure could not be detected for cancer morbidity and cancer mortality (prostate, lung, intestines) for this on average 10 times higher exposure situation compared to the Blair et al. study.

This evaluation (ten Berge, 1998, personal communication) supports the conclusion that acrylonitrile at historical and present exposure levels is probably not carcinogenic to man. This conclusion is additionally supported by the decision of the International Agency for Research on Cancer (IARC) to lower the classification of acrylonitrile from a 2A to a 2B carcinogen (IARC, 1999), indicating that IARC considers the available epidemiologic studies (while extensive) as inadequate evidence of a relationship between acrylonitrile exposure and cancer in man.

4.1.2.8.7 Summary of carcinogenicity studies

Acrylonitrile is carcinogenic to rats following either oral administration or via inhalation. Common target organs identified were the central nervous system (brain and spinal cord), Zymbal gland, gastrointestinal tract (tongue, non-glandular stomach and small intestine) and mammary gland. Also, as a result of irritation due to inhalation of acrylonitrile, inflammatory and degenerative changes (hyperplasia and metaplasia of the respiratory epithelium) were present in the nasal turbinates and a significantly increased number of rats at 80 ppm exposure levels showed focal gliosis and perivascular cuffing in the brain.

Acrylonitrile has been shown to be weakly mutagenic, primarily through its metabolism to CEO. Acrylonitrile itself hardly, if at all, interacts with DNA. The epoxide is a direct acting mutagen which binds with DNA with a much greater affinity than acrylonitrile. Adducts on guanine have been detected at very low levels in the liver of rats treated with CEO, but the significance of these adducts to the carcinogenic process is not clear. Acrylonitrile at a nearly lethal dose has been found to interact with DNA in the liver and stomach but an interaction of acrylonitrile with the brain DNA has not been demonstrated. This may point to an epigenetic rather than a genetic mechanism involved in the induction of astrocytomas in the brain of rats exposed to acrylonitrile.

However while there is no doubt that acrylonitrile is an animal carcinogen the mechanism of action with respect to inducing carcinogenicity is still relatively unclear. Based on current information and with no definitive contrary evidence acrylonitrile must be considered to be a carcinogen for which a threshold cannot be reliably identified. As such therefore, it is not possible to establish a safe threshold regarding exposure to acrylonitrile and a NOEL cannot in practice be estimated or established for this particular end point.

Very little human information is available which could help in determining the mechanism of possible carcinogenicity in man from exposure to acrylonitrile. Thiess and Fleig (1978) analysed chromosomal damage in peripheral lymphocytes of 18 workers exposed to acrylonitrile for an average of 15.4 years. Co-exposure existed to styrene, ethylbenzene, butadiene and butylacrylate. Under normal conditions air concentrations of acrylonitrile of 5 ppm (11 mg/m^3) were measured, although higher peak exposures will have been present due to faults and manual operation. The frequency of chromosomal aberrations in peripheral lymphocytes was not enhanced in workers as compared to the unexposed controls. However this information reflects a co-exposure rather than exposure due specifically to acrylonitrile and also resulted from higher peak exposures.

There was some indication of excess bladder cancer in three (“new”) epidemiological studies, a finding not reported in the “old studies”. However the excess seemed to be associated with exposure to aromatic amines and is unlikely to be related to acrylonitrile exposure. Furthermore, a reanalysis of the 1998, Collins meta-analysis in which two outlier studies, Kiesselbach et al. (1979) and Siemiatycki et al. (1994) were excluded, resulted in the bladder cancer meta relative risk (mRR) being reduced overall from 1.4 (95% CI 0.9-2.0) to 1.1 (95% CI 0.7-1.7).

Regarding the human epidemiological evidence available both the meta-analysis by Rothman et al. (1994) (on the “old studies”) and the 1998 meta-analysis performed by Collins and Acquavella, (which included the 4 most recent studies), no excess of all cancer or lung cancer was found among acrylonitrile workers. One advantage of the “old studies” is the much higher average levels of exposure experienced compared to current levels. Cancer excesses were not obtained even at these levels which reinforces the assessment that the current levels reflect probable safe limits. The Blair et al. (1998) study had almost 5 times more person years of

exposure than the Wood et al. (1998) study, but Wood et al. had considerably more expected deaths from lung cancer than Blair et al. in the highest exposure group. It is possible that this difference in the highest exposure group was caused by different methods in exposure assessment. However, the Wood study has older workers with longer durations of exposure than the Blair study.

Furthermore the plants in the Wood study were fibre plants, which typically have highest acrylonitrile exposures, and were much older plants than any plants in the Blair study which indicates the potential for higher exposures. The larger number of expected deaths in the Wood study in the higher exposure categories relative to Blair et al. may have been due to the fact that the workers were older and had longer durations of exposure to higher levels of acrylonitrile. Therefore the Wood study may currently provide more information about higher cumulative exposure to acrylonitrile than Blair et al. With regard to lung cancer it remains possible that there is an increased risk of lung cancer in workers exposed to acrylonitrile, but this is likely to apply only at high levels of exposure and requires a lengthy exposure period to manifest itself as an effect.

The excess prostate incidence reported by O'Berg et al. (1985), Chen et al. (1987) and confirmed by Wood et al. (1998) raised the concern that exposure to acrylonitrile may increase prostate cancer incidence risk. However, there is no increase in cancer rates with increasing exposure and this finding has not been seen in the mortality studies. Also, the excess of prostate cancer in the Wood et al. (1998) study is limited to a narrow reporting period i.e. 1978-1983, when improved diagnostic procedures were introduced. A deficit is observed (SIR = 0.3, 95% CI 0.0-1.4) from 1983 to 1991. This indicates the potential for diagnostic bias as cases may have been "harvested" early. Accordingly, overall the evidence does not support an association between prostate cancer and acrylonitrile exposure.

Excess cancer at multiple sites were observed in rats exposed to relatively low levels of acrylonitrile. However, there is little evidence that acrylonitrile workers have increased cancer rates even though exposures in some groups of workers were at levels which caused tumours in rats. Also by excluding the two possible outliers (Kiesselbach et al., 1979; Siemiatycki et al., 1994) from the more recent meta-analysis (Collins and Acquavella, 1998) heterogeneity was reduced as evidenced by the change in p-values from 0.18 to 0.45.

To summarise the excess risk of lung cancer from acrylonitrile exposure, if any, is small. For the less common cancers such as brain and prostate it is only possible to evaluate consistency across the available studies. In doing so a relatively imprecise estimate of risk was found for prostate and brain cancers in acrylonitrile workers and acrylonitrile cannot be completely ruled out as the causes of these cancers. However given all the evidence available, in particular the recent studies, there is little or no evidence to support a causal relationship between acrylonitrile exposure and cancer.

Considering the epidemiological studies available and their known limitations in conjunction with the animal study findings, the current classification of acrylonitrile as a Category 2 carcinogen, R 45 as required under the EU legislation on classification and labelling of dangerous substances remains appropriate for acrylonitrile. However it was confirmed in February 1998, that IARC has downgraded acrylonitrile to a category 2b (IARC, 1999). Their decision to do this was based mainly on the information and lack of carcinogenic evidence found in the four "new studies". By giving acrylonitrile the category of 2b instead of the former 2a, IARC now deem the data relating to potential carcinogenicity to humans, to be 'inadequate' or that the studies do not present evidence of a causal association. Based on the evidence presented,

in particular the epidemiological information available, while acrylonitrile is an animal carcinogen the risk to humans is low considering current exposure in the workplace and the lack of association arising from the large cohort studies performed. This is the conclusion taken forward for risk characterisation purposes.

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Effects on fertility

Studies in animals

Studies in rats

A 3-generation reproduction study in rats was carried out by Litton Bionetics (Beliles et al., 1980), with administration of acrylonitrile in drinking water at nominal levels of 0, 100 or 500 ppm (approximately 8.5 and 35 mg/kg/day). Analysis of actual levels of acrylonitrile in the drinking water indicated stability in water, with the exception of the 100 ppm level between weeks 44 and 65. Actual average weekly acrylonitrile concentration in the high-dose group was 522 ppm (SEM 11.6), and in the lower-dose group was 106 ppm (SEM 2.3). This latter value did not include the values determined between weeks 44 and 65, when mean levels ranged between 0 and 100 ppm, with an average of 37 ppm (SEM 6.2). This reduction in measured levels was associated with bacterial contamination of the water, which was controlled by disinfection of water bottles and storage vessels post week 65.

Study design involved groups of 15 male and 30 female rats (post-weanling) in the F0 parental generation, administered acrylonitrile for 100 days before mating. During this period, observations were limited to daily clinical observations with particular emphasis on signs of neurotoxicity, and measurement of body weight, food and water consumption. Limited histopathology was performed on the F0, F1b and F2b parents, with emphasis on neoplastic changes. At the end of the 100-day dosing period, 20 females and 10 males were paired for mating over a 6-day period, and any females not bred at the end of 6 days, as evidenced by the absence of vaginal plugs, were mated to another proven male. Exposure to acrylonitrile continued throughout the mating period and the subsequent gestation and lactation phases in females.

The results of the first and second matings of the F0 generation were analysed for the following:

- male Fertility Index (Number of males producing a litter/number mated),
- female Fertility Index (Number of pregnant females/number mated),
- gestation Index (Number of litters born/ number of females pregnant),
- viability Index (Number of live pups at 4 days/ number of pups born alive),
- lactation Index (Number of pups weaned/ number of live pups at 4 days),
- duration of mating and gestation,
- pup weight,
- live pups per litter.

F1a offspring were examined on days 0, 4 and 21 of lactation, with body weights being recorded on day 4 (litter) and day 21 (individual). Litters were reduced to 10 pups per litter on day 4, with equal numbers of males and females being retained. Although the original study design called for discarding of the F1a pups at weaning, due to pup mortality at the 500 ppm level, surviving pups were retained beyond weaning.

The female F0 rats were remated to produce the F1b offspring, the previously unmated F0 females also being mated at this time in order to ensure sufficient numbers of offspring to be selected for the F2 generation. Half of these pups were fostered at birth to untreated females, while at weaning (21 days) 1 male and 1 female from each unfostered litter were selected as breeders for the F2 generation.

F2 breeders were administered acrylonitrile in drinking water for 100 days and then mated with production of an F2a and an F2b litter as described for the F0 generation. Reproductive performance was assessed using the parameters described above for F0. Due to high pup mortality in the 500 ppm F1b offspring, some F1a animals were used as replacements to ensure a sufficient number of parental animals. Similarly, F3 breeders were selected from the F2b litters, with additional animals being used from surviving litters in order to achieve required breeding numbers. Following production of the F3a and F3b litters, with reproductive performance being assessed using the parameters described above for F0, 10 weanlings of each sex in the control and 500 ppm dose groups were selected for histopathological examination.

The study report available to the authors of this report is limited in the information provided, although the data presented on reproductive outcome are considered to be valid for risk assessment purposes. The report did not include results of the clinical investigations, and it is not clear therefore whether any clinical signs of toxicity were observed during the study. The study showed no effect of acrylonitrile on male or female fertility in the F1, F2 or F3 generations, as assessed by male or female Fertility Index. Fertility in some experimental groups was occasionally low (e.g. 50-60% in F2 generation for controls and the 100 ppm groups), however slightly higher fertility was consistently recorded in the 500 ppm groups. There was also no indication of an embryotoxic effect, gestation index being similar across all groups and numbers of live pups per litter also being reasonably consistent across the dose groups.

Viability Index was however reduced in the 500 ppm litters, with statistical significance being attained in the F1a (94%, $p < 0.05$), F1b (91% $p < 0.05$) and F3a (95%, $p < 0.05$) generations. There was a trend towards reduced viability also at 100 ppm, statistically significant in the F1b generation (90%, $p < 0.05$). This effect on pup viability was confirmed by reductions in the Lactation Index at both the 500 ppm (66% in F1a, $p < 0.05$, 88% in F1b, 94% in F2a, $p < 0.05$, 99% in F3a) and 100 ppm level. Bodyweights of pups at 500 ppm in all 3 generations at 21 days were also reduced (mean control pup weight in grams on day 4 over all generations was 10.5 ± 0.55, mean pup weight at 500 ppm was 9.0 ± 1, $p < 0.05$).

Although pup survival rate at 500 ppm was reduced, fostering of pups to untreated mothers lessened their mortality rate indicating that the effect was attributable to maternal toxicity. This was confirmed by findings in the F0 parental generation, in which acrylonitrile at 500 ppm caused reduced bodyweight gain in the first generation parent rats (this parameter was not investigated in F2 and F3 parents). Evidence of a possible neurotoxic effect, as evidenced by abnormal gait was also reported for some rats in the F0 and F1 generations. A dose-related tumorigenic effect occurred in female rats held 20 weeks after weaning of the second litter and histopathological examination of these dams showed an increase in astrocytomas and zymbal gland tumours.

Overall, this study can be regarded as valid for risk assessment purposes and the results indicate no obvious effect of acrylonitrile on fertility in the rat at an average level of 552 ppm in drinking water (approximately 35 mg/kg/day). The experimental design does however have limitations, in particular the absence of histopathological examination of gonads in the male rats, other than in the F3b offspring in which no abnormality was reported. Sperm parameters were also not investigated.

In a more recent study, Abdel Naim and co-workers (Abdel Naim et al, 1994; Abdel Naim, 1995) administered acrylonitrile orally by gavage at dose levels of 11.5, 23 and 46 mg/kg in saline daily over a period of 2 and 4 weeks. These authors demonstrated a dose-dependent decrease in body weight gain and in testicular weight. Decreases in testicular weight were paralleled by decreases in weight of the cauda epididymis and caput epididymis, however there was no significant effect on the weights of ventral prostate and seminal vesicles.

Histopathological examination of testes in the studies of Abdel Naim et al. indicated that spermatogenesis was affected after 4 weeks treatment with 23 or 46 mg/kg acrylonitrile, as evidenced by a decreased number of spermatocytes and spermatids. Sperm count and sperm motility were significantly decreased at all dose levels, and testicular LDH-X, a marker of pachytene spermatocytes, was inhibited at dose levels of 23 and 46 mg/kg. Flow cytometric analysis of testicular aspirates from rats treated with 46 mg/kg showed a reduction in the proportion of haploid cells (22% reduction after 4 weeks) and tetraploid cells (65% reduction), while diploid cells were increased (83%). Overall the results in this study indicate that repeated administration of acrylonitrile produces testicular damage in the rat. The dose levels are however relatively high, approaching the acute oral toxicity dose, and the effects seen may well have been secondary to systemic toxicity. The limited study report provides no detail on the condition of the animals. The dose level of 11.5 mg/kg/day was a LO(A)EL in this study, since effects on sperm count and sperm motility were seen at this dose level.

The results of this study suggest that acrylonitrile may be able to alkylate testicular DNA and induce DNA repair. It should be noted, however, that the dose level used in this study is very high, approaching an oral LD_{50} for the rat. It also represents a single bolus dose, as opposed to the 3-generation fertility study where administration in drinking water will provide lower plasma levels, sustained over a longer period. Additionally, the incorporation of radio-label into DNA in this study is not definitive proof of DNA alkylation. Since acrylonitrile reacts preferentially with thiol groupings in proteins to give stable protein adducts, even slight contamination of isolated DNA with protein may lead to erroneous results in quantifying possible DNA adduct formation.

In contrast to the above studies, which report testicular effects including DNA damage and DNA repair following administration of acrylonitrile in the rat histopathological examination of the testis and epididymis indicated no treatment-related testicular degeneration in the Quast et al. (1980a) carcinogenicity study in Sprague Dawley rats. The latter study used dose levels of 0, 20 and 80 ppm acrylonitrile by inhalation (equivalent oral uptake 0, 4.3 or 17 mg/kg/day). The incidence of testicular changes was similar across all groups, as shown in **Table 4.40**. The sperm content of the epididymis in treated rats was also comparable to controls.

Table 4.40 Results of histopathological examination of the testis and epididymis from male rats exposed to acrylonitrile by inhalation for up to 2 years

	Exposure level of acrylonitrile (ppm)		
	0	20	80
Histopathological findings in testis			
Decreased spermatogenesis of seminiferous tubules	30/100	3/17	25/100
Testicular atrophy, bilateral	25/100	7/17	13/100
Vascular degeneration of the testes	55/100	15/17	36/100
Mineralization or sclerosis of atrophic seminiferous tubules	12/100	1/17	5/100
Histopathological findings in epididymis			
Change in sperm count decreased sperm content, increased nucleated cells, or both)	55/98	3/15	38/100

Furthermore, Working et al. (1987), as already reported in Section 4.1.2.7.2, carried out a dominant lethal study in which acrylonitrile in saline was dosed by gavage at 60 mg/kg/day for 5 days to groups of 50 male Fisher 344 rats. Beginning on day 1 after dosing, each male was caged with one female (untreated) weekly for 10 weeks. Females were removed after 6 days and replaced by a new female 1 day later. Females were killed 13 days after the end of each respective mating week and examined for numbers of viable foetuses, early foetal deaths (resorptions), late foetal deaths and corpora lutea. Pre-implantation losses were calculated from the number of corpora lutea minus the total number of implants, while post-implantation losses were considered to be the sum of both early and late foetal deaths.

The results of the study showed that acrylonitrile was toxic to the male rats under study, as evidenced by significant reductions in bodyweight during the exposure period, body weights not returning to normal until week 5 after dosing. However no effects on male fertility were seen, and there was no increase in either pre- or post implantation loss in the females mated with the treated males indicative of a dominant lethal effect (see also Section 4.1.2.7). In respect of possible DNA-damaging effects of acrylonitrile, Hurtt et al. (1987) and Butterworth et al. (1992) used an autoradiographic method for determination of unscheduled DNA synthesis (UDS) in the spermatocytes of rats exposed to acrylonitrile to one single gavage dose of 75 mg/kg or to a repeated gavage dose of 60 mg/kg daily for 5 days. There was no significant difference in thymidine incorporation into spermatocyte DNA in treated and control animals, and the authors concluded that UDS in spermatocytes was apparently not induced by acrylonitrile.

Studies in mice

Tandon et al. (1988) reported testicular damage and a decrease in epididymal spermatozoa in CD-1 mice following oral administration of acrylonitrile at 10 mg/kg/day in saline for 60 days, this dose representing over 30% of the LD_{50} in mice. The testicular damage consisted of tubular atrophy and degeneration in approximately 40% of seminiferous tubules, with cytolysis and nuclear pyknosis of spermatids, formation of multinucleate giant cells and interstitial oedema. These changes were accompanied by a decrease in testicular sorbitol dehydrogenase (22% decrease, $p < 0.05$) and acid phosphatase (16% decrease $p < 0.05$) and an increase in lactate dehydrogenase (12% increase, $p < 0.05$) and B-glucuronidase (36.7% increase, $p < 0.05$).

Glucose-6-phosphatase was unaffected. These changes were seen in the absence of overt signs of toxicity or any effect on body weight or testicular weight. A dose of 1 mg/kg/day acrylonitrile did not produce any biochemical changes or histopathological evidence of damage in the testis.

A single dose of acrylonitrile administered intraperitoneally to AB Jena-Halle and DBA mice at a dose level of 32 mg/kg on days 5, 7 or 9 of pregnancy caused a significant decrease in post implantation losses in the Jena-Halle mouse (46.2% after administration on day 5, $p < 0.01$ and 24.4% after administration on day 7, $p < 0.05$, no effect following administration on day 9) (Scheufler, 1980). However no effect was seen in the DBA mouse. Schuefler also examined the effect of repeat dose intraperitoneal administration of acrylonitrile at levels of up to 26 mg/kg/day on days 1-14 of pregnancy in Jena-Halle mice or levels of up to 16-32 mg/kg/day respectively on days 7-14 in DBA or C57B1 mice. The author found no effects in any of these experimental groups.

A recent 90-day gavage study in B6C3F1 mice administered acrylonitrile in saline to groups of 10 mice per dose level at dose levels of 0, 1.2, 2.4, 4.8, 9.6 and 12.0 mg/kg/day acrylonitrile in saline (Serota et al, 1996). The study included a reproductive substudy with evaluation of testicular weights, epididymal weights, epididymal sperm density and motility and testicular spermatid counts, the left testis and accessory gonads being used in this study while the right testis and accessory gonads were processed for histopathological examination.

All reproductive parameters assessed were unaffected by acrylonitrile administration with the exception of epididymal sperm motility, which was significantly decreased at both the 1.2 and 12.0 mg/kg dose levels. No effect was detected at the intervening dose levels. Histopathological examination of the testis in the control and 12 mg/kg/day groups did not reveal any difference between treated and control mice.

Overall, while the results of the Tandon study indicated that acrylonitrile may have an effect on the testis in the mouse, testicular damage was not confirmed in the 90-day study in B6C3F1 mice administered 12 mg/kg/day. The significance of the epididymal sperm motility finding in the latter study is unclear, but the absence of a dose-response relationship should be noted.

4.1.2.9.2 Developmental toxicity

Studies in animals

Studies in rats

Murray and coworkers (1978) administered doses of 0, 10, 25, and 65 mg/kg acrylonitrile in water daily to groups of 29-39 mated Sprague Dawley female rats on days 6-15 of gestation, with daily clinical examination and periodic determination of body weight, food and water consumption. The animals were killed on day 21 and the numbers and positions of implantation sites, live, dead and resorbed foetuses were recorded. All foetuses were examined macroscopically for external abnormalities and cleft plate, one-third were then examined for visceral abnormalities under a dissecting stereo-microscope and the heads were examined by the razor-section technique of Wilson. All remaining foetuses were examined for skeletal alterations.

Animals receiving 65 mg/kg/day showed hyperexcitability and excessive salivation, and body weight was significantly decreased compared with controls between days 6 and 9 of the study and between days 10 and 15. Food consumption was decreased in the early stages of the study while water consumption was increased in the later stages. One dam at this dose level died on

day 1 of the study. Body weight was unaffected by acrylonitrile administration at the lower dose levels. Thickening of the glandular forestomach was observed in the majority of animals receiving 65 mg/kg/day and in 3/39 animals receiving 25 mg/kg/day. Sialodacryadenitis, as diagnosed by the presence of swollen salivary glands was seen in many animals in the study, including controls.

The incidence of pregnancy was significantly decreased in rats given 65 mg/kg/day (69% compared with 88% in controls, $p < 0.05$) and implantation sites were detected in 4 apparently non-pregnant dams at this dose level (14%). No effect on the incidence of pregnancy was seen at lower dose levels, and no effect was detected on numbers of implantations per dam, live foetuses per litter or resorptions per litter at any dose level. However foetal body weight was signficantly decreased at 65 mg/kg/day (7.4% decrease, $p < 0.05$, indicative of a foetotoxic effect and crown-rump length was also decreased (1.9% decrease, $p < 0.05$).

In foetuses examined for skeletal and visceral abnormalities, short tail occurred significantly more often among the litters of dams given 65 mg/kg/day than in control litters (in 8/212 foetuses examined at 65 mg/kg/day, compared with 1/443 in controls, $p < 0.05$). Short-tailed foetuses also had missing vertebrae, ranging from lack of one lumbar vertebra to lack of all sacral, lumber and most thoracic vertebrae, with associated ribs. Additional malformations in these foetuses included short trunk (3/212 foetuses, with 0/443 in controls) imperforate anus (2/212), right-sided aortic arch (1/212), missing kidney (1/212) and anteriorly-placed ovaries (1/212). There was also a increased incidence of minor skeletal abnormalities in the 65 mg/kg/day offspring compared with controls, these included delayed ossification of sternebrae, split sternebrae and delayed ossification of cervical vertebrae. At 25 mg/kg/day no single malformation occurred with an incidence statistically different to that in the controls, although a number of the same malformations seen in the 65 mg/kg/day group also occurred at this dose level.

Murray et al. (1978) exposed groups of 30 pregnant rats to dose levels of 0, 40 or 80 ppm acrylonitrile by inhalation for 6 hours per day from day 6-15 of gestation, the 80 ppm/6-hour exposure being stated to be equivalent to a single gavage administration of 23 mg/kg/day. The experimental parameters assessed were as for the gavage study described above. Dams in this study showed little clinical evidence of the toxicity seen in the high-dose group in the gavage study, although maternal body weight was significantly decreased in both the 40 ppm and the 80 ppm groups compared with control between days 6 and 9 of the study and between days 10 and 15. Food consumption was decreased in the early stages of the study while water consumption was increased in the later stages.

No effects on incidence of pregnancy, numbers of implantations per dam, live foetuses per litter, resorptions per litter, foetal body weight and crown-rump length was detected at any dose level when acrylonitrile was given by inhalation. The incidence of total major malformations was slightly increased (from 8/421 in controls to 11/416 at 80 ppm, $p=0.06$), however no single major abnormality occurred at an incidence significantly different than that in the controls. There was a decrease in the incidence of delayed ossification of skull bones at 80 ppm but not at 40 ppm.

It can be concluded on the basis of the results of these studies that acrylonitrile has a potential to interfere with embryonic and foetal development, but apparently only at doses producing significant maternal toxicity. Although the Murray studies, carried out by the Toxicology Research Laboratory of Dow Chemical Company, were comprehensive, it has to be recognised that the developmental effects seen in the gavage study were only seen at a dose level approaching the LD_{50} in rats, which produced significant maternal toxicity including death of

one dam. Interpretation of the results is also clouded by the outbreak of sialodacryadenitis in the study.

Saillenfait et al. (1993) examined the development toxicity of acrylonitrile as one of a series of eight aliphatic mononitriles. Groups of 20-23 previously mated Sprague Dawley rats were exposed to dose levels of 0, 12, 25, 50 or 100 ppm acrylonitrile by inhalation for 6 hours/day from day 6-20 of gestation, with daily clinical examination and periodic determination of body weight. The animals were killed on day 21 and the numbers and positions of implantation sites, live, dead and resorbed foetuses were recorded. Live foetuses were sexed and examined macroscopically for external abnormalities, one-half were then examined microscopically following fixation in Bouins, while the other half were examined for skeletal alterations following clearance and staining with alizarin red S.

Body weights of dams exposed to 25, 50 or 100 ppm acrylonitrile were significantly depressed compared with control from the commencement of the acrylonitrile exposure period (13% decrease at 100 ppm, $p < 0.01$, 4.3% at 50 ppm, $p < 0.01$, 1.8% at 25 ppm, $p < 0.01$). No adverse effects on the pregnancy rate, average number of implantations, live foetuses, or in incidences of non-surviving implants or resorptions per litter were noted in any of the groups exposed to acrylonitrile. There was a dose-dependent reduction in foetal weight in the litters from dams exposed to 25, 50 or 100 ppm acrylonitrile, a 5% decrease being seen at 25 ppm, reaching 13-15% at 100 ppm. Evaluation of the incidences of external, visceral and skeletal variations among foetuses from acrylonitrile-exposed dams gave no indication of any effect. One foetus from a 25 ppm litter had a missing thoracic centre, but there was no other evidence of major malformation in any acrylonitrile-exposed litter.

The results of this study indicate that although acrylonitrile was foetoxic, at exposure levels which were also maternally toxic, there was no evidence of a developmental effect. 12 ppm represented a No Effect Level for the foetotoxic effect.

Mehrotra et al. (1988) investigated the prenatal effects of acrylonitrile on early morphological and neurobehavioural development in rats. Groups of 30 pregnant Charles-Wistar rats were administered oral doses of 0, 1 or 5 mg/kg acrylonitrile daily from days 5-21 of gestation. Dams were weighed daily, and food and water consumption was recorded. At parturition litters were culled to 8, with equal numbers of males and females. Pups were evaluated post-partum for morphological development and functional teratology using a screening protocol suggested by Vorhees (1979). On day 21 post-partum the pups were killed and a range of neurochemical analyses were carried out on the brain.

No effect of acrylonitrile was detected on maternal body weight, length of gestation, numbers of litters, sex within litters and in pup weight at parturition and post partum. Nor was any effect detected on post-natal development of neonates and behaviour and reflexes appeared normal. However, there were alterations in brain levels of noradrenaline, 5-hydroxytryptamine and monoamine oxidase, which the authors suggested could be indicative of a derangement in synaptic transmission.

The results of this study contribute very little to the risk assessment of acrylonitrile, since dose levels used were very low, and the significance of the brain chemistry changes reported are unclear.

Studies in hamsters

Willhite at al. (1981) administered doses of 4.8, 10, 25, 65, 80 or 120 mg/kg of acrylonitrile in saline via intraperitoneal injection to pregnant golden hamsters on day 8 of gestation. Separate groups of animals received intraperitoneal injections of 1 g/kg sodium thiosulfate 20 minutes before and 80 minutes after administration of acrylonitrile. Dams were killed on day 14 of gestation and numbers of live foetuses, implantation sites and resorptions were recorded. Foetuses were examined macroscopically and after fixation for evidence of malformations.

No clinical signs of toxicity or developmental effects were seen in the offspring of dams administered up to 65mg/kg acrylonitrile. Animals receiving 80 mg/kg showed dysponoea, gasping, incoordination, hypothermia, salivation, and convulsions 1-5 hours after the injection, while those administered 120 mg/kg all died. The dose of 80 mg/kg resulted in encephalocoeles (7/51 foetuses), rib fusions and bifurcations in many of the offspring. Percentage of abnormal foetuses was 15.7%, compared with 0.8% in controls. Administration of sodium thiosuphate prevented overt signs of maternal toxicity but developmental effects were still seen in the offspring, indicating that the effects of acrylonitrile seen in this study may be due to the metabolic release of cyanide.

Overall the study suggests that acrylonitrile may have developmental effects in the hamster, but only at dose levels which are maternally toxic.

In vitro studies

Saillenfait et al. (1992) cultured 10-day rat embryos in rat serum for 26 hours in the presence of acrylonitrile at concentrations of 76 to 760 µM. Survival of embryos was unaffected at any concentration. Normal growth was observed at the lowest concentration tested, 76 µM. However at higher concentrations there were decreases in growth parameters such as yolk sac diameter, crown-rump length, head length and number of somite pairs. At concentrations above 152 µM there were significant increases in the number of malformations observed, shortened caudal extremity and a reduction of brain and head length being the predominant malformations seen. When a hepatic microsomal preparation (S9) was added, an increase in malformations was observed, suggesting that maternal mono-oxygenase metabolism may contribute to the developmental toxicity of acrylonitrile. The results of this study indicate a potential for acrylonitrile to interfere with embryonal and foetal development.

Studies in humans

There are no reports of effects on fertility in acrylonitrile-exposed workers, however no specific epidemiological studies have been carried out. A recent case control study of 475 female workers exposed to acrylonitrile compared with 527 controls in a fabric plant (Weiai et al., 1995) suggested a higher incidence of premature delivery (RR 1.55, logistic regression analysis), birth defects (RR 1.84), pernicious vomiting during pregnancy (RR 1.64) and anaemia (RR 2.79) in the acrylonitrile-exposed population. An increased incidence of miscarriage was also reported, although the increase was not statistically significant. The exposed population worked in a plant which is involved in the manufacture of acrylonitrile itself and also butadiene rubber, ABS plastic and polyacrylonitrile fibre. Monitoring in the plant during the period 1988-1990 indicated that exposure levels were in excess of the OEL of 2 mg/m^3 (0.87 ppm). Llevels as high as 92 mg/m^3 (40 ppm) were reported. The authors indicate that confounding factors such as age of the parents at pregnancy, drinking, smoking, health history, medication and X-ray have been taken into account in their analysis. There was, however, concomitant exposure to other

chemicals in the workplace, while it appears that the controls were involved in fabric processing, e.g. tailoring, and had little or no chemical exposure. Little confidence can be placed in this poorly-reported study, and no conclusions can be drawn regarding a possible effect of acrylonitrile on pregnancy outcome.

4.1.2.9.3 Summary of toxicity for reproduction

The results of a 3-generation reproduction study, which is considered to be valid for risk assessment purposes despite some methodological deficiencies, did not show any effects on fertility, although effects were seen on pup viability and bodyweights of pups in all 3 generations at 21 days were also reduced. These effects could be attributed to maternal toxicity. A number of other studies have also indicated that acrylonitrile is foetoxic, as evidenced by dose-dependent reductions in pup weight at exposure levels which are also maternally toxic. A No Effect Level of 12 ppm for the foetotoxic effect was established in the study of Saillenfait et al. (1993).

Other studies have reported that acrylonitrile causes testicular toxicity in the rat (at doses approaching the LD_{50}), although no such effect was seen in a recent 90-day study in mice or in other repeated dose toxicity studies. There are no data on fertility in humans.

A gavage study in rats and a study in hamsters using intraperitoneal administration indicated some developmental toxicity potential of acrylonitrile, and this was supported by the findings of an *in vitro* study in 10-day rat embryos. However, developmental effects *in vivo* were only seen in the presence of significant maternal toxicity, and there was little evidence for a developmental effect following exposure of rats by inhalation. An absence of developmental effects following inhalation exposure was confirmed by another group of researchers using comparable exposure levels.

Overall, it can be concluded that existing animal data do not show any clear indication of fertility, dominant lethal, reproductive or teratogenic effects of acrylonitrile at doses below those producing parental toxicity. Therefore, classification as toxic for reproduction with R63 is not appropriate, given the maternal toxicity seen in the Murray et al. study and the confounding influence of disease, the route of administration used in the hamster study of Willwhite et al. (1981), and the negative outcome of the study of Saillenfait et al. (1993).

4.1.3 Risk characterisation

4.1.3.1 General aspects

Acrylonitrile is a highly volatile liquid which has a wide range of uses. Exposure of humans to acrylonitrile is possible in the workplace, during production of acrylonitrile and its use in the manufacture of acrylic fibres, ABS-SAN plastics, nitrile rubbers, other intermediates such as acrylamide and adiponitrile and other end uses. Exposure of consumers is possible as a consequence of use of products manufactured from acrylonitrile, while the general public may be exposed via the environment to low levels of acrylonitrile released from point and diffuse sources.

In relation to workplace exposure, the odour threshold has been reported to be between 1.5-18 ppm. After prolonged inhalation the odour perception becomes less. Inhalation exposure of a single volunteer to 370-460 ppm acrylonitrile for 70 seconds did not result in an intolerable reaction (Grahl, 1970). After prolonged exposure irritation of the mucous membranes occurs. Based on human data and studies in animals, acrylonitrile has been shown to be toxic by ingestion, inhalation of the vapour or by absorption of the liquid through the skin. Estimates of skin penetration have shown that acrylonitrile may permeate the skin at a rate of 0.03 mg/cm^2 per minute.

Symptoms of acrylonitrile poisoning, by whatever route of entry are, in order of onset: limb weakness, laboured breathing, dizziness and impaired judgement, cyanosis and nausea, collapse and loss of consciousness, irregular breathing, convulsions, respiratory arrest and possible death. When symptoms include collapse, irregular breathing, or convulsions, cardiac arrest can occur without warning. Liquid coming in contact with the skin will be readily absorbed and will also cause irritation or induce sensitising effects. Exposure to acrylonitrile vapour results in mild eye irritation, but instillation of acrylonitrile liquid into the eye will result in severe irritation and permanent eye damage may result.

In animals, administration of acrylonitrile resulted in damage to the gastrointestinal tract (oral administration only), central nervous system and adrenal gland. There are also occasional reports of liver and kidney damage. The respiratory tract is also affected following inhalation exposure, based on lung pathology on and histopathological changes in the nasal turbinates of rats seen in the Quast et al. (1980a) two-year study. A LO(A)EL of 20 ppm was established in this study and this is used as a starting point in the risk assessment in relation to inhalation exposure. A No Adverse Effect Level (NAEL) of 4 ppm for the inhalation route has been derived from the LO(A)EL of 20 ppm, by application of a safety factor of 5. This is considered to be justifiable because of the local nature of the effect and the conclusion that other systemic, non neoplastic findings in acrylonitrile-treated rats were secondary to its tumorigenic effects, rather than due to direct systemic toxicity. The suggested NAEL is supported by the evidence from the study of Sakurai et al. (1978) that levels in excess of 10 ppm in acrylonitrile plants did not cause notable irritancy. In relation to oral administration of acrylonitrile, the N(A)OEL is estimated to be 3 ppm in drinking water, based on the information from the Biodynamics study (1980b) in rats.

Acute signs of neurotoxicity were seen in rats, including effects on cholinergic transmission, possibly due to an inactivation of acetylcholinesterase by cyanoethylation of the hydroxyl group of one serine residue or because of damage to the acetylcholine receptors by acrylonitrile or its metabolites. Evidence of neurotoxicity was more marked in glutathione-depleted rats. Two distinctive phases of acute neurotoxic effects were observed in animals treated by gavage or

subcutaneously with doses of 20, 40, or 80 mg/kg of acrylonitrile. The early phase (1 hour after dosing) included characteristics such as salivation, lacrimation, miosis, diarrhoea, polyuria, and peripheral vasodilation. The later phase (> 4 hours after dosing) included central nervous system abnormalities such as respiratory depression, convulsions and, for the high-dose animals, death.

In humans, specific case reports and workplace surveys indicate that chronic exposure to acrylonitrile is associated with neuropathological effects following exposure to acrylonitrile via inhalation or by physical contact with the substance. In general the effects reported with respect to worker exposure include irritation to the skin and eye, nausea, vomiting, diarrhoea, gastritis, general weakness, heart and chest pain, headaches, poor sleep and irritability, irritation of the mucosa and respiratory tract. Depression and lability of autonomic functions have also been reported in workers involved in acrylonitrile production.

Neuropathological effects are in the main due to metabolism to cyanide, as shown *inter alia* by the studies of Benesh and Cerna (1959) and Hashimoto and Kanai (1965) in rats and mice and by the reported case history in a man by Vogel and Kirkendall (1984). This is the main effect of acrylonitrile at sublethal dose levels and may exhibit reversibility of effect. However in the case of lethal dose levels there is also a direct effect on the central nervous system, which cannot be counteracted by cyanide antidotes. Irreversible damage occurs, probably by cyanoethylation of vital structures in the central nervous system.

The results of the mutagenicity and genotoxicity tests indicate that the DNA active compound is the metabolite epoxide CEO, with at best weak evidence of a direct mutagenic effect of acrylonitrile. The interpretation is clearly in accordance with the observations that acrylonitrile is mutagenic mainly after metabolic activation. CEO is mutagenic *in vitro*, but acrylonitrile is negative in *in vivo* genotoxicity tests. The lack of *in vivo* mutagenicity may be due to inactivation of CEO via glutathione conjugation resulting in a failure of acrylonitrile or its active metabolite to reach the target tissues. This inactivation pathway may not exist in *in vitro* test systems.

Acrylonitrile is classified as carcinogenic (Cat. 2) on the basis of the results of a number of animal studies, following either oral administration or via inhalation. The common target organs identified were the central nervous system (brain and spinal cord), zymbal gland, gastrointestinal tract (tongue, non-glandular stomach and small intestine) and mammary gland. Both in the inhalation and drinking water studies a linear relationship was observed between the incidence of astrocytomas and the dose level of acrylonitrile used. Given the positive mutagenicity data for the metabolite CEO, acrylonitrile is currently considered to be a carcinogen for which a threshold cannot be reliably identified, and a safe exposure level cannot therefore be estimated for this end point.

Epidemiological data presented may indicate a slight excess risk of lung cancer from acrylonitrile exposure. However for the less common cancers such as brain and prostate it is only possible to evaluate consistency across the available epidemiological studies. In so doing, a relatively imprecise estimate of risk was found for both prostate and brain cancers in acrylonitrile workers and acrylonitrile cannot therefore be completely ruled out as the cause of these cancers. However, given all the evidence available, however, in particular the recently completed epidemiological studies, there is little or no evidence to support a causal relationship between acrylonitrile exposure and cancer in humans. It should be noted that IARC have recently revised their categorisation of acrylonitrile as a carcinogen from category 2A to category 2B on the basis of the recent epidemiological data.

In relation to reproductive toxicity, effects on the testis in mice and rats have been reported by some authors in short-term studies (up to 60 days, doses of 23 or 46 mg/kg/day in the rat or 10 mg/kg/day in the mouse). This may however have been a secondary effect associated with systemic toxicity. No Effect Levels of 11.5 mg/kg/day in the rat and 1 mg/kg/day in the mouse were reported in these studies. Testicular toxicity has however not been reported in a 2-year inhalation study in rats at 80 ppm (equivalent oral uptake 17 mg/kg/day) or in a 90-day oral gavage study in mice at a top dose of 12 mg/kg/day. No effects were seen on fertility in a 3-generation reproduction study. There are no reports of impaired fertility in exposed workers. Overall, there is no clear evidence that acrylonitrile has an effect on fertility, and a N(A)OEL of 12 mg/kg/day has been adopted in relation to this end point, on the basis of the recent 90-day study in mice.

The results of a number of developmental toxicity studies indicate that acrylonitrile is foetotoxic, this effect showing a strong correlation with maternal toxicity. The lowest NO(A)EL established in these studies was 12 ppm/6 hours daily by inhalation and 25 mg/kg/day following gavage dosing. A gavage study in rats indicated some developmental toxicity potential of acrylonitrile at dose levels of 65 mg/kg/day. A non-significant trend was also reported in an inhalation study at a dose level of 80 ppm (stated to be equivalent to a single gavage administration of 23 mg/kg). This was not confirmed in an inhalation study by other authors using dose levels of up to 100 ppm/6 hours daily. A study in hamsters given a single dose of 80 mg/kg acrylonitrile by intraperitoneal administration also resulted in developmental abnormalities in the offspring. However the route of exposure in this study makes interpretation difficult. The reported effects in these studies were only seen at dose levels where there was significant maternal toxicity and a NO(A)EL for developmental effects in the rat is established at 25 mg/kg/day following gavage administration and 40 ppm following inhalation exposure.

The key potential health hazards in humans, as assessed from the information above, are acute toxicity, irritation, corrosivity, skin sensitisation, repeated dose toxicity (including neurotoxicity), carcinogenicity and mutagenicity. Sensitisation has been reported in exposed workers, and in line with current hypotheses regarding the idiosyncratic nature of skin sensitisation in humans, it is not possible to reliably characterise the risk of sensitisation related to acrylonitrile exposure in quantitative terms. Although data in humans are not indicative of significant chronic toxicity or reproductive effects, animal data suggest that both of these end points should be considered in the risk characterisation for humans together with local irritation effects on mucous membranes. The neurotoxic effects observed in animals occurred in high-dose exposure scenarios, and in relation to risk characterisation this end point is considered to be adequately covered by the N(A)OELs estimated for chronic toxicity. Neurotoxic effects have been described in humans based on specific incidents where very high exposures occurred. Reproductive toxicity in animals occurred as a secondary consequence of general toxicity and is not therefore regarded as a key health hazard for humans.

4.1.3.2 Workers

Workers are potentially exposed to acrylonitrile during production of the monomer and use of the monomer to produce acrylonitrile polymers. Although minor differences in exposure could potentially exist between these two scenarios, reflecting the extent of enclosure of the process, in practice this is not borne out by recent exposure data provided by industry for both production and further processing facilities (see Section 4.1.1.2.2). This indicates that maximum exposure levels lie well below the Occupational Exposure Limit of 2 ppm applying in most European countries. Risk characterisation has therefore been carried out for the combined category of

production and further processing workers, and a reasonable worst-case exposure level of 2 ppm has been chosen.

Although workers may also be exposed to acrylonitrile during manufacturing processes using acrylonitrile polymeric products such as fibres, ABS-SAN plastics and nitrile rubbers, levels of free monomer in such products are low, and the potential for exposure of workers is correspondingly low. For the purposes of characterisation of risk for workers, it is therefore assumed that the analysis carried out for workers in production and further processing represents a worst-case scenario.

4.1.3.2.1 Acute toxicity

Acrylonitrile is classified as acutely toxic by inhalation, in contact with skin and if swallowed (R 23/24/25). Risk characterisation is therefore necessary for this end point. Exposure by the oral route is expected to be minimal assuming normal good hygiene practices in the workplace. Following oral dosing the mouse appeared to be the most sensitive species, with an oral LD_{50} ranging from 28 to 48 mg/kg body weight. The reported range in the guinea pig was 50-85 mg/kg, an oral LD_{50} of 93 mg/kg was reported in the rabbit, while in the rat the range was 72-186 mg/kg. No acute oral toxicity data exist for the dog. The reported dermal LD_{50} for the rat lay between 148 and 282 mg/kg bodyweight, the dermal LD_{50} in the rabbit was 226 mg/kg and that in the guinea pig was between 260-690 mg/kg.

EASE predicts low to negligible exposure via the dermal route. The possibility of acute toxic effects mediated via exposure by the dermal route is anticipated to be negligible, reflecting also the control measures including personal protective equipment which apply in relation to the handling of acrylonitrile and its polymeric products (see Section 4.1.1.2).

Inhalation studies provided an approximate LC_{50} of 200 mg/m^3/4 hours in the dog, 300 mg/m^3/4 hours in the mouse and 990 mg/m^3/4 hours in the guinea pig while in rats the data of Dudley and Neal and those of Appel et al. provided a figure of between 1,030 and 1,210 mg/m^3/4 hours, although a lower value of 470 mg/m^3/4 hours was reported by Knobloch et al. (1971). With regard to acute lethality of acrylonitrile in animals, dogs appeared to be the most sensitive species following exposure via inhalation. However as outlined previously the acute toxicity of acrylonitrile is for the greater part caused by the release of cyanide. Dogs are more susceptible to cyanide toxicity because as a species they have considerably lower levels of the cyanide-detoxifying enzyme rhodanase in the liver than other mammals. Although exposure to low levels of acrylonitrile via the inhalation route is possible, the magnitude of such exposure is not predicted to give rise to acute toxic effects. Additionally, the classification of acrylonitrile as acutely toxic represents a risk reduction measure that is already in place.

Regarding short-term exposure of humans to acrylonitrile this cannot occur other than via accidental releases which are not covered in this risk assessment report with respect to risk characterisation. Any potential short-term exposure to acrylonitrile has been built into the long-term worst-case scenario whereby the value of 2 ppm has been assumed and used.

Therefore, acrylonitrile is of no concern for workers in relation to acute toxicity: **conclusion (ii)**.

4.1.3.2.2 Irritation and corrosivity

Acrylonitrile is classified as irritating to skin (R 38). Although direct dermal contact with acrylonitrile liquid is theoretically possible (the accident scenario is not considered in this assessment), in practice control measures would indicate that acrylonitrile is of no concern for workers in relation to acute skin irritation: **conclusion (ii)**. These control measures include the personal protective equipment known to be used in the handling of acrylonitrile and its polymeric products. Also, classification of acrylonitrile as irritating to skin represents a risk reduction measure that is already in place.

Exposure of eyes to acrylonitrile vapour is possible and accidental splashing is also a possibility. Although quantitative characterisation of the effect (dose-response relationship) is not possible, the results of a number of animal studies together with limited human data would indicate a risk of serious damage to eyes. Although this should not occur in the workplace, given the control measures including personal protective equipment known to apply in relation to the handling of acrylonitrile and its polymeric products, classification as R41 (risk of serious damage to eyes) is appropriate. However, based on current controls in the workplace together with no reports of such exposure and the strict use of personal protective equipment, indicates that acrylonitrile is of no concern for workers in relation to acute eye irritation: **conclusion (ii)**.

Irritation of the respiratory tract has been reported in exposed workers, and results in inhalation studies in animals support this finding. Although this should not occur in the workplace, given the exposure levels currently encountered in industry and the control measures known to apply, classification of acrylonitrile as R37 (irritating to the respiratory system) is appropriate. However, given that the cases reported in workers are not of recent occurrence and considering the exposure levels currently in the workplace and the controls measures being adopted, it is concluded that acrylonitrile is of no concern for workers in relation to acute respiratory irritation: **conclusion (ii)**.

For classification, see Section 1.4.

On a weight of evidence approach, it is concluded that corrosivity is not an issue of concern for acrylonitrile, despite isolated reports of skin blistering after accidental human contact and skin necrosis in a small number of animal studies of doubtful validity. It is concluded that acrylonitrile is of no concern for workers in relation to corrosivity: **conclusion (ii)**.

4.1.3.2.3 Sensitisation

Skin sensitisation has been reported in workers exposed to acrylonitrile, and results in a guinea pig maximisation test support this observation. It should be noted however that only a handful of reported cases exists, in reports from industry rather than in scientific papers, among the many thousands of workers who have been exposed to acrylonitrile. In line with current hypotheses regarding the idiosyncratic nature of skin sensitisation in humans, quantitative characterisation of the effect (dose-response relationship) is not possible, and a NO(A)EL for this end point cannot be derived. Classification of acrylonitrile as R43 (may cause sensitisation by skin contact), is appropriate. No recent cases of skin sensitisation have been reported (confirmed by industry) and given the level of control adopted so as to avoid skin contact, together with the current exposure levels in the workplace, acrylonitrile is of no concern for workers in relation to skin sensitisation: **conclusion (ii)**.

In relation to respiratory sensitisation, given that acrylonitrile shows reactivity for proteins and is a skin sensitiser, and that inhalation is the primary route of exposure, this could be regarded as an end point of concern. In practice, there are no reports of respiratory sensitisation in exposed workers which would confirm this concern, although reports of irritation of the respiratory tract exist, indicative that exposure to relatively high levels of acrylonitrile has occurred in the past. It is also recognised that the control measures that have been in place for many years to protect workers against the carcinogenic effects of acrylonitrile will also protect against possible induction of sensitisation. It is concluded therefore that acrylonitrile is of no concern for workers in relation to respiratory sensitisation: **conclusion (ii)**.

4.1.3.2.4 Repeated dose toxicity

In animals, repeated exposure to relatively high levels of acrylonitrile results in damage to the gastrointestinal tract, central nervous system and adrenal gland. There are occasional reports of liver and kidney damage. Prolonged exposure of rats and mice by, for example, inhalation of acrylonitrile or administration in drinking water results in general loss of condition, body weight loss and increased mortality without consistent target organ toxicity. Local effects in the respiratory tract are also seen following inhalation exposure. This is evidenced by the histopathological changes occurring in the nasal turbinates of rats in the Quast et al. (1980a) two-year study in rats or the lung changes seen in female dogs at an exposure level of 24 ppm in the 90-day study of Brewer (1976).

Repeated dose toxicity following inhalation exposure

Local effects

As indicated above chronic exposure of rats by inhalation to 20 or 80 ppm acrylonitrile in the Quast et al. (1980a) two-year study resulted in histopathological changes in the nasal turbinates of rats. 20 ppm represented a LO(A)EL for this effect, while an approximate No Adverse Effect Level (NAEL) of 4 ppm has been derived from the LO(A)EL by application of an assessment factor of 5 (see Section 4.1.2.6.1). A risk characterisation for local irritant effects is necessary, and both the LO(A)EL and the NAEL derived from the Quast study can be compared with the reasonable worst-case exposure level of 2 ppm proposed for workers in the industry to give MOSs of 10 and 2, respectively, as also shown in **Table 4.41**.

In the Brewer (1976) 90-day inhalation study in dogs, lung changes (multifocal bronchopneumonia) were seen in female dogs at an exposure level of 24 ppm, representing a LO(A)EL for these local irritant effects. A NO(A)EL was not established. This study provides a MOS of 12, based on the LO(A)EL. The duration of this study was shorter than that in the rat study. Also, as indicated in Section 4.1.2.6.1, the quality of the Brewer study is questionable, and its value as a pivotal study for risk assessment is limited. The result is reasonably comparable with that in the Quast rat study, and the latter study is therefore regarded as the key study in relation to risk characterisation for local irritant effects in workers.

Although a MOS of 10 (based on LO(A)EL) or 2 (based on NAEL) is low, it is concluded nevertheless that acrylonitrile is of no concern for workers in relation to local effects following repeated exposure (**conclusion (ii)**), on the basis of the reported sensitivity of the rat, an obligate nose breather, to upper respiratory tract irritants and the strict controls on exposure which apply in the industry. The former is supported by comparison with formaldehyde, which produces significant irritancy at exposure levels of 6 ppm yet has an OEL in a number of EU countries of

1.5 ppm, 25% of the LO(A)EL. In contrast, the irritant effects of acrylonitrile at 20 ppm in rats were relatively slight, as shown in **Table 4.16**. Application of a safety factor of 5 to the level of 20 ppm to give a suggested No Adverse Effect Level (NAEL) of 4 ppm is considered justifiable and reflects a conservative approach with respect of the nature of this local irritancy effect. Quast (2001, personal communication) anticipated that the No Observed Effect Level for these effects in this rat study probably lay in the region of 10 ppm. The NAEL of 4 ppm is consistent with the levels reported in the Brewer (1976) study, although as stated the Brewer study could not be considered a key study on its own due to the confounding factor of disease, identified in the test animals.

The data available on repeated dose exposure of humans are limited mainly to case reports of specific incidents and epidemiological type reports and studies. Many of the reports indicating comparative local effects in workers are old reports (when it is acknowledged that exposure levels were much higher and occupational hygiene methods and controls in their infancy). In addition, these effects could not be attributed to acrylonitrile alone, since at many of these workplaces exposure to mixtures of chemicals occurred. The information from the Sakurai et al. study (1978) must be put in context. Local effects did occur among the workers but on reappraisal of the study and the exposure levels involved it was found that levels less than 10 ppm did not cause notable irritancy. Also the author stated that the “exposure levels were not reliably reported” in this 1978 study. Furthermore, it should be noted that current exposure levels in industry are reported to be well below the level of 2 ppm chosen as a reasonable worst-case exposure level for risk characterisation for workers.

Chronic systemic effects

The results of the Quast et al. (1980a) study (Section 4.1.2.6.1) showed that prolonged exposure of rats to acrylonitrile by inhalation resulted in general loss of condition, body weight loss and increased and early mortality without consistent target organ toxicity. The likely explanation of these chronic systemic effects is the continual release of low levels of cyanide, derived from the metabolism of acrylonitrile to cyanoethylene oxide (CEO) with subsequent conjugation with glutathione and release of cyanide from the GSH-conjugate. Effects were seen in both sexes at 80 ppm and in females only at 20 ppm (the lowest dose in the study). As with local irritant effects, therefore, 20 ppm represents a LO(A)EL and an estimated NAEL of 4 ppm can be derived by application of an assessment factor of 5 to the LO(A)EL.

A systemic dose for the rats in this study can be derived from the LO(A)EL of 20 ppm using the physiological default values of the US EPA, with an assumed breathing rate in the rat of 0.011 m^3/hour, an exposure duration of 6 hours/day, an assumed absorption factor of 0.5 and a body weight (female) of 0.35 kg. This gives a systemic LO(A)EL dose of 4.1 mg/kg/day in the female rat. The comparable figure based on the NAEL of 4 ppm is 0.82 mg/kg/day.

A similar approach is adopted in derivation of a daily systemic dose for workers, assuming the reasonable worst-case exposure level of 2 ppm, a breathing rate of 0.833 m^3/hour (default used by the US EPA), an exposure duration of 8 hours/day, an assumed absorption factor of 0.5 and a body weight of 70 kg (US EPA default physiological values). This gives a systemic dose of 0.2 mg/kg/day due to inhalation of 2 ppm. However in considering the possible sources of exposure of workers to acrylonitrile, the possibility of dermal exposure in addition to exposure by inhalation cannot be discounted. A worst-case estimate of systemic exposure by this route can be derived using the EASE-model prediction. With regard to the EASE-model the following assumptions are made:

1. acrylontrile is a liquid,
2. non-dispersive use,
3. direct-handling,
4. incidental contact.

A worst-case dermal deposition can be estimated to be 10.6 mg, assuming a dermal deposition of between 0.0 and 0.1 mg/cm^2/day (average of 0.05 mg/cm^2/day) and a potential dermal surface of 850 cm^2, with an assumption that only 25% of this surface area is affected in incidental contact. Acrylonitrile deposited on the skin will partly evaporate and partly penetrate through the skin. Assuming a 0.5 absorption factor for dermal penetration, a dermal dose of 5.3 mg/day can be estimated. In the case of a 70 kg worker, this results in a daily dermal absorbed dose of 5.3/70 = 0.076 mg/kg/day.

It should be noted that this estimated dermal absorption is in practice improbable due to the precautionary measures taken to avoid direct contact with acrylonitrile. The estimated dermal dose of 5.3 mg/day or 0.076 mg/kg/day would indicate that the dermal route is a significant route of exposure in the workplace. If this were so, it would be expected that many cases of occupational skin sensitisation during the production and processing of acrylonitrile would be reported, and this is not the case.

Nevertheless, in estimating a daily systemic dose for workers, a total dose of 0.2 mg/kg/day (inhalation) + 0.076 mg/kg/day (dermal) can be derived, or 0.28 mg/kg/day. This assumes exposure to 2 ppm (which is a worst-case scenario based on the evidence/measured data provided by industry) and incidental dermal contact. Comparison of this systemic dose with the systemic dose of 4.1 mg/kg/day in the female rat based on the LO(A)EL of 20 ppm or 0.82 mg/kg/day based on the NAEL of 4 ppm gives MOSs of 14.6 and 2.9 respectively, as also shown in **Table 4.41**.

The Quast et al. inhalation study is regarded as the key study for the risk assessment and the risk characterisation in relation to workers. In the Maltoni et al. (1977) study, in which rats were exposed to levels of up to 40 ppm acrylonitrile for 4 hours daily, 5 days a week for a 12-month period, no effect was seen on mortality or on body weight gain. In the Brewer (1976) 90-day study in dogs, deaths were seen at an exposure level of 54 ppm. In contrast, in the parallel 90-day studies in rats and mice carried out by Brewer, effects on mortality and body weight gain were only apparent at an exposure level of 108 ppm. The increased sensitivity of dogs to the toxic effects of acrylonitrile is attributed to lower activity of the detoxifying enzyme rhodanase, which converts cyanide to thiocyanate. The LO(A)EL in the 90-day studies in rats, mice and dogs are however higher than that derived in the Quast 2-year study in rats (as might be expected), and this, coupled with the doubtful validity of the Brewer studies for risk assessment purposes, indicates that the risk characterisation for workers exposed by inhalation based on the Quast study is appropriate.

Consideration of inter-species variation could alter the MOS derived for workers in relation to chronic systemic effects and hence the appropriate conclusion to be reached in relation to this end point. The data on inter-species differences in metabolism (Section 4.1.2.1.1) indicate that mice excrete a higher percentage of administered acrylonitrile as thiocyanate and hence metabolise more to cyanide. This is related in turn to a higher rate of formation of CEO than for rats or humans. Yet the long-term inhalation data on which the risk characterisation is based relates to the rat. The results of Kedderis et al. (1995), (Section 4.1.2.1.1), based on *in vitro* studies in rat, mouse and human microsomal fractions indicate that the rate of conjugation of either ACN or CEO with GSH is lower in humans than in either rats or mice. However

hydrolysis of CEO by epoxide hydrolase is very high, while this detoxification pathway is apparently absent in rodents. This indicates that CEO is detoxified by GSH in rats or mice, but predominantly by epoxide hydrolase in humans. The metabolite from this latter pathway, glycolaldehyde cyanohydrin, $CH(OH_2)$-CH(OH)-CN, is rapidly converted to hydroxyacetaldehyde and hydrogen cyanide, as shown in **Figure 4.1**.

Assuming that the toxicity seen after long-term repeated dosing is mainly due to release of cyanide, the results outlined above indicate that inter-species variation must be taken into consideration in deriving a conclusion on risk for this end point. Based primarily on the work of Kedderis and co-workers, it can be estimated that:

1. CEO formation in humans is approximately 0.67 of that in rats and 0.17 of that in mice (**Table 4.10**);
2. 70% of CEO in the rat is metabolised by the cyanide-releasing pathway (conjugation of CEO in the 3-position of CEO), i.e. a molar fraction of acrylonitrile of 0.7;
3. 95% of CEO in humans is metabolised by the cyanide-releasing pathway, i.e. a molar fraction of acrylonitrile of $0.67 \cdot 0.95 = 0.64$.

The toxicokinetic data of Kedderis et al. would therefore indicate that cyanide levels in the rat and in human should be similar, given a similar absorbed dose. Reflecting, however the lower inhalation rate of human compared with the rat, the absorbed dose for human can be predicted to be lower than that in the rat under the same exposure conditions. Cyanide levels in mice could be anticipated to be appreciably higher due to a higher rate of formation of CEO, although the absence of toxicity at an exposure level of 12 ppm in a recent 90-day study in the mouse should be noted. Cyanide levels will also be higher in the dog, due to the lower activity of rhodanase. It is concluded that the risk assessment for human is reasonably based on the Quast et al. inhalation study, without application of a further assessment factor for inter-species variation, providing a MOS of 14.6 (based on LO(A)EL) or 2.9 (based on NAEL).

It is acknowledged that strict controls on exposure to acrylonitrile apply in the industry for this classified carcinogen, and industry confirm that exposure levels in the workplace are much less than 2 ppm. For example, in the 1990s in production and processing sectors the levels achieved were less than 1 ppm and 0.1–1ppm, respectively. In addition the EU Working Group on Classification and Labelling agreed that acrylonitrile should not be classified with R 48 (risk of serious damage to health on prolonged exposure) based on the information available. Overall however, the human data are difficult to assess in relation to establishment of a dose-response relationship. Many of the findings in the animal repeated dose studies reflect the reported findings in workers. Therefore, for the purposes of this risk assessment, given the difficulties in assessing the human data and the low MOSs achieved, it is concluded that there is concern for workers in relation to the repeated dose (systemic) toxicity end point: **conclusion (iii)**.

Table 4.41 Calculation of margins of safety (MOS) for workers in relation to local, systemic or reproductive toxicity following inhalation exposure

Effect	Reasonable worst case exposure level for workers	NAEL	LO(A)EL	MOS (NAEL)
Local toxicity	2 ppm	4 ppm [1)]	20 ppm [1)]	2
Systemic toxicity	0.28 mg/kg/day	0.82 mg/kg/day [1)]	4.1 mg/kg/day [1)]	2.9
Reproductive toxicity	0.28 mg/kg/day	2.46 mg/kg/day [2)] (NO(A)EL)	5.13 mg/kg/day [2)] (LO(A)EL)	8.8

1) 2-year inhalation study in rats (Quast et al., 1980a)
2) Saillenfait et al. (1993)

Neurotoxicity of acrylonitrile following inhalation exposure

Neurotoxicity and neurofunctional disturbances have been identified as a potential effect of acrylonitrile, and this aspect has been investigated in some detail by several researchers. The neurotoxic effects observed in animals occurred in high-dose exposure scenarios, while neurotoxic effects described in humans have also been related to specific incidents where very high exposures occurred. In relation to risk characterisation this end point is considered to be adequately covered by the N(A)OELs estimated for chronic toxicity.

Therefore, acrylonitrile is of no concern for workers in relation to neurotoxicity following repeated dose exposure: **conclusion (ii)**.

Repeated dose toxicity following dermal exposure

No repeated dose studies using the dermal route have been carried out with acrylonitrile. The possibility of dermal exposure in the workplace cannot however be discounted, although the strict controls in place in the industry to prevent this must be recognised. The possibilities for dermal exposure have been discussed for polymerisation of acrylonitrile to ABS/SAN plastics in Section 4.1.1.1.1 ("Potential for Occupational Exposure"). A worst-case estimate of systemic exposure by this route can be derived using the EASE-model prediction, as outlined above, giving a potential systemic dose of 0.076 mg/kg/day. Workers will, however also be potentially exposed via inhalation, and absorption by this route will contribute to the total body burden, giving a potential worst-case total systemic dose similar to that derived for inhalation exposure (0.28 mg/kg/day). Consideration of the physico-chemical properties of acrylonitrile, its extensive absorption and distribution following exposure by any route and the likelihood that metabolism following dermal absorption will be broadly similar to that following inhalation lead to the conclusion that in relation to the animal studies route-to-route extrapolation is valid and that the LO(A)EL and NAEL derived for the rat from the Quast inhalation study can be used in deriving a MOS. The MOS will therefore be the same as that derived for the inhalation scenario above, i.e. 14.6 (based on LO(A)EL) or 2.9 (based on NAEL). This is comparatively low, and it is concluded as for inhalation exposure above that there is concern for workers in relation to the repeated dose (systemic) end point following dermal exposure: **conclusion (iii)**. The same justification for this conclusion is considered to apply.

Repeated dose toxicity following oral exposure

The Biodynamics study (1980b) provides a NO(A)EL of 3 ppm (0.25 mg/kg/day). The risk characterisation regarding these study data is however dealt with in detail under the section for

consumers, where the oral route of exposure is considered. With regard to workers the oral route is not considered as a possible route concern as exposure via this route should not occur in the workplace. Exposure of workers by the oral route is expected to be minimal assuming normal good hygiene practices in the workplace.

Therefore, acrylonitrile is of no concern for workers in relation to repeated dose toxicity following oral exposure: **conclusion (ii)**.

4.1.3.2.5 Mutagenicity

The results of the mutagenicity and genotoxicity tests indicate that the DNA active compound is the metabolite epoxide CEO, with at best weak evidence of a direct mutagenic effect of acrylonitrile. The interpretation is clearly in accordance with the observations that acrylonitrile is mutagenic mainly after metabolic activation. CEO is mutagenic *in vitro*, but acrylonitrile is negative in *in vivo* genotoxicity tests. The lack of *in vivo* mutagenicity may be due to inactivation of CEO via glutathione conjugation resulting in a failure of acrylonitrile or its active metabolite to reach the target tissues. This inactivation pathway may not exist in *in vitro* test systems.

Recognising that the starting point for the risk characterisation of acrylonitrile in respect of carcinogenicity is that it is a carcinogen for which a threshold cannot be reliably identified, a conclusion (iii) could be considered to be appropriate. In relation to the qualitative likelihood of a mutagenic effect being expressed in exposed workers, a number of factors are relevant:

1. as indicated above, acrylonitrile itself is a weak *in vitro* mutagen, the *in vitro* genotoxicity being largely attributable to the epoxide metabolite CEO. The database in this risk assessment report does not support classification of acrylonitrile as a mutagen;
2. there is no definite evidence of *in vivo* mutagenicity, although the fact that acrylonitrile produces tumours at a number of sites including the brain would indicate that the mutagenic species can reach target tissues *in vivo*. Current research on possible non-genotoxic mechanisms for the carcinogenicity of acrylonitrile may throw more light on this aspect and indeed may eventually result in a reappraisal of the risk characterisation of the carcinogenic effects;
3. *in vitro* metabolism studies have indicated that man metabolises a lower proportion of absorbed acrylonitrile to CEO and has an efficient detoxifying pathway for CEO, epoxide hydrolase, not found in rodents.

Given the above factors, in particular the fact that acrylonitrile is not classified as mutagenic, overall acrylonitrile is of no concern for workers in relation to mutagenicity: **conclusion (ii)**.

4.1.3.2.6 Carcinogenicity

Acrylonitrile is classified as carcinogenic (Cat. 2) on the basis of the results of a number of animal studies, following either oral administration or via inhalation. Given the positive mutagenicity data for the metabolite CEO, acrylonitrile is currently considered to be a carcinogen for which a threshold cannot be reliably identified, and a safe exposure level cannot therefore be estimated for this end point. However, the evidence available, in particular the most recently completed epidemiological studies, does not support a causal relationship between

acrylonitrile exposure and cancer in human. It should be noted that IARC have revised their categorisation of acrylonitrile as a carcinogen from category 2A to category 2B. This was on the basis of the recent epidemiological data, which in IARC's opinion did not permit a conclusion regarding the presence or absence of a causal association between acrylonitrile exposure and cancer.

On the basis of systemic dose following exposure by inhalation, an estimate of the T_{25} has been derived from the Quast data, taking the incidence of the most common tumour type, malignant astrocytomas, as a basis for the calculation. The incidence in males, adjusted for mortality, was 0/97 at 0 ppm, 4/93 at 20 ppm (4.3%) and 15/83 at 80 ppm (18%), and in females was 0/99, 4/99 (4%) and 17/99 (17.2%). The incidence at 80 ppm was statistically significant in both sexes and was used to derive the T_{25}. The daily dose in animals exposed to 80 ppm can be derived as follows: 6 hours · inhalation volume · mg acrylonitrile/m^3 · (5/7) (average over 7 days a week) = 6 hours · 6 l/hr · 180 mg/m^3 · 1/1,000 · (5/7) = 4.63 mg/rat/day. Given a mean bodyweight of 400 g for males and 300 g for females, the daily dose per kg body weight is therefore 11.6 mg for males and 15.4 mg for females. The T_{25} after 2 years is then estimated to be 25/18 · 11.6 mg/kg/day = 16.1 in males and 25/17.2 · 15.4 = 22.4 mg/kg/day for females (see also Section 4.1.2.8.2, Study 2).

As derived in the section on risk characterisation (Section 4.1.3.2.4) for repeated dose toxicity above, a total daily dose for workers exposed by inhalation to 2 ppm acrylonitrile and incidental skin contact of 0.28 mg/kg/day has been estimated. Comparison of this figure with the T_{25} of 16.1 mg/kg/day in the male rat gives a Margin of Exposure of 57.5. It should be noted however that the use of 2 ppm is very much a worst-case scenario. Actual recently measured occupational exposure levels indicate that for production the mean exposure level is < 1.0 ppm and for processing the exposure levels range from 0.1 to 1.0 ppm, even when "high" risk tasks are undertaken such as maintenance and loading.

Alternatively, considering the tumour incidence data presented in **Table 4.21** (Section 4.1.2.8.2), the T_{25} can be estimated to be approximately 125 ppm. Assuming this value for T_{25}, using the US EPA default values (1996) and a worst-case exposure level of 2 ppm (OEL) a Margin of Exposure of 125/2 = 62.5 is derived.

Level of carcinogenic risk related to inhalation of acrylonitrile

Bell and Salem (1990) derived a risk estimate of $3.3 \cdot 10^{-8}$ for a lifetime continuous exposure scenario where a 70 kg man inhales 20 m^3 of air a day, containing 1 part per trillion acrylonitrile. Assuming a linear extrapolation, a rough estimate of risk for workers of approximately $2.4 \cdot 10^{-3}$ can be derived, related to exposure of 2 ppm for 8 hours a day, 5 days a week and a working life of 40 years. Felter and Dollarhide (1997) have recently re-evaluated the database available to support an inhalation cancer risk assessment, using the methodology of the EPA's 1996 cancer risk assessment guidelines. They have derived a risk estimate of between $8.2 \cdot 10^{-6}$ to $1.1 \cdot 10^{-5}$ associated with lifetime continuous exposure to 1 µg/m^3 ($0.44 \cdot 10^{-3}$ ppm).

The estimates of both Beall and Salem (1990) and Felter and Dollarhide (1997) are consistent and closely reflect the factors used to convert occupational exposure to continuous lifetime exposure. These authors comment that that these estimates are 6-8 fold lower than the US EPA's previous estimates of risk, reflecting the conclusion that the weight of evidence of the human studies does not support the conclusion that there is a causal association between exposure to acrylonitrile and lung cancer. However, assuming there is a risk of carcinogenicity in human and using a linear extrapolation approach, an estimate of risk for workers exposed to 2 ppm for 8 hours a day, 5 days a week and a working life of 40 years of between $1.3 \cdot 10^{-4}$ to $1.8 \cdot 10^{-2}$ is

derived (Section 4.1.2.8.2). Alternative approaches to assessment of level of risk are presented in Appendices B (Norway) and C (industry). It should be noted that estimates of risk presented in Appendix B are $4.7 \cdot 10^{-3}$ at an exposure level of 2 ppm, $1.2 \cdot 10^{-3}$ at an exposure level of 0.40 ppm, and $7.0 \cdot 10^{-3}$, when the systemic dose in processing is taken to be 0.6 mg/kg/day. It should also be noted that these risk calculations are based on the supposition that acrylonitrile is a non-threshold carcinogen and a linear extrapolation approach therefore applies. They do not take into account the effect of a possible epigenetic mechanism in the carcinogenicity of acrylonitrile, as discussed in Section 4.1.2.8.5.

On balance, given the Margin of Exposure of 57.5 and while acknowledging the fact that this is a well-controlled carcinogen handled (during production and processing) predominantly in closed systems and with the lower levels of exposure achievable as confirmed by industry, **conclusion (iii)** is considered appropriate.

4.1.3.2.7 Toxicity for reproduction

Although, as indicated above, reproductive toxicity is not considered to be a key health effect in relation to the risk characterisation since the effects seen in animal studies occurred in the same dose range and as a secondary consequence of general toxicity, a characterisation of the risk has nevertheless been carried out. The Saillenfait et al. (1993) study of developmental toxicity in Sprague Dawley rats exposed to acrylonitrile by inhalation has been used as pivotal study. This study showed a dose-dependent reduction in foetal weight in the litters from dams exposed to 0, 12, 25, 50 or 100 ppm acrylonitrile, a 5% decrease being seen at 25 ppm, reaching 13-15% at 100 ppm. Since the reduction in foetal weight was already significant at the 25 ppm level, this represents a LO(A)EL, with a NO(A)EL of 12 ppm being established in the study.

A systemic NO(A)EL dose for the rats in this study can be derived from the NO(A)EL of 12 ppm, using the physiological default values of the US EPA, with an assumed breathing rate in the rat of 0.011 m^3 /hour, an exposure duration of 6 hours/day, an assumed absorption factor of 0.5 and a body weight (female) of 0.35 kg. This gives a systemic NO(A)EL dose of 2.46 mg/kg/day in the female rat. Comparison of this dose with the worst-case daily systemic dose for workers of 0.28 mg/kg/day, assuming exposure to 2 ppm and incidental dermal contact, gives a MOS of 2.46/0.28 or 8.8, as shown in **Table 4.41**. It is concluded on the basis of this MOS and the fact that the effects seen were associated with maternal toxicity that acrylonitrile is of no concern for workers in relation to reproductive toxicity following inhalation (and by inference, dermal) exposure: **conclusion (ii)**.

4.1.3.2.8 Summary of the risk characterisation for workers

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

This conclusion is reached in relation to the following end points: (1) repeated dose (systemic) toxicity, (2) carcinogenicity.

In relation to conclusion (iii) for repeated dose (systemic) toxicity by the inhalation and by route-to-route extrapolation, the dermal route, this primarily reflects the toxicity seen in chronic studies in rats and the relatively low Margins of Safety (MOSs) between anticipated exposure levels and doses producing toxicity. Many of the findings in the animal repeated dose studies are mirrored in reported findings in workers. Overall, however, the human data are difficult to assess

in relation to establishment of a dose-response relationship. The EU Working Group on Classification and Labelling agreed that acrylonitrile should not be classified with R 48 (risk of serious damage to health on prolonged exposure) based on the information available. Nevertheless, for the purposes of this risk assessment, given the difficulties in assessing the human data and the low MOSs achieved, it is recommended that conclusion (iii) be applied to the repeated dose (systemic) toxicity end point. It is however acknowledged that strict controls on exposure to acrylonitrile apply in the industry for this classified carcinogen, and industry confirm that exposure levels in the workplace are much less than 2 ppm. For example in the 1990s, in production and processing sectors, the levels achieved were less than 1 ppm and 0.1-1 ppm, respectively.

In relation to conclusion (iii) for carcinogenicity, it is accepted that there is a risk at any level of exposure, given that acrylonitrile is currently regarded as a carcinogen for which a threshold cannot be reliably identified. The magnitude of this risk has been estimated to lie between $1.3 \cdot 10^{-4}$ to $1.8 \cdot 10^{-2}$ for workers exposed to 2 ppm (the current OEL in a number of EU countries) for 8 hours a day, 5 days a week and a working life of 40 years. A Margin of Exposure (MOE) of 57.5 has been derived, based on a T_{25} of 16.1 mg/kg/day in the male rat obtained from the Quast 2-year inhalation study.

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

This conclusion is reached for the end points of acute toxicity, skin, eye and respiratory irritancy, skin sensitisation, corrosivity, repeated dose (local) toxicity by the inhalation route, neurotoxicity, mutagenicity and reproductive toxicity.

4.1.3.3 Consumers

Acrylonitrile monomer is not sold to the general public/consumer as a pure liquid or as part of a preparation. Within the EU there is no exposure of consumers directly to acrylonitrile monomer. There is however the potential for indirect exposure due to the presence of residual monomer in consumer products (plastics and fibres) produced from acrylonitrile.

The major potential sources for consumer exposure are via the use of/wearing of materials, textiles, furnishings (including carpets), etc. These may contain trace quantities of unreacted acrylonitrile monomer, or via food which is packaged in containers made from acrylonitrile plastics, such as margarine tubs, fruit juice containers, vegetable oil bottles etc. The main routes of exposure are dermal contact due to slow release of acrylonitrile monomer from acrylic fibre clothing and ingestion of foodstuffs containing residual acrylonitrile from the plastic food packaging into the food itself.

Given the potential continuous nature of this exposure, the relevant end points to be addressed in relation to risk characterisation for consumers are as follows: skin sensitisation, repeated dose toxicity, carcinogenicity, mutagenicity and toxicity for reproduction. The risk characterisation for the end points of acute toxicity, irritation and corrosivity is not necessary, given that consumers will never come in contact with acrylonitrile liquid.

4.1.3.3.1 Skin sensitisation

As indicated in Section 4.1.3.2.3 above, in line with current hypotheses regarding the idiosyncratic nature of skin sensitisation in humans, quantitative characterisation of the effect (dose-response relationship) is not possible, and a NO(A)EL for this end point cannot be derived. In practice, however, the risk for consumers related to exposure of monomeric acrylonitrile in clothing or from handling plastics will be extremely low. This reflects the very low levels of residual monomer contained in such products (see Section 4.1.3.3.2 below). Also, the levels allowed in these products are already regulated for under Directive 76/769/EEC with respect to restrictions on the marketing and use of certain dangerous substances and preparations. Therefore acrylonitrile is of no concern for consumers in relation to possible skin sensitising effects: **conclusion (ii)**.

4.1.3.3.2 Repeated dose toxicity

As already indicated, consumers may be exposed to acrylonitrile via ingestion of foodstuffs containing residual acrylonitrile from the plastic food packaging into the food itself and via dermal contact with acrylic fibre clothing. The relevant routes of exposure for the risk characterisation are therefore oral and dermal.

Repeated dose toxicity following oral exposure

Data presented in Section 4.1.1.3.2 indicate that the level of acrylonitrile monomer in foodstuffs packed in acrylonitrile plastics is very low. Commission Directive 90/128/EEC lays down a specific limit for acrylonitrile in food of 20 µg/kg, based on the analytical detection limit, and Specific Migration Limits are laid down for release of monomer from the plastic in order to achieve this standard. In practice, based on the levels of residual monomer in acrylonitrile-derived products (<10 ppm) levels are anticipated to be well below 20 µg/kg. This has been demonstrated by Gawell et al. (1979). Assuming an intake of 1 kg food per day, a maximum of 5% of which has been packaged in an acrylonitrile-derived product, a maximum (worst case) estimate of intake would be 1 µg/person/day. A UK government study (1982) predicted that the likely daily intake of acrylonitrile in soft margarine would be a maximum of 0.3 µg/person/day. Intake from beverages packaged in acrylic plastic such as ABS may be somewhat higher, although the indications are that ABS is not used to any great extent for this end use, PET being the dominant plastic packaging for beverages. Assuming that 50% of all beverages are packed in acrylic-derived plastics (which is regarded as a very high estimate), a daily intake of such beverages of 500 ml and an acrylonitrile content of 20 µg/l, intake from this source could be 5 µg/person/day. This may be added to the intake from food to give a total intake of 6 µg/day. In relation to an adult man weighing 70 kg, this represents an intake of 0.09 µg/kg/day, for a 50 kg woman the intake would be 0.12 µg/kg/day, while for a 30 kg child, it would be 0.2 µg/kg/day.

The Biodynamics (1980b) drinking water study in rats is regarded as the key study for risk characterisation in relation to consumers exposed by the oral route. In this study, as described in Section 4.1.2.6.2 ("2-year drinking water study in rats"), the NO(A)EL for mortality in male rats was 3 ppm. Mortality in female rats was statistically significantly increased at 3 and 30 ppm but not at 10 ppm (**Table 4.17**). Derivation of a dose-response curve for females in the study was complicated by the fact that mortality in female controls was unusually low (29/140 compared with 20/70 at 1 ppm, 23/70 at 3 ppm and 20/70 at 10 ppm). An effect on body weight was also seen, significant in both sexes at 100 ppm acrylonitrile and in males at 30 ppm. It was concluded for the purposes of this risk assessment that 10 ppm represented the more likely NO(A)EL for

females, since there is no substantial body of evidence to suggest that females are more sensitive than males to the toxic effects of acrylonitrile, with 3 ppm being the NO(A)EL in males. The results from this study can be compared with those from the Gallagher et al. (1988) drinking water study, in which 20 ppm represented a NO(A)EL for both sexes, and the 1-year oral gavage study of Maltoni, in which 5 mg/kg/day did not cause any effect on survival or body weight gain.

In the Biodynamics (1980b) study the average daily dose for males receiving 3 ppm in drinking water was calculated to be 0.25 mg/kg/day. The figure for females at 10 ppm was 1.25 mg/kg/day, at 3 ppm was 0.36 mg/kg/day and at 1 ppm was 0.12 mg/kg/day. These systemic doses are comparable with the systemic dose of 0.82 mg/kg/day derived from the estimated No Adverse Effect Level of 4 ppm in the Quast 2-year inhalation study and that of 4.1 mg/kg/day based on the LO(A)EL in that study (see Section 4.1.3.2.4 above). Comparison of these systemic NO(A)EL doses with the estimated intake for consumers from food and beverages gives a MOS of 250/0.09 = $2.78 \cdot 10^{3}$ for an adult male, using the NO(A)EL and estimated systemic dose for the male rat and a MOS of 1,250/0.12 = $1.04 \cdot 10^{4}$ for an adult female, using the NO(A)EL and estimated systemic dose for the female rat, as shown in **Table 4.41**.

For a 30 kg male child the MOS would be 250/0.2 = 1,250, while if the worst-case scenario of a NO(A)EL of 1 ppm in the female rat is chosen, the MOS becomes 120/0.12 = 1,000. The derived MOS thus ranges from 1,000 to 10,400, depending on the sex-specific NO(A)EL chosen and the human population (adult male, adult female, child) for which the risk characterisation is being carried out.

Repeated dose toxicity following dermal contact with acrylic textiles

It is assumed that a consumer wears 1 kg of acrylic fibre (containing 1 mg of acrylonitrile) in clothing during a period of 30 days. During these 30 days 1 mg acrylonitrile is assumed to be fully released from the fibre, 33µg a day. About 0.4 % of this will be absorbed by the skin, contributing to an average daily load of 0.13 µg or 1.8 ng/kg/day for a 70 kg man. Since there are no animal data related to repeated dose toxicity via the dermal route, the NO(A)EL of 3 ppm for male rats established in the Biodynamics (1980b) has been taken as a first approach to be applicable to the dermal route, since acrylonitrile is absorbed efficiently by all routes and the toxicokinetics following dermal absorption are anticipated to be reasonably similar to those following oral administration via drinking water. The NO(A)EL of 3 ppm has been calculated to be equivalent to a daily systemic dose of 0.25 mg/kg/day in the male rat, and comparison of this with the anticipated daily dose from the wearing of fibres by consumers as outlined gives a MOS of $1.39 \cdot 10^{5}$. Consumer products of this nature (clothes) are not therefore considered to present a risk to the consumer with respect to migration and extraction of acrylonitrile.

While the conclusion reached does not give rise to concern, it should be also be noted that the above is a worst-case analysis. The 1 ppm residual acrylonitrile level used in the calculation is considerably in excess of the levels detectable in even freshly spun fibre, and a figure of < 0.1 ppm is more realistic (industry, personal communication). Any residual acrylonitrile on fibre is likely to be greatly reduced before it reaches the consumer because of subsequent processing of the fibre into textiles and then into a garment. This process includes wet dying and washing stages at elevated temperatures. The assessment also assumes that a garment is worn continuously for 30 days, and that all residual acrylonitrile is released within those 30 days.

Repeated dose toxicity following inhalation of acrylonitrile released from acrylic carpets

For the purposes of this risk assessment it is assumed that there is an acrylic fibre content of more than 90% bulk weight and that carpets contain 0.8-1.2 kg/m^2 of acrylic fibres. The residual acrylonitrile monomer content should be ≤ 1 mg per kg fibres and the average weight of the carpet is estimated at 1 kg acrylic fibres/m^2. The acrylonitrile diffusion coefficient in the carpet fibres was estimated by applying the AMEM programme (OECD, 1984) (see Section 4.1.1.3.1). The turnover time can be calculated from the time for release of 50% of total acrylonitrile from the fibre. On the basis of the turnover time, it can be estimated that every 36 days the acrylonitrile level is decreased by a factor of 10. Thus, after 144 days more than 99.99% of the original content will have been lost, resulting in a final level of $5.32 \cdot 10^{-7}$ mg/m^2 at day 144.

The average level in the room over a year (8,760 hours) is estimated as follows. The room is ventilated over a year with $0.2 \cdot 2.5 \cdot 8{,}760 = 4{,}380$ m^3 of air and a total of 1 mg could be released into this volume, giving an average level of 0.23 µ/m^3 ($0.1 \cdot 10^{-3}$ ppm). This assumes that the residual acrylonitrile is completely released in one year. An internal dose for a consumer of 8.2 ng/kg/day due to inhalation of 0.23 µ/m^3 can be calculated, assuming a breathing rate of 0.833 m^3/hour, an exposure duration of 6 hours/day, an assumed absorption factor of 0.5 and a body weight of 70 kg (US EPA default physiological values). A MOS of 0.82/0.0000082 = $1 \cdot 10^5$ can be derived from the estimated NAEL of 4 ppm (= 0.82/mg/kg/day) in rats.

4.1.3.3.3 Summary of repeated dose toxicity for consumers

On the basis of the MOSs derived for repeated dose toxicity via the oral ($2.78 \cdot 10^3$, adult male), dermal ($1.39 \cdot 10^5$) or inhalation ($1 \cdot 10^5$) routes, it is concluded that acrylonitrile is of no concern for consumers in relation to possible effects of repeated dosing via any route of exposure: **conclusion (ii)**.

4.1.3.3.4 Mutagenicity

Reflecting the absence of *in vivo* mutagenicity, the rapid detoxification of the mutagenic metabolite CEO in humans by epoxide hydrolase and the fact that acrylonitrile is not classified as mutagenic, acrylonitrile is of no concern for consumers in relation to mutagenicity: **conclusion (ii)**.

4.1.3.3.5 Carcinogenicity

Using the approach already described for workers, a T_{25} of 16.1 mg/kg/day in the male rat and 22.4 mg/kg/day in the female rat has been estimated (Section 4.1.2.8.2, Study 2) in the Quast 2-year inhalation study in rats. Comparison of this figure with the exposure estimates derived for consumers via the oral, dermal and inhalation routes for repeated dose toxicity above gives MOSs of 16.1/0.00009 = $1.8 \cdot 10^5$ for males, 22.4/0.00012 = $1.9 \cdot 10^5$ for females and 16.1/0.0002 = $8.1 \cdot 10^4$ for a male child via the oral route, 16.1/0.0000018 = $8.9 \cdot 10^6$ for the dermal route (based on the TD_{25} in male rats) and 16.1/0.0000082 = $2.0 \cdot 10^6$ for the inhalation route.

Regarding the level of the carcinogenic risk for consumers related to inhalation of acrylonitrile, as indicated for workers Bell and Salem (1990) derived a risk estimate of $3.3 \cdot 10^{-8}$ for a lifetime continuous exposure scenario where a 70 kg man inhales 20 m^3 of air a day, containing 1 ppt acrylonitrile. Felter and Dollarhide (1997) have recently re-evaluated the database available to

support an inhalation cancer risk assessment, using the methodology of the EPA's 1996 cancer risk assessment guidelines, and have derived a risk estimate of between $8.2 \cdot 10^{-6}$ to $1.1 \cdot 10^{-5}$ associated with lifetime continuous exposure to 1 µg/m^3 ($0.44 \cdot 10^{-3}$ ppm).

Both the Committee on Carcinogenicity of Chemicals in Food (UK) and the Food Additives and Contamination (UK) considered the likelihood of contamination of foodstuffs by acrylonitrile monomer following potential migration of the acrylonitrile from the plastic packaging into the food. Their conclusion was that the levels of contamination were very low and they considered the general public were not at measurable risk from exposure to acrylonitrile via this route/potential source of contamination.

However, as acrylonitrile is a carcinogen for which a threshold cannot be reliably identified, it is considered that **conclusion (iii)** is appropriate, since risks cannot be excluded for all exposure scenarios. However, since the predicted exposures are very low, the risks will be already very low, and this should be taken into account when considering the adequacy of controls feasibility and practicability of further specific risk reduction measures.

4.1.3.3.6 Toxicity for reproduction

Reflecting the very low concentrations of free acrylonitrile to which consumers will be exposed, toxicity for reproduction is not likely to present a risk to this group. MOSs can be derived by comparison of the exposure estimates derived for consumers via the oral, dermal and inhalation routes for repeated dose toxicity with the NO(A)EL of 12 ppm established in the Saillenfait et al. (1993) study of development toxicity in Sprague Dawley rats exposed to acrylonitrile by inhalation, equivalent to 2.46 mg/kg/day in the female rat. This gives a MOS of 2.46/0.00012 = $2.05 \cdot 10^4$ for the oral route, 2.46/0.0000018 = $1.37 \cdot 10^6$ for the dermal route and 2.46/0.0000082 = $3.0 \cdot 10^7$ for the inhalation route. It is concluded that acrylonitrile is of no concern for consumers in relation to toxicity for reproduction following oral intake, dermal contact or inhalation: **conclusion (ii)**.

4.1.3.3.7 Summary of risk characterisation for consumers

MOSs for consumers in relation to potential exposure to acrylonitrile monomer via ingestion, dermal contact with products made from acrylic fibres or polymerised acrylonitrile monomer, or from inhalation as a consequence of release from carpets/textiles in the home are presented in **Table 4.42**. Overall it is considered that there is negligible risk to the consumer.

Table 4.42 Calculation of margins of safety (MOS) for consumers in relation to repeated dose toxicity and toxicity for reproduction

Effect	Reasonable worst-case exposure level for consumers	NO(A)EL	MOS (LO(A)EL)
Systemic toxicity following oral ingestion	0.09 µg/kg/day [1)]	0.25 mg/kg/day [2)]	$2.78 \cdot 10$ [3)]
Systemic toxicity following dermal contact	0.0018 µg/kg/day	0.25 mg/kg/day [2)]	$1.39 \cdot 10$ [5)]
Systemic toxicity following inhalation of acrylonitrile evolved from carpets	0.0082 µg/kg/day	0.82 mg/kg/day [3)]	$1 \cdot 10$ [5)]
Reproductive toxicity	0.00012 mg/kg/day [4)]	2.46 mg/kg/day [5)]	$2.05 \cdot 10$ [4)]

1) Estimated oral systemic dose for males. See text for corresponding values for adult females and children
2) Biodynamics (1980a) drinking water study in rats
3) 2-year inhalation study in rats (Quast al., 1980b)
4) Estimated oral systemic dose for females
5) Saillenfait et al. (1993)

Overall the following conclusions are reached:

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

This conclusion is reached for the end point carcinogenicity.

Acrylonitrile is a carcinogen for which a threshold cannot be reliably identified; therefore it is considered that **conclusion (iii)** is appropriate, since risks cannot be excluded for all exposure scenarios. However, since the predicted exposures are very low, the risks will be already very low, and this should be taken into account when considering the adequacy of controls feasibility and practicability of further specific risk reduction measures.

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

This conclusion is reached for the end points of skin sensitisation, repeated dose toxicity by the inhalation or (by route-to-route extrapolation) the dermal route, mutagenicity and reproductive toxicity.

4.1.3.4 Humans exposed via the environment

Indirect exposure of the general public via the environment from consumption of biota or drinking water and exposure to air containing residual acrylonitrile is theoretically possible although considered to be of low risk due to the extremely low levels calculated or derived from actual monitoring data. Assessment of exposure via this route is addressed in Section 4.1.1.4, and indicates that two populations may be addressed: (1) populations exposed to background levels on a regional or continental basis, (2) populations exposed to potentially higher levels which may pertain near industrial production and processing sites.

In the former case EUSES (Sections 3.1.4.1.5, 3.1.4.2 and 3.1.7) provides values of 2.81 µg/l for the regional concentration of acrylonitrile in water (assuming inherent rather than ready biodegradability), 0.071 µg/m^3 in air, 3.96 µg/kg in wet fish, $1.30 \cdot 10^{-4}$ µg/kg in meat,

$1.66 \cdot 10^{-2}$ µg/kg in plant leaves and $1.30 \cdot 10^{-3}$ in milk. The Mackay level 3 model for predicting environmental concentrations of acrylonitrile (Section 3.1.4.1.5) gives an estimate of $2.37 \cdot 10^{-3}$ µg/l drinking water.

While acrylonitrile is acutely toxic, irritant and sensitising, it is considered that there is no concern for populations in either category (1) or category (2) in relation to these hazards, by any route of exposure, given the very low levels of exposure anticipated: **conclusion (ii)**.

In relation to mutagenicity, reflecting the absence of *in vivo* mutagenicity, the rapid detoxification of the mutagenic metabolite CEO in human by epoxide hydrolase and the fact that acrylonitrile is not classified as mutagenic, acrylonitrile is of no concern for this end point: **conclusion (ii)**.

The end point of concern remaining is carcinogenicity. In relation to risk characterisation for both populations exposed via drinking water, taking the Mackay level 3 model estimate of $2.37 \cdot 10^{-3}$ µg/l acrylonitrile in drinking water, and assuming consumption of 1 litre per day by a 70 kg man, an acrylonitrile intake of 0.034 ng/kg/day can be estimated. Application of the NOEL of 3 ppm (0.25 mg/kg/day in male rats) derived from the 24-month carcinogenicity assay carried out by Biodynamics indicates a MOS of 250/0.000034 = $7.4 \cdot 10^{-8}$. The EUSES estimate for daily intake per day is $8.02 \cdot 10^{-5}$ mg/kg/day or 0.08 ng, approximately three times that derived using the Mackay estimate, and giving a MOS of approximately $2.5 \cdot 10^{-8}$.

A risk estimate (EPA, 1983) was derived for the carcinogenic risk due to acrylonitrile in drinking water, based on the results of three positive drinking water studies in rats. Applying the linear non-threshold dose extrapolation model, an upper-bound lifetime risk of cancer of $1.5 \cdot 10^{-5}$ associated with ingestion of 1 µg/l acrylonitrile was calculated.

In relation to exposure by inhalation of air on a regional basis (population (I)), EUSES estimates a level of 0.071 µg/m^3 in air. Felter and Dollarhide (1996) have derived a risk estimate of between $1.1 \cdot 10^{-5}$ and $8.2 \cdot 10^{-6}$ for an exposure to 1 µg/m^3 acrylonitrile in atmospheric air on a continuous lifetime basis, based on the result of human epidemiological studies and the Quast inhalation study. Adopting the most conservative estimate, exposure to 0.071 µg/m^3 in air results in a risk estimate of $0.8 \cdot 10^{-6}$. Populations living near to industrial production and processing sites may potentially be exposed to higher local levels, and predicted environmental concentrations of acrylonitrile in air for individual plants ranged from 0 to 0.24 mg/m^3, based on the reported emissions (Appendices A.3 and A.4), with the majority lying below 0.03 mg/m^3.

Taking a worst-case figure of 30 µg/m^3 would provide an estimate of risk of $0.4 \cdot 10^{-4}$. It is considered, however that this is an unrealistically high estimate of potential exposure levels in the vicinity of plants, with fenceline monitoring studies from one plant providing an average figure of 0.6 µg/m^3 in air, and another plant indicating no detectable acrylonitrile. It is considered therefore that the estimate of Felter and Dollarhide (1996) of between $1.1 \cdot 10^{-5}$ to $8.2 \cdot 10^{-6}$ is the more realistic for this scenario. Beall and Salem (1990) derived a risk estimate of $3.3 \cdot 10^{-8}$ for a lifetime continuous exposure scenario where a 70 kg man inhaled 20 m^3 of air a day, containing 1 ppt acrylonitrile.

It is concluded however that there could be some concern for carcinogenicity for humans exposed via air, with respect to the immediate vicinity of plants (**conclusion (iii)**), based mainly on potential for local exposure to a carcinogen for which a threshold cannot be reliably identified. This conclusion however should be qualified indicating that risks are already very low. This should be taken into account when considering the adequacy of controls feasibility and practicability of further specific risk reduction measures.

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Workers

Exposure of workers to acrylonitrile has already been discussed in Section 4.1.1.2. Potential exposure to levels of the monomer likely to present a physico-chemical hazard under normal handling and uses could arise during production, polymerisation to acrylonitrile-containing polymers, and during manufacture of acrylic fibres. These processes take place in closed or partially closed systems and stringent exposure controls are in place. Exposure of workers to high levels of acrylonitrile could occur in the accident situation, but such accidental exposure is not addressed in this risk assessment.

4.2.1.2 Consumers

Exposure of consumers to acrylonitrile has already been discussed in Section 4.1.1.3. Consumers may be exposed to acrylonitrile via use of a wide range of acrylonitrile-containing polymers, levels of free monomer are however very low with any unreacted acrylonitrile monomer tightly bound and only released upon application of elevated temperatures.

4.2.1.3 Humans exposed via the environment

Exposure of humans to levels of free monomer via the environment is anticipated to be extremely low.

4.2.2 Effects assessment: Hazard identification

4.2.2.1 Explosivity

Although the standard Annex V tests for explosivity have not been performed on this liquid substance, vapours of acrylonitrile form explosive mixtures with air (Erdölchemie, 1994). The explosive substance:air ratio of acrylonitrile stabilised with 30-40 ppm ammonia has been reported by Nabert and Schön (1980) to lie between 2.8-28 vol/vol at ambient temperature. American Cyanamid (1959), Nabert and Schön (1970) and Groet and Schipper (1974) had earlier (1970) reported the explosive limits to lie between 3.05% and 17%, and these figures have also been cited by Langvardt (1985). The IUCLID datasheet uses the figures of Nabert and Schön (1980).

Spontaneous exothermic polymerisation of acrylonitrile may occur at elevated temperatures, or in the presence of light, acid or alkali (Erdölchemie, 1994), resulting in an explosion. This is prevented by the addition of stabilisers such as ammonia/water or hydroquinone monomethylether (MHQ)/water.

4.2.2.2 Flammability

Nabert and Schön (1970), Groet and Schipper (1974) and Langvardt (1985) all report a flash point of -5°C (open cup method), the experimental origins of this figure not being cited. American Cyanamid (1959) reported a value of 0°C (Tagliabue open cup method), based on unpublished company data and the work of Davis and Wiedeman (1945). A flash point of -1°C is reported by BASF (1994), using the Closed Cup method. These results indicate that acrylonitrile should be classified as highly flammable according to the EU classification criteria.

Nabert and Schön (1970) and BASF (1994) report an autoignition temperature of 480°C, the BASF reference citing the method used as DIN 51 794. A slightly higher figure of 481°C has been reported by American Cyanamid (1959), Groet and Schipper (1974) and Langvardt (1985), no methodological details being available. Unstabilised acrylonitrile undergoes exothermic polymerisation on heating.

4.2.2.3 Oxidising potential

On structural grounds, acrylonitrile will not have oxidising properties.

4.2.3 Risk characterisation

4.2.3.1 Workers

Assessment of physico-chemical hazards has indicated that acrylonitrile is highly flammable and has explosive properties when mixed with air in certain proportions. Spontaneous exothermic polymerisation of acrylonitrile also presents a risk of explosion. The use of closed systems and stringent safety controls indicates that the potential risk to workers is minimal under conditions of normal handling and use. The highest level recorded for a workplace in recent years was 17.1 ppm, which is far below the lower limit in air for explosion of 2.8% (vol/vol).

The risk of fire and explosion is addressed in the safety datasheets of the major producers (e.g. EC Erdölchemie, 1994. BASF AG, 1984). Controls include:

- stabilisation of acrylonitrile monomer,
- exclusion of direct heat and sunlight,
- exclusion of sources of ignition,
- storage in tightly closed containers under cool and well ventilated conditions,
- production and polymerisation in closed systems,
- avoidance of incompatible materials such as stainless steel, aluminium, trace contaminants.

It is concluded that acrylonitrile is unlikely to present a risk to workers due to physico-chemical hazard: **conclusion (ii)**.

4.2.3.2 Consumers

Given the very low levels of free (unreacted) monomer to which consumers are likely to be exposed, it is concluded that acrylonitrile is unlikely to present a risk to this population due to physico-chemical hazard: **conclusion (ii)**.

4.2.3.3 Humans exposed via the environment

Exposure of humans to levels of free monomer via the environment is anticipated to be extremely low and risk due to physico-chemical hazards is minimal, other than in the accident situation: **conclusion(ii)**.

5 RESULTS

5.1 ENVIRONMENT

Acrylonitrile monomer released to the environment as a consequence of production or further processing will distribute primarily to the atmosphere (approximately 66.3%, Mackay level 1, **Table 3.10**) and to the aqueous environment (approximately 33.6%). Redistribution to other environmental compartments is anticipated to be negligible (0.002% estimated to sediment). There is rapid photodegradation, while in the aquatic environment acrylonitrile, while not readily biodegradable based on available information, appears to degrade rapidly in wastewater treatment plants following acclimation, and also degrades in surface water. Up to 99% biodegradation has been reported in simulation tests.

Acrylonitrile is toxic to fish, *Daphnia* and algae, with long-term NOEC's for algae and fish (early life stage test) below 1 mg/l (0.41, 0.5 and 0.17 mg/l, respectively). A Predicted No Effect Concentration (PNEC) of 17 µg/l (assessment factor of 10) in the aquatic environment is derived by application of the assessment factor to the NOEC determined in the fish early life study. Derivation of $PEClocal_{water}$ for acrylonitrile production facilities and for facilities involved in further processing to acrylonitrile-containing polymers and other monomers provided values ranging from 2-53 µg/l, the majority of values lying in the 2-10 µg/l range.

The data for all but one of the 43 sites involved in production and processing of acrylonitrile in Europe, most of which have industrial WWTPs, result in PEC:PNEC ratios of less than 1 for surface water, using a PNEC of 17 µg/l. PEC:PNEC ratios for sediment for these sites are similarly below 1, indicative overall of low concern for the aquatic environment. One site had a PEC:PNEC ratio for surface water of 3.1. This site is located on a large estuary, does not have a wastewater treatment plant and the levels acrylonitrile in effluent were comparatively high compared with other sites, at 35 mg/l. It is concluded that there are concerns for effects on the local aquatic environmental sphere as a consequence of exposure arising from production of acrylic fibres at this site.

Predicted atmospheric concentrations ($PEClocal_{air}$) of acrylonitrile in the vicinity of acrylonitrile production facilities and facilities involved in further processing to acrylonitrile-containing polymers and other monomers were between 0.001 and 0.240 mg/m^3, while results of monitoring have indicated average levels of below 1 $\mu g/m^3$ at the perimeter of acrylonitrile plants. There is a paucity of data about the effects of these low levels of acrylonitrile on species exposed via the atmospheric environment, although the results of the mammalian toxicology reported in Section 4 would indicate a low level of concern. The PEC:PNEC ratios for all the sites included in this report were below 1. In addition, information made available to the authors of this report regarding a catastrophic event which happened outside the EU and during which the contents of a large storage tank containing acrylonitrile was released very rapidly, showed damage to vegetation observed within a 100 m zone of the spill. No damage to vegetation was observed greater than 100 m from the spill where acrylonitrile concentrations of up to 20 ppm were measured, a concentration far greater than the expected fence-line value.

In relation to risk assessment for microorganisms in wastewater treatment plants, PEC:PNEC ratios were in general below 1, indicative of little risk for microorganisms in WWTP. The magnitude of the PEC:PNEC ratio calculated for soil indicates that there is little risk for the soil compartment. The estimate of $PECregional_{soil}$ reflects primarily point source emissions from production or further processing, and diffuse emissions from car exhausts etc. have not been

taken into account. However, even with a significant contribution to $PECregional_{soil}$ from such sources, the PEC:PNEC ratio will still be well below 1. Exposure of species relevant for the food chain to low levels of acrylonitrile in the environment is theoretically possible. Physicochemical considerations and experimental evidence suggest that acrylonitrile is unlikely to bioaccumulate in exposed biota, and toxicity studies in mammalian species provide little evidence of cumulative toxicity in a range of species. Concentrations of acrylonitrile in biota are expected to be very low, and it is therefore concluded that the potential for secondary poisoning is very small.

5.1.1 Aquatic compartment (incl. sediment)

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

This conclusion is reached because of:

- concerns for effects on the local aquatic sphere (including sediment) as a consequence of exposure arising from production of acrylic fibres at a particular site.

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

This conclusion applies to:

- the aquatic compartment including sediment and microorganisms, for production of acrylonitrile and further processing to fibres and other plastics, with the exception of processing to acrylic fibres at one site only.

Since acrylonitrile is toxic to aquatic organisms and is not readily biodegradable, release into the aquatic environment could present some risk to aquatic species in the vicinity of plants producing or further processing acrylonitrile. Information from simulation tests and on the performance of wastewater biotreatment plants in a number of companies indicates, however, that greater than 90% biodegradation is achieved in acclimated WWTPs. The data for virtually all sites involved in production and processing of acrylonitrile in Europe, numbering 43 in all, most of which have industrial WWTPs, indicate PEC:PNEC ratios of less than 1 for surface water, using a PNEC of 17 µg/l. PEC:PNEC ratios for sediment for these sites are similarly below 1, indicative overall of low concern for the aquatic environment. It should be noted, however, that this conclusion applies only at a particular point in time to 42 out of the total of 43 European sites, existing at that time, which provided aquatic release data relating to the period 1994-1996, and cannot be extrapolated generally for the aquatic environment. The specific risk reduction measures (e.g. wastewater treatment) or particular characteristics of the assessed sites (e.g. high dilution factors due to effluent emissions into very large rivers or estuaries) cannot be extrapolated to sites not covered by this risk assessment, for example new sites starting up after the data for this assessment were gathered, or sites located outside the European Union.

One site, located in a coastal position, had a PEC:PNEC ratio of 3.1, and it is concluded that there are concerns for effects on the local aquatic environmental sphere as a consequence of exposure arising from production of acrylic fibres at this site.

In relation to risk assessment for microorganisms in wastewater treatment plants, PEC:PNEC ratios were in general below 1, indicative of little risk for microorganisms in WWTP.

5.1.2 Atmosphere

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

This conclusion applies to:

- the atmosphere for the production and further processing of acrylonitrile.

All 43 production and further processing companies provided data on atmospheric emissions. These showed that emissions were generally low, being reduced by scrubbing of gaseous and volatile wastes before discharge to the atmosphere. Derivation of PEC:PNEC rations for the atmospheric environment provided values of below 1.0 for all sites. Acrylonitrile is also rapidly photodegraded.

5.1.3 Terrestrial compartment

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

This conclusion applies to:

- soil for production and all uses of acrylonitrile.

Risk characterisation for the terrestrial compartment has excluded the possibility of sludge application to land, given information from industry that little or no industrial sludge from acrylonitrile production and processing facilities is spread on land in Europe. The majority of companies providing information on this aspect indicated that contaminated sludge is incinerated together with other wastes. Risk characterisation has therefore been based on the values obtained from EUSES for $PECregional_{soil}$, which results in a very low PEC:PNEC value for soil. This conclusion is however based on the assumption that sludge from the WWTP is not applied to soil, an assumption which is supported for the European Union, based on the data supplied. It cannot be extrapolated to sites not covered by this risk assessment.

5.1.4 Secondary poisoning

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

5.2 HUMAN HEALTH

5.2.1 Human health (toxicity)

Acrylonitrile is a highly volatile liquid with a wide range of uses. Exposure of humans to acrylonitrile is possible in the workplace, during production of acrylonitrile and its use in the manufacture of acrylic fibres, ABS-SAN plastics, nitrile rubbers, other intermediates such as acrylamide and adiponitrile and other end uses. Exposure of consumers is possible as a consequence of use of products manufactured from acrylonitrile, while the general public may be

exposed via the environment to low levels of acrylonitrile released from point and diffuse sources.

Acrylonitrile is acutely toxic to humans, causing irritation of the eyes and nose, weakness, laboured breathing, dizziness, impaired judgement, cyanosis, nausea, and convulsions following accidental exposure to high levels. Neuropathological effects have been reported at high doses. The main toxic effects seen in animals include respiratory changes, cyanosis, convulsions and death. Reported LD_{50} in a number of species range from 28-186 mg/kg, it is also acutely toxic via inhalation and via dermal exposure and is a skin irritant. Acrylonitrile is classified in accordance with EU criteria for all these end points. Data presented in this risk assessment also supports classification as a respiratory irritant (R37), a severe eye irritant (R41) (Risk of serious damage to eyes) and as a skin sensitiser (R43).

In animals repeated exposure to acrylonitrile results in damage to the gastrointestinal tract, central nervous system and adrenal gland. There are occasional reports of liver and kidney damage. The respiratory tract is also affected following inhalation exposure, based on histopathological changes in the nasal turbinates of rats in the Quast et al. (1980b) two-year study. A LO(A)EL of 20 ppm was established in the study, treatment-related local nasal changes being evident at this exposure level, while it was concluded that other systemic, non neoplastic findings in rats exposed to 20 ppm acrylonitrile were secondary to its tumorigenic effects, rather than due to direct systemic toxicity. This LO(A)EL was used as a starting point in the risk assessment in relation to inhalation exposure. A No Adverse Effect Level (NAEL) of 4 ppm for the inhalation route was derived from the LO(A)EL of 20 ppm, by application of a safety factor of 5. In relation to oral administration of acrylonitrile, the N(A)OEL is estimated to be 3 ppm (0.25 mg/kg/day) in drinking water, based on the information from the Biodynamics study (1980a) study in rats.

The results of a range of mutagenicity and genotoxicity tests indicate that acrylonitrile interacts only weakly with DNA and that the DNA-active compound is the metabolite epoxide cyanoethylene oxide, CEO. The negative results obtained in *in vivo* genotoxicity tests with acrylonitrile may be due to metabolism of CEO by glutathione and by (in humans) epoxide hydrolase to produce non-DNA-reactive species. This metabolic detoxification of the epoxide may not occur *in vitro*.

Acrylonitrile is classified as carcinogenic (Carc. Cat.2; R45), based on the results of studies in the rat following either oral (drinking water or gavage) administration or via inhalation. The common target organs identified were the central nervous system (brain and spinal cord), zymbal gland, gastrointestinal tract (tongue, non-glandular stomach and small intestine) and mammary gland. A linear relationship was observed both in the inhalation and the drinking water studies between the incidence of astrocytomas and the dose level of acrylonitrile used. Acrylonitrile is considered to be a carcinogen for which a threshold cannot be reliably identified, and a safe exposure level cannot therefore be estimated.

In relation to reproductive toxicity, at 65 mg/kg via the oral route embryotoxicity and foetotoxicity occurred in the presence of maternal toxicity, but there was also evidence of an effect on foetal development. Given the maternal toxicity, the developmental effects seen may not indicate a true teratogenic hazard. Inhalation of 80 ppm acrylonitrile also caused developmental effects, while foetotoxicity was observed at exposure levels as low as 25 ppm, an exposure level which was again maternally toxic. While acrylonitrile has been reported to damage the testes of rats, no effects on fertility were seen in a 3-generation fertility study.

The assessment of physico-chemical hazards has indicated that acrylonitrile is highly flammable and has explosive properties when mixed with air in certain proportions. Spontaneous exothermic polymerisation of acrylonitrile also presents a risk of explosion, which is minimised by the use of closed systems and stringent safety controls.

5.2.1.1 Workers

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

This conclusion is reached because of:

- concerns for general systemic effects and carcinogenicity as a consequence of exposure arising during the production and processing of the substance.

In relation to conclusion (iii) for repeated dose (systemic) toxicity by the inhalation and, by route to route extrapolation, the dermal route, this primarily reflects the toxicity seen in chronic studies in rats and the relatively low MOSs between anticipated exposure levels and doses producing toxicity. Many of the findings in the animal repeated dose studies are mirrored in reported findings in workers. Overall, however, the human data are difficult to assess in relation to establishment of a dose-response relationship. The EU Working Group on Classification and Labelling agreed that acrylonitrile should not be classified with R 48 (risk of serious damage to health on prolonged exposure) based on the information available. Nevertheless, for the purposes of this risk assessment, given the difficulties in assessing the human data and the low MOSs achieved, it is recommended that conclusion (iii) be applied to the repeated dose (systemic) toxicity end point. It is however acknowledged that strict controls on exposure to acrylonitrile apply in the industry for this classified carcinogen, and industry confirm that exposure levels in the workplace are much less than 2 ppm. For example in the 1990s, in production and processing sectors, the levels achieved were less than 1 ppm and 0.1–1 ppm, respectively.

In relation to carcinogenicity, it is recognised that there is a low risk risk at any level of exposure, given that acrylonitrile is currently regarded as a carcinogen for which a threshold cannot be reliably identified. The magnitude of this risk has been estimated to lie between $1.3 \cdot 10^{-4}$ to $1.8 \cdot 10^{-2}$ for workers exposed to 2 ppm (the current OEL in a number of EU countries) for 8 hours a day, 5 days a week and a working life of 40 years. A Margin of Exposure (MOE) of 57.5 has been derived, based on a T_{25} of 16.1 mg/kg/day in the male rat obtained from the 2-year inhalation study carried out by Quast (1980).

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

This conclusion applies to:

- the end points of acute toxicity, skin, eye and respiratory irritancy, skin sensitisation, corrosivity, repeated dose (local) toxicity by the inhalation route, neurotoxicity, mutagenicity and reproductive toxicity.

5.2.1.2 Consumers

The major potential sources for consumer exposure are via the use of/wearing of materials, textiles, furnishings (including carpets) etc., which may contain a small percentage of unreacted

acrylonitrile monomer, or via food which is packaged in containers made from acrylonitrile plastics such as margarine tubs, fruit juice containers, vegetable oil bottles etc. The main routes of exposure are dermal contact due to slow release of acrylonitrile monomer from acrylic fibre clothing and ingestion of foodstuffs containing residual acrylonitrile from the plastic food packaging into the food itself.

MOSs for consumers in relation to potential exposure to acrylonitrile monomer via ingestion, dermal contact with products made from acrylic fibres or polymerised acrylonitrile monomer, or from inhalation as a consequence of release from carpets/textiles in the home are in the range of 3,000 to 140,000. Overall it is considered that there is a negligible risk to the consumer. However, given that acrylonitrile is considered to be a carcinogen for which a threshold cannot be reliably identified, the following conclusions are reached:

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

This conclusion is reached because of:

- concerns for carcinogenicity.

Risks cannot be excluded for all exposure scenarios, as the substance is identified as a non-threshold carcinogen. The adequacy of existing controls and the feasibility and practicability of further specific measures should be considered. However, the risk assessment indicates that risks are already low. This should be taken into account when considering the adequacy of existing controls and the feasibility and practicability of further specific risk reduction measures.

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

This conclusion applies to:

- the end points of skin sensitisation, repeated dose toxicity by the inhalation or (by route-to-route extrapolation) the dermal route, mutagenicity and reproductive toxicity.

5.2.1.3 Humans exposed via the environment

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

This conclusion is reached because of:

- concerns for carcinogenicity after highest predicted atmosphere concentrations at a local level.

There could be some concern for carcinogenicity for humans exposed via air, with respect to the immediate vicinity of plants, based mainly on potential for local exposure to a carcinogen for which a threshold cannot be reliably identified. This conclusion however should be qualified indicating that risks are already very low. This should be taken into account when considering the adequacy of controls feasibility and practicability of further specific risk reduction measures.

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

This conclusion applies to all other endpoints.

5.2.2 Human health (risks from physico-chemical properties)

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

This conclusion is reached because:

- the risk assessment shows that risks to workers, consumers and humans exposed via the environment related to physico-chemical properties are not expected. Risk reduction measures already being applied are considered sufficient.

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BOD	Biochemical Oxygen Demand
bw	body weight / *Bw*, *bw*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CEPE	European Committee for Paints and Inks
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 90 percent dissipation / degradation

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

IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
OC	Organic Carbon content
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic

P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex IV of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
ThOD	Theoritical Oxygen Demand

UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
VOC	Volatile Organic Compound
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organization
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

Appendix A.1 Local PEC and other emission parameters for surface water for acrylonitrile production plants in Europe

Table A.1 Local PEC and other emission parameters for surface water for acrylonitrile production plants in Europe

In this and subsequent PEC tables, some values are based on detection limits. Thus their PEC is a 'less than' value.

Site	Production t/y	Released t/y	Daily release t/d	WWTP	C, influent mg/l	C effluent mg/l *	Discharge to	Dilution factor	C, water mg/l	C water an mg/l	PEC, wate mg/l	PEC wat.a mg/l	RCR water	PEC, sed mg/kg	RCRstp	RCR sed
1	***120500***	***0.100***	0.0003	Yes	0.167	**0.1000**	***sea***	10	0.010000	0.00822	0.0128	0.0110	0.754	0.01058	0.0200	0.8399
2 (+BB)	***190000***	***4.000***	0.0133	Yes	***3.800***	***0.0013***	***river***	***30***	0.000043	0.00004	0.0029	0.0028	0.168	0.00236	0.0003	0.1871
3	***85000***	***0.043***	0.0001	Yes	***2.500***	0.0036	***sea***	10	0.000363	0.00030	0.0032	0.0031	0.187	0.00262	0.0007	0.2080
4 (+D+EE)	***300000***	***0.031***	0.0001	Yes	0.052	***0.0020***	***river***	**1529**	0.000001	0.00000	0.0028	0.0028	0.165	0.00232	0.0004	0.1843
5	***280000***	***9.300***	0.0310	No	15.500	***5.8000***	***estuary***	***500***	0.011600	0.00953	0.0144	0.0123	0.848	0.01190	n.a.	0.9448
6	***60000***	***0.040***	0.0001	Yes	0.067	***0.3700***	***river***	***2000***	0.000185	0.00015	0.0030	0.0030	0.176	0.00247	0.0740	0.1964
7	***110000***	***0.024***	0.0001	Yes	0.040	***0.0500***	***river***	**4154**	0.000012	0.00001	0.0028	0.0028	0.166	0.00233	0.0100	0.1850
8	***105000***	***0.053***	0.0002	Yes	***2.500***	0.0044	***sea***	10	0.000439	0.00036	0.0032	0.0032	0.191	0.00268	0.0009	0.2130
Total	1250500	13.59														

Note
Numbers in bold italics actual figures
Where a production facility and further processing facility(s) are located at the same site, the production figure in column 2 is for the production site only, but emissions relate to all facilities at the site (as in 4 (+D+EE)
Production figures rounded to nearest 100.
Where an influent concentration was lower than an effluent concentration calculated, the influent was used as the effluent concentration
p.a.release / 300 = daily release t/d
[Daily release (t/d) / STP volume (default 2,000,000 l/d)] x 1,000,000,000 (convert t to mg) = C, influent mg/l
C, effluent = C, influent * fraction to water (EUSES 0.116). This accounts for fraction of 0.85 being degraded, 0.0324 discharged to air, and a fraction of 0.00132 in sludge.
Note that the fraction effluent degraded in STP of 0.885 is from EUSES for readily biodegradable, as agreed for industrial sites with WWTP, but does not apply to site 5
However, it was only necessary to apply these fractions for sites which had not provided effluent concentrations.
For sites 3 and 8, C, effluent = C, influent/80*0.116 and C, influent/66*0.116 respectively, since the acrylonitrile waste stream is further diluted by other aqueous waste (240 m3 in 19.200 m3 and 360 m3 in 24,000 m3 respectively) before discharge into WWTP
C, effluent / dilution factor (default 10) = C, water.
Where a site discharges to an estuary the dilution factor has been derived from the main river flow into the estuary only.
Where additional dilution data has been available for WWTP this has been used in calculating effluent concentrations.
C water + PEC regional = PEC water (PEC regional (EUSES) = 0.00281)
PEC water, annual = C, water annual + PEC regional (PEC regional (EUSES) = 0.00281)
C water * 300/365 = C, water annual
RCR = PEC/PNEC
PNEC surfacewater = 0.017 where NOEC / 10
* C local effluent = PEC STP
RCR stp = PEC stp / PNEC where PNEC = 5 mg/l
PEC sediment = (0.95/1150)*1000*PECwater = 0.8260869 * PECwater
PNEC sediment = 0.0126 mg/kg (Section 3.2.1.4)

Appendix A.2 Local PEC and other emission parameters for surface water for acrylonitrile processing plants in Europe

Table A.2 Local PEC and other emission parameters for surface water for acrylonitrile processing plants in Europe

Site	Processing capacity t/y	Process releases t/y	Daily release t/d	C, influent mg/l	WWTP	C,effluent mg/l	Dilution factor		C, water mg/l	C water. annual mg/l	PEC, water mg/l	PECwater annual mg/l	RCR water	PEC, sed mg/kg	RCR stp	RCR sed
Fibre																
B (scenario 2)	70000	5.750	0.0192	9.583	No	7.200	1123	2nd river	0.0064	0.005	0.0092	0.0081	0.54	0.0076	n.a.	0.60
C (scenario 2)	40000	0.200	0.0007	0.333	No	0.200	38681	estuary	0.0000	0.000	0.0028	0.0028	0.17	0.0023	n.a.	0.18
D (+EE+4)	112000	0.031	0.0001	0.052	Yes	0.002	1529	river	0.0000	0.000	0.0028	0.0028	0.17	0.0023	0.00	0.18
E	78000	294.000	0.9800	490.000	No	35.000	701	estuary	0.0499	0.041	0.0527	0.0438	3.10	0.0436	n.a.	3.46
F	130000	0.235	0.0008	35.000	Yes	0.250	2000		0.0001	0.000	0.0029	0.0029	0.17	0.0024	0.05	0.19
G	62000	0.200	0.0007	80.000	Yes	0.100	20		0.0050	0.004	0.0078	0.0069	0.46	0.0065	0.02	0.51
H	40000	2.134	0.0071	3.557	Yes	0.500	100		0.0050	0.004	0.0078	0.0069	0.46	0.0065	0.10	0.51
J	49000	0.008	0.0000	0.013	Yes	0.002	10		0.0002	0.000	0.0030	0.0029	0.17	0.0024	0.00	0.19
K	78000	0.350	0.0012	10.000	Yes	0.250	59		0.0042	0.003	0.0070	0.0063	0.41	0.0058	0.05	0.46
Total	659000	302.908														
ABS/SAN																
AA	10300	3.600	0.0120	6.000	No	1.160	1400	estuary	0.0008	0.001	0.0036	0.0035	0.21	0.0030	n.a.	0.24
BB (+2)	26000	4.000	0.0133	3.800	Yes	0.001	30		0.0000	0.000	0.0028	0.0028	0.17	0.0023	0.00	0.19
CC	18000	9.000	0.0300	0.500	Yes	0.058	10		0.0058	0.005	0.0086	0.0076	0.51	0.0071	0.01	0.56
DD	5000	0.500	0.0017	0.833	Yes	0.100	151		0.0007	0.001	0.0035	0.0034	0.20	0.0029	0.02	0.23
EE (+D+4)	30000	0.031	0.0001	0.052	Yes	0.002	1529		0.0000	0.000	0.0028	0.0028	0.17	0.0023	0.00	0.18
FF	4000	0.500	0.0017	0.833	No	0.833	7213	river	0.0001	0.000	0.0029	0.0029	0.17	0.0024	0.17	0.15
GG	16000	0.004	0.0000	0.007	Yes	0.009	573	estuary	0.0000	0.000	0.0028	0.0028	0.17	0.0023	0.00	0.19
HH	25000	0.004	0.0000	0.007	Yes	0.001	818		0.0000	0.000	0.0028	0.0028	0.17	0.0023	0.00	0.18
II	27000	0.010	0.0000	0.017	Yes	0.002	10		0.0002	0.000	0.0030	0.0030	0.18	0.0025	0.00	0.16
JJ	12000	0.000	0.0000	0.000	Yes	0.000	10	sea	0.0000	0.000	0.0028	0.0028	0.17	0.0023	0.00	0.18
KK	4500	5.720	0.0191	6.200	Yes	0.032	10	sea	0.0032	0.003	0.0060	0.0054	0.35	0.0050	0.01	0.39
LL (+HHH)	48000	13.200	0.0440	22.000	Yes	0.050	156		0.0003	0.000	0.0031	0.0031	0.18	0.0026	0.01	0.21
MM	16000	0.100	0.0003	0.167	Yes	0.050	125		0.0004	0.000	0.0032	0.0031	0.19	0.0027	0.01	0.21
Total	241800	36.669														

Table A.2 continued overleaf

Table A.2 continued Local PEC and other emission parameters for surface water for acrylonitrile processing plants in Europe

Site	Processing capacity t/y	Processing release t/y	Daily release t/d	C, influent mg/l	WWTP	C,effluent mg/l	Dilution factor		C, water mg/l	C water annual mg/l	PEC, water mg/l	PECwater annual mg/l	RCR water	PEC, sed mg/kg	RCR stp	RCR sed
NB COPOLYMERS																
AAA	***4500***	***8.139***	0.0271	13.565	No	***11.760***	1250	***sea***	0.0094	0.008	0.0122	0.0105	0.72	0.0101	n.a.	0.80
BBB	***1200***	***5.260***	0.0175	***50.000***	Yes	5.800	***24570***		0.0002	0.000	0.0030	0.0030	0.18	0.0025	1.16	0.20
CCC	***1100***	***0.028***	0.0001	0.047	Yes	0.005	10		0.0005	0.000	0.0034	0.0033	0.20	0.0028	0.00	0.22
DDD	***1500***	***0.090***	0.0003	0.150	Yes	***0.018***	10		0.0018	0.001	0.0046	0.0043	0.27	0.0038	0.00	0.30
EEE	***10450***	***3.150***	0.0105	5.250	No	5.250	***14800***		0.0004	0.000	0.0032	0.0031	0.19	0.0026	n.a.	0.21
FFF	***12000***	***2.700***	0.0090	4.500	Yes	***0.050***	10		0.0050	0.004	0.0078	0.0069	0.46	0.0065	0.01	0.51
GGG	***4400***	***3.200***	0.0107	5.333	Yes	***0.050***	10	***sea***	0.0050	0.004	0.0078	0.0069	0.46	0.0065	0.01	0.51
HHH (+LL)	***600***	***13.200***	0.0440	22.000	Yes	***0.100***	***156***		0.0006	0.001	0.0035	0.0033	0.20	0.0029	0.02	0.23
JJJ	***10000***	***4.000***	0.0133	***2.480***	Yes	0.008	10		0.0008	0.001	0.0036	0.0035	0.21	0.0030	0.00	0.24
Total	45750	39.767														
ACRYLAMIDE + ADIPONITRILE																
L	***39000***	***0.000***	0.0000	0.000	Yes	0.000	10		0.0000	0.000	0.0028	0.0028	0.17	0.0023	0.00	0.18
M	***40000***	***0.030***	0.0001	***1.000***	Yes	***0.100***	***150000***	***river***	0.0000	0.000	0.0028	0.0028	0.17	0.0023	0.02	0.18
N	***161000***	***0.000***	0.0000	0.000	Yes	0.000	***500***	***estuary***	0.0000	0.000	0.0028	0.0028	0.17	0.0023	0.00	0.18
O	***23000***	***0.000***	0.0000	0.000	Yes	0.000	10		0.0000	0.000	0.0028	0.0028	0.17	0.0023	0.00	0.18
Total	263000	0.030														

Where a production facility and further processing facility(s) are located at the same site, the production figure in column 2
is for the processing site only, but emissions relate to all facilities at the site (as in 4 (+D+EE)
p.a.release / 300 = daily release t/d
[Daily release (t/d) / STP volume (default 2,000,000 l/d)] x 1,000,000,000 (convert t to mg) = C, influent mg/l
C, effluent = C, influent * fraction to water (EUSES 0.116). This accounts for fraction of 0.85 being degraded, 0.0324 discharged to air, and a fraction of 0.00132 in sludge.
Note that the fraction effluent degraded in STP of 0.85 is from EUSES for 'ready biodegradable'.
For site KK, C, effluent = C, influent/22.5*0.116, since the acrylonitrile waste stream is further diluted by other aqueous waste (40 m3 in 900 m3) before discharge into WWTP.
For site JJJ, C, effluent = C, influent/35*0.116, for the same reason as cited for site KK
The fraction to water (less that lost to air and sludge) has only been applied to sites which have been reported to have an STP.
For other sites the C, effluent = C influent.
It was only necessary to apply these fractions to sites which had not provided actual effluent concentrations.
C, effluent / dilution factor (default 10) = C, water.
C water + PEC regional = PEC water (PEC regional (EUSES) = 0.0028)
C water * 300/365 = C, water annual
RCR = PEC/PNEC PNECwater = 0.017
PEC sediment = (0.95/1150)*1000*PECwater = 0.8260869 * PECwater
PNEC sediment = 0.0126 (Section 3.2.1.4)
Site B, Scenario 2 = discharge to main river via 100 m canal
Site C, Scenario 2 = dilution factor proposed by industry

Appendix A.3 Local PEC and other emission parameters to air for acrylonitrile production plants in Europe

Table A.3 Local PEC and other emission parameters to air for acrylonitrile production plants in Europe

Site	Production t / y	Released t/y	Daily relea t/d	E,air Kg/d	E,effluent mg/l	E air STP kg/d	C, air mg/m3	C air ann. mg/m3	PEC, air mg/m3	PECair,anı mg/m3	RCR air
1	***120500***	***1.235***	0.004	4.12	***0.1000***	0.0032	0.0011	0.001	0.001	0.001	0.002
2 (+BB)	***190000***	***12.400***	0.041	***99.00***	***0.0013***	0.0000	***0.0006***	0.000	0.001	0.001	0.001
3	***85000***	***5.000***	0.017	16.67	0.0036	0.0001	0.0046	0.004	0.005	0.005	0.009
4 (+D+EE)	***300000***	***3.200***	0.011	10.67	***0.0020***	0.0001	0.0030	0.002	0.003	0.003	0.006
5	***280000***	***259.000***	0.863	863.33	***5.8000***	n.a.	0.2400	0.197	0.240	0.240	0.480
6	***60000***	***0.054***	0.000	0.18	***0.3700***	0.0120	0.0001	0.000	0.000	0.000	0.000
7	***110000***	***2.300***	0.008	7.67	***0.0500***	0.0016	0.0021	0.002	0.002	0.002	0.004
8	***105000***	***2.000***	0.007	6.67	0.0044	0.0001	0.0019	0.002	0.002	0.002	0.004
Total	1250500	283									

Note
Numbers in bold italics are figures provided by industry (emissions provided for all sites)
Where a production facility and further processing facility(s) are located at the same site, the production figure in column 2 is for the production site only, but emissions relate to all facilities at the site (as in 4(+D+EE)
n.a. = not applicable
p.a.release / 300 = daily release t/d
Indirect emission to air from STP (EUSES) as fraction of effluent = 0.0324.
This only applies to sites with an STP, i.e. sites 1, 2, 3, 4, 6, 7 and 8.
C air = (direct + indirect emissions to air)* 0.000278 (from TGD)
C air + PEC regional = PEC air
PEC regional (EUSES) = 0.0000708 mg/m3
C air * 300/365 = C air, annual
RCR = PEC/PNEC, where PNEC = 0.5 mg/m3
The total release for site 5 represent 62 tonnes from production (monitored) and 197 tonnes from storage (modelled)

Appendix A.4 Local PEC and other emission parameters to air for acrylonitrile processing plants in Europe

Table A.4 Local PEC and other emission parameters to air for acrylonitrile processing plants in Europe

Site	Processing	Released	Daily releas	E a	E, effluent	E air STP	C, air	C air ann	PEC, air	PEC air, anı	RCR
	t / y	t/y	t/d	Kg/d	mg/l	Kg/d	mg/m3	mg/m3	mg/m3	mg/m3	air
FIBRES											
B	***70000***	***14.000***	0.047	46.67	***7.200***	n.a.	0.013	0.011	0.0130	0.0107	0.0261
C	***40000***	***20.400***	0.068	68.00	***0.200***	n.a.	0.019	0.016	0.0190	0.0156	0.0379
D (+EE+4)	***112000***	***0.085***	0.000	0.28	***0.002***	0.0001	0.000	0.000	0.0001	0.0001	0.0003
E	***78000***	***154.000***	0.513	513.33	***35.000***	n.a.	0.143	0.117	0.1428	0.1174	0.2856
F	***130000***	***26.200***	0.087	87.33	***0.250***	0.0081	0.024	0.020	0.0244	0.0200	0.0487
G	***62000***	***5.000***	0.017	16.67	***0.100***	0.0032	0.005	0.004	0.0047	0.0039	0.0094
H	***40000***	***41.175***	0.137	137.25	***0.500***	0.0162	0.038	0.031	0.0382	0.0314	0.0765
J	***49000***	***13.300***	0.044	44.33	0.002	0.0001	0.012	0.010	0.0124	0.0102	0.0248
K	***78000***	***16.000***	0.053	53.33	***0.250***	0.0081	0.015	0.012	0.0149	0.0123	0.0298
Total	***659000***	***290.160***									
ABS/SAN											
AA	***10300***	***35.000***	0.117	116.67	***1.160***	n.a.	0.032	0.027	0.0325	0.0267	0.0650
BB (+2)	***26000***	***23.500***	0.078	***99.00***	***0.001***	0.0000	***0.0006***	0.000	0.0007	0.0006	0.0013
CC	***18000***	***1.000***	0.003	3.33	0.058	0.0019	0.001	0.001	0.0010	0.0008	0.0020
DD	***5000***	***3.000***	0.010	10.00	***0.100***	0.0032	0.003	0.002	0.0029	0.0024	0.0057
EE (+D+4)	***30000***	***3.100***	0.010	10.33	***0.002***	0.0001	0.003	0.002	0.0029	0.0024	0.0059
FF	***4000***	***4.300***	0.014	14.33	0.833	n.a.	0.004	0.003	0.0041	0.0033	0.0081
GG	***16000***	***73***	0.243	243.0	***0.009***	0.0003	0.068	0.056	0.0676	0.056	0.13525
HH	***25000***	***1.450***	0.005	4.83	***0.001***	0.0000	0.001	0.056	0.0014	0.0556	0.0028
II	***27000***	***0.585***	0.002	1.95	0.002	0.0001	0.001	0.000	0.0006	0.0005	0.0012
JJ	***12000***	***17.000***	0.057	56.67	***0.000***	0.0000	0.016	0.013	0.0158	0.0130	0.0316
KK	***4500***	***11.000***	0.037	36.67	0.032	0.0010	0.010	0.008	0.0103	0.0085	0.0205
LL (+HHH)	***48000***	***5.500***	0.018	18.33	***0.050***	0.0016	0.005	0.004	0.0052	0.0043	0.0103
MM	***16000***	***0.000***	0.000	0.00	***0.050***	0.0016	0.000	0.000	0.0001	0.0001	0.0001
Total	***241800***	***178.435***									

Notes on Appendix 1.4 are provided on the following page

Table A.4 continued overleaf

Table A4 continued Local PEC and other emission parameters to air for acrylonitrile processing plants in Europe

AAA	***4500***	***21.600***	0.072	72.00	***11.760***	n.a.	0.020	0.016	0.0201	0.0165	0.0402
BBB	***1200***	***0.018***	0.000	0.06	5.850	0.1895	0.000	0.000	0.0001	0.0001	0.0003
CCC	***1100***	***0.005***	0.000	0.02	0.005	0.0002	0.000	0.000	0.0001	0.0001	0.0002
DDD	***1500***	***1.000***	0.003	3.33	***0.018***	0.0006	0.001	0.001	0.0010	0.0008	0.0020
EEE	***10450***	***0.600***	0.002	2.00	5.250	n.a.	0.001	0.000	0.0006	0.0005	0.0013
FFF	***12000***	***1.100***	0.004	3.67	***0.050***	0.0016	0.001	0.001	0.0011	0.0009	0.0022
GGG	***4400***	***20.200***	0.067	67.33	0.050	0.0016	0.019	0.015	0.0188	0.0155	0.0376
HHH (+LL)	***600***	***5.500***	0.018	18.33	***0.100***	0.0032	0.005	0.004	0.0052	0.0043	0.0103
JJJ	***10000***	***4.000***	0.013	13.33	0.008	0.0003	0.004	0.003	0.0038	0.0031	0.0076
Total	***45750***	***54.023***									
ACRYLAMIDE + ADIPONITRILE											
L	***39000***	***4.500***	0.015	15.00	***0.000***	0.0000	0.004	0.003	0.0042	0.0035	0.0085
M	***40000***	***1.500***	0.005	5.00	***0.100***	0.0032	0.001	0.001	0.0015	0.0012	0.0029
N	***161000***	***95.000***	0.317	316.67	***0.000***	0.0000	0.088	0.072	0.0881	0.0724	0.1762
O	***23000***	***0.053***	0.000	0.18	***0.000***	0.0000	0.000	0.000	0.0001	0.0001	0.0002
Total	263000	101.053									

Note Numbers in bold italics are figures provided by industry (emissions were provided for all sites)

Where a production facility and further processing facility(s) are located at the same site, the production figure in column 2 is for the processing site only, but emissions relate to all facilities at the site (as in D (+4+EE)

p.a.release / 300 = daily release t/d

Indirect emission to air from STP (EUSES) = fraction 0.0324.

This only applies to sites with STP; i.e. D, F, G, H, J, K, BB, CC, DD, EE, GG, HH, II, JJ, KK, LL, MM, BBB,CCC, DDD, FFF, GGG, HHH, JJJ, K, M, N, O.

C air = (direct + indirect emissions to air)* 0.000278 (from TGD)

C air + PEC regional = PEC air

PEC regional (EUSES) = 0.0000709 mg/m3

The total release for site N represent 62 tonnes from production (monitored) and 54 tonnes from storage (modelled)

Appendix B

Calculation of T_{25} (Prepared by T. Sanner, 1998)

Administered in drinking water (Biodynamics, 1980a)

Male rats

Brain/spinal cord astrocytomas:	Control:	3/200
	2.49 mg/kg/day:	10/99
	Net:	$[(10/99 - 3/200) \cdot 100]/(1 - 3/200) = 9\%$

$$T_{25} = 2.49 \cdot 25/9 = 6.9 \text{ mg/kg/day}$$

Administered by inhalation (Quast et al., 1980b)

Male rats

Astrocytoma:	Control:	0/100
	80 ppm:	15/99
	Net:	15/99

1 ppm = 53.06/24.45 = 2.17 mg/m^3

80 ppm = $2.17 \cdot 80/1000$ = 0.174 mg/l

Daily dose per rat during exposure period
6 hours · inhalation vol per hour (6 l/h, default value) · mg acrylonitrile per l · (5/7) for 5 days a week

$6 \cdot 6 \cdot 0.174 \cdot 5/7$ = 4.47 mg per day

Daily dose per rat (500 g, default value) 4.47 · 1000/500 = 8.94 mg/kg/day

$$T_{25} = 8.94 \cdot 25/15 = 14.9 \text{ mg/kg/day}$$

In the calculation below it is assumed that the uptake in rats and human is the same.

Risk characterisation

Workers

Three scenarios are used for calculation:

1. Occupational exposure limit: 2 ppm = 4.34 mg/m^3
2. Occupational exposure in production: 0.49 ppm = 1.06 mg/m^3 **(Table 4.3)**
3. Occupational exposure (systemic dose) in processing: 0.60 mg/kg/day

In scenarios 1 and 2
Inhalation for a working day of light work is 10 m^3. Working 5 days/week, lifetime of 70 years, working time of 45 years. Weight 70 kg.

1. 2 ppm: $(4.3 \cdot 10 \cdot 5/7 \cdot 45/70)/70$ = 0.28 mg/kg/day
2. 0.49 ppm: $(1.06 \cdot 10 \cdot 5/7 \cdot 45/70)/70$ = 0.07 mg/kg/day

Inhalation
T_{25} = 14.9 mg/kg/day; Dose giving lifetime cancer risk of 10^{-3} = 0.06 mg/kg/day

Scenario 1 represents a lifetime cancer risk of : $0.28/0.06 = 4.7 \cdot 10^{-3}$
Scenario 2 represents a lifetime cancer risk of : $0.07/0.06 = 1.2 \cdot 10^{-3}$

In scenario 3
Working week 5 days. Lifetime 70 years, working time 45 years.

3. 0.60 mg/kg/day: $(0.065/7 \cdot 45/70) = 0.28$ mg/kg/day

Skin and inhalation nearly same doses. T_{25} used to represent mean of T_{25} inhalation and T_{25} oral.

$T_{25} = (14.9+6.9)/2 = 10.9$ mg/kg/day; Dose giving lifetime cancer risk of 10^{-3} = 0.04 mg/kg/day.

Scenario 3 represents a lifetime cancer risk of : $0.28/0.04 = 7.0 \cdot 10^{-3}$

Consumers

Wearing acrylic textiles
Skin absorbency 0.0018 µg/kg/day

T_{25} for oral intake is used.
T_{25} = 6.9 mg/kg/day. Dose giving lifetime cancer risk of 10^{-5} = 0.28 µg/kg/day

Lifetime cancer risk of (0.0018/0.28) $<10^{-6}$

Carpets
Air concentration 0.23 µg/m^3
Inhalation 20 m^3 per day, 70 kg
Inhalation $[(0.23 \cdot 20)/70]$ 0.066 µg/kg/day

T_{25} = 14.9 mg/kg/day. Dose giving lifetime cancer risk of 10^{-5}

Lifetime cancer risk of $(0.066/0.6) = 1.1 \cdot 10^{-6}$

Food packing
Intake from soft margarine 0.3 µg/day = (0.3/70) = 0.004 µg/kg/day.
Oral intake
Lifetime cancer risk of (0/004/0.28) $< 10^{-6}$

Humans exposed via the environment

$2.37 \cdot 10^{-3}$ µg/l drinking water. Daily intake 2 l, body weight 70 kg
$(2.37 \cdot 10^{-3} \cdot 2)/70 = 6.8 \cdot 10^{-5}$ µg/kg/day.

Lifetime cancer risk of (0.000068/0.28) $< 10^{-6}$

Comparison with other risk estimates

The draft report of Beall & Salem (1990) published the following estimates:

Inhalation

Lifetime risk $3.3 \cdot 10^{-3}$. 1 ppt lifetime exposure 70 kg bodyweight 20 m^3 air.
$(1 \cdot 2.2 \cdot 10^{-3} \cdot 20/70)$ $0.63 \cdot 10^{-3}$ µg/kg/day.
$1 \cdot 10^{-5}$ = 0.19 µg/kg/day; Risk at 1 µg/kg/day = $5.3 \cdot 10^{-5}$

Oral intake

1 µg/day gives a lifetime risk of $3.4 \cdot 10^{-6}$. 1 µg/day = 0.014 µg/kg/day.
Lifetime risk of $1 \cdot 1^{-5}$ = 0.04 µg/kg/day; Risk at 1 µg/kg/day = $25 \cdot 10^{-5}$

Table B.1 shows a comparison between the risk characterisation by Beall and Salem (1990) and the risk characterisation carried out in this Appendix B based on T_{25}. The risk is calculated as lifetime cancer risk after uptake of 1 µg/kg/day. The risk after inhalation of acrylonitrile is 3.1 times higher in the paper by Beall and Salem (1990) than that obtained by the use of T_{25}. This is similar to previous findings when comparing results based on the unit risk as used by EPA and T_{25}. The difference is mainly due to the fact that the unit risk represents an upper-bound estimate while no statistical uncertainty is used in the T_{25} calculations.

Table B.1 Risk characterisation using different methods

Study	**Risk at 1 µg/kg/day · 10^{-5}**		**$Risk_{oral}/Risk_{inhalation}$**
	Inhalation	Oral	
Beall and Salem (1990)	5.3	25	4.7
Present (T_{25})	1.7	3.6	2.1
Risk Beall and Salem/RiskT_{25}	3.1	6.9	

The T_{25} based on inhalation is 2.1 times lower than that found for oral intake. On the other hand Beall and Salem (1990) found that the risk for inhalation was 4.7 times lower than that found for oral intake. This indicates that the T_{25} for oral intake is based on other data than those used in the risk characterisation by Beall and Salem (1990). **Table B.1** shows that the risk after oral uptake obtained by Beall and Salem (1990) is 7 times higher than that based on T_{25}.

(Source: Sanner T (1998). Dept. of Environment and occupational Cancer, Oslo, Norway.)
(Dybing et al., 1997).

Appendix C Evaluation of the dose-response of microscopic brain tumour incidence in F344 rats, exposed to acrylonitrile via drinking water

(Biodynamics study, 1980b)

Introduction

The evaluation set out below as performed by ten Berg (1998) provides an extensive analysis of the microscopic brain tumour incidence in F344 rats, exposed to acrylonitrile via drinking water at 5 different concentrations.

The Biodynamics study is the only appropriate study for dose-response modelling because of the following reasons:

- F344 rats were exposed to 5 different dose levels;
- two dose levels were clearly below and two definitely above the maximum tolerated dose;
- the data on individual rats are known (day of death, specific tumour present or not).

The evaluation will not be done according to the linearised multistage model (LMS). Recently Lovell and Thomas (1996) showed that,

- the Maximum Likelihood Estimate (MLE) of the low-dose slope (q1) was unstable and extremely sensitive to small changes in the data;
- the 95% Upper Confidence Limit (UCL) estimate ($q1^*$) preferred by the US Environmental Protection Agency (EPA) was insensitive with only small changes in values being obtained for large changes in data;
- data sets, were there was no statistical significance could give risk estimates similar to those obtained from data sets with clear dose related effects;
- the size of the values of the Virtually Safe Dose (VSD) obtained did not necessarily relate to the biological interpretation of the data sets;
- the value of $q1^*$ obtained was closely related to the top dose used in the study.

The one hit model with an assumed linear relationship between dose and tumour response will not be used for dose-response estimations. It is shown that a linear dose-response is not supported at all by the available data.

An alternative method will be presented below, in which survival and specific tumour rate control the crude observed tumour incidence. It will be shown that the specific tumour rate is related to the dose to a power of about 2. This has a great impact on the low-dose extrapolation within the experimental dose range.

Mortality and brain tumour data

In order to avoid large random fluctuations in low observed tumour incidence the pooled data of the Biodynamics study will be presented. **Table C.1** presents the mortality data.

Table C.1 Mortality data of rats exposed to acrylonitrile via drinking water

Male and female rats, Biodynamics study, mortality						
ppm in drinking water	**0**	**1**	**3**	**10**	**30**	**100**
Groupsize	**279**	**140**	**140**	**140**	**140**	**140**
Day of study						
180	0	0	1	0	0	0
210	0	0	1	0	0	0
240	0	0	1	0	0	0
270	1	0	1	1	0	0
300	1	0	2	1	0	2
330	1	0	2	1	0	4
360	1	0	2	2	0	6
390	1	0	2	3	0	9
420	3	0	2	3	0	11
450	4	0	3	4	0	17
480	4	1	6	5	0	22
510	6	1	10	6	5	30
540	8	2	11	8	6	44
570	10	3	15	9	15	52
600	13	6	19	14	23	61
630	24	12	24	18	30	72
660	35	21	27	21	32	89
690	48	26	33	32	36	96
720	60	32	35	36	47	99
750	69	36	40	44	49	102
780	76	38	47	53	55	110
Interim kill (day 188)	40	20	20	20	20	20
Interim kill (day 369)	40	20	20	20	20	19
Interim kill (day 552)	40	20	20	20	20	20
Terminal kill (day 705, 775)	203	102	93	87	85	30
Total group size F + M rats	399	200	200	200	200	199

The encircled row at day 720 stresses, that till this time both male and female rats were alive and that after day 720 only male rats were present.

Table C.2 Incidence of brain tumour in rats exposed to acrylonitrile via drinking water

ACN brain tumour incidence, male and female rats Biodynamics study						
ppm in drinking water	**0**	**1**	**3**	**10**	**30**	**100**
Groupsize	**279**	**140**	**140**	**140**	**140**	**140**
Day of study						
510	0	0	0	1	0	1
540	0	0	0	1	0	7
570	0	1	0	1	0	9
600	0	1	0	2	1	13
630	0	1	0	2	3	17
660	0	1	0	2	4	23
690	0	1	1	2	5	23
720	0	2	1	2	5	25
750	0	2	1	2	5	27
780	0	2	1	3	7	30
Interim kill (day 188)	0 / 40	0 / 20	0 / 20	0 / 20	0 / 20	0 / 20
Interim kill (day 369)	0 / 40	0 / 20	0 / 20	0 / 20	0 / 20	0 / 19
Interim kill (day 552)	0 / 40	0 / 20	0 / 20	0 / 20	2 / 20	2 / 20
Terminal kill (day 705, 775)	4 / 203	1 / 102	2 / 93	3 / 87	7 / 85	13 / 30
Total groupsize F + M rats	399	200	200	200	200	199
Total brain tum. inc.	4	3	3	6	16	45

These data show that the microscopic brain tumours could only be observed only from day 510 of the study while mortality already started at day 180. The first microscopic brain tumour at the highest dose level was observed at day 510 and at that time already 29 mortalities without any microscopic brain tumours were observed. At a first glance the relationship between dose and microscopic brain tumour incidence does not seem to be very linear. In fact, this table presents crude data and these data should be corrected for mortality. A simple way for correction of mortality is to estimate the Kaplan-Meyer (K-M) probability for having a tumour at death. The Kaplan-Meier probability of specific death cause is the preferred parameter for actuarial analysis of population vital statistics in establishing an annual rate for life insurance in case of specific ailments. Another advantage of the K-M probability is that the derivative to time provides an estimate of the specific tumour rate per age group. The estimation of the K-M-probability for having a tumour is shown in the example below.

In the following example a group of 10 experimental animals is followed. The first 2 mortalities without a tumour occurred in week 61 and 62. In week 63 eight animals are left and one dies with a tumour. So in week 63 the probability to remain tumour free is 7/8 and the probability to have a tumour is 1-7/8 = 1/8 or 0.125. In week 66 again a tumour is found in the 5 surviving animals. The probability to remain tumour free in week 66 is $7/8 \cdot 4/5 = 0.7$. So the probability to have a tumour is 1-0.7 = 0.3 in week 66. Note that the observed rough incidence rate of 2/10 or 0.2 is quite different from 0.3 and does not reflect the real tumour probability in week 66.

Table C.3 Calculation of the Kaplan-Meier probability for having a tumour at death for a population of 10 animals

Week	Death	Death with tumour	Crude tumour incidence	K-M * probability tumour free	K-M * probability of tumour	K-M * probability of tumour
61	1		0	1	0	0
62	1		0	1	0	0
63	1	1	0.1	7/8	1-7/8	0.125
64	1		0.1	7/8	1-7/8	0.125
65	1		0.1	7/8	1-7/8	0.125
66	1	1	0.2	7/8 · 4/5	1-7/8 · 4/5	0.300
67	1		0.2	7/8 · 4/5	1-7/8 · 4/5	0.300
68	1	1	0.3	7/8 · 4/5 · 2/3	1-7/8 · 4/5 · 2/3	0.533
69	1		0.3	7/8 · 4/5 · 2/3	1-7/8 · 4/5 · 2/3	0.533
70	1		0.3	7/8 · 4/5 · 2/3	1-7/8 · 4/5 · 2/3	0.533

* K-M = Kaplan-Meier

It is interesting to note the great difference between the crude observed tumour incidence and the Kaplan-Meier cumulative probability of having a tumour at death. The K-M cumulative probability is equal to the mortality incidence, if the tumour in the table is the only death cause of the population. The tumour and other diseases cause 100 % mortality at day 70. If the tumour were the only cause of death, only 53% of the population would have died from the tumour. Dose-response estimations are not permitted to perform with the crude specific tumour incidence, but should always be carried out with the Kaplan-Meier specific tumour incidence.

The Kaplan-Meier probability for microscopic brain tumours was estimated from the pooled data (male + female rats) at day 720 of the Biodynamics drinking water study.

Table C.4 Calculation of the Kaplan-Meier probability for microscopic brain tumours

Kaplan-Meier microscopic brain tumour probability at day 720						
ACN in drinking water	0 ppm	1 ppm	3 ppm	10 ppm	30 ppm	100 ppm
Crude observed incidence	0	0.014	0.007	0.014	0.036	0.179
Observed K-M incidence	0	0.024	0.009	0.014	0.043	0.291

From **Table C.2** it is also clear, that the K-M incidence does not show a linear relationship with the ACN level in the drinking water. However, it is possible to derive the regression coefficients of a Weibull model, which fit the K-M incidence for brain tumours with the drinking water level. This will be shown in the following text.

Modelling the Kaplan-Meier probability of microscopic brain tumours

The model used is presented in the equation below. The K-M probability of finding a microscopic brain tumour at death is assumed to be dependent on drinking water level *D* and the survival period *Ts*.

$$K/M\ tum.prob.(D,T_s) = 1 - \exp[-(A_0 + A_1 \cdot D^{A_2}) \cdot T_s^{A_3}] \quad (1)$$

D	=	drinking water concentration in ppm
T_s	=	survival period in days
A_{0-3}	=	regression coefficients tumour probability

The K-M-probability was fitted to the drinking water levels by means of iterative non-linear regression analysis. This resulted into the following values of the regression coefficients and their variances and covariances:

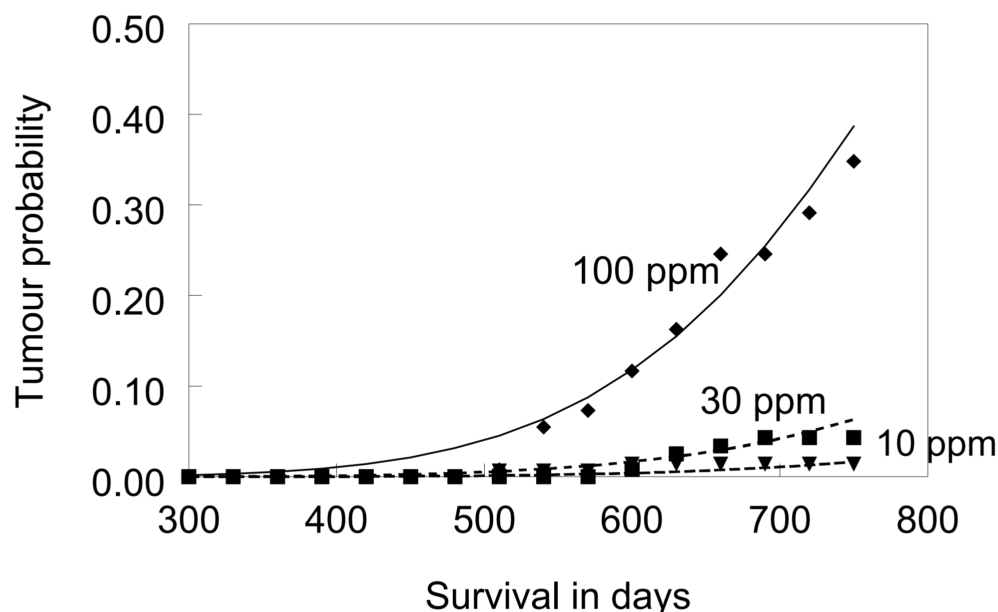
Residual variance = 1.893D-04
Degrees of freedom = 375

A 0 = 2.417D-20	Student t for A 0 = 1.623D+00
A 1 = 3.992D-22	Student t for A 1 = 1.540D+00
A 2 = 1.767D+00	Student t for A 2 = 3.031D+01
A 3 = 6.103D+00	Student t for A 3 = 6.887D+01

variance A 0 0	= 2.219D-40
covariance A 0 1	= 3.057D-42
covariance A 0 2	= 1.082D-22
covariance A 0 3	= -1.250D-21
variance A 1 1	= 6.725D-44
covariance A 1 2	= -6.847D-24
covariance A 1 3	= -2.092D-23
variance A 2 2	= 3.399D-03
covariance A 2 3	= 2.234D-04
variance A 3 3	= 7.854D-03

Remarkable is the rather small residual variance, which indicates that the model describes the K-M tumour probability dependent on drinking water level and survival time quite well.

The model estimated and the observed Kaplan-Meier microscopic brain tumour probability were plotted against the concentration in drinking water.



The smooth lines in the graphical picture are the model estimated and the markers are the observed Kaplan-Meier tumour probability. It is clear from the presented regression coefficients, that the tumour probability is related to the concentration in drinking water to the power of 1.77.

How can this plot be connected to a risk level of having a microscopic brain tumour in 1 of 1,000 over lifetime? It is proposed, that the risk level of 1 on 1,000 over lifetime is more or less identical to 5 on 10,000 at 50% survival of the population. The Kaplan-Meier tumour probability can be estimated at 50% survival if the 50% survival time is known. The 50% survival period was estimated to be 851 days. The risk level of 5 on 10,000 at 50% survival at the 95% confidence level was estimated to occur at a drinking water level of 1.1 ppm. In case of F344 rats this is equivalent with a daily dose of 0.11 mg/kg/day. It should be noted, that the low estimated risk level at 1.1 ppm is within the experimentally tested range of drinking water levels.

In case of extrapolation to occupational exposure the daily dose have to be corrected for number of working days per year and the number of working years during life. It is assumed, that a worker will be occupationally exposed during 240 days per year of 365 days for a period of 40 years on a lifetime of 70 years. This produces a correction factor of $365/240 \cdot 70/40 = 2.66$. This means, that an additional risk of finding a microscopic brain tumour is 1 on 1,000 over lifetime at a daily occupational dose level of 0.29 mg/kg/day. In case of a body weight of 70 kg this is equivalent with a daily intake of 20.5 mg.

Acrylonitrile is only for 50% retained by inhalation. This means that of 41 mg inhaled acrylonitrile only 20.5 mg will be retained in the body. A worker inhales over a working day 10 m^3 of air. If 4 mg acrylonitrile is present in workroom air (1.8 ppm) and a worker is as sensitive as an F344 rat, the worker would have an additional risk of 1 on 1,000 that a microscopic brain tumour will be found at death in his brain without neurological deficiencies. It

should be noted, that the microscopic brain tumours in rats were never the cause of death and that signs of neurological deficiencies were never observed during the Biodynamics study compared to the control population of rats. On the basis of the vital statistics of the Dutch population in 1992 the lifetime probability of dying from a fatal brain tumour was 3 to 4 on 1,000 persons of the general population. A fatal brain tumour is much larger in size than the microscopic brain tumours in the Biodynamics study and is easily to be detected by gross pathology or by a PMR scan. In this connection it is not surprising, that brain tumours are not detected in epidemiological studies in which workers were exposed to 2 ppm for a working life.

Final remarks

The Kaplan-Meier incidence of specific death causes is used by actuaries to establish the specific death probability of persons, who would like to have a life insurance. So the K-M incidence is a generally accepted concept in human epidemiological risk analysis providing reliable probability estimates of dying from specific causes. It is a pity, that this concept was never used in analysing chronic animal toxicity studies and that the probability of dying from specific causes was directly derived from the crude observed specific tumour incidence. Generally, this is not an acceptable procedure in epidemiology.

It is of course possible to simulate the crude observed tumour incidences, but this is not possible with a simple model. This is only possible when the total mortality and the specific tumour rate are considered simultaneously according to the equation below.

The specific crude observed tumour incidence ($P_{spec.tum.incid.}$) may be estimated according to equation 2 on the condition, that the specific tumour rate and the survival can be described as a function of the daily dose and the survival period. The specific tumour rate as a function of daily dose and survival period is the derivative to the time of the Kaplan-Meier tumour probability. The survival as function of daily dose and time can be directly derived from the observed mortality.

$$P_{spec.tum.incid.}(D,T_s) = \int_0^{T_s} Spec.tum.rate(D,t) \cdot Surv(D,t) \cdot dt \quad (2)$$

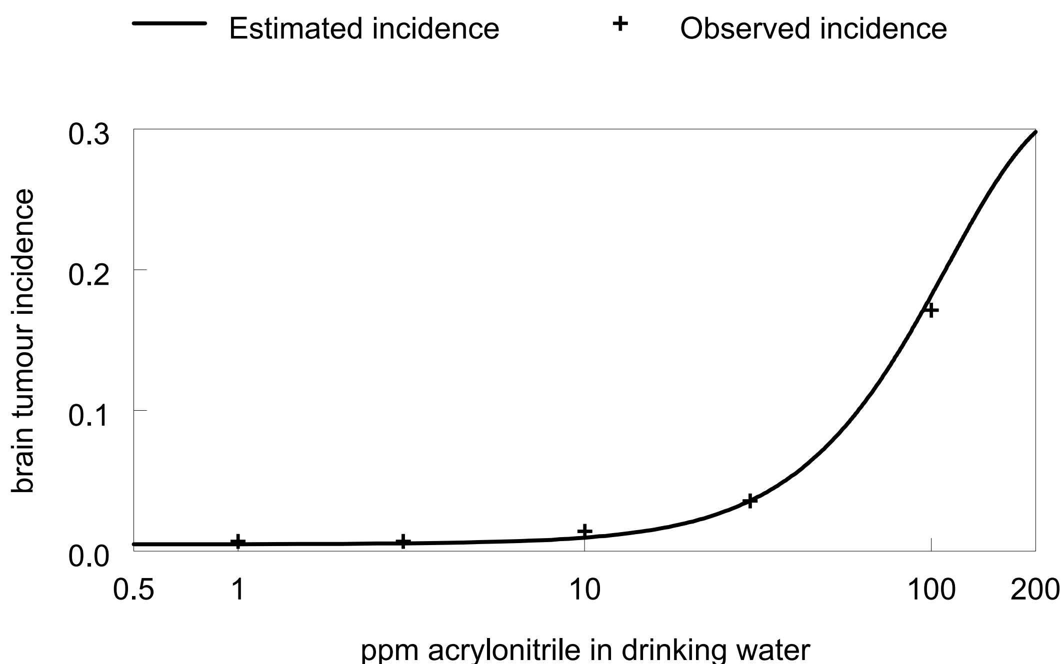
$$K - M\,tum.inc.(D,T_s) = 1 - \exp[-(A_0 + A_1 \cdot D^{A_2}) \cdot T_s^{A_3}] \quad (3)$$

$$Spec.tum.rate(D,T_s) = \frac{dKM/dt}{1 - KM} = A_3 \cdot (A_0 + A_1 \cdot D^{A_2}) \cdot T_s^{A_3 - 1} \quad (4)$$

$$Survival\ (D,T_s) = \exp[-(B_0 + B_1 \cdot D^{B_2})T_s^{B_3}] \quad (5)$$

KM	=	*K-M tum.inc.(D,Ts)*
D	=	drinking water concentration in ppm
T_s	=	survival period in days
A_{0-3}	=	regression coefficients tumour probability
B_{0-3}	=	regression coefficients survival

In the figure below the estimated crude tumour incidence according to equation 1 and the observed crude tumour incidence is plotted against the dose level.



One should realise, that the smooth line was the result of the integration over time of the product of the specific tumour rate and the survival, producing an excellent fit to the experimental observations. One should take notice of the flattening of the response curve at the higher dose level above 100 ppm. This reflects the empirical finding, that the microscopic brain tumour incidence will never achieve 1, because mortality by other causes will prevail. This observation supports the correctness of the quantitative evaluation, presented in this note.

Some objections could be raised against the pooling of tumour incidences of male and female rats. The justification for this is to avoid the influence of natural fluctuations in low incidences on the dose-response modelling.

The model analysis was also carried out with the measured dose levels in mg/kg/day for male and female rats. This resulted in an estimate of the dose level (corresponding with a 95% upper confidence of a risk of 1 on 1,000) somewhat higher (0.12 mg/kg/day) than using the drinking water concentration of acrylonitrile as a dose level (0.11 mg/kg/day).

Kaplan-Meier brain tumour probability in the Quast inhalation study (1980)

$$KM - tum.prob.(d,t) = 1 - \exp\left[-A_1 \cdot d^{A_2} \cdot t^{A_3}\right]$$

residual variance = 7.322D-05
Degrees of freedom = 5

A 1 = 9.149D-16 Student t for A 1 = 4.248D-01
A 2 = 1.450D+00 Student t for A 2 = 9.191D+00
A 3 = 4.111D+00 Student t for A 3 = 1.201D+01

variance A 1 1 = 4.638D-30
covariance A 1 2 = -1.044D-16
covariance A 1 3 = -7.054D-16
variance A 2 2 = 2.489D-02
covariance A 2 3 = 9.114D-04
variance A 3 3 = 1.173D-01

Table C.5 Quast inhalation study in Sprague Dawley rats

ppm	Estimated lifetime risk
67.05 (T_{25})	0.250
1.422	0.001
0.2905	0.0001
0.01213	0.000001

Table C.6 Quast inhalation study in Sprague Dawley rats (95% upper confidence limit)

ppm	Estimated lifetime risk (95% Upper Conf. Limit)
62.05 (T_{25})	0.250
0.7618	0.001
0.1356	0.0001
0.004586	0.000001

T_{25} according to Erik Dybing, Tore Sanner, Henk Roelfzema et al. (1997)

The analysis of the Quast inhalation study reveals, that an estimated level of 1.4 ppm in rats (0.76 ppm upper 95% confidence limit of a risk of 0.001) will cause an additional lifetime risk of about of 1 on 1,000.

Choice of chronic study for quantitative risk assessment

The inhalation study of Quast et al. (1980a) and the Biodynamics study (1980b) in Fisher 344 rats are more or less equivalent considering mortality and incidence and type of tumours. The Biodynamics study has to be preferred for quantitative risk assessment, because:

- the natural mortality was considerably smaller than in the Quast inhalation study,
- all exposure levels in the Quast inhalation study exceeded the maximum tolerable dose level,
- of the 5 exposure levels in the Biodynamics study were 3 below and only 2 above the maximum tolerable dose level,
- the number of animals exposed in the Biodynamics study were much more in number than in the Quast inhalation study.

Conclusion

Quantitative risk assessment of brain tumour incidence by exposure to acrylonitrile was carried out on the basis of the Biodynamics drinking water study and on the basis of the Quast inhalation study. In this analysis the brain tumour incidence data were corrected for mortality, which was considerable at the higher dose levels. The Kaplan-Meier brain tumour probability was derived from the data and mathematically modelled according to a Weibull model with dose and survival time as independent parameters.

The relation between dose and Kaplan-Meier tumour incidence appeared to be non-linear. The dose levels at 50 % survival time were estimated, which caused an additional brain tumour incidence of 0.125, $5 \cdot 10^{-4}$, $5 \cdot 10^{-5}$ and $5 \cdot 10^{-7}$ corresponding with a full lifetime risk of 0.25, 1 on 1,000, 1 on 10,000 and 1 on a million. The dose levels corresponding with a full lifetime risk of 1 on 1,000 are in the same order of magnitude as present occupational exposure levels.

However, some caution is required in using this data in the scope of risk assessment. The brain tumours in rats in the Quast inhalation and the Biodynamics drinking water study were tumours, that could only be detected by histopathology and were never the primary cause of death. Even clinical signs of neurological disturbance were hardly seen and not different from control animals.

Moreover, in the analysis no threshold is assumed to exist in the dose-response for brain tumours due to acrylonitrile exposure because of a supposed genotoxic mode of action via cyano-ethylene oxide. However, there remains the possibility for a non-genotoxic mechanism, because DNA-adducts were never detected in brain tissue. Finally, in retrospective cohort mortality studies in more than 30000 workers occupationally exposed to acrylonitrile no increase of cancer could be detected.

Appendix D Modelling of exposure

With regard to whether or not skin absorption of airborne acrylonitrile is an important route of exposure, Rogaczeska (1975), observed that the uptake of acrylonitrile vapour in rabbits via the dermal route was 1% of the uptake via the inhalatory route.

Rogaczweska and Piotrowski (1968) observed a dermal permeation rate in volunteers of 0.6 mg/cm^2/hour and van Hooidonk (1986) found a dermal permeation rate for human skin *in vitro* of 3.6 mg/cm^2/hour in the case of skin contact with pure acrylonitrile. It is possible to derive the permeation coefficient by dividing the permeation rate by the water solubility of acrylonitrile (Wilschut et al., 1995) of 73 mg/cm^3. This results in a permeation coefficient between 0.008 and 0.05 cm/hour for aqueous solutions of acrylonitrile. This may be converted into the permeation coefficient in air by multiplying with the water/air partition coefficient of acrylonitrile (+ 275 reciprocal dimensionless Henry coefficient). This results in an estimated permeation coefficient in air between 2.2 and 13.8 cm/hour. This is relatively small compared to the diffusive transfer of 400 cm/hour in air, so the permeation through the skin is the controlling factor. With the help of the permeation coefficient in air the dermal uptake from air may be compared to the uptake by inhalation. A rabbit inhales 0.015 m^3 of air per kg body weight per hour and has a dermal surface area of 725 cm^2 per kg body weight. At a concentration of 1,000 mg/m^3 in air the following absorption can be estimated:

- 15.5 mg via inhalation. In the case of 50 % retention this results in an actual uptake of 7.75 mg per kg body weight;
- between 1.6 and 10 mg per kg body weight via dermal uptake (= $0.001 \cdot 725 \cdot$ permeation coefficient [2.2 and 13.8 respectively]). This is 20 % to 129 % of the absorption by inhalation.

The estimated dermal uptake via air, derived from the permeation coefficient of pure acrylonitrile (or saturated acrylonitrile in water) in contact with skin is much higher than that experimentally observed by Rogaczewska (1975). The explanation for this finding might be, that by direct contact of liquid acrylonitrile with the skin (Van Hooidonk, 1986) a reaction possibly occurs with skin proteins, which increases the permeability. This is more or less supported by the relatively long lag time (reaction time with skin proteins?) of 20 to 20 mins, whilst most compounds in this class (low molecular weight and octanol/water partition coefficient) have a lag time between 5 and 10 minutes.

Modelling using the SKINPERM Programme (ten Berge, 1996) predicts absorption by vapour more appropriately, because the vapour of acrylonitrile will not result in a concentration in the stratum corneum sufficiently high so as to react with the tissue macromolecules. Presented below is an explanation of the SKINPERM Model used for the estimation of permeation of values through the skin.

Diffusion through the skin (Wilschut et al., 1995)

Permeation coefficient through the lipid fraction of the stratum corneum (K_{lip}):

$$\log K_{lip} = -1.326 + 0.6097 \cdot \log Kow - 0.1786 \cdot Mw^{0.5}$$

Permeation coefficient of the protein fraction of the stratum corneum (K_{pol}):

$$K_{pol} = \frac{0.0001519}{\sqrt{Mw}}$$

Permeation coefficient of the watery (epi) dermal layer (K_{aq}):

$$K_{aq} = \frac{2.5}{\sqrt{Mw}}$$

The water-air partition coefficient (K_{wa}):

$$K_{wa} = \frac{R \cdot T \cdot Wsb}{Vp \cdot Mw} \quad R = 8.314 J/Mol/°K \quad T = 298°K$$

K_{wa}	=	water/air partition coefficient
R	=	gas constant ($J/Mol/^0K$)
T	=	temperature (^0K)
Wsb	=	water solubility (g/m^3)
Vp	=	vapour pressure (Pa)
Mw	=	molecular weight

$$Kp_{water-skin} = \frac{1}{\frac{1}{K_{lip} + K_{pol}} + \frac{1}{K_{aq}}}$$

$$Kp_{air-skin} = \frac{1}{\frac{1}{K_{lip} + K_{pol}} + \frac{1}{K_{aq}}} \cdot K_{wa}$$

$Kp_{water-skin}$	=	permeation coefficient from aqueous solution to skin (cm/h)
$Kp_{air-skin}$	=	permeation coefficient from air to skin (cm/h)

<u>Diffusion through the air boundary layer on the skin</u>

The air diffusion constant (Dair, in cm^2/hour):

$$Dair = 360 \cdot \sqrt{\frac{76}{Mw}}$$

Permeation coefficient of the air boundary layer on the skin (Kp_{air}, hour/cm):

$$Kp_{air} = \frac{Dair}{\delta}$$

Based on a study of Lotens and Wammes (1993), d is assumed to be 3 cm. Finally, the overall $Kp_{air\text{-->}air\ layer\text{-->}skin}$ (in cm/hour) is controlled by the diffusion through the air boundary layer on the skin (Kp_{air}) and diffusion through the skin (Kp_{sk}):

$$Kp_{air->airlayer->skin} = \frac{1}{\frac{1}{Kp_{air-skin}} + \frac{1}{Kp_{air}}}$$

OUTCOME of SKINPERM programme

Substance name	ACRYLONITRILE
CAS number	107-13-1
Molecular weight	53
Vapour pressure (Pa, 25°C)	13,330
Solubility in water (25°C, mg/l)	75,000
Log[octanol/water part.] (25 °C)	0.16

Vapour of ACRYLONITRILE

Skin permeation coefficient (cm/hr)	0.7731
Latency time (minutes)	4.909
Concentration in air (mg/m^3)	1
Duration of skin contact (minutes)	60
Skin exposure surface (cm^2)	18,000
Thickness air layer (cm)	3
Without considering skinpeeling	

Permeation rate equil. (mg/cm^2/hr)	0.0000007731
Storage in Stratum Corneum (mg)	0.003309
Uptake permeation skin (mg)	0.01495
Ratio(skin permeation/lung retention)	0.01495

In SKINPERM it is assumed, that a worker inhales 1 m^3 of air per hour and that 100% of inhaled material is retained. In the case of 50% retention the ratio (skin permeation/lung retention) becomes about 3%. Therefore should occupational exposure levels exceed 30 times the threshold limit value, in fact, respiratory protection would not be deemed sufficient for protection of the worker against such a high exposure.

However modelling by SKINPERM does not take into account reaction with tissue macromolecules and so should not be used in estimating permeation of skin in contact with pure acrylonitrile. It is recommended that experimental observations should be used in preference when such data are available.

European Commission

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acrylonitrile, Volume 32

Editors: S.J. Munn, R. Allanou, K. Aschberger, F. Berthault, J. de Bruijn, M. Luotamo, C. Musset, S. O'Connor, S. Pakalin, A. Paya-Perez, G. Pellegrini, S. Scheer, S. Vegro.

2004 – X pp., 310 pp. – 17.0 x 24.0 cm

Environment and quality of life series

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

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

The environmental risk assessment for acrylonitrile concludes that there is a concern for the aquatic compartment as a consequence of exposure arising from production of acrylic fibres at one site.

The human health risk assessment for acrylonitrile concludes that there is a concern for workers, consumers and humans exposed via the environment as the substance is classified as a non-threshold carcinogen.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, private or national.

European Commission – Joint Research Centre
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European Union Risk Assessment Report

acrylonitrile

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