European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

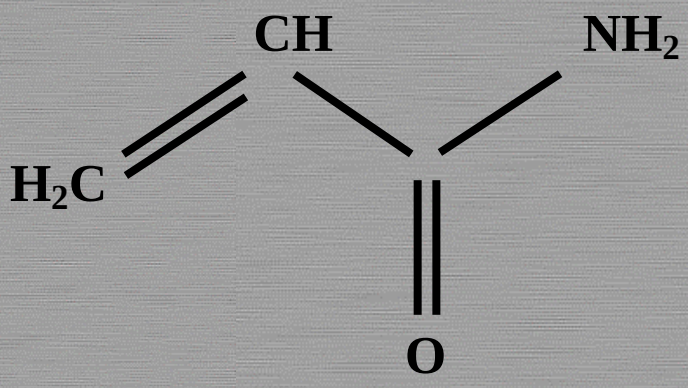
Existing Substances

European Union Risk Assessment Report

CAS No: 79-06-1

EINECS No: 201-173-7

acrylamide



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European Union Risk Assessment Report

ACRYLAMIDE

CAS No: 79-06-1

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RISK ASSESSMENT

Cataloguing data can be found at the end of this publication

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ACRYLAMIDE

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RISK ASSESSMENT

Final Report, 2002

United Kingdom

This document has been prepared by the UK rapporteur on behalf of the European Union. The scientific work on the environmental part was prepared by the Building Research Establishment (BRE), by order of the rapporteur.

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Date of Last Literature Search : **1995**
Review of report by MS Technical Experts finalised: **1999**
Final report: **2002**

(The last full literature survey was carried out in 1995 - targeted searches (for example on grouting) were carried out subsequently, and information found through scanning certain sources has also been included).

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

[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: 79-06-1
EINECS no: 201-173-7
IUPAC name: acrylamide

Environment

Conclusion (i) There is need for further information concerning the toxicity of the substance to terrestrial organisms.

This conclusion applies to the terrestrial ecosystem for use of acrylamide-based grouts in construction applications. Both the PEC and the PNEC for this use could be refined. However, the control strategy for the aquatic ecosystem is also expected to remove any risk to the terrestrial ecosystem, and hence no specific activity is considered necessary at this time. Any further information and/or testing requirements should await the outcome of the risk reduction measures on releases to the environment.

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

This conclusion applies to the aquatic and terrestrial ecosystems (production of acrylamide, production of polyacrylamides, use of polyacrylamides and use of acrylamide based grouts in pipeline and sewer repairs and manhole sealing operations), microorganisms in the sewage treatment plant, atmosphere and accumulation via the food chain (secondary poisoning).

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 ecosystem for use of acrylamide based grouts in construction applications, and to indirect exposure of other organisms through contaminated water from the same use.

Human health

Human health (toxicity)

Workers

In view of the carcinogenic and mutagenic nature of acrylamide and in view of the low MOS values obtained for neurotoxicity and reproductive toxicity in some exposure scenarios **conclusion (iii)** is 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 applies to

- concerns for mutagenicity and carcinogenicity as a consequence of exposure arising from production of the substance, use as an intermediate in the chemical industry for the

production of polyacrylamide, use of polyacrylamide, use of polyacrylamide gels for electrophoresis and use of acrylamide based grouts (small and large scale applications),

- concerns for neurotoxicity and reproductive toxicity as a consequence of exposure arising from the small and large scale use of acrylamide based grouts.

Consumers

Polyacrylamide enters a range of consumer products such as soap, shaving foam and hair gels, and gardening products. There are no measurements available, but estimations of consumer exposure lead to values approximately 50 times lower than those encountered occupationally with the major contribution thought to arise from the use of polyacrylamide in cosmetics. Although thresholds cannot be reliably identified, the risk of mutagenicity and carcinogenicity is considered to be very low, therefore **conclusion (iiia)** applies.

Conclusion (iiia) Risks cannot be excluded for all other 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.

Humans exposed via the environment

In relation to scenarios except where grouts have been used, acrylamide enters the environment directly via industrial emissions but also indirectly through the addition of polyacrylamide as a flocculating agent to drinking water. Although some measured data are available, these are not considered to be entirely adequate and the risk characterisation with respect to human health is based on the use of modelling techniques. The estimated exposures, for reasonable worst-case scenarios are low.

In relation to the use of acrylamide grouts in large-scale operations, the estimated exposures for reasonable worst-case scenarios are high. Thresholds for genotoxic and carcinogenic effects cannot be reliably identified, and these exposure levels give rise for concern. There is further concern for the threshold effects of neurotoxicity and reproductive toxicity. The result of the assessment of indirect exposure via the environment for the large-scale use of grouts is that **conclusion** (**iii**) applies.

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

- concerns for neurotoxicity, reproductive toxicity, mutagenicity and carcinogenicity as a consequence of exposure resulting from the use of acrylamide based grouts in large-scale construction applications.

In relation to the use of acrylamide grouts in small-scale operations, the estimated exposures for reasonable worst-case scenarios are low. Although there may be some residual risk of mutagenicity and/or carcinogenicity this is likely to be very low. The result of the assessment of

indirect exposure via the environment for scenarios except the large-scale use grouts is that **conclusion (iiia)** applies.

Conclusion (iiia) Risks cannot be excluded for all other 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.

Combined exposure

Human exposure to acrylamide indirectly via the environment from sources other than using grouts in large-scale operations is clearly negligible. In addition, exposure via consumer products is also very small. The most significant route of exposure is in occupational settings, the contribution from the environment and from consumer products is negligible in comparison and does not add significantly to the overall body burden.

Human health (risks from physico-chemical properties)

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

Conclusion (**ii**) is reached because there are no risks from physico-chemical properties arising from the use of acrylamide.

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

TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS-No.:	79-06-1
EINECS-No.:	201-173-7
IUPAC name:	acrylamide
Molecular formula:	C_3H_5NO
Structural formula	$CH_2 = CH - CONH_2$
Molecular weight	71.09
Synonyms:	2-propenamide, acrylic acid amide, ethylene carboxamide, propenoic acid amide, vinyl amide

1.2 PURITY/IMPURITIES, ADDITIVES

1.2.1 Purity

Acrylamide is available in a solid form or as an aqueous mixture. In its solid form the purity of acrylamide is reported to be greater than 98% w/w. Acrylamide may also be supplied as a 30-60% w/w aqueous solution.

The major impurities reported as present (w/w on basis of acrylamide solid) are as follows:

3-hydroxypropionitrile	< 0.5%
3-hydroxypropionamide	< 0.5%
acrylic acid	< 0.3%
tris-nitrilopropionamide	< 0.3%
acrylonitrile	< 0.1%
water	< 1%

Acrylic acid and tris-nitrilopropionamide are by-products of the production process or the polymerisation reaction. Acrylonitrile is present as unconverted material from the production process.

1.2.2 Additives

Copper is added typically in amounts less than 100 ppm as an inhibitor to polymerisation.

1.3 PHYSICO-CHEMICAL PROPERTIES

1.3.1 Physical state (at ntp)

Acrylamide is a stable white crystalline solid which sublimes slowly at room temperature. At temperatures up to its melting point, in the absence of light, it does not polymerise significantly. However, above its melting point it can polymerise rapidly and exothermically.

1.3.2 Melting point

The melting point from the standard reference books (Merck Index, CRC Handbook, Kirk-Othmer, etc.) is 84.5°C. The IUCLID data set quotes 84-84.5°C from earlier sources (Van der Burg, 1922; Carpenter and Davies, 1957). The most reliable data are considered to be quoted by Carpenter and Davies (1957) at 84.5±0.3°C which was compiled from a study of many investigators at the American Cyanamid Company. The test method used is clearly described (constant temperature differential melting point apparatus using a platinum resistance thermometer as the sensing element).

The normal melting point can be accepted as 84.5°C.

1.3.3 Boiling point

The boiling point has been quoted as 125°C at 25 mm Hg or 3.3 kPa (Merck Index, Lange's Handbook etc.).

Kirk-Othmer (1991) quotes a range of boiling points at various pressures including 136°C at 3.3 kPa, 116.5°C at 1.4 kPa, 103°C at 0.67 kPa (5 mm Hg) and 87°C at 0.27 kPa (2 mm Hg). The IUCLID data set quotes the values presented in Kirk-Othmer. The CRC Handbook quotes a boiling point of 192.6°C at 1 atmosphere (101.3 kPa) although only general references are given. As acrylamide polymerises at temperatures above its melting point this figure should be viewed with some caution. In the original source of these values (Carpenter and Davies, 1957 and a bulletin issued by the American Cyanamid Co., 1969) a graph only is given, rather than actual results which are inferred. However, because of the tendency of acrylamide to polymerise above its melting point the boiling point has often been established at lower than normal atmospheric pressure and it is likely that polymerisation inhibitors may have been used. It is likely that the quoted values in the literature have their origin in the Carpenter paper and the work of the American Cyanamid Company.

Consequently, although the above values may be accepted as correct, it should be noted that, since it has a tendency to polymerise, acrylamide does not have a normal boiling point.

1.3.4 Density

The secondary sources consulted (Merck, Lange, Kirk-Othmer etc.) all quote a relative density of 1.122 at 30°C. The IUCLID data set quotes a relative density value at 25°C of 1.122 (Jung et al., 1980). The most reliable source (Carpenter and Davies, 1957) quotes 1.127 g cm^{-3} at 25°C and a description of the test method is given. This value can be considered to be reliable.

The vapour density of acrylamide is quoted (Dow, 1989) as 2.46 (air=1). Acrylamide vapour is heavier than air and as such is likely to accumulate at low levels rather than disperse in the air.

1.3.5 Vapour pressure

The vapour pressure of acrylamide has been quoted as 0.9 Pa at 25°C, 4.4 Pa at 40°C and 9.3 Pa at 50°C (Kirk-Othmer, 1994 quoting "Chemistry of Acrylamide", American Cyanamid Co., 1969).

The IUCLID data set quotes 3.9 Pa at 40°C (Thomas, 1964), 4.4 Pa at 40°C, 11 Pa at 50°C (Carpenter et al., 1957), 213 Pa at 84.5°C (Sax, 1965), 267 Pa at 87°C (Thomas, 1964), ca 270 Pa at 87°C (Dow, 1989) and 3,333 Pa at 125°C (Thomas, 1964), although the original data were in mm Hg.

The data referred to in Thomas (1964) and indeed in the handbooks have their origins in the work of Carpenter and Davies (1957) and the American Cyanamid Company and although this data is considered reliable (the test method is described) no other source of data for this parameter has been established. The one value from a different source (Dow, 1989) is unverified work and its origins not stated.

Although the IUCLID data appears to refer to pure acrylamide as opposed to a solution, the figures obtained at or above the melting point (84.5°C) should be treated with caution because of the polymerisation occurring above this temperature. Carpenter states that it is not possible to get vapour pressure measurements on liquid acrylamide unless polymerisation inhibitors are present.

Carpenter gives vapour pressure measurements for temperatures above the melting point where significant quantities of inhibitors have been added. These are likely to be accurate values as a description of the test procedure is given in the original paper. However, the usefulness of data above the melting point is questionable due to the amounts of inhibitors needed to obtain the results.

The IUCLID data set also quotes 2,000 Pa at 20°C for a 30-50% water solution (Dow, 1989) and 2,500 Pa at 25°C for a 50% water solution (MacWilliams, 1973). It is not clear from the information presented, what were the separate contributions of the water and the acrylamide to the overall vapour pressure at the temperatures quoted.

Acrylamide tends to sublime and hence exibits a vapour (sublimation) pressure at room temperature.

The values if 0.9 Pa 25°C for solid acrylamide and 2,500 Pa at 25°C for 50% water solution have been used for the purposes of the risk assessment.

1.3.6 Solubility

Acrylamide is very soluble in water and a figure of 2,155 $g \cdot l^{-1}$ at 30°C is quoted in the chemical data handbooks (origin is Carpenter and Davies, 1957). The IUCLID data set quotes a literature figure of 2,155 $g \cdot l^{-1}$ at 30°C and this is also referenced in the Carpenter paper. Thomas (1964) quotes 2,040 $g \cdot l^{-1}$ at 25°C but this data is referenced to the American Cyanamid Company for which Carpenter established the most reliable data. It is likely that all the handbooks quote values originating from this source.

Acrylamide (Carpenter and Davies, 1957) is soluble in methanol (1,555 $g \cdot l^{-1}$), and ethanol (862 $g \cdot l^{-1}$), acetone (631 $g \cdot l^{-1}$), ethyl acetate (126 $g \cdot l^{-1}$) and chloroform (26.6 $g \cdot l^{-1}$). These values were obtained using recrystallised, vacuum-dried acrylamide and dried solvents, hence the results can be regarded as accurate.

A value of 2,155 $g \cdot l^{-1}$ at 30°C can be accepted as a true value for water solubility.

1.3.7 N-Octanol-water partition coefficient (K_{ow})

The IUCLID data set quotes from the original literature references and the log K_{ow} values are -1.65 (calculated, US EPA, 1980) and -1.24 (measured, Fujisawa and Masuhara, 1981). A value of -1.04 (calculated) is also quoted (Hermans et al., 1982) and - 0.9 (measured, HPLC method, Fujisawa and Masuhara, 1980) along with - 0.86 (calculated, Lipnick et al., 1987) and - 0.67 (measured, Hansch and Leo, 1979 and Dow, 1989).

Although the values are not consistent it is considered not necessary for an agreed standard test to be carried out to assign an accurate value.

A value of -1.0 has been used for the purposes of the risk assessment.

1.3.8 Flash point

No data have been supplied in the IUCLID data set and only one text (Dictionary of Substances and their effects, Vol.1, 1994) quotes a value (138°C). However no reference is given to its origin nor is a method quoted.

Acrylamide is a solid and is known to polymerise at temperatures above its melting point (84.5°C). Consequently the concept of a flash point is not relevant and the unsubstantiated figure quoted can be ignored particularly as there does not appear to be any evidence to support it.

1.3.9 Autoignition

Acrylamide polymerises exothermically above its melting point in the absence of stabilisers and does not undergo autoignition. No data on autoignition are supplied in the IUCLID data set.

1.3.10 Explosivity

Acrylamide polymerises exothermically (heat of polymerisation of -82.8 kJ mol^{-1}) above its melting point in the absence of stabilisers. This can be considered as a significant evolution of heat such that precautions should be taken with molten acrylamide. A quantity of acrylamide at its melting point of 84.5°C polymerised spontaneously resulting in an exothermic reaction with a temperature rise to 165°C after about 2 minutes (Carpenter and Davies, 1957).

As previously stated, at elevated temperatures, acrylamide is likely to be handled as a solution or gel in water. No data on explosivity are supplied in the IUCLID data set. However, there is no evidence in the literature of any explosive properties.

1.3.11 Oxidising properties

Not an oxidising agent. See Sections 1.3.9 - 10.

1.3.12 Summary

Much of the physicochemical data available for acrylamide have their origins in the work of Carpenter and Davies (1957) and The American Cyanamid Company, latterly published as a bulletin in 1969. These data are reliable enough for the purposes of the risk assessment. Properties such as vapour pressure could be usefully re-measured to confirm the data.

Acrylamide exhibits a sublimation pressure (albeit low) at room temperature and the vapour is denser than air.

The heat evolved on polymerisation is such that it is recommended that no more than a few grams of solid acrylamide should be heated above the melting point (84.5°C) without due precautions (American Cyanamid Co., 1969).

The partition coefficient values from a variety of sources range from -0.67 to -1.65. A value of -1 has been used throughout. It is not necessary to re-measure.

The basic physicochemical properties of acrylamide can be accepted as valid.

Table 1.1 below summarises the physicochemical properties of acrylamide.

Table 1.1 Summary of the physico-chemical properties of acrylamide

Parameter	Value	Reference
CAS No	79-06–1	
Physical State	white crystalline solid at ntp	Carpenter and Davies (1957)
Melting Point	84-84.5°C	Carpenter and Davies (1957)
Boiling Point	125°C at 25 mmHg (3.3 kPa) 103°C at 0.67 kPa see text for more values bpt. not applicable at normal pressure due to polymerization	Carpenter and Davies (1957)
Density	1.127 $g \cdot cm^{-3}$ at 30ºC	Carpenter and Davies (1957)
Partition Coefficient Log P_{ow}	- 0.67 to -1.65 (use -1.0 as a value if needed)	Various-see text.
Vapour Pressure	0.9 Pa at 25°C 4.4 Pa at 40°C 9.3 Pa at 50°C 213 Pa at 84.5°C (mpt) with 5% $CuCl_2$ see text for further values	Carpenter and Davies (1957) - refers to *sublimation* pressure
Solubility	2,155 $g \cdot l^{-1}$ at 30°C	Carpenter and Davies (1957)
Flash Point	n/a – however can polymerize exothermically above mpt	-
Autoignition	as above	-
Explosivity	as above	-
Oxidising Properties	not an oxidising agent	-

1.4 CLASSIFICATION

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

Classification

Carc.Cat. 2; R45	May cause cancer
Muta.Cat. 2; R46	May cause heritable genetic damage
Repr.Cat. 3; R62	Possible risk of impaired fertility
T; R25-48/23/24/25	Also toxic: danger of serious damages to health by prolonged exposure through inhalation, in contact with skin and if swallowed
Xn; R20/21	Also harmful by inhalation and in contact with skin
Xi; R36/38	Also irritating to eyes and skin
R43	May cause sensitisation by skin contact

Note D
Note E

Specific concentration limits: None

Labelling

T;
R45-46-20/21-25-36/38-43-48/23/24/25-62
S53-45

S53	Avoid exposure – obtain special instructions before use
S45	In the case of accident of if you feel unwell seek medical advice immediately (show the label where possible)

No classification for the environment.

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

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

Acrylamide is produced by a catalytic hydration process. In this process a water/acrylonitrile mixture is heated to 100-150^{o}C in a solid bed reactor, with a copper catalyst, to form acrylamide. The unreacted acrylonitrile is recovered by distillation and recycled in the process. In this process a 30-50% aqueous solution is produced.

Other production methods include the sulphuric acid hydration process and the hydration of acrylonitrile by bacteria in a bioreactor (BUA, 1992). Neither of these processes is thought to be in use in the EU.

Three companies are reported as producing acrylamide within the EU (UK, Germany and the Netherlands) while a further two companies are involved in the import of acrylamide. The total plant capacity within the EU is estimated at between 80,000-150,000 tonnes per annum. Individual plant capacities are in the range of 10,000-50,000 tonnes per annum. Total EU production is estimated at between 80,000-100,000 tonnes, the largest amount at a single plant being approximately 40,000 tonnes per annum. The above production figures are calculated for acrylamide solids. Acrylamide is usually made as a 30-50% aqueous solution although a crystalline form of acrylamide is produced at one European production plant.

German production in 1991 was 16,000 tonnes, of which 1,000 tonnes was exported (BUA, 1992). Acrylamide is imported primarily by the major manufacturers of polyacrylamides for their own use. Small amounts of acrylamide are also exported outside the EU. Figures were not available for these imports and exports, although it is understood that the majority of EU production is used within the EU.

2.2 USES

2.2.1 Acrylamide use

It is estimated that up to 99.9% of acrylamide in the EU is used in the production of polyacrylamides. These high molecular weight polymers are produced to give non-ionic, cationic and anionic properties for specific uses. Polymerisation (homopolymerisation and copolymerisation) is brought about through a free radical mechanism in aqueous solution. The residual content of acrylamide in the polymers is kept below 0.1% w/w to avoid classification as a Category 2 carcinogen under the Dangerous Preparations Directive (88/379/EEC and adaptations). Many polymers are marketed with acrylamide concentrations significantly lower than this. This is discussed further in the section titled “Occupational exposure during the use of polyacrylamides”.

There are seven producers of polyacrylamide within the EU, two of which are also acrylamide manufacturers. The seven companies operating these plants form the Polyacrylamide Producers Group. There are also a number of smaller polyacrylamide producers throughout the EU. BUA (1992) states that there are a total of eight polyacrylamide manufacturing plants in Germany. Each producer generally has different product ranges, although the smaller producers are understood to concentrate on particular grades for particular applications.

Solid and aqueous grades of acrylamide are used in polymer manufacture to produce various solid and liquid grade polymers. Solid grades (bead, granular, powder etc.) are supplied as about 95% polymer and liquid grades (oil and water dispersions) as about 50% polymer. These solid and liquid grades account for 70% and 30% of the market, respectively.

Acrylamide monomer may also be sold for on-site preparation of polyacrylamide gels. About 0.1% of the acrylamide produced in the EU is used to produce polyacrylamide electrophoresis gels which are used as a research tool for separating nucleic acids in research establishments, universities and hospitals.

Acrylamide can also be used in the formulation of grouting agents. Acrylamide grouts are no longer thought to be produced within the EU but are imported from outside the EU. The one known EU producer of an acrylamide grout stopped production at the end of 1997 and has no plans to restart production.

Table 2.1 Breakdown of the percentage of acrylamide used in each application

Application	Production volume (Acrylamide solids)		
	Range	Value used in exposure assessment	Tonnes per annum (EU)
Production of polyacrylamide and copolymers of acrylamide	95-100%	100%	100,000
On site production of polyacrylamide gels	<1%	0.1%	100
Grouting agent	n/a	n/a	n/a

2.2.1.1 Use of acrylamide grouts and acrylamide based grouting agents

The major end uses of acrylamide grouts are sewer line sealing and manhole sealing. Acrylamide grouts were the most widely used chemical grouts for sewer rehabilitation because of their low cost, quick and controllable “gel” or “set up” time (the time it takes for the grout to polymerise), long history of reliable performance, and very low viscosity. They tend to be used when other, less expensive cementitious grouts are unsuitable.

Acrylamide grouts were first introduced to the US market in 1955. They were popular because of their low cost and superior performance properties compared to other grouts then on the market. In the 1970s, demand for acrylamide grout grew as a result of an increase in sewer repair (rehabilitation) activities. In 1978, production of acrylamide grout in the US ceased because of the producers concern for its potential risk to human health. Acrylamide grout continues to be the chemical grout selected most often for use in sewer operations in the US. About 386 tonnes of acrylamide grout were consumed in 1989, which constituted about 43% of the total US chemical grout usage (EPA).

Acrylamide grouts or acrylamide-based grouting agents are no longer thought to be produced in the EU. The one known producer of an acrylamide-based grout stopped production at the end of 1997 and has no plans to restart production. This producer appears to have been the only supplier of an acrylamide-based grout within the EU in recent years. To the best of their knowledge the European producers and importers of acrylamide into Europe no longer supply acrylamide for use in this application.

Acrylamide grouts generally consist of a 19:1 mixture of acrylamide and a cross-linking agent. When preparing the grout for use, water and small amounts of other chemicals are added. These chemicals include catalysts, activators or accelerators, and inhibitors. When the acrylamide grout polymerises or "gels", it solidifies into a stiff gel that is impervious to water. In gel form, the grout contains less than 0.05% free acrylamide.

Grouters typically inject acrylamide grout in and around concrete, rock and soil to increase the absolute strength of the mass and to restrict the flow of water through a structure or the grouted area. In the US approximately 87% of all acrylamide grout is used in sewer rehabilitation: 76% in sewer line repair and 11% in manhole sealing. Sewer rehabilitation helps minimise the demands on sewage treatment capacity and wastewater treatment costs by reducing the inflow of rainwater and non-point run-off and the infiltration of groundwater through cracks, holes, and joints in the sewer system. In sewer rehabilitation, leaking pipes and joints can be sealed remotely using appropriate equipment. In manhole sealing applications this is usually done manually.

Acrylamide grout has two other minor uses: 8% is used for structural water control and 5% for geotechnical applications. In structural water control the grout is used to repair leaking concrete structures. These projects include seepage control, sealing cracks in sewage aeration basins, and repairing dams. In geotechnical applications, the grout is applied to soil or rock formations. Geotechnical grouting includes water cut-off in mines and reservoirs, sealing underground salt domes and potash mines, and isolating hazardous waste sites. Both structural water control and geotechnical grouting operations involve manual injection techniques.

2.2.1.2 N-methylolacrylamide grouts

A derivative of acrylamide, N-methylolacrylamide (NMA) may also be used in grouting applications. The method of application is the same as for acrylamide but a different catalyst is used. NMA accounted for approximately 3% of the chemical grouting market in the USA in 1989, and was used exclusively in sewer rehabilitation and manhole sealing. The product sold in the EU and now withdrawn (following the incident at Hallandsås, see Section 2.2.1.3, Sweden) was a NMA-based grout.

NMA has the potential to regenerate acrylamide but the extent to which this happens in practice is not known and is dependent on the environmental conditions. NMA may also be partially transformed to acrylamide when sodium silicate is added (in two solution grouting systems sodium silicate is often used in one part and acrylamide or NMA in the other part). It is possible that when the two components are mixed to initiate polymerisation, the pH of the mixture changes to levels that allow transformation of NMA into acrylamide. Exposure to mixed, but not yet polymerised, product might therefore lead to exposure to higher concentrations of acrylamide than expected.

The product used within the EU until recently (Rhoca Gil) was prepared by mechanical mixing of two solutions, designed to be diluted with water and mixed on site. Solution 1 contained a maximum of 1.5% acrylamide, approximately 37% NMA and approximately 0.9% formaldehyde (with cross-linking agents, silicate hardeners and stabilisers). Solution 2 contained sodium silicate and sodium persulphate (an initiator for the polymerisation reaction). The accelerator (ACS), which contained dimethyl adipate, dimethyl glutarate, dimethyl succinate and triethanolamine, was initially added to the first solution at a level of ~10%. The mixed solution consisted of 3.75 parts of water, 0.125 parts of concentrated solution 1, and 0.125 parts of

concentrated solution 2. Once mixed and polymerisation had begun the manfacturer stated that the residual acrylamide monomer should be consumed. In laboratory experiments the manufacturers found that the acrylamide content in the final resin was 1% after 3 hours and 0.04% after 7 days.

The manufacturers guidelines for the use of NMA gels gave the following information on the chemical properties of the grout. The setting of the grout occurs in two distinct stages: gelling of the silicate and then polymerisation of the arcylamide monomer. To ensure that the gel sets successfully, the process to render the silicate insoluble needs to begin before polymerisation. The grout is designed for use at temperatures between 5 and 30°C. Above 30°C the polymerisation of the acrylic monomer may occur before the silicate gel sets. This causes the gel formed to have a lower elasticity and plasticity. Below 5°C polymerisation of the monomer may be considerably delayed in relation to the gelling of the silicate. Consequently the grout will not be properly adhered to the fissures and may leak out of the zone being treated. Air, which is entrained and dissolved during vigorous mixing of the solutions, will retard the gel time of the resin. In water the grout undergoes considerable reversible swelling. In dry conditions the grout contracts.

2.2.1.3 Use of acrylamide based grouts in the EU

Denmark

In Denmark approximately 1.5 tonnes of acrylamide were used in the construction industry in 1997.

UK

In the UK four companies used NMA based grouts prior to 1998. All four companies have now switched to alternatives that are not based upon NMA or acrylamide, following withdrawal of the product they were using by the producing company in late 1997. Of the four companies, three used the grouts for sewer repairs. The grout was applied by remote injection equipment to fill cracks in mainline sewers and hence effect repairs. The remaining company used acrylamide grouts for structural repairs within buildings. The typical tonnage used by these companies was small with approximately 1-3 tonnes used per year per company. In the UK the use appears to have been in quite specialised applications.

Finland

In Finland an acrylamide containing product (Spirogel 110) has been used to seal cracks in a dam. This use has occurred in one location between 1995-1997. Since the incidents in Sweden and Norway (see below) the import of the product has ceased. Previously the company concerned had imported 1,004 kg in 1995, 1,102 kg in 1996 and 345 kg in 1997 and had found the product effective in stopping the leakage of water. Similar products have been used to seal drain pipes. About 4,860 kg were used between 1990 and 1996. This kind of use no longer occurs in Finland.

Sweden - Construction of a tunnel at Hallandsås

In Southern Sweden (Hallandsås) an 8.6 km long tunnel was being built through a bed-rock ridge. The ridge had a very high water content. In early 1997 several different products were tested as grouting materials. The product chosen for use was a NMA based grout containing NMA and acrylamide. The large-scale use of the product started in August 1997.

A few weeks after use of the grout commenced, adverse effects symptomatic of acrylamide poisoning were observed in fish and cows downstream of the construction works. At the same time, symptoms characteristic of exposure to acrylamide were observed in workers at the tunnel.

Norway - Construction of tunnel at Romeriksporten

NMA grout was also used in tunnel construction in Norway, 1995-1997. In the construction of the Romeriksporten tunnel, about 350 tonnes of the NMA grouting agent Rhoca–Gil was used; this includes 110-210 tonnes acrylamide and NMA. In the tunnel the pressures and the water flow leaking into the tunnel influenced the hardening of the chemical. (In subsequent work the boreholes with the highest amount of unhardened material were found to be those with the lowest water flow through.) Acrylamide based grouts have been used in the past in Norway for construction applications with no apparent problems occurring.

2.2.2 Polyacrylamide use

Polyacrylamides can be produced so that they have non-ionic, cationic or anionic properties making them suitable for a range of uses. The polymerisation reactions (homopolymerisation or copolymerisation) are free-radical reactions in aqueous solution or emulsion. The solubility and polyelectrolyte characteristics of these polymers are imparted by residual carboxyl, quaternary ester or amide groups. Water-soluble cationic polymers can be produced by copolymerisation with unsaturated quaternary ammonium compounds, such as diallyl dimethyl ammonium chloride or vinylbenzyltrimethyl ammonium chloride. Acrylamide cationic polymers are also produced by other reactions. Anionic polyacrylamides are formed by copolymerisation with carboxylic or sulphonic acid.

The three largest uses of polyacrylamide are in wastewater treatment, paper and pulp processing and mineral processing - estimated by Industry to be 80% of the market. Detailed figures for the EU were not provided. In 1991, 65% of polyacrylamide used in the USA went into water treatment, with a further 20% used for paper and pulp processing and 5% for mineral processing (IARC, 1994). These industries generally dilute the polymer to give a stock solution of about 0.5% w/w, which may be further diluted (1:10) before use. Polyacrylamides may be supplied to formulators before reaching the end user. However in general, the largest industries purchase direct from the polymer manufacturer.

These and other uses (some of which may be historical) of polyacrylamide are described below.

Water treatment and wastewater treatment

The largest use for polyacrylamides is in the treatment of municipal drinking water and wastewater. Approximately 50,000 tonnes of polyacrylamides are used per annum. Polyacrylamides act as flocculants or coagulants to condition sludge, to clarify raw water and to treat effluent streams from sewage plants. Polyacrylamides can also be used to remove suspended solids in industrial wastewater prior to discharge, re-use or disposal.

The polymers bind with colloidal particles to form heavy aggregates. The polymer/particle flocculants quickly settle out from solution to leave a clear supernatant. When polyacrylamides are used as sludge conditioning/dewatering agents, they allow a more concentrated sludge than do inorganic coagulants.

Paper and pulp processing

Polyacrylamides are used in the pulp and paper production industry as binders and as retention aids for fibres. They are also used as drainage aids/flocculants. Cationic polymers increase the pigment retention on paper fibres. Approximately 12,000 tonnes of polyacrylamides per annum are used in this application.

Mineral processing

Polyacrylamides are used for clarification of wastewater, the recovery of tailings and the flocculation of ores in mineral processing. They are also used to thicken mineral concentrates and to permit re-use of water in the extraction process.

Crude-oil production processes

Polyacrylamides are used to increase water viscosity in oil recovery processes. In addition, partially hydrolysed polyacrylamides and acrylamide-acrylic acid copolymers can be used to control fluid loss in oil-well drilling muds.

Cosmetic additives

Polyacrylamides are used in soap and cosmetic preparations as thickeners. They are also used in pre-shave lotions and hair grooming preparations.

Soil and sand treatment

Polyacrylamide resins can be used to stabilise soil as the polymers bind loose grains when injected under the surface or when applied mixed into the soil. Polyacrylamides can also be used to allow foundry sand to flow freely into moulds.

Coating applications

Polyacrylamides are used as dispersants and bindings in coatings. Water-based paints containing 0.1-0.5% polyacrylamides have better pigment suspension and flow. Since 1952, numerous patents have been granted describing the use of polyacrylamide resins in surface coatings and thermosetting acrylics. These resins are used as coatings in home appliances, building materials and automotive parts.

Textile processing

Polyacrylamides have been used as sizing agents for cotton and as shrink-proofing agents for wool. They have also been used to bind textile fibres and as water repellents.

Miscellaneous uses

Polyacrylamide may also be used as a thickener. Examples include as a thickener for latex, emulsion stabilisers for printing inks, gelling agents for explosives, electrophoretic gels and retardants for crystal growth in the production of diazo compounds.

Adhesives and adhesive tapes may contain acrylamide polymers as binders. Polyacrylamides are used to clarify solutions in the chemical and food manufacturing industries.

Some polyacrylamides can be used as dispersants, anti-precipitants and deflocculants in aqueous systems. These properties are used for fluidising pigment press-cakes and reducing the viscosity of latexes. When added to herbicidal gels, polyacrylamides allow them to sink before breaking up thereby limiting the treatment to the bottom of a lake or reservoir.

Recently crosslinked polyacrylamides, so-called hydrogels, have been introduced as agricultural and horticultural aids for improved water management. Their market share is thought to be small at present.

2.3 Legislative controls

Acrylamide is an existing substance with a long history of production and use. A number of effective controls to reduce emissions currently exist, whether simply adopted as part of plant design or added later in response to demands to reduce emissions into the workplace or environment.

Within the EU, under the Dangerous Preparations Directive 1999/45/EC (replaces Directive 88/379/EEC and adaptations), any preparation containing greater than 0.1% w/w acrylamide would require classification and labelling as a Category 2 carcinogen. All polyacrylamides in the EU contain less than 0.1% w/w free acrylamide monomer and are classified as either non-hazardous or irritant (reflecting the hazardous properties of any additional formulants). Free monomer levels may also be lower than 0.1% depending on additional regulations covering specific uses as detailed below.

Registration of polyacrylamides is required for certain specified uses in Europe. Certain countries (e.g. UK, Netherlands) require the registration of polyacrylamide products for drinking water treatment, typical free acrylamide levels being less than 0.025% acrylamide (w/w of polymer). These levels are calculated within the regulations based on acceptable daily intake of acrylamide from treated drinking water. Drinking water treatment customers in other European countries may request prior approval under UK or USA (National Sanitation Foundation) procedures before permitting their use.

Polyacrylamides in general have approval as direct and indirect food additives such as in paper and paperboard food packaging and coating under US (FDA), Germany (BgVV) and the Netherlands (VGB) regulations/recommendations. Polyacrylamide is also approved for use in sugar clarification. In some cases the permissible concentration of free acrylamide in the polyacrylamide or in the end product is restricted. Since the Dangerous Preparations Directive was enacted in the EU in 1988, no polyacrylamides containing more than 0.1% free acrylamide (w/w) have been supplied for any use.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

The level of exposure of the environment to a chemical is dependent upon the quantities and compartments of release and subsequent degradation, distribution and accumulation. Section 3.1.2 discusses the releases of acrylamide to the environment and its subsequent behaviour. Detailed information on the resulting levels in the different compartments is given in Sections 3.1.4 to 3.1.7. In deriving these figures, the methods and calculations described in the Technical Guidance Document (TGD) for the risk assessment of new and existing substances have been followed.

3.1.2 Environmental releases

Chemicals may potentially be released to the environment during each stage of their lifecycle. For acrylamide the main lifecycle stages leading to releases to the environment are:

- Production
- Processing - Polyacrylamide production
- Processing - Polyacrylamide use
- Processing - Use of acrylamide-based grouts (such grouts are not produced in Europe).

Releases from these stages are considered in Sections 3.1.2.1 to 3.1.2.3.

3.1.2.1 Releases from production and processing sites

Acrylamide is produced by the catalytic hydration of acrylonitrile in closed systems. There are three producers within the EU, located in the United Kingdom (UK), Germany and the Netherlands. A fourth company based in Germany stopped producing acrylamide in 1993. The total acrylamide production capacity within the EU is estimated at 101,780 tonnes/year (1994).

Typically an aqueous form of acrylamide is produced (50% water, 50% acrylamide). A crystalline form is also made at one EU site. The acrylamide produced is stored on site prior to use or shipping (shipping covers both sale to other companies within the EU and export to non-EU countries).

The main use of acrylamide is in the production of acrylamide polymers and copolymers. The production of these involves different polymerisation processes depending upon the product required. There are estimated to be seven large-scale users of acrylamide within the EU, located in the UK, Germany, the Netherlands, France and Finland. The total amount of polyacrylamide produced within the EU is estimated at 80,000-100,000 tonnes/year (solids). A BUA report (1992) listed eight acrylamide processors, but five of these are thought to no longer process acrylamide.

Table 3.1 lists the main acrylamide production and processing sites within the EU. Individual sites and site locations have not been identified, to preserve company confidentiality. There are eight main production or processing sites within the EU, and two of these undertake both production and processing. For these sites the emissions reported refer to the combined emissions from both activities. From the data presented it is possible to calculate site-specific environmental concentrations for all of the sites.

Continental and regional releases are used to calculate predicted environmental concentrations (PECs) for the continental and regional environments. These PECs are then used as background concentrations and added to the local environmental concentrations to give local PECs. For acrylamide the continental releases to water are taken as the sum of the releases from each production and processing plant currently in operation (see **Table 3.1**). Where releases are given as kg/year, emission is assumed to take place over 300 days (where no other information on days in operation is available). **The continental emission to water is therefore 6.3 kg/day**. In calculating emissions to air, the release estimate for site B will be used for the three production plants (A, B and C) and processing site D (since it is difficult to convert concentrations to releases in kg/d). This release estimate will be related to tonnage for the other three production and processing plants. For processing sites E, F, G and H the emissions are summed together. **This gives a continental emission to air of 103 kg/year (0.38 kg/day)** (total emissions from all sites).

For the regional scenario the TGD recommends that 10% of the continental emission is used. It should be noted that for acrylamide there are less than 10 large-scale production or processing plants, so the emissions in a region may therefore be more than 10% of the continental emissions. Therefore the largest local emissions to water and air from production and processing plants will be used. **This gives a regional emission of 3 kg/d to water and 0.22 kg/d to air**.

Table 3.1 Acrylamide production and processing site data

Site	Production capacity (t/year)	Use as intermediate (t/year)	Release to air or concentration in air	Release to water or concentration in water	Comments
A	36,000 (in 1994)	31,090 (in 1994)	0.43 mg/m^3	3 kg/d (in effluent going to on-site treatment plant)	Effluent discharges to on-site treatment works then municipal treatment works. Concentration in air measured in reactor prior to release to atmosphere
B	24,000 (in 1994)	No	9.5 kg/year	0 (after on-site treatment)	Waste from acrylamide production recycled back into process. Effluent from site monitored for acrylamide but not detected at detection limit. Releases to air include fugitive emissions from pipes, valves and storage
C	42,000 (in 1994)	Yes		Maximum concentration in plant effluent 1 mg/l. Based upon typical flow rates this gives a release of 2 kg/day	Plant produces solid and aqueous acrylamide. No information on releases to air or tonnage processed
D	4,000 (in 1991)	Yes	0.1 mg/m^3 (legal limit)	365 kg/year to wastewater In wastewater 1-12 mg/l In effluent from municipal treatment plant <10 μg/l	Measured concentrations supplied cover emissions from production and processing operations. No on-site treatment. Discharge to municipal wastewater treatment plant. Information from BUA report (1992) Production of acrylamide ceased in 1993
E	No	22,000 (in 1996)	55.2 kg/year (54.6 kg/year drying; 0.6 kg/year tanks) (250 days operation per year: 0.2208 kg/day)	2.25 kg/year (50 weeks operation per year: 0.009 kg/day)	Effluent pretreated at plant before discharge to municipal wastewater treatment plant. The concentration of acrylamide is monitored in the effluent of the plant before discharge to municipal wastewater treatment plant. Acrylamide concentrations measured at outlets of air scrubbers and dryers
F	No	Yes	0 kg/year	5 kg/year	No information on tonnage processed Emissions from BUA report (1992)

Table 3.1 continued overleaf

Table 3.1 continued Acrylamide production and processing site data

Site	Production capacity (t/year)	Use as intermediate (t/year)	Release to air or concentration in air	Release to water or concentration in water	Comments
G	No	Yes	5 kg/year	9 kg/year (after on-site treatment)	On-site treatment then release to municipal wastewater treatment plant. No information on tonnage processed Emissions from BUA report (1992)
H	No	Yes	1 kg/year	10 kg/year	Release estimates based upon measured levels. Wastewater discharges to a municipal WWTP
I	No	No	< 30 kg/year	60 kg/year (to wastewater treatment plant)	No longer a processor. Emissions from BUA report (1992)
J	No	No	< 25 kg/year	50 kg/year (to wastewater treatment plant)	No longer a processor. Emissions from BUA report (1992)
K	No	No	< 5 kg/year	0	No longer a processor. Emissions from BUA report (1992)
L	No	No	0.1 kg/year	20 kg/year (to wastewater treatment plant)	No longer a processor. Emissions from BUA report (1992)
M	No	No	< 10 kg/year	<1 kg/year (to wastewater treatment plant)	No longer a processor. Emissions from BUA report (1992)

3.1.2.2 Releases of acrylamide monomer from polyacrylamide

Information in this section is based upon information provided by industry and the TEGEWA Polyelectrolyte Producers Group.

Approximately 100,000 tonnes of polyacrylamides are used in the EU each year (Section 2.2.2). Of this amount about 50,000 tonnes are used in the water industry for water clarification, waterworks sludge treatment and sewage sludge thickening. They may also be used to assist in primary settlement, though this use is minor compared to the others. The remaining 50,000 tonnes are used in other industries, primarily in mineral processing (to aid the removal of fine particles from wash waters) and pulp and paper treatment (to improve the efficiency of the separation of paper fibres from water).

Polyacrylamide is supplied from the manufacturer to the end user as either a solid or a liquid depending on the application. The end user will then prepare an active stock of polyacrylamide in water, at a typical concentration of 0.5% (w/w). Polyacrylamide flocculants are generally of a high molecular weight and may be either anionic or non-ionic in nature. The flocculants cause stabilised particles or coagulated suspensions to form aggregates, which can be removed by settlement or filtration. They may also be added to the resultant solids to facilitate dewatering.

The maximum concentration of residual acrylamide monomer in polyacrylamides is 0.1% (w/w), though for some applications (e.g. drinking water treatment) the maximum permitted amount of free acrylamide in the polymer is 0.025% (w/w).

The potential for release of monomer from the polymer needs to be considered. The breakdown of the polyacrylamide backbone into monomer units is energetically unfavourable, and is therefore unlikely to occur. Soponkanaporn and Gehr (1989) studied the degradation of polyelectrolytes in the environment using size exclusion chromatography. They failed to observe the degradation of the polymer backbone and acrylamide was not identified as a degradation product. The maximum amount of acrylamide available for release from polyacrylamides is therefore taken to be the maximum concentration of residual acrylamide monomer in the polymer.

Drinking water treatment

The United Kingdom Drinking Water Inspectorate has provided the following information on use of polyacrylamides in drinking water treatment. While the data are based upon the UK situation it is taken as representative of the European situation in deriving a $PEC_{drinking\ water}$.

In the UK polyacrylamides are approved for the treatment of water for drinking supplies provided that:

- no batch of polyacrylamide contains more than 0.025% of free acrylamide monomer based on the active polymer content; and
- the dose used must average no more than 0.25 mg/l and never exceed 0.50 mg/l of the active polymer.

Therefore the minimum dilution of the stock material must be 1:10,000 to give a maximum dose of 0.50 mg/l during treatment of drinking water supplies. If the average dose of no more than 0.25 mg/l is met the dilution in the stock material would need to be 1:20,000. (Note that for other applications such as wastewater treatment the dilution rate of the stock material is thought to be 1:10).

Using these typical dilution rates and the total tonnage of material used it is possible to calculate a worst-case release of acrylamide monomer from polyacrylamides. In calculating this release it is assumed that the total amount of free acrylamide in the polymer is released, although in practice this is unlikely to be the case. Of the 50,000 tonnes polyacrylamide used in wastewater treatment application 10,000 tonnes are thought to be used for drinking water treatment and 40,000 tonnes for sewage/sludge treatment. For the continental and regional scenarios all water treatment is assumed to be wastewater treatment as a worst case.

Potential release of residual acrylamide monomer from polyacrylamides used in drinking water treatment:

Total amount of polyacrylamide used = 10,000 tonnes per annum
Total amount of free acrylamide in polymer (0.025%) = 2.5 tonnes

Dilution in stock solution (0.5% w/w)
Total amount of polyacrylamide stock solution = 2,000,000 m^3

Dilution in treated material (1:10,000)
Total amount of polyacrylamide-treated material = 20,000,000,000 m^3

Concentration of free acrylamide in polyacrylamide-treated material
= 2.5 tonnes /20,000,000,000 m^3 = 0.125 μg/l

This concentration is the maximum possible concentration of acrylamide in drinking water from water treatment works using polyelectrolyte flocculants. If the average dose of the polymer is 25 mg/l the potential concentration of acrylamide in drinking water becomes 0.0625 μg/l.

Note that the release of acrylamide during the preparation of drinking water in Germany is estimated to be a maximum of 5 kg/year (BUA, 1992).

Sludge and sewage treatment

Polyacrylamides for use in sludge and sewage treatment have a maximum free residual monomer content of 0.1% (w/w), and the typical dilution of stock solutions is 1:10. Polyacrylamides used for the thickening and dewatering of sludges are preferentially absorbed to the sludge. The water from sludge dewatering and thickening is usually fed back into the head of the treatment works. As a worst-case scenario it is assumed that all the free monomer in the polymer is lost to the water phase during sludge dewatering. The water is then fed back into the treatment works where it undergoes degradation before being discharged into the receiving watercourse. Note that BUA (1992) report an emission to water of 1 tonne/year from the use of polyacrylamide as a flocculation aid in the dehydration of sewage sludge in Germany.

Total amount of polyacrylamide used = 40,000 tonnes per annum
Total amount of free acrylamide in polymer (0.1%) = 40 tonnes
Dilution in stock solution (0.5% w/w)
Total amount of polyacrylamide stock solution = 8,000,000 m^3
Dilution in treated material (1:10)
Total amount of polyacrylamide-treated material = 80,000,000 m^3

Concentration of free acrylamide in polyacrylamide-treated material
= 40 tonnes /80,000,000 m^3 = 0.5 mg/l.

This is the maximum concentration of acrylamide monomer in wastewater from sludge and sewage treatment. In calculating the PECs for this application this concentration will be taken as an influent concentration as the wastewater is typically fed into the head of the wastewater treatment plant.

Other applications

For polyacrylamides used in industrial applications the total amount of free acrylamide in the polymer is less than 0.1% and the dilution in stock solutions is thought to be 1:10. The two main industrial applications of polyacrylamide are in the pulp and paper industry and in mineral processing. The amounts used in each application are not known.

Pulp and paper industry

The Use Category Document on water treatment (UCD, 1996) gives the following information on the use of coagulants and flocculants in pulp and paper production. Losses of water during the process are typically 30-35 m^3/tonne. Lower levels than these are possible where internal recycling of water takes place. Polyacrylamide is used as a coagulant and a flocculant at a concentration of 0.2-10 mg/l. If the total amount of free acrylamide in the polymer is taken as 0.1% this gives a concentration of acrylamide monomer in the process waters of 0.2-10 μg/l. Polyacrylamides may also be used as a drainage and retention aid, when the amount used is typically 0.1-2 kg/tonne. If the total amount of free acrylamide in the polymer is 0.1% this gives a concentration of acrylamide monomer of 0.1-2 g/tonne. Assuming that 30 m^3 of water is treated the concentration of acrylamide in the process waters is 0.003-0.06 mg/l.

Mineral processing

The following calculation assumes that 50,000 tonnes of polyacrylamides are used each year in mineral processing.

Total amount of polyacrylamide used = 50,000 tonnes per annum
Total amount of free acrylamide in polymer (0.1%) = 50 tonnes

Dilution in stock solution (0.5% w/w)
Total amount of polyacrylamide stock solution = 10,000,000 m^3

Dilution in treated material (1:10)
Total amount of polyacrylamide-treated material = 100,000,000 m^3

Concentration of free acrylamide in polyacrylamide-treated material
= 50 tonnes /100,000,000 m^3 = 0.5 mg/l.

This concentration is the maximum possible concentration of acrylamide in process waters from mineral processing.

Kirk-Othmer (1997) contains some information on the possible use of polyacrylamide in irrigation water to reduce erosion of soils. It should be noted that this is based upon practice in the USA and it is not in widespread use. There is no information on similar uses in the EU and the following information is presented here for illustration only. Polyacrylamides for this use have a molecular weight greater than 10^7 g/mole. The concentration of polyacrylamide in irrigation water suggested is 1-10 ppm, giving an application rate for the polymer of 1 lb per acre ($1.1 \cdot 10^{-4}$ kg/m^2). Assuming a residual monomer content of 0.1% gives an application rate for the monomer of

$1.1 \cdot 10^{-7}$ kg/m^2 or 0.11 mg/m^2. The initial concentration in soil calculated from this is 0.32 μg/kg with a soil pore-water concentration of 2.6 μg/l.

Continental and regional emissions

In calculating the continental and regional emissions of acrylamide a worst-case assumption of 0.1% residual acrylamide is assumed. This gives a continental emission of acrylamide from polyacrylamides of 100 tonnes/year (274 kg/day). The regional emission is taken as 10% of the continental emission, which gives a regional emission of 10 tonnes/year (27.4 kg/day). In calculating daily releases it is assumed that emissions occur for 365 days a year.

3.1.2.3 Releases from the use of acrylamide and N-methylolacrylamide grouts

There is no known current production of acrylamide grouts or acrylamide-based grouts in the EU. The grouts are used mainly for sewer line sealing and manhole sealing, with minor uses in structural water control and geotechnical applications.

Acrylamide grouts generally consist of a 19:1 mixture of acrylamide and cross-linking agent. When the grout solidifies it contains less than 0.05% free acrylamide. A derivative of acrylamide, N-methylolacrylamide (NMA) may also be used in grouting applications.

As discussed in Section 2.2.1.1, NMA has the potential to be hydrolysed to acrylamide at low pH and may also be transformed to acrylamide when sodium silicate is added. Exposure to mixed, but not yet polymerised, product might therefore lead to exposure to higher concentrations of acrylamide than expected.

The product used within the EU until recently contained NMA as well as acrylamide and formaldehyde as impurities. When properly mixed and the polymerisation process had started the manufacturer stated that the residual acrylamide monomer should be consumed. In laboratory experiments the manufacturers found that the acrylamide content in the final resin was 1% after 3 hours and 0.04% after 7 days. Depending upon the experimental conditions the amount of acrylamide diffusing in water was 0.03% to 0.17% with respect to the prepared resin.

The manufacturer's data suggest that under suitable conditions the amount of acrylamide released to the environment should be quite small. In pipeline repairs and manhole sealing operations where the quantities used in one place are likely to be in the order of kilograms per day the amount of acrylamide released is likely to be in the order of grams per day. The acrylamide released is only likely to be observed immediately after application of the gel to the crack to be sealed. Once the polymerisation process is completed the amount of acrylamide released is likely to be negligible. As a worst-case scenario a point source release for less than 7 days is considered. There is not likely to be any long-term release from the polymerised gel.

Since there is very little information on the tonnage of acrylamide grout or NMA grout used within the EU either at present or historically it is not possible to calculate predicted environmental concentrations for this application on a regional scale.

On a local scale it is possible to perform a generic calculation based upon the data supplied by UK companies who used to use NMA-based grouts. In the UK the grout was applied by remote injection equipment to fill cracks in mainline sewers and hence effect repairs. The typical tonnage used per company was 1-3 tonnes/year. Taking a value of 3 tonnes/year and assuming that grouting operations occur for 250 days a year (5 days operation per week) gives the amount

used per day as 12 kg/day. From the data supplied by the EU producer of the grout, the residual concentration of acrylamide monomer is a maximum of 5% (0.6 kg).

For the larger-scale use of these grouts, for example in tunnelling, it is not considered possible to describe a generic scenario, because each situation will be different. Information on environmental levels arising from actual use in these applications is discussed in Section 3.1.4.2.1.

3.1.2.4 Releases from other uses

A maximum of 1,000 tonnes of acrylamide is used in other applications, primarily the production of electrophoresis gels in laboratories. No information is available on the release from this use although it is thought to be negligible when compared to release during production and as free monomer from polyacrylamides.

3.1.2.5 Regional and continental releases

The total continental and regional releases are calculated by adding the releases from production and processing sites to the maximum potential release of residual acrylamide monomer from polyacrylamides. This gives a total continental release of 280 kg/day to water and 0.38 kg/day to air and a total regional release of 30.4 kg/day to water and 0.22 kg/day to air.

3.1.3 Environmental fate

3.1.3.1 Degradation in the environment

On release to the environment acrylamide may undergo a number of degradation and removal processes, depending upon the compartments into which the release occurs.

3.1.3.1.1 Atmospheric degradation

Acrylamide released into the atmosphere may undergo reaction with reactive species, the most significant reaction being with hydroxyl radicals. The half-life for the reaction of acrylamide with hydroxyl radicals at room temperature is calculated as 8.3 hours. This is based upon a hydroxyl radical concentration of $5 \cdot 10^5$ molecules^{-1} cm^3 and a reaction rate constant of $k_{OH} = 46.3 \cdot 10^{12}$ cm^3 molecule$^{-1} \cdot$ s^{-1} calculated using structure activity relationships developed by Atkinson (1987). No information is available as to the likely reaction products formed.

Due to its high solubility in water it is probable that acrylamide will be removed from the atmosphere by rain out. Acrylamide is not known to contribute to the formation of low-level ozone or to ozone depletion. The half-life of acrylamide in the troposphere is short enough that it is unlikely that acrylamide will be transported to the stratosphere.

3.1.3.1.2 Aquatic degradation

Abiotic

Acrylamide is reported to undergo hydrolysis in water under strongly acid or alkaline conditions. The extent to which this contributes to the overall removal of acrylamide from aquatic systems is uncertain and is dependent upon the pH of the water course. The reaction rate also appears to be dependent upon temperature.

Acrylamide is reported as being hydrolysed to acrylic acid and ammonia with the reaction being catalysed by hydroxyl ions (OH^-) and hydrogen (H^+) ions (Jung et al., 1980).

Moens and Smets (1957) measured second order rate constants for the acid and alkaline hydrolysis of acrylamide at various temperatures. They found that the rate constant increased as the temperature increased. The rate constants for alkaline hydrolysis were: $k_2(OH^-)$ $1.47 \cdot 10^{-4}$ $l \cdot mole^{-1} \cdot s^{-1}$ at 55°C and $k_2(OH^-)$ $13.8 \cdot 10^{-4}$ $l \cdot mole^{-1} \cdot s^{-1}$ at 85°C. The rate constants for acid hydrolysis were: $k_2(H^+)$ $1.48 \cdot 10^{-4}$ $l \cdot mole^{-1} \cdot s^{-1}$ at 80°C and $k_2(H^+)$ $16.6 \cdot 10^{-4}$ $l \cdot mole^{-1} \cdot s^{-1}$ at 110°C. These values give half-lives of >1 year at 55°C in the pH range 5-9, and so hydrolysis in the environment would not be significant.

Brown et al. (1980b) studied the degradation of acrylamide in river water under varying conditions. Acrylamide monomer was added to sterilised river water at concentrations of 0.5 and 5 mg/l. The pH of the river water was then adjusted to give acidic or alkaline conditions as required by the addition of sulphuric acid or sodium hydroxide. The samples were then stored under anaerobic conditions. No degradation was observed in any of the samples after 2,000 hours. In river water that had not been sterilised, degradation was observed. The absence of degradation in sterilised waters suggests that the removal of acrylamide occurs via a biotic route, with abiotic removal mechanisms such as hydrolysis and photolysis being negligible.

Acrylamide is reported as undergoing photodegradation in surface waters. The half-life for the reaction based upon the reaction with hydroxyl radicals at pH 10.7 is reported as about 1 year. This is based upon a reaction rate for acrylamide with hydroxyl radicals of $k_{OH} = 2.3 \cdot 10^9$ $l \cdot mole^{-1} \cdot s^{-1}$ at a pH of 10.7 (Anbar and Neta, 1967) and a hydroxyl radical concentration of $6 \cdot 10^3$ radicals cm^{-3} (Mill et al., 1979).

Biodegradation

A number of biodegradation test results are reported for acrylamide and these are discussed below. Generally they show that acrylamide undergoes biodegradation after a period of acclimation. Based upon these results acrylamide will be taken as readily biodegradable and meeting the 10-day test window (OECD 301D Readily Biodegradable test). The first order rate constant for biodegradation in a sewage treatment plant is taken as 1 h^{-1} (Section 2.3.6, Chapter 3 of the TGD). The first order rate constant for biodegradation in surface water is taken as $4.7 \cdot 10^{-2}$ d^{-1} (Section 2.3.6, Chapter 3 of the TGD). For acrylamide the level of abiotic degradation is negligible compared to biodegradation and so the first order rate constant for biodegradation in water will be taken as the overall first order rate constant for degradation in surface water.

Acrylamide has been tested for ready biodegradability in the OECD 301D “Ready Biodegradability: Closed Bottle Test” (United States Testing Company Inc., 1991). In the test the BOD (Biological Oxygen Demand) is measured and compared with the ThOD (Theoretical Oxygen Demand). Acrylamide was dissolved in standard dilution water and then inoculated with an inoculum derived from activated sludge bacteria. The solution was then incubated in closed bottles in the

dark at 20°C for 28 days. The dissolved oxygen content was monitored over the 28-day period. At 2 mg/l acrylamide 100% degradation was observed after 28 days (degradation at 15 days and 5 days was 75.9% and 7.4%, respectively). At a higher concentration of 5 mg/l acrylamide 53.3% degradation was observed after 28 days (degradation at 15 days and 5 days was 57.0% and 7.4% respectively). This concentration was tested to confirm partially degradable material. At a lower concentration of 1 mg/l acrylamide 100% degradation was observed after 28 days (degradation at 15 days and 5 days was 100% and 7.4%, respectively). This concentration was tested to confirm readily degradable material. From the results of these tests acrylamide was classified as readily biodegradable at lower concentrations (<2 mg/l). At higher concentrations the results indicate that it may be toxic to microorganisms since a lower percentage degradation was seen. There are a small amount of data indicating toxicity in the Microtox test (See Section 3.2.1.4).

Brown et al. (1982) studied the *in situ* adsorption, degradation and toxicity of acrylamide by the spiking of the wastes of two sewage works and river water. The first sewage works utilised vertical flow primary sedimentation followed by diffuse plug flow surface aeration activated sludge, with retardation of returned activated sludge from radial flow secondary clarifiers. The second sewage works utilised radial flow primary sedimentation followed by percolating biological filters and radial flow secondary clarifiers. The river chosen for the study drained an upland granite moorland catchment and a valley of mixed woodland and rough pasture. The substrate of the river was predominantly bed rock, boulders and pebble embedded in a matrix of china-clay mine waste. The residence time of the river was 4-5 hours. Acrylamide was dosed into the river or sewage works inlet by a peristaltic pump at a chosen rate to achieve the required sewage or river concentration. The retention of the various tanks at the treatment works was assessed by the use of tracer studies. The average residence time was found to be 12 hours. Acrylamide was added continuously for 48 hours with analysis of acrylamide concentrations conducted after 24 and 48 hours. The river was continuously dosed with acrylamide for 2-3 months at a concentration of 6 mg/l, 6 hours before sampling the concentration was increased to 50 mg/l. Samples were collected during the experiment from both the sewage works and river, and incubated in the laboratory under aerobic conditions in daylight to assess their ability to degrade acrylamide. Brown et al. found that there was little *in situ* degradation of acrylamide in primary or final settlement tanks of the sewage works. Degradation rates of 50% (based upon mass flow) were observed in the activated sludge tanks and biological filter beds. Laboratory incubations of samples of sewage discharging from the biological filter bed, activated sludge tank and primary and final settlement tanks showed no loss of acrylamide after 24 hours. Laboratory incubations of acrylamide-spiked tapwater dosed with varying quantities of activated sludge and filter bed clinker suggested that significant degradation of acrylamide might occur. This was thought to be due to the presence of microbes on the surface of the clinker. No *in situ* loss of dosed acrylamide was observed in the river during the study period.

Brown et al. (1980b) studied the degradation of acrylamide in natural and polluted waters. Representative water samples were spiked with 0.5 mg/l and 5.0 mg/l acrylamide and incubated under naturally illuminated aerobic conditions for 2,000 hours. The degradation mechanisms were further studied by experiments with spiked river water samples under varying conditions (aerobic and anaerobic, light and dark, sterilisation, pH). Acrylamide was found to be degraded in all the unsterilised natural and polluted water samples. Under aerobic conditions illumination did not affect the rate of degradation, but under anaerobic conditions degradation was slower in the dark. Algal growth was observed in aerated samples only. **Table 3.2** details the degradation rates observed for different water systems.

Table 3.2 Degradation of acrylamide (0.5 mg/l) in natural and polluted water samples

Sample	Degradation
China Clay Pit - Effluent	Lag phase of 3.1 days then complete primary degradation after 6.3 days
China Clay Pit - Hose pool	Lag phase of 4.3 days then complete primary degradation after 15.6 days
China Clay Pit - Process water	Complete primary degradation after 0.5 days
Wastewater treatment plant - Effluent	Lag phase of 2.3 days then complete primary degradation after 5.2 days
Estuarine water	Lag phase of 5.2 days then complete primary degradation after 7.3 days
Peat bog water	Lag phase of 2.3 days then complete primary degradation after 5.2 days
Riverwater	Lag phase of 1.5-1.9 days then complete primary degradation after 4.2-5.2 days
Seawater	Lag phase of 7.3 days then complete primary degradation after 10.4 days

Croll et al. (1974) studied the degradation of acrylamide in aerated sunlit water. They found that 8 mg/l acrylamide was rapidly lost after a lag period of 220 hours. Subsequent re-seeding of the culture with acrylamide, produced faster rates of decomposition with little or no lag period. The initial lag period indicated that acclimatisation of the organisms present was required before degradation could occur. To test this hypothesis two cultures were run in parallel, one with aerated river water and the other with aerated river water inoculated with 500 ml of a culture capable of degrading acrylamide. The inoculated culture started to degrade acrylamide within 5 hours while the aerated river water started to degrade acrylamide after 50 hours. The biodegradability of acrylamide in open and closed vessels seeded with settled sewage effluent (5 mg/l) was investigated. Degradation was found to occur more quickly in the closed vessel.

Yamada et al. (1979) isolated from activated sludge a bacterium (*Arthrobacter*) capable of utilising acrylonitrile as a sole source of carbon and nitrogen. The bacterium isolated was able to degrade 2-5 g of acrylamide within 7 days.

Arai et al. (1981) isolated a bacterium from a sewage sample obtained from an acrylamide plant. The bacterium was identified as a strain of *Rhodococcus* 10 021 R. The bacterium was capable of degrading acrylamide and utilising it as the sole source of carbon and nitrogen.

Klump et al. (1986) proposed a likely degradation pathway for acrylamide by heterotrophs capable of utilising both the carbon and nitrogen in acrylamide. The first step is the deamination of acrylamide to acrylic acid, followed by decarboxylation of acrylic acid to ethylene or ethanol. They used ^{14}C-labelled substances to determine the microbially-mediated mineralisation and uptake of acrylamide. Samples were obtained from a deep fresh water lake, harbour water and effluent from a metropolitan sewage plant. Microbial activity was defined as the difference in mineralisation observed between test samples and samples in which microbial activity was inhibited by formaldehyde. The samples were then incubated under varying conditions (aerobic and anaerobic, temperature). No uptake or mineralisation was detected in the harbour water sample. Uptake was measured in the sewage effluent sample (0.08 ng/ml/hr) though no respiration was detected. In sediment samples obtained from the harbour and lake mineralisation was observed. The rate was higher in the harbour sediment than the lake water sediment, and this was thought to occur because of different ambient temperatures of sediment and differing microbial populations.

Bridié et al. (1979b) measured the BOD and COD of acrylamide. The BOD was measured using the APHA Standard Method 219 (1971). 500 ml test solutions were seeded with 10 ml of filtered effluent from a biological sanitary waste treatment plant and incubated at 20^{o}C for 5 days. The

COD was determined using ASTM D 1252-67. The resulting BOD was 0.05 g/g (4% of ThOD), the COD was 1.33 g/g (99% of ThOD) and the ThOD without taking nitrification into account was 1.35 g/g. BOD and COD demand measurements may be used a screening test for biodegradation. The BOD result indicates little biodegradation.

Winter et al. (1982) measured the BOD using the APHA Standard Method 219 (1971). They adapted the test by adding 0.5 mg/l allyl thiourea to prevent nitrification. Effluent from a sewage treatment plant was used as seed material. The BOD for unadapted seed was 0.04 g/g (3% of ThOD) and for adapted seed 0.9 g/g (67% of ThOD). The ThOD was calculated without taking nitrification into account.

Batchelder (1975) reported a BOD of 1.08 g/g (50% of ThOD) and CODs of 1.38 g/g (Dichromate method) and 1.50 g/g (Permanganate method) (64% and 72% of ThoD respectively) for 100% acrylamide. The ThOD was 2.25 g/g and was calculated taking nitrification into account.

3.1.3.1.3 Degradation in soil

Acrylamide is degraded in soil and the rate of degradation appears to be dependent upon soil content, pH and temperature. Optimum degradation is observed in soils with alkaline pH and at higher temperatures. The available results are discussed below.

Lande et al. (1979) studied the degradation and leaching potential of acrylamide in a variety of different soil types. Acrylamide metabolism in soil was examined by the biometer flask procedure under aerobic and anaerobic conditions. Acrylamide degradation was monitored by the production of CO_2. The parameters evaluated in the aerobic degradation studies were soil type (loam, silt loam, loamy fine sand, silt clay), temperature and acrylamide concentration. Half-lives obtained varied between 18 to 45 hours (25 mg/kg acrylamide); at higher concentrations higher half-lives were measured (95 hours at 500 mg/kg). In anaerobic studies longer half-lives were observed.

Abdelmagid et al. (1982) studied the conversion of acrylamide to inorganic nitrogen by soil microorganisms under aerobic and waterlogged conditions. Surface soil samples were collected from a variety of soil types, and the samples were prepared by either air drying for 48 hours or storing in the refrigerator. Acrylamide solution (10 mg acrylamide) was added dropwise to the soils (10 g samples) until the sample was moist. The resulting moisture contents of the soils ranged from 40-60% of their water holding capacities. The waterlogged experiments were conducted with 10 g sample treated with 20 ml of water containing 10 mg acrylamide. The samples were then incubated at 10, 20 or 30^{o}C under aerobic conditions for between 2 to 21 days. They found that acrylamide is hydrolysed in soils to produce NH_4^+. The NH_4^+ produced is then oxidised to NO_2^- and NO_3^- under aerobic conditions and is accumulated as NH_4^+ under waterlogged conditions. They found that the rate of decomposition in soil was affected by temperature and time of incubation.

The first order rate constant for degradation in soil can be calculated from the Kp_{soil} (Section 3.1.3.2.1) and the biodegradation in surface water (Section 3.1.3.1.2). For acrylamide the half-life for biodegradation in soil is estimated at 30 days and the first order rate constant for biodegradation in soil $kbio_{soil}$ as 0.023 d^{-1} (Section 2.3.6, Chapter 3 of the TGD).

3.1.3.1.4 Degradation in use

No data are reported in the IUCLID document. The major use of acrylamide is in polymer processing. As detailed in Section 3.1.2.2 the main use of these polymers is as flocculation aids, and so free monomer may be released and undergo degradation as described in the above sections.

3.1.3.2 Distribution

3.1.3.2.1 Adsorption

Adsorption of acrylamide by natural sediments, industrial and sewage sludges, clays, peat, and cationic, anionic and hydrophobic resins is thought to be negligible. Acrylamide is relatively mobile in most soil types.

Brown et al. (1980c) studied the removal of acrylamide from water columns by adsorption on to sludge and sediment particles. Sediment or sewage sludge (containing 5 g solids) was added to filtered natural waters spiked with acrylamide (0.5 mg/l or 10 mg/l). Solutions were also prepared without the addition of sediment or sewage sludge. The solutions were stored under aerobic conditions for 168 hours and acrylamide concentrations measured after 4, 24 and 168 hours. The authors found that the presence of sediment or sewage sludge did not significantly alter acrylamide loss. Acrylamide was completely lost after 168 hours from estuarine water and river water samples, while between 40-75% loss was observed in the seawater and sewage works effluent samples. The experiments were repeated using different substrates (Kaolinite clay, montmorillonite clay, peat, activated carbon, cation exchange resin, anion exchange resin and hydrophobic resin) and sterilised river water. No loss of acrylamide from sterile river water samples in contact with montmorillonite, kaolinite, anionic, cationic or hydrophobic resins was observed. Activated carbon adsorbed acrylamide but appeared to have a limited capacity. The loss of acrylamide in the peat solution was thought to be due to microbial degradation.

Lande et al. (1979) studied the degradation and leaching potential of acrylamide in a variety of different soil types. The soil leaching rate was measured by thin-layer chromatography. The values measured indicated that acrylamide was relatively mobile in soil, being most mobile in loamy fine sand (*Rf* values 0.846-0.880) and least mobile in silt clay (*Rf* values 0.637-0.657).

The organic carbon-water partition coefficient value (K_{oc}) for acrylamide can be calculated using the octanol-water partition coefficient (K_{ow}) (Chapter 4 of the TGD). The K_{oc} value can then be used to derive the solid-water partition coefficient (Kp) of acrylamide for each compartment (soil, sediment, suspended matter) (Equation 9, Chapter 3 of the TGD). These may also be expressed as dimensionless partition coefficients (Equation 10, Chapter 3 of the TGD). For acrylamide the following partition coefficients are calculated based on a log K_{ow} of -1:

K_{oc}	0.195 l/kg	Organic carbon-water partition coefficient (Chapter 4, TGD)
Kp_{susp}	0.0195 l/kg	Solid-water partition coefficient in suspended matter (Equation 9, Chapter 3, TGD)
Kp_{sed}	0.00976 l/kg	Solid-water partition coefficient in sediment (Equation 9, Chapter 3, TGD)
Kp_{soil}	0.0039 l/kg	Solid-water partition coefficient in soil (Equation 9, Chapter 3, TGD)
$K_{soil\text{-}water}$	0.206 m^3/m^3	Soil-water partition coefficient (Equation 10, Chapter 3, TGD)
$K_{susp\text{-}water}$	0.905 m^3/m^3	Suspended matter-water partition coefficient (Equation 10, Chapter 3, TGD)
$K_{sed\text{-}water}$	0.805 m^3/m^3	Sediment-water partition coefficient (Equation 10, Chapter 3, TGD)

In summary the data show that adsorption of acrylamide to soil and sediment is not significant.

3.1.3.2.2 Volatilisation

Acrylamide is unlikely to volatilise from water due to its high water solubility and low vapour pressures. The Henry's Law constant has been calculated as $2.97 \cdot 10^{-5}$ $Pa \cdot m^3 \cdot mol^{-1}$ at 25°C from the vapour pressure and water solubility (Equation 7, Chapter 3 of the TGD). The air-water partitioning coefficient ($K_{air,\ water}$) (also known as the dimensionless Henry's Law constant) can be calculated from the Henry's Law constant (Equation 8 Chapter 3 of the TGD), and is $1.25 \cdot 10^{-8}$ m^3/m^3. These values indicate that it is unlikely that acrylamide will volatilise from water at ambient temperatures.

3.1.3.2.3 Modelling

Using the FUGMOD (OECD workshop) Mackay Level I model, the distribution of acrylamide in the environment is calculated as:

Air	<0.1%
Water	99.99%
Soil	<0.1%
Sediment	<0.1%
Biota	<0.1%

Using the FUGMOD (OECD workshop) Mackay Level III model, the distribution of acrylamide in the environment is calculated as:

Air	<0.1%
Water	99.95%
Soil	<0.1%
Sediment	<0.05%

The level III model calculation uses a release rate of 1,000 kg/hour and assumes that releases are to water only. The results show that water is the most important compartment for acrylamide.

3.1.3.3 Accumulation and metabolism

Several studies have been reported on the uptake and elimination of acrylamide in fish, and these are detailed below. The studies show a low bioconcentration factor (BCF) for acrylamide in fish. Generally uptake is rapid upon exposure, and then slows until a steady state is reached. Elimination has been found to be biphasic with most of the acrylamide being lost in an unchanged form. The low log K_{ow} value indicates a very low likelihood of accumulation.

Fujiki et al. (1982) studied the accumulation of acrylamide in fish. In the first study they exposed carp (*Cyprinus carpio*) to acrylamide solutions of 1 mg/l and 10 mg/l for between 20 and 40 days under static conditions. The solutions were renewed daily to maintain the acrylamide concentrations. Fish were removed at 1, 5, 10, 15 and 20 days from the 1 mg/l test solution tank and at 1, 10, 20, 30 and 40 days from the 10 mg/l solution and control tanks. They found that carp exposed to 1 mg/l acrylamide accumulated acrylamide slowly during the first 10 days then increased rapidly until it reached 0.26 mg/kg on day 20. In carp exposed to 10 mg/l acrylamide accumulation was rapid to day 10, then slow until day 30, and then rapid until day 40 when it reached a concentration of 7.65 mg/kg. In a second study they exposed Japanese medaka (*Oryzias latipes*) to acrylamide solutions of 1 mg/l and 10 mg/l for 20 days under static conditions. Fish were removed on days 1, 5, 10, 15 and 20 for analysis. They found that accumulation of acrylamide in fish exposed to 1 mg/l acrylamide was slow in the first 10 days of exposure then rapid between days 10-20, the resulting concentration being 0.31 mg/kg. In fish exposed to 10 mg/l acrylamide accumulation was rapid to day 15 then slow until day 20 when a concentration of 25.3 mg/kg was obtained. In a third study they exposed carp and Japanese medaka to polyacrylamide (20 mg/l) for 60 days. In each case no accumulation of acrylamide monomer in the fish was detected. The BCFs estimated from these results are 0.26 and 0.77 for carp and 0.31 and 2.53 for Japanese medaka.

Petersen et al. (1985) studied the uptake, deposition and elimination of acrylamide in rainbow trout (*Oncorhynchus mykiss*). Fish were exposed to a steady state concentration of acrylamide and 2,3-^{14}C acrylamide under static conditions for 72 hours. This was followed by a depuration period in fresh water for 96 hours. Samples of fish for analysis were taken at regular intervals. In fish exposed to 0.710 mg/l acrylamide, uptake was rapid in the carcass and viscera in the first 24 hours. Uptake was then slower until a steady concentration was reached at 72 hours. The BCFs calculated from these exposures were 1.44 for the carcass and 1.65 for the viscera. The elimination of acrylamide from the carcass and viscera was found to be biphasic with an initial phase being rapidly followed by a slower second phase. The $t_{½}$ values for elimination of acrylamide from the viscera were 16 hours for the initial elimination and 5.7 days for the second elimination, and for the carcass, the values were 10 hours for the first elimination and 7.7 days for the second elimination. After a 96-hour depuration period acrylamide concentrations in both the carcass and viscera had declined to approximately 25% of the steady state values. Further studies indicated that acrylamide uptake was greatest in the kidneys with elimination greatest from the blood and gills and slower from the muscles and intestines. Acrylamide was found to be excreted via the gills, urine and bile, 90% of it in an unchanged form.

Waddel et al. (1981) studied the distribution of acrylamide in the rainbow trout (*Oncorhynchus mykiss*). Acrylamide was administered by intraperitoneal injection of 3.2 mg/kg body weight of 2,3-^{14}C acrylamide into fish weighing 7 g. The fish were transferred to a fresh water pond and autoradiographs were taken on sagittal sections of the body after 22 hours and 5 days respectively after injection. A large amount of radioactivity was present in the kidney, urinary bladder, gall bladder, contents of the small intestine, and the lens of the eye after 22 hours. Smaller amounts were present in the central nervous system, liver and gills. A large amount of

radioactivity was still present in the gall bladder and lens after 5 days, whereas smaller amounts were present in the sclera, spinal column, central nervous system, kidneys, wall of the small intestine and at isolated sites in the subcutaneous tissue. A very small amount of radioactivity was present in the muscles for up to 5 days.

In summary, uptake of acrylamide by aquatic organisms is generally rapid upon exposure, then slow until a low steady state concentration is achieved. Elimination from the organisms then occurs via a biphasic mechanism with most of the acrylamide being lost in an unchanged form. Very low BCFs (<1) have been measured for acrylamide in aquatic organisms. The data suggest that biomagnification is unlikely to occur.

3.1.4 Aquatic compartment (including sediment)

Releases of acrylamide to water may occur during production, production of acrylamide polymers and copolymers, as monomer from acrylamide-based polymers used as water treatment flocculants, and from acrylamide-based grouts.

The data reported in Section 3.1.3.1.2 suggest that in surface waters biodegradation is likely to be the main removal mechanism. Hydrolysis may occur under alkaline or acidic conditions and photolysis may also occur, though the extent of these removal mechanisms is thought to be negligible when compared to biodegradation. Volatilisation of acrylamide from surface waters is unlikely due to the high water solubility of acrylamide.

Acrylamide is unlikely to adsorb to sediment. The sediment pore water concentrations will therefore be similar to the surface water concentrations.

3.1.4.1 Predicted environmental concentrations in water

The predicted environmental concentrations (PECs) of acrylamide in water have been calculated using the TGD and related computer modelling programs.

3.1.4.1.1 Calculation of $PEClocal_{water}$

Full details on how to calculate a $PEClocal_{water}$ may be found in the TGD (Chapter 3, Sections 2.3.7, and 2.3.8.3). In summary the following equations are used:

$$Clocal_{\text{inf}} = \frac{Elocal_{water} \cdot 10^{6}}{EFFLUENT_{stp}} \qquad \text{(17 TGD)}$$

$$Clocal_{eff} = Clocal_{\text{inf}} \cdot Fstp_{water} \qquad \text{(18 TGD)}$$

$$EFFLUENT_{stp} = CAPACITY_{stp} \cdot WASTEW_{inhab} \qquad \text{(19 TGD)}$$

$$Clocal_{water} = \frac{Clocal_{eff}}{\left(1 + Kp_{susp} \cdot SUSP_{water} \cdot 10^{-6}\right) \cdot DILUTION} \qquad \text{(30 TGD)}$$

$$Dilution = \frac{EFFLUENT_{stp} + FLOW}{EFFLUENT_{stp}} \quad \text{(31 TGD)}$$

$$Clocal_{water,ann} = Clocal_{water} \cdot \frac{Temission}{365} \quad \text{(32 TGD)}$$

$$PEClocal_{water} = Clocal_{water} + PECregional_{water} \quad \text{(33 TGD)}$$

$$PEClocal_{water,ann} = Clocal_{water,ann} + PECregional_{water} \quad \text{(34 TGD)}$$

Explanation of symbols:

$Clocal_{eff}$	Concentration of the chemical in the STP-effluent [mg/l]
$Clocal_{inf}$	Concentration in untreated wastewater [mg/l]
$Clocal_{water}$	Local concentration in surface water during emission episode [mg/l]
$Clocal_{water,ann}$	Annual average local concentration in surface water [mg/l]
$CAPACITY_{stp}$	Capacity of the STP [10,000]
DILUTION	Dilution factor [Default 10]
$EFFLUENT_{stp}$	Effluent discharge rate of stp [Default 2,000,000 l/d]
$Elocal_{water}$	Local emission rate to (waste) water during episode [kg/d]
$Fstp_{water}$	Fraction of emission directed to water by STP [Chapter 3, Appendix 2 TGD: 0.13]
FLOW	Flow rate of the river [l/d]
Kp_{susp}	Solids-water partitioning coefficient of suspended matter [0.0195 l/kg - see Section 3.1.3.2.1]
$PEClocal_{water}$	Predicted environmental concentration during episode [mg/l]
$PEClocal_{water,ann}$	Annual average predicted environmental concentration [mg/l]
$PECregional_{water}$	Regional concentration in surface water [mg/l]
$SUSP_{water}$	Concentration of suspended matter in water [15 mg/l]
Temission	No of days per year that emission takes place [d/year]
$WASTEW_{inhab}$	Sewage flow per inhabitant [200 l/d]

The $PEC_{regional}$ is taken as a background concentration and added to the local concentration to give the PEC_{local}. The $PEC_{regional}$ is calculated in Section 3.1.4.1.2. as 0.05 μg/l.

$PEC_{local, water}$ from acrylamide production and processing plants

The $PEClocal_{water}$ is calculated for each production and processing site detailed in **Table 3.1** (Section 3.1.2.1). Where information on releases from the plant is not available default release estimates are used.

Site A

Effluent from production and processing is treated at an on-site treatment works. The effluent from the on-site treatment works is then treated in a municipal wastewater treatment plant. The release to the on-site treatment works is estimated at 3 kg/d. No information is available on the operating parameters of the on-site or municipal sewage treatment plant.

$PEClocal_{water}$ for Site A:

$Clocal_{inf}$	= 1.5 mg/l
$Clocal_{eff}$	= 0.195 mg/l

The $Clocal_{eff}$ from on-site treatment is taken as the $Clocal_{inf}$ in the municipal wastewater treatment plant:

$Clocal_{inf}$	= 0.195 mg/l
$Clocal_{eff}$	= 0.025 mg/l
$Clocal_{water}$	= 2.54 μg/l (Based upon 300 days operation per year)
$Clocal_{water,ann}$	= 2.08 μg/l
$PEClocal_{water}$	= 2.59 μg/l
$PEClocal_{water,ann}$	= 2.13 μg/l

Site B

Effluent from acrylamide production at site B is recycled back into the production process on site. The effluent is monitored for acrylamide, but it is not detected. The $PEClocal_{water}$ for site B is therefore taken as the background concentration of acrylamide in the environment ($PECregional_{water}$).

$PEClocal_{water}$	= 0.05 μg/l
$PEClocal_{water,ann}$	= 0.05 μg/l

Site C

The maximum concentration of acrylamide in plant effluent is set as a discharge consent of 1 mg/l. Monitoring data from the plant confirm that on most days the concentration of acrylamide is below 1 mg/l, and so this will be taken as the $Clocal_{eff}$ in calculating the $PEClocal_{water}$ for acrylamide. Effluent from the plant is discharged directly to an estuary; the dilution rate of the receiving waters is therefore likely to be significantly higher than the default value of 10. A dilution factor of 100 will be used in this assessment.

$PEClocal_{water}$ for Site C:

$Clocal_{eff}$	= <1 mg/l
$Clocal_{water}$	= <10 μg/l (Based upon 300 days operation per year)
$Clocal_{water,ann}$	= <8.22 μg/l
$PEClocal_{water}$	= <10 μg/l
$PEClocal_{water,ann}$	= <8.22 μg/l

Site D

Effluent from the acrylamide production and processing plant at site D is treated at a municipal sewage treatment plant. The effluent is monitored for acrylamide, and concentrations are less than 10 μg/l. This value will be used as the $Clocal_{eff}$ in calculating the $PEClocal_{water}$.

$PEClocal_{water}$ for Site D:

$Clocal_{eff}$	= <10 μg/l
$Clocal_{water}$	= <1 μg/l (Based upon 300 days operation per year)
$Clocal_{water,ann}$	= <0.82 μg/l
$PEClocal_{water}$	= <1.05 μg/l
$PEClocal_{water,ann}$	= <0.87 μg/l

Site E

Effluent at site E is treated on site prior to release to a municipal wastewater treatment plant. The effluent is monitored for acrylamide prior to treatment at the municipal wastewater treatment plant. The average concentration of acrylamide in emulsion wash water is 0.9 mg/l. This is treated on site by hydrolysis to give an average acrylamide concentration of 0.09 mg/l. This concentration is taken as $Clocal_{inf}$ for input into the municipal sewage treatment plant. No information is available as to the operating parameters of the municipal sewage treatment plant so the default parameters will be used. The number of days operation at the plant is reported as 250 days per year.

PEClocal, $_{water}$ for Site E:

$Clocal_{inf}$	= 0.09 mg/l (Measured)
$Clocal_{eff}$	= 11.70 μg/l
$Clocal_{water}$	= 1.17 μg/l (Based upon 250 days operation per year)
$Clocal_{water,ann}$	= 0.80 μg/l
$PEClocal_{water}$	= 1.22 μg/l
$PEClocal_{water,ann}$	= 0.85 μg/l

Site F

The release of acrylamide during processing from site F is reported as 5 kg/year. There is no indication from the BUA report (1992) if this emission is direct to surface water or to a wastewater treatment plant, so it will be assumed that releases are direct to surface water. Assuming operations are for 300 days a year gives a daily release of 0.02 kg/day. The $Clocal_{eff}$ may be derived if the effluent discharge rate from the plant is known - due to lack of information the default effluent discharge rate of 2,000,000 l/d will be used.

$PEClocal_{water}$ for Site F:

$Clocal_{eff}$	= 0.01 mg/l
$Clocal_{water}$	= 1 μg/l (Based upon 300 days operation per year)
$Clocal_{water,ann}$	= 0.82 μg/l
$PEClocal_{water}$	= 1.05 μg/l
$PEClocal_{water,ann}$	= 0.87 μg/l

Site G

Effluent from processing at site G is treated on site. The effluent from the on-site treatment plant is then discharged to a municipal sewage treatment plant. The emission of acrylamide in the effluent after on-site treatment is 9 kg/year. Assuming 300 days operation a year gives a release of 0.03 kg/day.

$PEClocal_{water}$ for Site G:

$Clocal_{inf}$	= 15 μg/l
$Clocal_{eff}$	= 1.95 μg/l
$Clocal_{water}$	= 0.19 μg/l (Based upon 300 days operation per year)
$Clocal_{water,ann}$	= 0.16 μg/l
$PEClocal_{water}$	= 0.24μg/l
$PEClocal_{water,ann}$	= 0.21 μg/l

Site H

Effluent from the acrylamide production and processing plant at site H is treated at a municipal sewage treatment plant. Emissions are estimated at 10 kg/year from the production plant to the municipal sewage treatment plant. Assuming 300 days operation a year gives a daily release of 0.03 kg/day. The $Clocal_{eff}$ may be derived if the effluent discharge rate from the plant is known - due to lack of information the default effluent discharge rate of 2,000,000 l/d will be used.

$PEClocal_{water}$ for Site H:

$Clocal_{inf}$	= 15 μg/l
$Clocal_{eff}$	= 1.95 μg/l
$Clocal_{water}$	= 0.2 μg/l (Based upon 300 days operation per year)
$Clocal_{water,ann}$	= 0.16 μg/l
$PEClocal_{water}$	= 0.25 μg/l
$PEClocal_{water, ann}$	= 0.21 μg/l

$PEC_{local, water}$ due to release of monomer from polyacrylamides

In Section 3.1.1.1 the concentration of free acrylamide monomer in polyacrylamide-treated waters is calculated, based upon data provided by the TEGEWA Polyelectrolyte Producers Group. The maximum concentration of acrylamide in treated water is calculated as 10 μg/l from

use as a coagulant and flocculant in the pulp and paper industry, 60 μg/l for use as a drainage and retention aid in the pulp and paper industry and 0.5 mg/l for other industrial processes using polyacrylamide flocculants. These concentrations are equivalent to a $Clocal_{eff}$, and they are then subject to further dilution in the receiving waters. For these applications it is assumed that use is for 300 days a year.

$PEClocal_{water}$ for polyacrylamide-treated process water from use as a coagulant and flocculant in the pulp and paper industry discharged to surface water:

$Clocal_{eff}$	= 10 μg/l
$Clocal_{water}$	= 1 μg/l (Based upon 300 days operation per year)
$Clocal_{water,ann}$	= 0.82 μg/l
$PEClocal_{water}$	= 1.05 μg/l
$PEClocal_{water,ann}$	= 0.87 μg/l

$PEClocal_{water}$ for polyacrylamide-treated process water from use as a drainage aid in paper and pulp production discharged to surface water:

$Clocal_{eff}$	= 60 μg/l
$Clocal_{water}$	= 6 μg/l (Based upon 365 days operation per year)
$Clocal_{water,ann}$	= 4.93 μg/l
$PEClocal_{water}$	= 6.05 μg/l
$PEClocal_{water,ann}$	= 4.98 μg/l

$PEClocal_{water}$ for polyacrylamide-treated process water discharged to surface water:

$Clocal_{eff}$	= 0.5 mg/l
$Clocal_{water}$	= 50 μg/l (Based upon 300 days operation per year)
$Clocal_{water,ann}$	= 41.10 μg/l
$PEClocal_{water}$	= 50.05 μg/l
$PEClocal_{water,ann}$	= 41.15 μg/l

This scenario, involving the direct discharge of treated water to surface water, is not considered to be common practice; information from the industry indicates that this happens rarely if at all. Therefore this calculation is included for information, but will not be taken through to the risk characterisation.

The effluent from processes using polyacrylamide flocculants will in most cases enter a municipal wastewater treatment plant. In this case the maximum concentration of free acrylamide monomer in treated water of 0.5 mg/l may be treated as a $Clocal_{inf}$.

$PEClocal_{water}$ for polyacrylamide-treated process water discharged to a municipal wastewater treatment plant:

$Clocal_{inf}$	= 0.5 mg/l
$Clocal_{eff}$	= 65 μg/l
$Clocal_{water}$	= 6.5 μg/l (Based upon 300 days operation per year)
$Clocal_{water,ann}$	= 5.34 μg/l
$PEClocal_{water}$	= 6.55 μg/l
$PEClocal_{water,ann}$	= 5.39 μg/l

The resultant $PEClocal_{water}$ for polyacrylamide-treated process waters from pulp and paper production discharge to a municipal wastewater are 0.18 μg/l for use as a coagulant and flocculant and 0.83 μg/l for use as a drainage and retention aid.

For sewage sludge treatment processes where the water is fed into the head of the sewage treatment plant the maximum concentration of free acrylamide monomer in treated water is calculated as 0.5 mg/l. As the water is fed back into the sewage treatment plant it is taken as equivalent to a $Clocal_{inf}$. The $PEClocal_{water}$ will therefore be the same as the $PEClocal_{water}$ calculated for polyacrylamide-treated process water discharged to a municipal wastewater treatment plant ($PEClocal_{water}$ 6.55 μg/l).

In calculating these PECs a number of assumptions have been made as follows:

- the residual monomer content of the polymer is 0.1%;
- all of this is potentially released to the environment;
- the dilution in the stock solution is 0.5%; and
- the dilution in the treated material is 1:10.

The dilution of stock solution in treated material appears to be quite low when compared to the dilution in drinking water treatment (1:10,000). It is likely to be higher for a number of applications, especially wastewater treatment.

$PEC_{local, water}$ due to the use of acrylamide and NMA-based grouts

Sewer repair use

Very little information on concentrations of acrylamide in water is available following pipeline repairs or manhole sealing operations using acrylamide-based grouts. However under normal operating conditions, acrylamide concentrations due to use of grouts should be relatively small and localised in their nature. The highest concentrations are likely to occur directly after application of the grout and before the polymerisation process is complete. Based upon information supplied by UK companies that used to use NMA-based grouts the daily amount of grout used was 12 kg/day. From the data supplied by the EU producer of the grout, the residual concentration of acrylamide monomer is 5% (0.6 kg) maximum.

As a worst case this amount will be assumed to be released upon application of the grout to the crack to be sealed. In calculating a $PEClocal_{water}$ this amount will be taken as an input to a local wastewater treatment plant. No account is taken of acrylamide that may be formed during the gelling procedure due to the reaction of NMA with the silicate or of biodegradation in the pipeline before it reaches the wastewater treatment plant. It also assumes that all the residual

acrylamide monomer in the grout is released, whereas in practice the majority of it is likely to be incorporated into the grout as it polymerises.

Using the equations given in the TGD, a $Clocal_{water}$ of 3.9 μg/l is calculated. The $PEClocal_{water}$ is obtained by adding the $PECregional_{water}$ (0.05 μg/l) to $Clocal_{water}$. This gives a $PEClocal_{water}$ of 3.9 μg/l.

Construction use

Not enough information is currently available to predict local concentrations due to the use of acrylamide-based grouts in construction applications such as tunneling. However, measured levels in the environment as a result of use in tunneling applications are available and these are presented in Section 3.1.4.2.1.

Summary of $PEClocal_{water}$

Table 3.3 summarises the $PEClocal_{water}$ calculated for acrylamide from each production and processing site and due to potential release of acrylamide monomer from polyacrylamides.

Table 3.3 Summary of $PEClocal_{water}$

Scenario	$PEClocal_{water}$ (μg/l)	$PEClocal_{water,ann}$ (μg/l)
Site A	2.59	2.13
Site B	0.05	0.05
Site C	< 10	< 8.2
Site D	< 1.05	< 0.87
Site E	1.22	0.85
Site F	1.05	0.87
Site G	0.24	0.21
Site H	0.25	0.21
Wastewater from use as a coagulant and flocculant in the pulp and paper industry	1.05	0.87
Wastewater from use as a drainage aid in pulp and paper production	6.05	4.98
Wastewater treated with polyacrylamide discharged to surface water	50.05	41.15 *
Wastewater treated with polyacrylamide discharged to sewage treatment plant	6.55	5.39
Use of NMA grouts (sewer repair)		3.9

Note: * not considered further in this report

3.1.4.1.2 Calculation of $PECregional_{water}$ and $PECcontinental_{water}$

The regional and continental PECs for acrylamide have been calculated using the EUSES model. The input to the model for the continental and regional scenarios is detailed in Section 3.1.2.5. The following regional and continental PECs are calculated for acrylamide:

$PECregional_{water}$ 0.05 μg/l
$PECcontinental_{water}$ 0.007 μg/l

3.1.4.1.3 Calculation of PECstp

The sewage treatment plant PEC (PECstp) is taken as being equivalent to the $Clocal_{eff}$. For acrylamide the following $Clocal_{eff}$ are calculated:

$Clocal_{eff}$ (Production and processing plants - highest value Site A)	0.195 mg/l
$Clocal_{eff}$ (Use of polyacrylamide flocculants in wastewater treatment)	0.065 mg/l
$Clocal_{eff}$ (Use of NMA grouts in sewer repair)	0.039 mg/l

3.1.4.1.4 Calculation of PECsediment

The $PEClocal_{sediment}$ can be derived from the $PEClocal_{water}$ assuming a thermodynamic partition equilibrium. The following equation is used to derive the $PEClocal_{sediment}$:

$$PEClocal_{sed} = \frac{K_{susp,water}}{RHO_{susp}} \cdot PEClocal_{water} \cdot 1{,}000 \qquad \text{(TGD 35)}$$

Explanation of symbols:

$K_{susp,water}$	Suspended matter-water partition coefficient [0.905 m^3/m^3 Section 3.1.3.2.1]
$PEClocal_{sed}$	[mg/kg]
$PEClocal_{water}$	Highest value of 6.55 µg/l used [Section 3.1.4.1.1.]
RHO_{susp}	Bulk density of suspended matter [1,150 kg/m^3]

The worst-case $PEClocal_{sediment}$ for acrylamide is calculated as 0.005 mg/kg using this method, based on the default data for wastewater treated with polyacrylamide discharged to a sewage treatment plant. All other sites and uses would give values lower than this.

3.1.4.2 Measured levels in water

Acrylamide has been measured in a number of water systems and these levels are detailed in **Tables 3.4 - 3.9**. The background levels of acrylamide are generally low and in most cases below the level of detection. Higher values have been observed in water systems near production sites.

Table 3.4 Measured levels of acrylamide in surface (river) waters

Location	Concentration	Notes	Reference
UK: River Tavy	3.4 μg/l	d.l. 0.2 μg/l	Brown and Rhead (1979)
UK: River Erme	n.d	d.l. 0.2 μg/l	Brown and Rhead (1979)
UK: River Calm	n.d	d.l. 0.2 μg/l	Brown and Rhead (1979)
USA: Tributary James River	n.d	Sampling point 1.6 km downstream of discharge point from a production site	Going (1978)
USA	n.d.	d.l. 0.8 μg/l. 4 samples: 1 acrylamide producer, 2 acrylamide producers and processors, 1 polyacrylamide user	Going (1978)
UK	0.3 μg/l	Sample downsteam from China clay quarry	Croll et al. (1974)
UK	n.d	d.l 0.2 μg/l. Sample from water works using polyacrylamide	Brown et al. (1980a)
UK	n.d	d.l. 0.2 μg/l. 600 m downstream of discharge point from a paper factory using polyacrylamide	Brown et al. (1980a)

Note: n.d = not detected; d.l = detection limit

Table 3.5 Measured levels of acrylamide in estuarine and sea waters

Location	Concentration	Notes	Reference
UK: Estuarine water	n.d.	d.l. 0.2 μg/l	Brown and Rhead (1979)
UK: Sea water	n.d.	d.l. 0.2 μg/l	Brown and Rhead (1979)

Note: n.d = not detected; d.l = detection limit

Table 3.6 Measured levels of acrylamide in drinking water

Location	Concentration	Notes	Reference
UK: Bradinch	n.d.	d.l. 0.2 μg/l	Brown and Rhead (1979)
UK: Ivybridge	n.d.	d.l. 0.2 μg/l	Brown and Rhead (1979)
UK: Plymouth	< 4.5 μg/l	Polyacrylamide used as flocculant in water treatment works	Brown and Rhead (1979)
Japan	400 mg/l	Sample 2.5 m from a drain grouted with a substance containing acrylamide	Igisu et al. (1975)
USA	n.d.	d.l. 1 μg/l. Polyacrylamide used as a flocculant in water treatment works	Going and Thomas (1979)
USA	n.d.	d.l. 25 μg/l	Going (1978)

Note: n.d = not detected; d.l = detection limit

Table 3.7 Measured levels of acrylamide in wastewater treatment plants

Location	Concentration	Notes	Reference
UK	2.3-17.4 μg/l (Effluent)	Polyacrylamide not used in water treatment works	Brown and Rhead (1979)
UK	n.d. (Sludge)	d.l. 4 μg/l. Sewage sludge from plant using polyacrylamide flocculants	Brown et al. (1980a)
UK	1,100 μg/l (Influent) 280 μg/l (Effluent)	Wastewater treatment plant receiving water from factory processing acrylamide	Croll et al. (1974)
UK	1 μg/l (Sludge)	Sewage sludge from plant using polyacrylamide flocculants	Croll et al. (1974)

Note: n.d = not detected; d.l = detection limit

Table 3.8 Measured levels of acrylamide in process waters

Location	Concentration	Notes	Reference
UK	n.d. (Process water) n.d. (Effluent)	d.l. 0.2 μg/l. Waters sampled from China clay works using polyacrylamide as a flocculant	Brown and Rhead (1979)
UK	39 & 42 μg/l	Settlement tanks at colliery washery	Croll et al. (1974)
UK	1.8 μg/l (Effluent)	Sample from a colliery	Croll et al. (1974)
UK	16 μg/l (Effluent) 1.2 μg/l (Discharge)	Sample from China clay works	Croll et al. (1974)
UK	0.47 & 1.2 μg/l (Effluent)	Paper works.	Croll et al. (1974)
USA	25-125 μg/l (Effluent)	Effluent from production site.	Going (1978)

Note: n.d = not detected; d.l = detection limit

Table 3.9 Measured levels of acrylamide in sediment

Location	Concentration	Notes	Reference
USA	n.d.	d.l. 20-80 μg/kg. Sampling carried out in vicinity of factories producing or using acrylamide or polyacrylamide	Going (1978)

Note: n.d = not detected; d.l = detection limit

3.1.4.2.1 Measured exposure data relating to the use of acrylamide-based grouts

Very few measured exposure data are available relating to the use of acrylamide-based grouts in sewer and pipeline repairs within the EU. In the United States most monitoring data are based upon occupational exposure, not environmental exposure. In Japan in 1975, a drinking water sample taken 2.5 metres from a pipeline grouted with acrylamide, contained acrylamide at a concentration of 400 mg/l (Igisu et al., 1975). It should be noted that this result is not typical of concentrations found in drinking water and appears to have arisen due to the particular circumstances of the incident - it is more representative of contamination of water due to use of acrylamide in construction.

Acrylamide-based grouts are known to have been used in large tunnelling projects in Norway and Sweden in recent years, and these have lead to high levels of acrylamide being detected in watercourses downstream of the construction operations.

1. In Southern Sweden at the Hallandsås ridge an 8.6 km tunnel was driven through bed-rock. The ridge has a very high water content, and the chosen grout contained NMA and residual acrylamide. The large-scale use of the product started in August 1997.

   River water samples taken from the Vadbäken creek immediately downstream from the construction site at the end of September 1997 had acrylamide concentrations of 92 mg/l and NMA concentrations of 342 mg/l. At the same time samples taken in fish ponds that were connected to the Vadbäcken creek contained 2 mg/l acrylamide and 180 mg/l NMA. By the end of December 1997 the concentration had dropped to below 0.1 mg/l for both acrylamide and NMA in the creek. At the furthest point monitored downstream the acrylamide concentration was below the detection limit of 0.005 mg/l.

   River water samples taken from the Stensån creek, near the construction site, were 19 mg/l acrylamide and 59 mg/l NMA at the end of September 1997. Further downstream the concentration of acrylamide was below the detection limit of 0.005 mg/l.

   In the part of the tunnel that had its outlet to the Stensån creek much lower amounts of grout had been used compared to the part of the tunnel where the outlet was directed to the Vadbäcken creek.

   Water samples were also taken from well water from the area surrounding the construction site. At most of the sites the concentration of acrylamide and NMA was below the detection limit of 0.005 mg/l. The highest concentration of NMA observed was 50 mg/l and the highest concentration of acrylamide was 5.1 mg/l.

2. In Norway NMA grout has been used in the construction of a tunnel called Romeriksporten. Acrylamide and NMA have been detected in the water leaking from the tunnel. 6 weeks after the grout was last used the concentration of acrylamide in the drainage water was measured at about 100 μg/l (equivalent to 4 kg/week). The concentration of NMA in the drainage water was about 60 μg/l.

   The composition and use of these grouts was discussed in section 3.1.1.1.3. In these two cases the formation of the gel and the polymerisation process did not match the manufacturer's expectation. The observations would suggest that the NMA was breaking down to acrylamide and formaldehyde (e.g. due to pH changes on mixing the grout components). The high water pressures and flow rates in the tunnels may also have given the gel insufficient time to polymerise properly.

3.1.4.3 Comparison of PECs with measured data

The monitoring data suggest that surface water concentrations are generally below the level of detection (typically <1 μg/l). In some cases higher levels have been observed though these are generally in waters near production plants or processes using polyacrylamide flocculants.

The local PECs calculated for surface waters are in the region of 1-50 μg/l. These are higher than the measured levels. The high-value PECs for water are due to the default release estimation made for one processing plant. The PECs for acrylamide monomer release from polyacrylamides

assume that all the free monomer in polyacrylamides is released to the environment. In practice the release of all the free acrylamide monomer is very unlikely to occur. The PECs for the other production and processing plants are all significantly lower than the default release estimate. It is therefore unlikely that the high value PECs will occur in practice.

The local PEC calculated for wastewater treatment plants using polyacrylamide flocculants agrees well with that measured by Brown and Rhead (1979). For other process waters higher levels have been measured (up to 125 mg/l). The PEC calculated for process waters discharged directly to receiving waters is in the range of these measured levels.

It should be noted that many of the measured data were taken before 1980. Since then the level of free acrylamide monomer in polyacrylamides has been reduced, particularly in applications involving wastewater treatment. Current levels are therefore likely to be lower than those measured before 1980.

The regional and continental PECs are all very low (<0.2 μg/l). This appears to be in agreement with the majority of measurements that are below the detection limit.

In the assessment the calculated PECs will be used for risk characterisation because these represent a worst-case scenario for acrylamide levels.

3.1.5 Terrestrial compartment

Acrylamide is relatively mobile in soil (Section 3.1.3.2) and adsorption to soil particles is negligible. It also undergoes biodegradation (Section 3.1.3.1) with optimum degradation observed in alkaline soils and at elevated temperatures. The concentrations of acrylamide in various soil types are likely to be very low.

The main use of acrylamide is in the production of polyacrylamides. Direct releases to soil during production are unlikely to occur, unless as a result of accidental spillage. Polyacrylamides have a variety of uses (Section 2.2), the major use being as a flocculant in the water treatment industry. It is possible that these polyacrylamide flocculants will be present in sludge which is then applied to land. The amount of free acrylamide monomer in the polyacrylamide is small (<0.1% w/w) and it has been assumed in this assessment that it is lost from the polymer in the wastewater treatment plant (Section 3.1.2.2). Hence the amount of acrylamide that may potentially be applied to soil is expected to be very small.

Some minor use of polyacrylamides in the direct treatment of soils has been reported. Kirk Othmer (1997) indicates that polyacrylamides can be used to reduce erosion in irrigated soils. Some information on this is included in Section 3.1.2.2. As such use has not been reported for the EU it is not discussed further in this report.

3.1.5.1 Calculation of PEC_{soil}

A number of steps need to be followed in calculating the PEC, and these include calculating the concentration due to dry and wet deposition in the soil and the concentration due to sewage sludge application. The calculations for each of these steps are given below.

3.1.5.1.1 Removal from top soil

The first-order rate constant for removal from top soil is estimated by summing together the first order rate constants for volatilisation, leaching and biodegradation in soil. The first order rate constant for biodegradation in soil is 0.023 d^{-1} for acrylamide. The first order rate constants for volatilisation and leaching can be calculated as follows:

k_{volat} is estimated as follows

$$\frac{1}{k_{volat}} = \left[\frac{1}{kasl_{air} \cdot K_{air,water}} + \frac{1}{kasl_{soil,air} \cdot \mathrm{K}_{air,water} + kasl_{soil,water}} \right] \cdot K_{soil,water} \cdot DEPTH_{soil} \qquad \text{(TGD 42)}$$

$kasl_{air}$	Partial mass transfer coefficient at air-side of the air-soil interface [120 m/d]
$kasl_{soil, air}$	Partial mass transfer coefficient at soil air-side of the air-soil interface [0.48 m/d]
$kasl_{soil, water}$	Partial mass transfer coefficient at soil water-side of the air-soil interface [0.000048 m/d]
$K_{air, water}$	Air-water equilibrium distribution coefficient [Section 3.1.3.2.2: $1.25 \cdot 10^{-8}$ m^3/m^3]
$K_{soil, water}$	Soil-water partitioning coefficient [section 3.1.3.2.1: 0.206 m^3/m^3]
$DEPTH_{soil}$	Mixing depth of soil [0.2 m local and agricultural, 0.1 m grassland]

This gives $k_{volat} = 3.53 \cdot 10^{-5}$ d^{-1} (local and agricultural soils) and $7.06 \cdot 10^{-5}$ d^{-1} (grassland).

k_{leach} is estimated as follows:

$$k_{leach} = \frac{Finf_{soil} \cdot RAINrate}{K_{soil,water} \cdot DEPTH_{soil}} \qquad \text{(TGD 43)}$$

$Finf_{soil}$	Fraction of rainwater that infiltrates into soil [0.25]
RAINrate	Rate of wet precipitation (700 mm/year) [0.00192 m/d]
$K_{soil,water}$	Soil-water partitioning coefficient [Section 3.1.3.2.1. 0.206 m^3/m^3]
$DEPTH_{soil}$	Mixing depth of soil [0.2 m local and agricultural, 0.1 m grassland]

This gives k_{leach} = 0.0116 d^{-1} (local and agricultural soils) and 0.0233 d^{-1} (grassland).

The total first order rate constant for removal from top soil is:

$$k = k_{volat} + k_{leach} + k_{bio,soil} \qquad \text{(TGD 41)}$$

= 0.035 d^{-1} (local and agricultural soils) and 0.046 d^{-1} (grassland)

3.1.5.1.2 Aerial deposition

The aerial deposition is calculated using the annual average total deposition flux calculated in Section 3.1.6.1 ($DEPtotal_{ann}$: $9.05 \cdot 10^{-5}$ $mg/m^2/d$), the mixing depth of soil ($DEPTH_{soil}$: 0.2 m agricultural, 0.1 m grassland) and the bulk density of soil (RHO_{soil}: 1,700 kg/m^3).

$$D_{air} = \frac{DEP_{total,ann}}{DEPTH_{soil} \cdot RHO_{soil}} \qquad \text{(TGD 37)}$$

The aerial deposition flux for acrylamide is calculated as $2 \cdot 10^{-7}$ mg/kg/d for agricultural and local soils and $5 \cdot 10^{-7}$ mg/kg/d grassland.

The resultant concentration in soil due to aerial deposition after 10 years is given by:

$$Cdep_{soil10}(0) = \frac{D_{air}}{k} - \frac{D_{air}}{k} \cdot e^{-365 \cdot 10 \cdot k} \qquad \text{(TGD 44)}$$

The first-rate order constant for removal from top soil is defined above. This equation gives a concentration in local and agricultural soil of $5.7 \cdot 10^{-6}$ mg/kg and in grassland of $1.08 \cdot 10^{-5}$ mg/kg.

3.1.5.1.3 Sewage sludge application

The concentration of acrylamide in sewage sludge can be calculated from the fraction of emission directed to sludge by the wastewater treatment plant; for acrylamide this is zero. This means that for acrylamide the concentration in soil is derived from aerial deposition only.

3.1.5.1.4 Concentration in soil

The overall concentration of acrylamide in soil after ten years is taken as the concentration due to deposition only. This gives a total concentration in local and agricultural soils of $5.7 \cdot 10^{-6}$ mg/kg and in grassland of $1.08 \cdot 10^{-5}$ mg/kg. The average concentration over the period is given by the following equation:

$$Clocal_{soil} = \frac{D_{air}}{k} + \frac{1}{kT}\left[C_{soil}(0) - \frac{D_{air}}{k}\right] \cdot \left[1 - e^{-kT}\right] \qquad \text{(TGD 40)}$$

In the equation, T is the averaging time (local soil 30 days, agricultural soil 180 days, grassland soil 180 days). For acrylamide this gives an average concentration in soil of $5.7 \cdot 10^{-6}$ mg/kg for local and agricultural soil and $1.08 \cdot 10^{-5}$ mg/kg for grassland. The fraction of steady state achieved in soil has been calculated as 1 for all soil types.

3.1.5.1.5 PEC_{soil}

The $PECregional_{soil}$ and $PECcontinental_{soil}$ have been calculated using EUSES. The $PECregional_{natural\ soil}$ is taken as a background concentration and added to the local concentrations in soil to give the $PEClocal_{soil}$. **Table 3.10** summarises the PEC_{soils} calculated for acrylamide.

The $PEClocal_{soil,\ porewater}$ can be calculated from the $PEClocal_{soil}$ using the soil-water partitioning coefficient ($K_{soil\text{-}water}$ 0.206 m^3/m^3) and the bulk density of soil (RHO_{soil} 1,700 kg/m^3).

$$PEClocal_{soil,porewater} = \frac{PEClocal_{soil} \cdot RHO_{soil}}{K_{soil,water} \cdot 1000} \qquad \text{(TGD 52)}$$

For acrylamide the $PEClocal_{soil,\ porewater}$ is calculated as $1.7 \cdot 10^{-4}$ mg/kg. The PEC for groundwater is taken as the same as the PEC for soil porewater.

Table 3.10 PEC_{soil}

	Concentration (mg/kg)	Calculation
$PECcontinental_{natural,soil}$	$1.1 \cdot 10^{-8}$	EUSES
$PECcontinental_{agricultural,soil}$	$7.13 \cdot 10^{-9}$	EUSES
$PECcontinental_{industrial,soil}$	$1.1 \cdot 10^{-8}$	EUSES
$PECregional_{natural,soil}$	$5.51 \cdot 10^{-7}$	EUSES
$PECregional_{agricultural,soil}$	$3.48 \cdot 10^{-7}$	EUSES
$PECregional_{industrial,soil}$	$5.51 \cdot 10^{-7}$	EUSES
$PEClocal_{soil}$	$5.7 \cdot 10^{-6}$	TGD
$PEClocal_{agricultural,soil}$	$5.7 \cdot 10^{-6}$	TGD
$PEClocal_{grassland}$	$1.08 \cdot 10^{-5}$	TGD

The reports on the incidents relating to tunnelling operations do not contain any information on resulting levels in soil. It is understood that at the tunnel in Norway, drainage channels were in place so that acrylamide did not reach the soil directly. However, there is a possibility of acrylamide reaching soil from similar incidents, and pore water levels could in theory be similar to those measured in surface water. This will be discussed in a qualitative way in the risk characterisation.

3.1.5.2 Measured levels

Going (1978) measured the levels of acrylamide in soils in the vicinity of factories producing or using acrylamide or polyacrylamide in the USA. In all the samples acrylamide concentrations were below the detection limit of 20-80 mg/kg.

3.1.6 Atmosphere

3.1.6.1 Calculation of $PEClocal_{air}$

The $PEClocal_{air}$ is calculated using a gaussian plume model with standard parameters. The method used is described in more detail in the TGD Chapter 3, Section 2.3.8.2. In calculating the $PEClocal_{air}$ the emissions from point sources and sewage treatment plants need to be considered.

The sewage treatment plant is assumed to be a point source and the concentration of the chemical is calculated at a 100 m distance from it. The indirect emission of a chemical from a sewage treatment plant to air is calculated from the fraction of emission to water directed to air

by the sewage treatment plant. For acrylamide this is zero and so there is no indirect emission to air from a sewage treatment plant.

Therefore the concentration in local air is calculated from direct emissions to air. The direct emission to air is multiplied by the concentration in air at a source strength of 1 kg/d ($Cstd_{air}$ $2.78 \cdot 10^{-4}$ mg/m^3) to give the local air concentration. The local concentration can be converted to an annual concentration if the number of days emission per year are known.

$$Clocal_{air} = max(Elocal_{air}, Estp_{air}) \cdot Cstd_{air} \qquad \text{(TGD 25)}$$

$$Clocal_{air,ann} = Clocal_{air} \cdot \frac{Temission}{365} \qquad \text{(TGD 26)}$$

Explanation of symbols:

$Elocal_{air}$	Local direct emission rate to air during emission episode
$Estp_{air}$	Local indirect emission to air during emission episode [0]
Temission	Number of days per year that the emission takes place

Direct releases to air are reported for six production and processing sites. For three of these sites releases are reported as an average concentration due to monitoring of emissions from vents and stacks. The concentrations reported are 0.43 mg/m^3 for site A, 0.1 mg/m^3 for site D and 0.01 mg/m^3 from drying operations and 6 mg/m^3 from storage tanks for site E. Three sites report emissions on a kg/year basis, and these have been converted to kg/day by assuming 300 days operation for sites B, G and H and 250 days operation for site E (company data). These are 9.5 kg/year (0.032 kg/d) for site B (includes fugitive emissions due to storage), 55.2 kg/year (0.22 kg/day) for site E (concentrations also reported), 0.03 kg/day for site H and 5 kg/year (0.016 kg/day) for site G. For sites C and H no information is reported on releases to air so the default release value of 0.1 kg/d (30 kg/year) is used.

The PEC_{air} for the regional and continental scenarios is calculated using EUSES. The regional PEC is taken as a background concentration and added to the local concentrations to give the PEClocals. **Table 3.11** summarises the PEC_{air} calculated for each production and processing site and at the regional and continental level.

Table 3.11 PEC_{air}

	$Clocal_{air}$ (mg/m³)	$Clocal_{air,ann}$ (mg/m³)	$PEC_{air,ann}$ (mg/m³)	Notes
Site A	0.43	0.43		Measured value. Dilution after release not taken into account.
Site B	$8.8 \cdot 10^{-6}$	$7.3 \cdot 10^{-6}$	$7.3 \cdot 10^{-6}$	
Site C	$2.8 \cdot 10^{-5}$	$2.3 \cdot 10^{-6}$	$2.3 \cdot 10^{-6}$	Default
Site D	0.1	0.1		Measured value. Dilution after release not taken into account.
Site E	$6.1 \cdot 10^{-5}$	$4.2 \cdot 10^{-5}$	$4.2 \cdot 10^{-5}$	
Site F			$3.56 \cdot 10^{-11}$	Releases to air reported as zero.
Site G	$4.4 \cdot 10^{-6}$	$3.6 \cdot 10^{-6}$	$3.6 \cdot 10^{-6}$	
Site H	$9.2 \cdot 10^{-6}$	$7.6 \cdot 10^{-6}$	$7.6 \cdot 10^{-6}$	
$PEC_{regional}$			$3.56 \cdot 10^{-11}$	EUSES
$PEC_{continental}$			$7.11 \cdot 10^{-13}$	EUSES

Aerial deposition

Acrylamide in the atmosphere may be deposited on the soil. The deposition flux emission is therefore calculated to consider soil concentrations due to deposition (see Section 3.1.5.1.2). In calculating the deposition flux the emissions from the two sources (direct and via sewage treatment plant) are summed.

$$DEPtotal = \left(Elocal_{air} + Estp_{air}\right) \cdot \left(Fass_{aer} \cdot DEPstd_{aer} \cdot \left(1 - Fass_{aer}\right) \cdot DEPstd_{gas}\right) \qquad \text{(TGD 28)}$$

$$DEPtotal_{ann} = DEPtotal \cdot \frac{Temission}{365} \qquad \text{(TGD 29)}$$

Explanation of symbols:

$DEPstd_{aer}$	Standard deposition flux of aerosol-bound compounds at a source strength of 1 kg/d [0.01 mg/m²/d]
$DEPstd_{gas}$	Deposition flux of gaseous compounds as a function of Henry's Law coefficient, at a source strength of 1 kg/d. [0.0005 mg/m²/d]
DEPtotal	Total deposition flux during emission episode [mg/m²/d]
$DEPtotal_{ann}$	Annual average total deposition flux [mg/m²/d]
$Elocal_{air}$	Local direct emission rate to air during emission episode [0.22 kg/d Site E]
$Estp_{air}$	Local indirect emission to air during emission episode [0]
$Fass_{aer}$	Fraction of the chemical bound to aerosol [Calculated as 0.000111]
Temission	Number of days per year that the emission takes place [300 days/year]

For acrylamide the DEPtotal is calculated as $1.1 \cdot 10^{-4}$ mg/m²/d and the $DEPtotal_{ann}$ is calculated as $9.05 \cdot 10^{-5}$ mg/m²/d.

3.1.6.2 Measured levels of acrylamide in air

Releases to air during production and polymer production are controlled to limit human exposure. Details of occupational monitoring data are reported in Section 4 (Human Health Effects). **Table 3.12** gives details of measured levels of acrylamide in air. These measurements are mainly based upon personal monitoring campaigns and are not directly relevant to levels in the environment.

Table 3.12 Measured levels of acrylamide in air

Location	Concentration	Notes	Reference
USA	n.d. (vapour) n.d. (particles)	d.l. 0.1-1.1 μg/m³ (vapour) d.l. 0.4-0.7 μg/m³ (particles) Sampling carried out in vicinity of factories producing or using acrylamide or polyacrylamide	Going (1978)
USA	Personal monitoring Range: 100-3600 μg/m³ Mean weekly: 100-400 μg/m³ (Process area) 100-900 μg/m³ (Packing room) 100-400 μg/m³ (Inspection room) 4 hour period: 480, 520 μg/m³ (Process area) 760, 520 μg/m³ (packing room) Stationary sampling: 100-300 μg/m³	Manufacturing site	NIOSH (1976)
USA	8-hour TWA 10-390 μg/m³ (Mean 110 μg/m³)	Manufacturing site	Hills (1985)
USA	10-8,291 μg/m³ (8-hour TWA 1-393 μg/m³)	Manufacturing site	Hills and Greife (1986)
USA	n.d.	Manufacturing site for chemicals and flocculating agents for paper manufacture and water treatment	IARC (1986)

Notes: n.d. = not detected; d.l. = detection limit; TWA = Time Weighted Average

3.1.7 Secondary poisoning

No data have been reported concerning acrylamide concentrations in biota or food products. Accumulation and metabolism of acrylamide are discussed in Section 3.1.3.3. The data show that the potential for accumulation of acrylamide in aquatic organisms from exposure to water is very low. Because acrylamide is not accumulative or persistent a secondary poisoning assessment is not required.

For plants and food products, exposure may occur via air or contaminated water either during growth or manufacture. Atmospheric concentrations of acrylamide are very low (Section 3.1.4) and it is unlikely that plants and food products are contaminated to any significant degree via this route. Based upon the low log K_{ow} value, absorption of acrylamide by plants grown in contaminated waters is likely to be negligible. Contamination of food products during manufacture is assumed to occur by accidental contamination of water supplies only.

In view of these data and the low log K_{ow} value, concentrations of acrylamide in biota from eating food or drinking natural water would be expected to be negligible.

Domestic animals might be exposed to acrylamide additionally via water that has been treated with polyacrylamide flocculants in a treatment works. **Table 3.6** in Section 3.1.4.2 details measured levels of acrylamide in drinking water. The maximum level measured in drinking water was 4.5 μg/l (Brown and Rhead, 1979), though non-standard and non-validated methodology was used. Since this measurement was made changes in legislation restricting the residual monomer content of polyacrylamides have come in to force. This value is therefore thought to be significantly higher than the actual level in drinking water.

In Section 3.1.2.2 the maximum concentration of acrylamide in drinking water is calculated to be 0.125 μg/l, based upon the maximum allowed dose of active polymer. Average concentrations in drinking water are expected to be 0.0625 μg/l. These values are based upon the current legislative position in the UK. As discussed in Section 3.1.2.2 this is a worst-case figure and assumes that all the potential free acrylamide monomer in the polyacrylamide is released to the environment. The actual level is likely to be significantly lower. No information is available as to the legislative position in other countries, and so these data are taken to be representative of the worst-case scenario.

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

3.2.1 Aquatic compartment (including sediment)

3.2.1.1 Toxicity to fish

3.2.1.1.1 Acute toxicity

Available studies are summarised in **Table 3.13**. The most sensitive freshwater species appears to be the bluegill sunfish (*Lepomis macrochirus*) with a 96-hour LC_{50} of 100 mg/l. The sensitivity of the other species appears to be in a similar range (96-hour LC_{50}s between 100 and 180 mg/l).

The validity of the acute toxicity tests to fish has been assessed in accordance with Chapter 3.2.1.1 and Appendices IV and V of the TGD. The results reported by ABC Labs (1982a, 1983a and 1982b) and the United States Testing Company are fully documented and meet with GLP guidelines, and are considered valid. The results by Petersen et al. (1985) and Batchelder (1975) do not contain sufficient information on the test methods employed and are not considered valid. The remaining results are for non-representative species and it is recommended that they are used in the assessment to support the valid data only.

3.2.1.1.2 Long-term toxicity

A limited number of long-term toxicity studies on fish have been reported. The information obtained from these results is not very well documented and is provided here for information only.

Hermans and Leeuwangh (1982) reported a 14-day LC_{50} of 34.8 mg/l for the Guppy (*Poecilia reticulata*). No details of the test method were given.

Edwards (1975) reported a 7-day LC_{100} of 100 mg/l and a 30-day NOEC of 50 mg/l for the goldfish (*Carassius auratus*). The test was carried out in static conditions, but no other test conditions were reported.

Petersen et al. (1987) studied the behavioural and histological effect of acrylamide on rainbow trout (*Oncorhynchus mykiss*). The fish were exposed under static conditions for 15 days to various concentrations of acrylamide, followed by a 7-day depuration period. Histological lesions were observed in the gill and liver in fish exposed to 25 mg/l for 15 days. Fish exposed to 50 mg/l developed lesions in the cephalic lateral line and peripheral lateral line in addition to the gill and liver. After the depuration period additional lesions were observed in the sagittal and proximal nerve plexus (25 mg/l and 50 mg/l exposure) and in the optic nerve (50 mg/l exposure only). Swimming behaviour of the fish was unaffected at exposure concentrations below 25 mg/l. At 50 mg/l fish had difficulty in orientating themselves when swimming, and based upon this effect an EC_{100} of 50 mg/l was quoted.

Table 3.13 Acute toxicity tests to fish

Species	Age/ Length	pH	Dissolved oxygen (mg/l)	Water hardness (mg/l $CaCO_3$)	Temperature (°C)	Flow/Static	Method	Effect concentration (mg/l)	Data validity	Reference
Freshwater species										
Lepomis macrochirus	0.23 g21 mm	8.1-8.2	8.3-8.8	255	22 (±1)	Flow	a	24-hr LC50 260 (m) 48-hr LC_{50} 160 (m) 96-hr LC_{50} 100 (m) 96-hr NOEC 35 (n) 96-hr EC50 85 (n)	Valid	ABC Labs (1982a)
Pimephales promelas	0.11 g 17 mm	8.0-8.3	8.4-9.8	255	22 (±1)	Flow	a	24-hr LC50 320 (m) 48-hr LC_{50} 230 (m) 96-hr LC_{50} 120 (m) 96-hr NOEC 41 (n) 96-hr EC50 86 (n)	Valid	ABC Labs (1983a)
						Static	b	96-hr LC50 124 96-hr NOEC 56	Not valid	Batchelder (1975)
Oncorhynchus mykiss	1 g 40 mm	8.0-8.3	7.4-8.0	255	12 (±1)	Flow	a	24-hr LC50 370 (m) 48-hr LC50 240 (m) 96-hr LC50 110 (m) 96-hr NOEC 37 (n) 96-hr EC50 88 (n)	Valid	ABC Labs (1982b)
	64 days < 50 mm	7.5-7.7	8.0-8.6	90	15 (±2)	Static	c	24-hr LC50 500 (n) 48-hr LC50 360 (n) 72-hr LC50 240 (n) 96-hr LC_{50} 180 (n)	Valid	United States Testing Company (1990)
	2-4 g				12	Static	b	24-hr LC50 300 (m) 48-hr LC50 210 (m) 72-hr LC50 170 (m) 96-hr LC50 162 (m)	Use with care	Petersen et al. (1985)
Salmo trutta	yearlings	7.6-8.0	>50% saturation	290-210	10	Static	b	48-hr LC_{50} 400 (n)	Use with care	Woodiwis and Fretwell (1974)

Table 3.13 continued overleaf

Table 3.13 continued Acute toxicity tests to fish

Species	Age / Length	pH	Dissolved oxygen (mg/l)	Water hardness (mg/l $CaCO_3$)	Temperature (°C)	Flow/Static	Method	Effect concentration (mg/l)	Data validity	Reference
Carassius auratus	3.3 g 620 mm	7.8	9-10		20 (±1)	Static	d	24-hr LC50 460 (m) 96-hr LC50 160 (m)	Use with care	Bridie et al. (1979a) and Bridie et al. (1973)
							b	72-hr LC_{50} 140	Not valid	Paulet and Vidal (1975)
Heteropneustes fossilis	80-110 g 160-200 mm	7.7	6.25	129	20-25	Static	d	24-hr LC50 104 48-hr LC_{50} 87 48-hr NOEC 15	Use with care	Shanker and Seth (1986)
Saltwater species										
Rasbora heteromorpha	10-30 mm	8.1		20	20	Flow	e	24-hr LC50 460 (n) 48-hr LC50 250 (n) 96-hr LC50 130 (n)	Use with care	Tooby et al. (1975)

Test Methods
a Standard method for acute toxicity test with fish, macroinvertebrates and amphibians.
b Acute toxicity test (No details)
c OECD Guideline 203 Fish, Acute Toxicity Test
d APHA Guideline
e MAFF Guideline Standard constant flow procedure
n = Nominal concentration
m = Measured concentration
EC_{50} and NOEC values based upon behaviour and mortality

3.2.1.2 Toxicity to aquatic invertebrates

Table 3.14 gives details of acute and long-term toxicity studies to aquatic invertebrates. In acute tests, the water flea *Daphnia magna* is the most sensitive species with a 48-hour LC_{50} of 98 mg/l. Tests with the salt water shrimp *Mysidopsis bahia* showed a very similar sensitivity (48-hour LC_{50} of 109 mg/l). Long-term toxicity data are only available for saltwater species, with a 28 day NOEC of 2.04 mg/l being reported for *Mysidopsis bahia*.

The validity of the toxicity tests to aquatic invertebrates has been assessed in accordance with Chapter 3.2.1.1 and Appendices IV and V of the TGD. The results reported by ABC Labs are fully documented and meet good laboratory practice guidelines. The results reported by E.G. & G Bionomics and Springborn Bionomics are fully documented. Of the species for which data are reported only *Daphnia magna* is recommended as a representative species. The data on *Daphnia* have therefore been classed as valid while the data on other species, although meeting the remaining quality criteria, should be used with care.

3.2.1.3 Toxicity to aquatic plants

A 72-hour EC_{50} of 67.7 mg/l (growth inhibition) and a NOEC of 32 mg/l (growth inhibition) are reported for the fresh water alga *Selenastrum capricornutum* with a 50% acrylamide solution (S.E.P.C., 1997). The test has been performed to OECD 201 Guidelines and EEC Directive 92/69 Method C.3. Full details of the test conditions and methodology are given in the report and the study is judged as valid. As the test was performed on a 50% acrylamide solution, the EC_{50} and NOEC values should be divided by two to give the toxic effect due to acrylamide. This gives a 72-hour EC_{50} of 33.8 mg/l (growth inhibition) and a NOEC of 16 mg/l (growth inhibition). The EC_{50} for growth rate was found to be greater than 100 mg/l 50% acrylamide solution (50 mg/l acrylamide).

Spraggs et al. (1982) reported an IC_{50} of 72 mg/l for *Selenastrum capricornutum* with acrylamide in an algal growth inhibition test. No details were reported as to the experimental conditions or length of exposure.

Table 3.14 Toxicity to aquatic invertebrates

Species	Age / Length	pH	Dissolved oxygen (mg/l)	Water hardness ($CaCO_3$ mg/l)	Temperature (°C)	Method	Effect concentration (mg/l)	Data validity	Reference
Freshwater species									
Daphnia magna	1st instar (< 24 hrs)	8.2-8.4	7.1-7.9	255	20	a	24-hr LC_{50} 230 (m) [Mortality] 48-hr LC_{50} 98 (m) [Mortality] 48-hr EC_{50} 98 (m) [Immobilisation] 48-hr NOEC 60 (m) [Immobilisation and mortality]	Valid	ABC Labs (1983b)
Paratanytarsus parthenogenetica	3rd and 4 thinstar (8-10 days)	8.4-8.5	7.7-8.3	255	19-20	a	24-hr LC50 570 (m) [Mortality] 48-hr LC_{50} 410 (m) [Mortality] 48-hr EC_{50} 230 (m) [Immobilisation] 48-hr NOEC 60 (m) [Immobilisation and mortality]	Use with care	ABC Labs (1983c)
Saltwater species									
Mysidopsis bahia	4 day	7.7-7.8			23-25	b	24-hr LC50 >161 (m) 48-hr LC_{50} 109 (m) 72-hr LC_{50} 94 (m) 96-hr LC_{50} 78 (m) 96-hr NOEC 5.2 (m)	Use with care	EG&G Bionomics (1986)
	1 st instar (< 26 hrs)	8.0-8.1	5.3-7.2	Salinity 28-31	22.9-26.9	c	96-hr NOEC 2.04 mg/l (m) [Mortality in F1 generation] 28-day NOEC 2.04 (m) [Mortality] 28-day NOEC >4.4 (m) [Reproduction]	Use with care	Spingborn Bionomics (1985)

Test Methods
a Standard method for acute toxicity test with fish, macroinvertebrates and amphibians.
b Acute toxicity test
c Prolonged toxicity test
n = Nominal concentration
m = Measured concentration

3.2.1.4 Toxicity to microorganisms

In an OECD 301D "Ready Biodegradability: Closed Bottle Test" acrylamide was found to be readily biodegradable at low concentrations (<2 mg/l) (United States Testing Company Inc., 1991). At higher concentrations the degradation rate was found to decrease, and this was thought to be due to acrylamide having a toxic effect on the microorganisms used within the test (see Section 3.1.3.1.2). Based upon this result it is suggested that 2 mg/l is taken as a NOEC for microorganisms.

Starostina et al. (1983) studied the effect of treating bacterial cells with acrylamide. The 16-hour EC_{100} for *Escherichia coli* was reported as 20 g/l in a cell division test. They found that action of acrylamide significantly decreases the viability of *E. coli* and *Pseudomonas putida* populations. Addition of acrylamide to the growth medium was found to inhibit the division of *E.coli* cells and cells of some other gram-negative bacterial species and at some concentrations to lead to their elongation. They also found that acrylamide disturbs the synthesis of DNA and to a lesser extent RNA in *E. coli* cells. The cell wall was found to be the primary target for acrylamide, which disturbs the cell envelope structure and penetrates inside the cell, thus inhibiting the synthesis of nucleic acids and disturbing the cell wall synthesis. The authors concluded that acrylamide was one of the major toxic factors affecting microbial cells during their immobilisation in polyacrylamide.

Spraggs et al. (1982) reported an EC_{50} of 13,500 mg/l for *Photobacterium phosphoreum* with acrylamide in a photoluminiscence test.

3.2.1.5 Toxicity to amphibians

Edwards (1975) studied the effects of acrylamide on frogs (*Rana temporaria*). The frogs were given acrylamide either by injection in saline solution into the dorsal sac or by exposing them to a solution containing acrylamide. Three doses of 50 μg/g in 7 days killed three out of five frogs and a 2-hour exposure to a 2% (w/v) solution of acrylamide killed two out of three frogs. No adverse effects were observed in the surviving frogs.

3.2.1.6 Other studies

Brown et al. (1982) studied the *in situ* adsorption, degradation and toxicity of acrylamide in a river. As part of the study they investigated the effect of acrylamide in stream water on the insect fauna living on stones covered with moss. In the study a solution of acrylamide was fed continuously for six hours into a small stream with the aim of achieving a concentration of 50 μg/l of acrylamide. After this time, the input was reduced to give 6 μg/l under conditions of average flow for seven days. The authors note that spates and high mica-dam discharges (the site was downstream from a china clay site) would lead to lower concentrations, and low flows and discharges would produce higher concentrations. There are no indications of monitoring over the lower concentration periods and the implication is that a set level of input was maintained irrespective of the actual flows. Hence the concentrations could have varied considerably from the nominal.

The pattern of high input for six hours, low for seven days was carried out for four high inputs, and then left at the lower level for six further weeks. The concentrations at the end of this period were close to the nominal 6 μg/l, with the flow at that time being ~0.9 m^3/s. Flow rates at the

earlier high input times (the only ones included in the paper) were around half to one third of this flow rate, which would have given higher concentrations for the same input rate.

A qualitative assessment of the insect fauna was performed. At the end of the initial 6-hour exposure period the density of the insects on the moss-covered stones was reduced (some of the insects were found free in the water lower down the stream). The insects included stone flies (*Amphinemura sulcicollis* and *Leuctra hippopus*) and the day-fly (*Baetis rhodani*). After 7 days, only small populations of the day-fly and the non-stinging midges of the genus *Chironomidae* were present. The following species were found to be absent: caddis flies (*Sericostoma personatum* and *Rhyacophila dorsalis*) and the stone flies (*Leuctra hippopus, Protonemura meyeri, Amphinemura sulcicollis, Nemura cambrica* and *Chloroperla torrentium*). However there is no indication in the paper that the larvae were killed by the exposure, and it is possible that they were displaying an avoidance reaction, which is an effect but not a directly toxic one. The majority of the species concerned are free living on stones, although one or two build nets or cases (*Hydropsyche* and *Sericostoma*).

Only partial recolonisation had taken place two months after exposure ceased compared to the species distribution before application began. Four months after exposure the population of some of the species of insects that had been studied were within the control range when compared to the composition of the natural population of the stream.

The same stream had been surveyed for similar species at two-month intervals over the year before the study. If the situation two months after the end of exposure (February 1979) is compared to that from the year before at a similar time of year then the same species are present and the only difference is an increased presence for one species. Most if not all of the species which disappeared during the exposure were not found over the winter months in the previous year, and some were only found either in September or November. Thus a reduction to similar levels might have been expected in any case. This is not to say that the high levels of acrylamide did not have some impact, but there does not seem to be any real evidence for a continuous effect of low-level exposure relative to the only related “control” information available.

In summary, the exposure levels during most of the study are not well defined, although the high 50 μg/l levels appear to have been reached by the end of the 6 hours of higher input. There was probably some immediate effect on the organisms, but no indication that this was lethality. The longer-term picture seems to be similar to that found over a natural cycle. No meaningful quantitative result can be obtained from this study. The authors themselves concluded that acrylamide appears to have a selective adverse effect on invertebrates, but more research was needed to adequately define the effect of acrylamide on rivers.

Chet and Mitchell (1976) studied the control of marine fouling by chemical repellents. Motile marine bacteria identified as *Pseudomonads* were isolated from seawater and grown on artificial seawater nutrient agar. Test materials were then placed in this seawater broth. Field studies were also conducted by placing metal panels coated with the test materials in seawater. Repulsion of bacteria was determined by counting bacteria or measuring slime production on immersed plates. Acrylamide was found to be effective at repelling the marine bacteria and hence inhibiting marine organism growth. In further studies (Mitchell et al., 1975) the colonisation by marine mussels of stainless steel plates which had been coated with paint containing acrylamide (acrylamide concentration <0.5% by weight in paint) before being immersed in the sea for 44 days was found to be inhibited. The plates were exposed to light and slowed the growth of algae. The colonisation by marine mussels was significantly retarded when the plates were stored in the dark.

Observations from grouting incidents

In fish exposed to acrylamide and NMA following grout application incidents in Scandinavia the following observations were made. Gill alterations were observed with a thickening of the epithelial cells, hyperplasia and fusion of the secondary lamellae. Between the covering epithelial cells and the underlying blood vessels odema and eosinophilic granular cells were seen. In the liver, alterations were observed in the necrotic liver cells. An increase in haemoglobin adduct levels was also observed (personal communication, 1997).

3.2.1.7 Predicted no-effect concentration (PNEC) for aquatic organisms

For most existing chemicals, the pool of data from which to predict ecosystem effects is limited. In these circumstances empirically derived assessment factors are used to calculate a PNEC. The PNEC is the level below which the probabilities suggest that an adverse environmental effect will not occur. It is not intended to be a level below which the chemical is safe. The PNEC is calculated by dividing the lowest $L(E)C_{50}$ or NOEC by the appropriate assessment factor.

For acrylamide, short-term $L(E)C_{50}$s are reported for fish, aquatic invertebrates, algae and microorganisms. The data reported for fish, *Daphnia* and algae are from validated sources, while the data on microorganisms are not valid. The lowest 96-hour LC_{50} reported for fish is 100 mg/l (*Lepomis macrochirus*), the lowest 48-hour EC_{50} for *Daphnia* is 98 mg/l (*Daphnia magna*), and the lowest 72-hour EC_{50} for algae is 33.85 mg/l (*Selenastrum capricornutum*) (based upon a 72 hour EC_{50} of 67.7 mg/l for a 50% acrylamide solution).

Long-term NOEC data are reported for fish, aquatic invertebrates and freshwater algae. The data for fish are not valid and will not be used to derive a PNEC. A long-term NOEC is not reported for *Daphnia* though a long-term NOEC is reported for the saltwater shrimp *Mysidopsis bahia*. From **Table 3.14** it can be seen that the acute toxicity of *Mysidopsis bahia* is similar to the toxicity observed in *Daphnia*. The lowest NOEC observed for *Mysidopsis bahia* is a 28-day NOEC of 2.04 mg/l based upon mortality. For algae a 72-hour NOEC of 16 mg/l is reported (based upon a NOEC of 32 mg/l for a 50% acrylamide solution). For algae it is generally accepted that a 72-hour NOEC value can be considered as a long-term result.

Therefore valid studies from acute toxicity tests for freshwater species are reported for three trophic levels and a valid long-term NOEC is reported for freshwater algae. Since long-term studies are not available for fish or *Daphnia* an assessment factor of 1,000 will be applied to the lowest acute toxicity test result. This gives a PNEC of 33.85 μg/l based upon the 72-hour EC_{50} for freshwater algae (the most sensitive species in short-term tests).

There is debate about the use of saltwater species toxicity data in deriving the PNEC. A long-term study is reported for a saltwater invertebrate and a long-term NOEC is reported for freshwater algae. If this was taken into account in deriving the PNEC a factor of 100 should be applied to the lowest long-term NOEC from species representing two trophic levels. This would give a tentative PNEC of 20.4 μg/l. The factor of 100 is applied because the most sensitive species in the long-term studies is not the most sensitive species in the acute studies.

Since the PNEC derived using all the available data is lower than the PNEC derived using freshwater data alone, the former will be used for risk characterisation. The PNECs derived are similar and the chosen value (20 μg/l) should also be protective of the species studied in the field study test.

There are no toxicity test results available for sediment organisms with acrylamide. The TGD recommends that where there are no test results for sediment and no measured levels for substances with a log K_{ow} <5 a separate sediment assessment is not required because the risk characterisation result is the same as for the water compartment (using the equilibrium partitioning approach). Therefore no PNEC is derived for sediment organisms in this assessment.

3.2.1.8 PNEC for microorganisms in wastewater treatment plants

Chemicals can have an adverse effect on microbial activity in wastewater treatment plants, and so a $PNEC_{microorganisms}$ is derived. The assessment factor depends upon the microbial effect data available. If the test has been performed on nitrifying bacteria the effect concentration may be used directly. For other tests assessment factors in the range of 10 to 100 may be applied.

For acrylamide the data set of toxic effects on microorganisms is limited. For *E. coli* a 16-hour EC_{100} of 20 g/l (based upon a cell division test) is reported and from the OECD 301D biodegradation test it appears reasonable to assume a NOEC of 2 mg/l for microorganisms in WWTP. Applying a factor of 10 to the NOEC of 2 mg/l gives a PNEC of 200 μg/l. The factor of 10 is chosen because at 2 mg/l no significant adverse effects were observed, and this assessment factor is felt to be adequate to derive a PNEC for microorganisms in wastewater treatment plants.

3.2.2 Terrestrial compartment

A wide range of mammalian toxicity test results are reported. These tests are reviewed in Section 4 (Human health effects).

3.2.2.1 Toxicity to terrestrial plants

Bilderback (1981) studied the effect of toxic substances on pollen germination and tube growth using the pollen of *Impatiens sultanii*. Pollen was transferred from the anthers of the plants to a basal medium on a depression, dispersed in the liquid and covered with a glass cover. During the experiment the slide was kept in a closed plastic box. The pollen was incubated for 15 minutes and photomicrographs taken at random periods after the incubation period. The total number of grains, the number of germinated grains and grains producing tubes longer than 40 mm were counted. To investigate the growth of pollen tubes on a solid medium 1% agar was added to the basal medium and autoclaved. When acrylamide was added to the basal medium at concentrations ranging from 10 to 2,000 ppm, there was no significant effect upon germination, tube formation, or tube growth.

Kuboi and Fujii (1984) studied the toxicity of cationic polymer flocculants to higher plants. About 50 seeds of turnip (*Brassica rapa* L. cv. Chuusei-kanamachi), rape (*Brassica rapa* L. cv. Tokiwa-jibai), chinese cabbage (*Brassica pekinensis*), sesame (*Sesamum indicum*) and cucumber (*Cucumis sativus*) were incubated in distilled water for 1 day at 30°C in the dark. Seeds of upland rice (*Oryza sativa*) and wheat (*Triticum aestivum*) were cultured for 2 days. 10 seedlings of similar growth rate were then transferred to a flask containing 20 ml of polymer flocculant or monomer and cultured for 2 more days. The EC_{50} was calculated as the concentration of flocculant or monomer where root elongation rate is equal to 50% of the control. The effect of polymer flocculants on germination was determined by dipping the seeds directly into the test solutions and shaking for 1 day. At 100 mg/l acrylamide was found to retard root elongation by

39% compared to the control and the EC_{50} was calculated as 220 mg/l. No significant effect on seed germination was observed.

Sonoda et al. (1977) studied the behaviour of polyacrylamide as a cohesive agent in soil-plant systems. (Note this paper is in Japanese - the following text was supplied by industry in the IUCLID). The seeds of the chinese cabbage (*Brassica pekinensis*) were allowed to germinate in soil treated with 5 to 100 mg acrylamide/kg. The plants were thinned-out after 40 days and harvested after a further 20 days. Germination was interfered with by concentrations of more than 50 mg acrylamide/kg soil and plants did not grow normally thereafter. Concentrations of less than 10 mg acrylamide/kg soil only affected the growth of the plants since growth was delayed.

The potential for the uptake and accumulation of acrylamide into plant tissue has been examined using lettuce plants (*Lactuca saliva* L) (Hazeleton Labs, 1987). ^{14}C labelled acrylamide monomer was added to 100 ml of nutrient solution and mixed with 4,000 g of air dried soil to obtain a uniform concentration of 5.0 ppm. The plants were analysed for ^{14}C after 18 days. The roots, soil, leachate and shoots were analysed separately. They found that in those soils treated with acrylamide germination and growth were slower and the plants showed signs of necrosis. ^{14}C was detected in the shoots and roots of treated plants, and was also present in the soil and leachate. The ^{14}C in the leachate and plant tissue did not appear to be acrylamide.

In conclusion, acrylamide shows a slight toxic effect on plant growth at concentrations of 10 mg/kg soil. No effect on seed germination was observed.

3.2.2.2 PNEC for terrestrial organisms

In calculating the PNEC for terrestrial organisms only data on bacteria, plants and earthworms are considered. Acrylamide shows a slight toxic effect to plant growth at concentrations of 10 mg acrylamide/kg soil. An EC_{50} of 220 mg/l (based upon root elongation) is reported for plant seedlings. As the EC_{50} is a short-term toxicity test, an assessment factor of 1,000 could be applied to it giving a PNEC for terrestrial species of 220 μg/l. However, this calculation is based on only one terrestrial toxicity result. In addition to this, therefore, the equilibrium partitioning method has been used to estimate a PNEC for terrestrial organisms. As concentrations in soil have been calculated as concentrations in pore water, the PNEC for the aquatic compartment can be used to compare with these pore water concentrations. The PNEC for the aquatic compartment is 20 μg/l; as this is lower than the value derived from the plant data, it will be used in the risk characterisation.

3.2.3 Atmospheric effects

There are no data on the effects of acrylamide through aerial exposure other than those from mammalian toxicity tests (see Section 4). The calculated concentrations of acrylamide in ambient air are low and it is not expected that these levels will cause effects on organisms exposed through this route. Acrylamide reacts with hydroxyl radicals in the atmosphere with a short half-life, and is not considered an important cause of photochemical air pollution. Acrylamide is not known to contribute to the formation of low-level ozone or to ozone depletion. The half-life of acrylamide in the troposphere is short enough that it is unlikely to be transported to the stratosphere.

3.2.4 Secondary poisoning

Acrylamide is not accumulative or persistent in the environment. Therefore according to the TGD an assessment for secondary poisoning does not need to be carried out, as acrylamide is unlikely to accumulate in the food chain and have an effect on higher organisms.

While acrylamide may not have an effect on higher organisms due to accumulation in the food chain it may have a direct effect if exposure occurs through drinking contaminated water. In cattle exposed to acrylamide and NMA following grout application incidents in Scandinavia the following observations were made. Cows that were grazing near the Vadbäcken creek and that used the creek for their water supply were examined for adverse effects following exposure to acrylamide and NMA from grouting application. The cows initially showed signs of poisoning, with paresis of the hind legs as the main symptom. Five cows had to be put down due to the adverse symptoms they displayed. After removal from the field seven cows were observed for further evidence of poisoning. One cow showed an unsteady gait and had difficulty in rising. Eleven days after exposure she was almost unable to rise at all. Sixteen days after removal from exposure her condition improved and she was able to stand again. The cow also showed dilation of the pupils. Of the seven cows that were kept for observation four were pregnant and later gave birth to healthy calves (personal communication, 1997).

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment (including sediment)

3.3.1.1 Water

A PNEC of 20 μg/l is obtained for aquatic species exposed to acrylamide as detailed in Section 3.2.1.7. Measured levels and PECs of acrylamide in water are detailed in Section 3.1.4. **Table 3.15** summarises these PECs and compares them with the PNEC. The PNEC used is derived from freshwater and saltwater species. If the PNEC derived from freshwater species data only (34 μg/l) is used the risk characterisation gives the same result.

Table 3.15 Comparison of PEC_{water} with $PNEC_{water}$

Scenario	Concentration (μg/l)	PEC/PNEC
$PEC_{local, water,ann}$		
Site A	2.59	0.1
Site B	0.05	0.003
Site C	<10	<0.5
Site D	<1.05	0.05
Site E	1.22	0.06
Site F	1.05	0.05
Site G	0.24	0.01
Site H	0.25	0.01
Wastewater from use of polyacrylamide flocculants and coagulants in the paper and pulp industry	1.05	0.05
Wastewater from use of polyacrylamide drainage aids in the paper and pulp industry	6.05	0.3
Wastewater treated with polyacrylamide discharged to sewage treatment plant	6.55	0.3
Use of NMA grouts (sewer repair)	3.9	0.2
$PEC_{regional, water}$	0.05	0.003
$PEC_{continental, water}$	0.007	0.0004
Measured levels		
Surface water	<0.2-3.4	<0.05-0.17
Estuarine and seawaters	<0.2 (detection limit)	<0.01

Under normal operating conditions, acrylamide concentrations due to use of grouts should be relatively small and localised in their nature. A $PEClocal_{water}$ of 3.9 μg/l has been estimated for sewer and manhole cover repair based upon data supplied by UK users and generic assumptions. Following withdrawal of the main product used for this application by the producer in late 1997, use is likely to decrease further.

The use of acrylamide-based grouts in the construction of tunnels in Norway and Sweden has lead to measured levels of acrylamide in waters downstream of the construction sites as high as 92 mg/l. In Norway, acrylamide concentrations up to 100 μg/l have been detected. Therefore at both sites environmental levels in excess of the $PNEC_{water}$ for acrylamide occurred (although the concentrations decreased after the use of the grout was stopped).

For microorganisms in the aquatic compartment a $PNEC_{microorganisms}$ of 200 μg/l is calculated. For acrylamide the PECstp is taken as equivalent to the $Clocal_{eff}$. This gives a worst-case PECstp of 0.195 mg/l for production and processing plants (Site A) and 0.065 mg/l for polyacrylamide flocculants used in wastewater treatment. In both cases the PEC/PNEC ratio is less than 1.

Result

For the assessment of surface water for production of acrylamide, production of polyacrylamides, use of polyacrylamide and use of acrylamide-based grouts in pipeline and sewer repairs and manhole sealing operations, and the assessment of risks to wastewater treatment plants for all scenarios:

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.

For the use of acrylamide-based grouts in construction applications:

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

This conclusion is derived from data generated from two specific incidents. The withdrawal of the main product used for this application within the EU at the end of 1997 should be taken into account, as should any risk reduction measures as a result of occupational exposure. The results of the national investigations into the incidents also need to be considered.

3.3.1.2 Sediment

For sediment no appreciable adsorption of acrylamide is expected and so exposure will be mainly via pore water. Consequently the PEC/PNEC ratio for sediment organisms will be similar to the PEC/PNEC ratio for aquatic organisms. Therefore the risk characterisation for surface water in Section 3.3.1.1 also applies to sediment-dwelling organisms.

Result

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

3.3.2 Terrestrial compartment

Acrylamide releases to the terrestrial compartment will chiefly enter the water phase (see Section 3.1.5). Therefore in the risk assessment it is appropriate to compare the $PECsoil_{pore\ water}$ with the PNEC. The PNEC for acrylamide is derived from data on plant growth. When the PNEC (20 μg/l) is compared to the $PECsoil_{pore\ water}$ (0.00017 μg/l), a PEC/PNEC ratio of $8 \cdot 10^{-6}$ is obtained. This suggests that adverse effects are unlikely to occur to terrestrial species due to

acrylamide exposure from the production of acrylamide, production of polyacrylamides, use of polyacrylamide and use of acrylamide-based grouts in pipeline and sewer repairs and manhole sealing operations. A PEC for soil from release from use in tunnelling cannot be calculated from the information available, but it is possible that high levels could be found in pore water under some circumstances. The effects data available for the terrestrial compartment are not sufficient to allow a PNEC to be derived, so the current PNEC is based on the aquatic PNEC - this could be refined by testing on terrestrial species.

Result

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

This applies to the production of acrylamide, production of polyacrylamides, use of polyacrylamide and use of acrylamide-based grouts in pipeline and sewer repairs and manhole sealing operations.

Conclusion (i) There is a need for further information and/or testing.

This applies to the use of acrylamide-based grouts in construction applications. Both the PEC and the PNEC for this use could be refined. However, the control strategy for the aquatic compartment is also expected to remove any risk to the terrestrial compartment, and hence no specific activity is considered necessary at this time.

3.3.3 Atmosphere

In the atmosphere acrylamide has a short half-life (8.3 hours based upon reaction with hydroxyl radicals) and due to its high solubility in water it is probable that acrylamide will be removed by rain out. Acrylamide is not known to contribute to the formation of low-level ozone or contribute to ozone depletion. The half-life of acrylamide in the troposphere is short enough that it is unlikely to be transported to the stratosphere.

The PECs of acrylamide in air from storage and transport are very low. At the local level emissions are generally controlled to limit human exposure. These controls should satisfactorily limit environmental exposure and it is unlikely that adverse environmental effects will be observed due to atmospheric releases of acrylamide.

Result

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

3.3.4 Secondary poisoning

The octanol-water partition coefficient suggests that the bioaccumulation potential of acrylamide is low. This is supported by reported data. Levels in organisms as a result of ingestion of food or natural water are expected to be negligible. Because acrylamide is not accumulative or persistent a secondary poisoning assessment is not required.

Direct exposure of species may occur where water is contaminated with acrylamide for example due to the use of acrylamide-based grouting products. Environmental levels of acrylamide have

been observed as a result of the use of acrylamide-based grouts in tunnelling operations that lead to adverse effects in cattle exposed to contaminated water. This indicates that there is a risk to species due to direct exposure to contaminated water from the use of acrylamide-based grouts. Although this is not "secondary poisoning", it is considered relevant to include it under this heading.

Result

For the production of acrylamide, production of polyacrylamides, use of polyacrylamide and use of acrylamide-based grouts in pipeline and sewer repairs and manhole sealing operations:

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.

For the use of acrylamide-based grouts in construction applications:

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

This conclusion is derived from data generated from two specific incidents. The withdrawal of the main product used for this application within the EU at the end of 1997 should be taken into account, as should any risk reduction measures as a result of occupational exposure. The results of the national investigations into the incidents also need to be considered.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 Occupational exposure

4.1.1.1.1 General discussion

Definitions and limitations

In this document, unless otherwise stated, the term exposure is used to denote personal exposure as measured or otherwise assessed without taking into account the effect of any personal protective equipment (PPE) which might be in use. This definition permits the effects of controls other than PPE to be assessed and avoids the problem of trying to quantify the actual protection provided by PPE in use.

The general discussion sections summarise the important issues arising from the exposure assessments and bring together measured exposure data and predictions from the EASE (Estimation and Assessment of Substance Exposure) model. EASE is a general-purpose predictive model for workplace exposure assessments. It is an electronic, knowledge based, expert system which is used where measured exposure data are limited or not available. The model is in widespread use across the EU for the occupational exposure assessment of new and existing substances.

All models are based upon assumptions. Their outputs are, at best, approximate and may be wrong. EASE is only intended to give generalised exposure data and works best in an exposure assessment when the relevance of the modeled data can be compared with and evaluated against measured data.

EASE is essentially a series of decision trees. For any substance, the system asks a number of questions about the physical properties of the substance and the circumstances of its use. For most questions, the EASE user is given a multiple-choice list from which to select the most appropriate response. Once all the questions have been answered, the exposure prediction is determined absolutely by the choices made. EASE can be used to estimate inhalation and dermal exposure - dermal exposure is assessed as the potential exposure rate to the hands and forearms (a total skin area of approximately 2,000 cm^2). The dermal model is less developed than the inhalation model, and its outputs should be regarded as no more than first approximation estimates.

The output ranges generated by EASE for inhalation exposure relate to steady-state conditions, and estimate the average concentration of the substance in the atmosphere over the period of exposure. The model will not directly predict short-term exposures, but predictions of values for these circumstances are possible by interpreting and modifying the output data using professional judgment. Although short-term exposures may be predicted by EASE in this way, such modifications to the model output should be regarded with caution.

Some information has been made available through the manufacturers and users of acrylamide, but detailed information regarding sampling techniques, frequency and duration of exposure were not always provided.

Where real exposure data are not available or scant, EASE has been used to predict exposures. Details of the reasoning behind any assumptions made during the course of EASE predictions are made clear in the relevant sections.

Overview of exposure

There are five industry sectors where occupational exposure to acrylamide may occur. These are:

1. Manufacture of acrylamide.
2. Manufacture of polyacrylamide (two manufacturers also produce acrylamide).
3. Polyacrylamide use.
4. Preparation and use of electrophoresis gels.
5. Use of acrylamide grouts:
   - Large-scale use of grouts.
   - Small-scale use of grouts.

The total number of workers in the EU exposed to acrylamide during its manufacture and use is estimated to be between 1,200-1,800. In addition to these figures are maintenance workers who are only intermittently exposed. This is estimated to number a further 2,500-3,500 workers. The total number exposed to residual acrylamide during the use of the polymers is far greater than these figures and difficult to estimate: it is likely to be tens of thousands. The number of workers exposed during the manufacture and use of electrophoresis gels is likely to be thousands. Acrylamide is a solid, although it is primarily used as 50% aqueous solution. The solid has a vapour pressure of 0.9 Pa (at 25^{o}C) and the 50% aqueous solution has a vapour pressure of 2.53 Pa (at 25^{o}C). The latter reflects primarily the volatility of water and not that of acrylamide. Therefore workers will be exposed to both water and acrylamide vapour from the aqueous solution. The results presented in the following sections, however, represent acrylamide exposure only.

During manufacture of solid acrylamide and its subsequent use in polymer manufacture exposure will be to dust and to vapour from sublimation of the solid. These situations will include bagging of the solid acrylamide at the Netherlands plant, and during bag opening at polyacrylamide plants. Other situations where exposures to acrylamide dust are likely include cleaning filters, spillages or maintenance. Exposure to dust may also arise during the use of aqueous acrylamide if it is allowed to dry out. The majority of exposures will be during the manufacture and use of the 50% aqueous solution owing to its large market share. During the use of aqueous acrylamide, exposure will be to vapour from the solution. As stated above, this vapour will consist of both water and acrylamide, although air sampling is specific to the latter. It is unlikely that the generation of aerosol will occur during any of its uses.

During the use of polyacrylamides, occupational exposure may occur to residual monomer. This may be to dust or vapour arising from solid grades or vapour from liquid grade polymers. Occupational exposure to residual acrylamide from the polymer may also occur at the polymer production plant during packaging.

Occupational exposure to acrylamide may occur during the preparation of electrophoresis gel packs at laboratory suppliers. Exposure may also occur during the preparation and use of the gels by research establishments, hospitals, universities etc.

Occupational exposure to acrylamide may occur during the large- and small-scale use of acrylamide grouts. Dermal exposure in large-scale use can result from injecting grout in the tunnels and leakage water. Inhalation exposure to acrylamide/NMA can occur during the grouting process. Dermal exposure in small-scale use will occur where workers come into contact with surfaces contaminated directly by the solid or solutions or by condensed vapour; or as a result of direct contact on to the skin. Inhalation exposure to acrylamide/NMA can occur during the grouting process.

The Health and Safety Executive has no acrylamide occupational exposure data; however, extensive air sampling has been carried out by industry. The following sections detail exposure data supplied by industry. In general, it shows that airborne exposure (inhalation) during manufacture averages about 0.2 mg/m^3 8-hour TWA and during its use in polymer manufacture, average about 0.05 mg/m^3 8-hour TWA. Occupational exposure to acrylamide during the use of polyacrylamides was found to be significantly lower than the above figures, primarily due to restrictions on the level of residual monomer in the polymer. Exposures at the one producer of electrophoresis gels who submitted data were about 0.03 mg/m^3 (not 8-hour TWAs). Results of <0.005 mg/m^3 and 0.067 mg/m^3 (not 8-hour TWAs) were obtained for the laboratory weighing out of acrylamide to make gel plates. Occupational exposure data available for the use of acrylamide grouts in Swedish tunnels exceeded the Swedish occupational exposure limit of 0.03 mg/m³ (8-hour TWA). The available data suggest that inhalation exposure even after injection ceased had the ability to exceed 0.012 mg/m^3 (8-hour TWA) acrylamide. The highest occupational exposure value during sewer work was 0.12 mg/m^3 (not 8-hour TWA).

Occupational exposure limits

An occupational exposure limit of 0.3 mg/m^3 8-hour TWA has been adopted by the majority of EU member states, with the exception of Germany and Norway (IARC, 1994). Germany has TRK (technical exposure limits) values of 0.06 mg/m^3 for exposure during the use of solid acrylamide and 0.03mg/m^3 for others (Deutsche Forschungsgemeinschaft, 1995). Where relevant, the results detailed in the following sections are compared to 0.3 mg/m^3 8-hour TWA, as the exposure limit adopted by most member states.

4.1.1.1.2 Occupational exposure during the manufacture of acrylamide

Work pattern

It is understood that about 60 people are exposed to acrylamide during its manufacture in the EU. In addition to these figures there are also maintenance personnel and contractors, of which at the UK plant there are about 50-100. These maintenance personnel work on all areas of the plant and are therefore exposed to a number of substances. Occupational exposure to acrylamide is therefore likely to be intermittent, although it may involve periods of higher exposure than that experienced by process operators. The number of maintenance personnel at each of the other two manufacturing plants was not established, but it is likely to be similar to the UK plant.

During the manufacture of solid acrylamide exposure will be to dust and to vapour from sublimation. The plant in the Netherlands is the only one in the EU manufacturing the solid grade. During the manufacture of the 50% aqueous solution, exposure will be to vapour. At the UK plant, feed stocks are delivered in closed systems to the reactor vessel, with subsequent processing also in closed pipelines and vessels. The final product is piped to storage for the polymer production plants. At the plants in the Netherlands and Germany, manufacture is also

understood to be in closed vessels. The latter, however, do not produce polymers, therefore it is assumed that the aqueous acrylamide is delivered to bulk tankers ready for transport to customers. This also occurs at the other plants where acrylamide is sold. Packaging of the product involves either bagging of the powder or the filling of bulk tankers with 50% aqueous solution. The former is only carried out at the manufacturing plant in the Netherlands, using an automated bagging machine. This machine is housed in an enclosed area, which the operators may have to enter occasionally. The company reports that exposure during this time is usually above the MAC (maximum allowable concentration) value of 0.3 mg/m^3 8-hour TWA. Therefore the wearing of respiratory protection is mandatory for this area. The air sampling is carried out to determine full shift exposure and not short-term exposure during this task. The short-term exposure whilst in the enclosure is therefore not known. **Table 4.1**, however, shows that high results have been experienced at the Netherlands plant, presumably as a result of entering the bagging area.

Occupational exposure may also occur during activities which involve breaching the closed system such as material sampling and maintenance. At the UK plant sampling involves drawing samples of acrylamide into plastic bottles at specified points. The company reported that it is changing to semi-enclosed sampling points to reduce this short-term exposure.

Inhalation exposure

Table 4.1 Occupational inhalation exposure to acrylamide during its manufacture at EU plants

Plant location	Task	No of samples	Arithmetic mean (mg · m^{-3})	Geometric mean (mg · m^{-3})	Range (mg · m^{-3})	% of results < mg · m^{-3})		
						0.1	0.2	0.3
UK 1992-1995	Plant operators	11	0.18	0.09	0.03-0.34	45	91	91
Germany (dates of sampling not known)	Plant operators	24	0.007	*	0.001-0.022	*	*	*
	Laboratory technicians	20	0.002	*	<0.001-0.005	*	*	*
	Total	44	0.005	*	<0.001-0.022	*	*	*
Netherlands Oct. 93 –94	Plant operators **	87	0.17	0.13	<0.05-1.3	38	71	90

Note: * Data not available;
** These air samples were collected over periods of between 3 and 7 hours. It is assumed that they representative of full shift exposure and therefore represent 8-hour TWAs

The results of air sampling carried out at the three plants are detailed in **Table 4.1**. It was not clear as to which tasks were included in the air sampling, although it is assumed that the results are typical of an operator's shift and include tasks such as sampling, filter cleaning (solid manufacture) and packaging. These results therefore represent exposure from these sources as well as any vessel or pipeline leaks. The arithmetic and geometric means for these plants were about 0.2 mg/m^3 8-hour TWA and 0.1 mg/m^3 8-hour TWA respectively. About 10% of results exceeded the occupational exposure limit of 0.3 mg/m^3 8-hour TWA, adopted by most member states. The higher results generally are due to bagging of the solid. The reason for the lower results at the German plant was not established. It is understood that it employs similar

technology to the UK plant (i.e. processing in closed plant with breaching during sampling, maintenance etc.). The limited number of results may be insufficient to conclude that exposure is lower at the German plant, although one possible influence is the stricter exposure limits that exist in Germany compared to the two other producer countries. These TRK (technical exposure limits) values are 0.06 mg/m^3 for exposure during the use of solid acrylamide and 0.03 mg/m^3 for others.

Short-term exposure

The results presented in **Table 4.1** indicate that there may be, on occasion, high short-term exposures. These result from tasks such as bagging, cleaning and maintenance. These short-term exposures are not known, although the three producers appear to have identified these tasks and instigated respiratory protective equipment programmes to control the exposure. In the case of cleaning and maintenance, steps are first taken to reduce contamination, by flushing through, before the worker comes into contact with the plant. It is therefore likely that the use of respiratory protection for these non-routine tasks is currently the best option. At the UK plant if maintenance or cleaning work requires the breaching of the closed system then a permit-to-work system is followed. The instructions detailed by the permit-to-work require the isolation of the pipeline or vessel from the plant, followed by automated cleaning through with water. The operators wear personal protective equipment including airline full-face breathing apparatus, although positive pressure respirators are used in some situations. Contractors may be used for some aspects of this work. Results were not available for exposure during such work; however, it is likely in most cases to be higher than the results in **Table 4.1**, although as stated, these exposures are likely to be reduced by the wearing of respiratory protective equipment.

Dermal exposure

During manufacture dermal exposure may occur when operators come into contact with surfaces contaminated by splashes or condensed vapour or as a result of direct splashes on to the skin. Maintenance work involving the removal of acrylamide is likely to be the task where operators have the greatest contact. The UK manufacturer of acrylamide has carried out dermal measurements by asking operators to wear cotton glove liners inside their PVC gloves. The purpose of this work was to identify the presence of acrylamide and use the information to help evaluate control strategies and housekeeping procedures and not to assess an individual's dermal exposure. The company reported that it had not yet validated the procedure and that the results should be used with caution because the cotton gloves may absorb acrylamide and it is possible that this is an overestimate of exposure. The results were, however, still used in this risk assessment as they provide a reasonable indication of dermal exposure. **Table 4.2** shows the results of the measurements.

Table 4.2 Dermal exposure during the manufacture of acrylamide at the UK plant

Area	No. of samples	Arithmetic mean mg/glove	Geometric mean mg/glove	Range mg/glove
Manufacture	32	1,832	698	0 - 8,427

These figures were used to calculate full shift dermal exposure based on the glove being the same surface area as a hand (410 cm^2). This shows dermal exposures to be up to 0.01 mg/cm^2/day (the lowest mg/glove value reported was 0). The geometric and arithmetic mean results of 0.002 mg/cm^2/day and 0.004 mg/cm^2/day respectively are likely to be representative of exposure where workers have taken reasonable steps to reduce exposure. The higher values may be the result of poor housekeeping or working practices (e.g. removing gloves in the process plant or wearing damaged gloves) and not indicative of routine exposure. These results represent actual dermal exposure resulting when workers were wearing gloves, although the degree to which glove permeation contributed to this exposure is not known.

4.1.1.1.3 Occupational exposure during the manufacture of polyacrylamides

Work pattern

It is estimated that the number of workers exposed during the manufacture of acrylamide polymers in the EU is 1,000-1,500. In addition to these figures are maintenance personnel who receive short-term intermittent exposure. At one UK plant there are 50-100 maintenance personnel who work on both the acrylamide and polymer plants. A further 2,000-3,000 workers in the EU are also likely to experience acrylamide exposure during maintenance.

Polyacrylamides are manufactured primarily from 50% aqueous acrylamide, although some plants do use the solid. Solid (bead, granular, powder etc.) and liquid (oil and water dispersions) grade polyacrylamides are manufactured. The stages for the production of the polymers follow essentially the same steps; addition of chemicals to a monomer make-up vessel where comonomers may also be added; transfer to a reaction vessel where further materials such as initiators may be added; final processing to produce the desired grade and then packaging.

In general the addition of the solid acrylamide to the monomer make-up vessel utilises a fully enclosed automated bag opening machine. Operators only need to enter this enclosed area occasionally for short periods. Plants using the aqueous grade deliver it to the monomer make-up vessel direct from storage. This storage receives bulk stock from the monomer production plant (if a producer) or road tankers.

There are a number of ways in which the monomer can be reacted, including in a vessel or drum, or continuously onto a conveyor. The resulting polymer is in the form of a gel which is either formulated into a liquid dispersion or size reduced and dried to form solid grades. When producing the solid grades the gel may be formed as beads which are subsequently dried or as a block which is size reduced and dried to produce granular polymer. Powder is produced by milling the dried granular polymer. The conveyor method involves feeding polymer, which is mixed continuously in a mixer nozzle with initiator, on to a moving conveyor. The monomer starts to polymerise in the mixer nozzle, and completes polymerisation on the conveyor belt. The nozzle head and conveyor are enclosed and maintained under a nitrogen atmosphere. The point where the sheet of polymer gel exits the enclosed part of the process is under extraction ventilation.

It is understood that all polymer production plants have all stages of the process enclosed until the monomer has been polymerised. Occupational exposures to acrylamide on the plant are likely to be intermittent and generally only as a result of breaching the system. These situations include tanker delivery (if aqueous), bag opening (if solid), material sampling, maintenance and packaging. The latter will only result in exposure to residual monomer in the polymer. Exposure to acrylamide dust will only occur at plants using the solid. Exposure to vapour from sublimation

of the solid may also occur. During all other situations exposure will be to vapour from the aqueous solution, although solid will be present if spillages or vessel contents are allowed to dry out.

Inhalation exposure

Table 4.3 shows the results of air sampling at a UK plant where a variety of polymer grades are produced. With the exception of the fine chemicals plant, which uses solid acrylamide, all plants use the 50% aqueous grade. The bead and powder grade plants require the addition of some materials (not acrylamide), such as initiators through reaction vessel manways. Some of these vessel manways are equipped with extraction ventilation. Visual checking of vessel levels by opening the manway is also carried out on some plants. The company is investigating the use of level indicators to avoid this, and reducing charging via open manways.

Occupational exposures in this UK plant ranged from below 0.01 mg/m^3 to 0.77 mg/m^3 8-hour TWA, although the majority of results, 98%, were less than 0.3 mg/m^3. Seven results were in excess of this value, three of which were for operators on the powder grade reactors prior to the installation of extraction ventilation on the vessels. The one result in excess of 0.3 mg/m^3 for the bead monomer plant was reported to be due to the vessel leaking. The company is currently investigating the other high results. The highest exposures appear to be during the manufacture of polymer powders, for which no cause was reported, although charging of materials via the manway is carried out. It was reported that operators wear respiratory protection during tasks where they are likely to come into contact with the monomer, such as opening manways.

Table 4.3 Occupational inhalation exposure to acrylamide during the manufacture of polyacrylamides in the UK (8-hour TWAs)

Polymer production		No of samples	Arithmetic mean (mg · m^{-3})	Geometric mean (mg · m^{-3})	Range (mg · m^{-3})	% of results < (mg · m^{-3})		
Grade	Plant					0.1	0.2	0.3
Powder	reactors	54	0.10	0.07	0 - 0.36	58	92	94
	monomers	23	0.15	0.09	0.02 - 0.77	43	83	
Bead	reactors	33	0.03	0.02	0.01 - 0.06	100	-	-
	monomers	49	0.08	0.05	0.01 - 0.34	79	96	98
	dryers	29	0.02	0.02	0.02	100	-	-
Powder	line 6	41	0.03	0.02	0.01 - 0.11	98	100	-
Liquid dispersions	line 1	15	0.02	0.02	0.01 - 0.04	100	-	-
	line 2	15	0.02	0.01	0.01 - 0.02	100	-	-
	line 3	22	0.02	0.03	0.01 - 0.03	100	-	-
	line 6	15	0.04	0.03	0.02 - 0.13	93	100	-
	line 4	33	0.02	0.01	0.01 - 0.09	100	-	-
General chemicals		6	0.04	0.02	0.01 - 0.13	83	100	-
Fine chemicals		25	0.04	0.04	0.02 - 0.14	88	100	-
Laboratories	research, QC, etc	63	0.02	0.03	0.01 - 0.07	100	-	-
Total	-	423	0.05	0.03	0.01 - 0.77	85	97	98

There is understood to be only one polyacrylamide production plant in the EU using the continuous conveyor method to produce solid grades. The plant also produces solid grades using the drum method.

Results were obtained for 1993 which showed exposure to be 0.02-0.08 mg/m^3 8-hour TWAs for monomer make-up, 0.04-0.05 mg/m^3 8-hour TWAs for the drum plant, 0.001- 0.06 mg/m^3 8-hour TWAs for the continuous conveyor plant. The total number of air samples for this exercise was ten and the arithmetic and geometric means were 0.03 mg/m^3 8-hour TWA and 0.02 mg/m^3 8-hour TWA respectively.

A small UK company manufacturing water based polyacrylamide emulsions for the surface coating industry also provided some air sampling results. Both personal and static air samples were taken. The highest result obtained was 0.01 mg/m^3 8-hour TWA. This plant receives 50% aqueous acrylamide in 1 tonne bulk containers. Acrylamide is metered to a monomer make-up vessel, before it is piped to the reaction vessel. Extraction ventilation is in place on the vessel to reduce exposure when the manway is opened.

Occupational exposure data were also received from a German polymer manufacturer. These data represent exposure when using solid grade acrylamide to manufacture polyacrylamide. No details were provided on how acrylamide is charged to vessels, although it was reported that the operators wear complete body protection (type not stated) and respiratory protection. These results are reproduced in **Table 4.4**. Another German manufacturer of polyacrylamide reported that the results of air sampling at its plant were all less than 0.03 mg/m^3 8-hour TWA. No further details were reported.

Table 4.4 Occupational inhalation exposure to acrylamide during polymer manufacture in Germany (8-hour TWAs)

Department	No. of samples	Arithmetic mean ($mg \cdot m^{-3}$)	Geometric mean ($mg \cdot m^{-3}$)	Range ($mg \cdot m^{-3}$)
Polymer production	16	0.04	0.05	0.01-0.099
Storage	3	0.02	0.02	<0.01-0.029
Pilot plant	2	0.02	0.03	0.01-0.022
Changing rooms	2	0.01	0.005	<0.001-0.022
Total	23	0.03	0.02	<0.01-0.099

Occupational exposures at polymer plants are likely to be highest during maintenance and cleaning activities. This may involve the removal of solidified polymer from vessels which may involve several weeks' work, although such extensive "dig outs" are infrequent. At the UK plant this work involves a permit-to-work system, which incorporates instructions which require measures such as the isolation of plant and provision of personal protective equipment. **Table 4.5** details the results of air sampling during such activities at the UK plant. The actual exposures will be less than these as they were attenuated by the respiratory protection.

It is understood that polymer producers provide respiratory protective equipment for tasks such as cleaning and maintenance where exposures may be relatively high and it is not practical to control by other means. Some plants operate mandatory schemes.

Table 4.5 Occupational inhalation exposure to acrylamide during infrequent tasks in the UK (8-hour TWAs)

Polymer plant – bead grade	Task	No. of Samples	Arithmetic mean ($mg \cdot m^{-3}$)*	Geometric mean ($mg \cdot m^{-3}$)*	Range ($mg \cdot m^{-3}$) *	% of results less than ($mg \cdot m^{-3}$)*		
						0.1	0.2	0.3
Monomers	Holding tank dig out	48	0.19	0.07	0 - 1.44	60	71	83
Reactors	Reactor dig out	4	0.27	0.24	0.14 - 0.42	-	50	50
Not stated	Cleaning pipe work	2	0.01	0.01	0.01 - 0.02	100	-	-
Reactors	Monomer egg dig out	3	0.01	0.01	0.01 - 0.02	100	-	-

* Actual exposures are likely to have been lower than these as operators wore respiratory protective equipment

Dermal exposure

During the use of polyacrylamides dermal exposure may occur when operators come into contact with surfaces contaminated by splashes or condensed vapour or as a result of direct splashes on to the skin. Maintenance work involving the removal of acrylamide is likely to be the task where operators have the greatest dermal contact.

A UK polymer manufacturer has carried out dermal measurements by asking operators to wear cotton glove liners inside their PVC gloves. The purpose of this work was to identify the presence of acrylamide and use the information to help evaluate control strategies and housekeeping procedures. It was not done to assess an individual's dermal exposure. The company reported that it had not yet validated the procedure and that the results should be used with caution because the cotton gloves may absorb acrylamide and it is possible that this is an overestimate of exposure. The results were, however, still used in this risk assessment as they provide a reasonable indication of dermal exposure. **Table 4.6** shows the results of measurements taken at the UK plant to estimate dermal exposure.

Table 4.6 Dermal exposure during the manufacture of polyacrylamide (UK)

Grade	Plant	No of samples	Arithmetic mean (mg/glove)	Geometric mean (mg/glove)	Range (mg/glove) *
Bead	Monomers	33	4,268	1,352	23,713
	Reactors	20	483	61	3,723
	Dryers	20	172	100	568
Powder	Monomers	24	4,297	1,697	34,707

* The lowest value quoted was 69 mg/glove, although 8 results were reported as below the limit of detection (BLD). To calculate means a figure of 1 was used for BLD

These figures were used to calculate dermal exposures for the full shift based on the glove being the same surface area as a hand (410 cm^2). This gives overall dermal exposures for all the plants of 0.0002-0.08 mg/cm^2/day. The geometric and arithmetic mean results of 0.0001-0.004 mg/cm^2/day and 0.0004-0.01 mg/cm^2/day respectively are likely to be representative of exposure where workers

have taken reasonable steps to reduce exposure. The higher values may be the result of poor housekeeping or working practices (e.g. removing the gloves whilst in the process plant or wearing damaged gloves) and not indicative of routine exposure. These results represent actual dermal exposure resulting when workers were wearing gloves. It is not known the degree to which glove permeation contributed to this exposure.

4.1.1.1.4 Occupational exposure during the use of polyacrylamides

Pattern of work

Polyacrylamides may contain residual monomer, to which workers may be exposed whilst using the polymer. This exposure may be to acrylamide vapour and dust from solid grade polymers, or vapour from liquid grades. Situations where polymer aerosol, and therefore acrylamide aerosol are generated are considered to be infrequent. Due to the low levels of residual monomer, users of the polymer do not carry out air sampling for acrylamide. It was therefore not possible to obtain any air sampling data from users. Regulations and guidelines are in place in the EU to control the level of monomer, and some polymer manufacturers have carried out investigations in an attempt to quantify the potential exposure from the residual monomer. These are discussed below.

Within the EU, under the Dangerous Preparations Directive (88/379/EEC and adaptations), any preparation containing greater than 0.1% w/w acrylamide requires classification and labelling as a Category 2 carcinogen. All polyacrylamides supplied in the EU are either unclassified or classified as irritant. This latter classification reflects the properties of other constituents in the formulation. The IARC monograph (1994) on acrylamide cites Brown et al. (1980) as stating that unregulated polyelectrolytes, containing up to 5% acrylamide, may be used for effluent treatment. It is understood, however, from the acrylamide manufacturers that with the advent of the Dangerous Preparations Directive, all polyacrylamides supplied in the EU have less than 0.1% w/w acrylamide. Many polyacrylamides are marketed with acrylamide levels very much lower than 0.1% w/w.

The UK and the Netherlands require the registration of polyacrylamide products used for water treatment. To obtain approval for these products the level of free monomer should not exceed 0.025%. Some polyacrylamides have been approved for use in paper and paperboard food packaging under German and Dutch regulations and guidelines. The IARC monograph (1994) reports a number of additional regulations and guidelines on the level of free monomer. However, as these are generally above 0.1%, it is assumed that these restrictions have been superseded by the requirements of the Dangerous Preparations Directive.

Inhalation exposure

Industries using polyacrylamides generally use the polymer in a diluted form. The paper industry, for example, uses a 40% emulsion of the polymer which is automatically mixed in a closed system to a final working solution of approximately 0.2% polymer. This final dilution of 1:500 is used on the paper mill as a fibre retention aid. During all stages up to the use on the mill the system is fully enclosed. Workers in the plant are therefore only likely to be exposed to this 0.2% polymer solution. During application to the paper a mist is generated, although workers remain remote from the mill for the majority of their shift (i.e. in the control room). Assuming the residual acrylamide concentration is 0.1% w/w, the final concentration of acrylamide will be at most 0.0002%. The EASE model predicted exposure to acrylamide mist during this scenario is 0.001-0.003 mg/m^3 8-hour TWA.

In many situations, even if neat polymer is handled during diluting, exposure will only be to the vapour. Occupational exposure to acrylamide vapour in these situations will be significantly lower than the figures predicted for exposure to the mist. For situations where solid polymers are used the EASE model predicts exposures to dust to be 0.0001 mg/m^3 8-hour TWA. These predictions are also based on a dilution of 1:500, although it is understood that the three major industries may use dilutions up to 1:2,000.

The highest exposure to acrylamide during the handling of polyacrylamides is likely to occur during packaging at polymer production plants. A UK manufacturer of polyacrylamide carried out both dermal measurements and air sampling to determine exposure during this work. The air sampling results were all less than the analytical limit of detection, which the company reported to be 20 times less than 0.3 mg/m^3 (i.e. 0.015 mg/m^3). The dermal exposure results are discussed below.

A study was carried out in 1984 by a polyacrylamide manufacturer to determine the potential for acrylamide vapour to arise from polymers. This was carried out by transferring 5 gallons of liquid polymer or 50 pounds of solid polymer into a 55-gallon drum. The drum was then sealed and idled for 24 hours to ensure equilibrium. Head space air sampling was then carried out to determine the concentration of airborne acrylamide in the drum. It was then opened in an unventilated room and left for several hours before air samples were collected. Air samples were collected directly above the drum and 5 feet above the ground. This study included four liquid polymers and four solid polymers. The results are presented in **Table 4.7**.

Table 4.7 Acrylamide emissions from polyacrylamide products

Polymer Product	Percentage monomer in the product	Results of the air sampling (mg/m^3)		
		drum head space	above open drum	5 feet above the ground
A (liquid)	0.15	0.114	0.011	<0.001
B (liquid)	0.091	0.022	0.012	0.002
C (liquid)	<0.01	0.002	<0.001	<0.001
D (liquid)	<0.001	<0.001	<0.001	<0.001
E (solid)	0.26	0.019	0.011	<0.001
F (solid)	0.031	0.01	0.003	<0.001
G (solid)	<0.02	0.002	<0.001	<0.001
H (solid)	<0.01	<0.001	<0.001	<0.001

Note: These results are representative of the concentrations at the time of sampling and are not 8-hour TWAs

Dermal exposure

Dermal exposure to acrylamide may occur during the handling of polyacrylamides. In general workers are likely to receive intermittent dermal exposure during their shift to the diluted polymer. The EASE model predicts exposure to be up to $2 \cdot 10^{-7}$-$2 \cdot 10^{-6}$ mg/cm^2/day assuming a 1:500 dilution. If workers come into contact with the neat polymer during dilution, the EASE model predicts dermal exposure to be up to $1 \cdot 10^{-4}$ mg/cm^2/day. A UK producer of polyacrylamide, as stated earlier, carried out dermal measurements during packaging of the polymer. This was carried out by requesting operators to wear cotton liners under their protective gloves for the duration of

their 8-hour shift. The results were all below the analytical detection limit of 5 mg acrylamide / glove which is equivalent to $1 \cdot 10^{-5}$ mg/cm^2/day, assuming a hand surface area of 410 cm^2.

Summary

The EASE model predictions and the work carried out by producers shows that occupational exposure to airborne residual acrylamide at plants using polymers is unlikely to exceed 0.003 mg/m^3, even where aerosol generation is possible. The highest dermal exposures predicted were during the handling of neat polymer at the producers or during manual dilution at the users. The data from the UK producers above and from EASE predictions suggest this to be about $1 \cdot 10^{-4}$-$1 \cdot 10^{-5}$ mg/cm^2/day.

4.1.1.1.5 Occupational exposure arising from the breakdown of polyacrylamide

Occupational exposure to acrylamide during the use of polymers results mainly from residual monomer. In addition the possibility of degradation of the polymer back to the monomer was considered. Very little research appears to have been carried out into this; manufacturers of the polymers report degradation back to the polymer to be unlikely, citing chemical theories as opposed to evidence. It does appear that degradation back to the monomer is unlikely based on the following chemistry.

Acrylamide polymers are formed by free radical polymerisation and would only degrade by scission of the bonds along the backbone. It is extremely unlikely that the two bonds either side of a monomer unit in a polymer would combine to form a double bond and thus reform a monomer unit. This is because the double bond represents a higher energy state requiring severe conditions for its reformation.

Many references support this theory, although none provide proof. Some studies have looked at breakdown of the polymer in the environment. Soponkanaporn and Gehr (1989) carried out an investigation into the effect on degradation of polyacrylamide of factors such as pH and temperature. Size exclusion chromatography measurements showed that complete degradation (to CO_2 and inorganic monomers) only occurred during biodegradation. Acrylamide was not produced.

In terms of occupational exposure it does seem unlikely that acrylamide would be reformed to a sufficient degree so as to significantly increase that already present as residual monomer and therefore the exposure assessments in the previous section are valid.

4.1.1.1.6 Occupational exposure during the preparation and use of polyacrylamide electrophoresis gels

Work pattern

Laboratory chemical suppliers supply acrylamide for gels as either the solid or as aqueous solutions. These may be purchased as pre-weighed or measured packs which contain the crosslinker, to which the user adds the initiator, water (for the powder), mixes and pours. Alternatively the user can buy ready-made gels, which result in negligible exposure to acrylamide.

Inhalation exposure

Occupational exposure may occur at laboratory suppliers during the preparation of these packs. At a UK plant acrylamide is recrystallised with ethyl acetate to increase purity. This involves manually pouring solid acrylamide from 25kg sacks into an extraction vessel. After extraction the wet acrylamide is scooped out into a mobile vessel for transfer to the drying vessel. The wet acrylamide is then scooped into the drier, and spun (closed). It is then scooped onto drying trays, where the remaining solvent evaporates off. These stages of the process are carried out in a ventilated enclosure with extraction to the vessels. The trays of acrylamide are moved to ventilated drying rooms for 24 hours. Blending of acrylamide with methylene-bis-acrylamide is carried out using an enclosed blending vessel in a tented area. Acrylamide is manually charged and discharged to this vessel. Packing of acrylamide powder and blending with methylene-bis-acrylamide are carried out in a down draught booth. Aqueous acrylamide products are prepared by adding water and then tapping into 1-litre bottles. The operators wear full body air fed suits or hoods for all tasks where they are exposed to acrylamide. This company stopped recrystallisation of acrylamide at the end of 1995. Acrylamide of the desired purity is purchased and packaged into appropriate containers. **Table 4.8** shows the results of recent air sampling at the plant.

Table 4.8 Airborne concentrations of acrylamide during the manufacture of electrophoresis gel packs - results represent actual concentrations during the task

Year of sampling	Activity and sample position	Result ($mg \cdot m^{-3}$)
1993	unloading blender, outside tent	<0.002
1993	unloading blender, outside tent	0.002
1993	recrystallisation – inside air fed suit	0.012
1993	recrystallisation – inside air fed suit	0.011
1993	hydro and lay up – inside air fed suit	0.004
1993	unloading blender – inside air fed suit	0.002
1993	recrystallisation	0.072
1993	unloading 1 rack tray, inside enclosure	1.29
1993	unloading 1 rack tray, outside enclosure	0.071
1993	unloading 3 rack trays, inside enclosure	0.67
1993	unloading 3 rack trays, outside enclosure	0.012
1993	unloading blender, outside tent	0.014
1993	unloading blender, inside tent	4.22
1993	unloading blender, inside tent	2.03
1993	unloading blender, outside tent	0.005
1994	preparing aqueous gel packs	0.003
1994	preparing aqueous gel packs	0.004
1994	preparing aqueous gel packs	0.001
1994	packing acrylamide	0.003
1994	packing acrylamide	0.013
1994	packing acrylamide	0.028

Note: All results are actual airborne concentrations - not exposures - except those detailed as inside air fed suits. All samples were personal (i.e. fixed to the outside of the air fed suit or lapel inside)

The high exposures shown in **Table 4.8** are in all cases mitigated by the wearing of full body air fed suits. The four results taken inside the air fed suits are representative of occupational exposure and show that actual exposures are relatively low when compared against 0.3 mg/m^3. These results also include the period of decontamination and removal of the suit, confirming that significant exposure does not occur during this period.

The degree of exposure during the use of the gels will depend on the method used to make them. Clearly exposure is likely to be highest when the user makes the gels from the raw materials. This will involve weighing, although only using a laboratory balance. It is understood that up to 20% of users may use this method, as it is cheaper. The use of prepared packs will only result in exposure whilst adding the water; this is likely to be minimal. Subsequent exposure will only be to vapour as will the use of aqueous acrylamide packs. Preparation of the gels takes only a few minutes, although the exposure may be up to 30 minutes. It is understood that users generally make the gel stock solution in a fume cupboard and then pour the plates at the laboratory bench. **Table 4.9** shows the results of measurements taken during the preparation of electrophoresis gels.

Table 4.9 Personal exposure during the preparation of electrophoresis gels

Task	Result (mg·m^{-3})
Weighing	<0.005
	0.067

These results represent exposure during weighing, which is likely to be the period of highest exposure and not full shift exposures. Therefore shift exposure is likely to be less than this.

Dermal exposure

Dermal exposure may occur during handling of the acrylamide and gels, although technicians wear gloves to avoid contaminating the gel. These gloves may only be general-purpose disposable gloves, however, the duration of the task is such that significant permeation is unlikely.

4.1.1.1.7 Occupational exposure during the use of acrylamide grout

The large-scale use of acrylamide grouts occurs for structural water control and geotechnical grouting operations and the small-scale use occurs for sewer line sealing and manhole sealing.

Both structural water control and geotechnical grouting operations involve manual injection techniques. Worker exposures may occur during grout mixing, injection equipment disassembly and clean up.

There are some similarities in the tunnelling and sewer processes in that they are both often done in confined spaces and they use similar injection equipment. The maintenance and cleaning procedures are also similar. However, some of the sewer procedures involve solid acrylamide as opposed to the liquid formulations used in tunnels such as Hallandsås. Therefore direct read-across from one exposure scenario to the other is not possible.

Occupational exposure during the large-scale use of acrylamide grout and acrylamide based grouting agents.

Investigations were unable to discover any company or other organisation that had undertaken air sampling or other checks during the use of acrylamide/NMA grouts in tunnels other than those undertaken at Hallandsås, Sweden (see below) and Romeriksporten, Norway. It is assumed that these grouts have been used elsewhere but there is no information on such uses. At the locations in Sweden and Norway environmental exposure to acrylamide was detected and at Hallandsås worker exposure led to symptoms consistent with acrylamide-induced effects on health. However, it is understood that in both instances the product was applied by the correct means and therefore the exposure data are interpreted as representative for correct usage.

The construction of the Hallandsås tunnel (The Hallandsås Tunnel, the Royal Commission, Stockholm, 1998.)

The construction of the Hallandsås tunnel came about due to a Swedish Government bill 1987/88:50 on Transport Policy for the 1990s which stated it was urgent that work started on a number of large rail projects, especially the construction of a double-track tunnel to extend the whole West Coast line. The importance of investment in infrastructure for the growth and competitiveness of industry was stressed and it was said that priority would be given to projects which were large, coherent and profitable to the economy. The thinking behind this was that it would lead to a better environment and conditions which would favour economic growth. In 1998 the then Swedish Railways was divided into two companies; one called SJ, which had responsibility for rail traffic and another (the Swedish National Rail Board) which had responsibility for infrastructure. The Swedish National Rail Board proposed that the double track Hallandsås tunnel be built.

The large-scale use of acrylamide grout and acrylamide based grouting agents occurred at Hallandsås, a ridge in the southeast of Sweden where a tunnel had been under construction since 1994. There were approximately 220 workers employed in the construction of the tunnel through the Hallandsås ridge. Because of major problems with water leaks, the grouting agent Rhoca Gil was used in late spring 1997 (Royal Commission Report, 1998).

Background information

The Hallandsås ridge, Sweden, consists of ribs of primary rock approximately 30 km long and 6-10 km wide, which run from the Sinarpsdalen Valley in the west to the district of Örkelljunga in the east. The Hallandsås ridge is a prominent area of high land with very steep sides to the north and south and to the Sinarpsdalen Valley in the west.

Extensive movements in the bedrock have resulted in zones which have brittle splits in the bedrock after steep fractures. The tunnel built through the Hallandsås ridge came across these fracture zones and this had an enormous effect on the tunnel building conditions. The deformation made the rock very poor in quality with only 30% of the rock of reasonable quality and approximately 40% of the bedrock was fissured and very permeable to water. Also, harmful effects on the ground water level were envisaged due to the tunneling but these effects could be prevented it was said by advance injection, i.e. sealing, before blasting or boring commenced.

When the tender for the construction of the tunnel was proposed to prospective contractors geological expertise was asked to be enlisted before judgements were made. The tender indicated that tunneling boring was to be carried out by conventional means, i.e. blasting, but a secondary bid was possible. The contractors chosen for the work, Svenska Kratbyggarna Entrprenad AB,

submitted a secondary bid to involve boring with the use of a very large drill of the same dimensions as the tunnel.

Sydkraft Konsult, a company that had experience of tunnel building, studied the proposals and was very critical of the very large drill method proposed. Sydkraft Konsult considered that it was not possible to use the proposed full-cut method in every part of the ridge as the rock was too soft and also the method would make injection more difficult. However, the proposal of using the full cut method was accepted by the National Rail Board.

Drilling began in June 1993 but ceased, as the rock was too soft and the drill bit stuck fast. However, as Kraftbyggarna employed the blasting method as well it was possible to drive a total of 500 m north inside the tunnel and 1200m south before the work was abandoned 1995. The contract to build the tunnel was worded in such a way that the contractor would be held responsible for any failure in the tunneling. After the work ceased in 1995 Kraftbyggarna tried to re-negotiate the contract on the basis that the details given to them regarding the condition of the rock were incorrect. In May 1995 Kraftbyggarna was released from the contract in return for a large compensation sum continued working until the National Rail Board found a new contractor. The new contractor, Skanska Stockholm AB, was contracted to complete the work by 25 November 1999.

Sealing methods

Sealing methods of various kinds were tried using both concrete and other chemicals apart from Rhoca Gil and linings were also considered. The first option was to continue building the tunnel without space for a lining. This would significantly increase strength and sealing but would fall short of satisfying the conditions stipulated by the Water Rights Court. (The Water Act (1983:291) contains regulations concerning the utilisation of surface and ground water. Under the Act, consent is required for water undertakings, which is given by the Water Rights Court.). The second option involved a combination of lining and sealing. This method would also fall short of the conditions stipulated by the Water Rights Court, although was closer to meeting them than was the first option. The third option was to make a complete lining. This method respected the provisions of the Water Rights Court. The second option was preferred and the option of a complete lining was put forward as an alternative. The National Rail Board decided that the northerly tunnel section should be widened to allow space for a lining but it would continue to look for an effective sealing method.

Sealing product - Rhoca Gil

In January 1997 Skanska acquired information about Rhoca Gil and in February 1997 the National Rail Board commissioned an independent consultant from the Swedish Cement and Concrete Institute to enquire into the long-term stability of Rhoca Gil. On 20 February the National Rail Board ordered Skanska to carry out four injections using Rhoca Gil. On 21 February the National Rail Board received a preliminary report from the Cement and Concrete Institute, which said that “the problem with acrylamide injection agents is their poisonous properties” and that “the degree to which a substance is poisonous after injection depends on how well it has polymerised”. In the final report it declared that Rhoca Gil was less toxic than earlier sealants since the pure acrylamide is replaced with N-methylolacrylamide. But no final conclusions were drawn regarding the toxic properties of Rhoca Gil. The final conclusion drawn was that if injection was found to be successful it would seal against leakage in the Hallandsås tunnel for at least 100 years. Neither the National Rail Board nor Skanska made any further enquiries into the

toxicity of Rhoca Gil. They relied on information from the supplier regarding toxicity and also about the conditions required for complete polymerisation.

The use of the acrylamide-based grouting agent

The specific grouting sealant used at Hallandsås was the product Spirogel produced by Rhone-Poulenc, based on a combination of Rhoca Gil with a sodium silicate solution.

The gel is obtained by combining sodium silicate solution plus an organic reagent with an acrylic monomer and its catalysts. The product is formed as a result of synthesis and co-formation of acrylic and silica gels.

The sealant is presented in the form of two concentrated aqueous solutions, which are diluted with water and mixed on site; an accelerator (ACS) may be added to solution 1, if required.

Solution 1, according to the declaration of contents contains:

- maximum 2% acrylamide,
- approx. 37-38% NMA,
- approx. 1% formaldehyde,
- accelerator/stabiliser for monomer (stated to contain dimethyl adipate, dimethyl glutarate, dimethyl succinate and triethanolamine),
- silicate hardener.

Solution 2, according to the declaration of contents contains:

- liquid sodium silicate,
- sodium persulphate.
  (Safety Data Sheet for Spirogel 110-25 dated 15/09/1994)

Although the safety data sheet declared an acrylamide content of 2% or less, analyses indicated a range of concentrations from 3.5-9% (The National Chemicals Inspectorate, Sweden). The analysis is, apparently, difficult to achieve reliably because of the unknown extent of transformation of NMA to acrylamide. The manufacturer admitted that the safety data sheet was inaccurate and issued a statement that the acrylamide content was about 4% (communication with HSE, UK).

Grouting formulation and injection

The grouting agent is made up in three stages by adding water to each of the two concentrates:

1. Add the required quantity of ACS accelerator to Solution 1
2. Dilute each solution with equal volumes of water:
   -1 volume of solution 1 + 1 volume of water
   -1 volume of solution 2 + 1 volume of water
3. Mix together equal volumes of each of these new solutions.

Therefore, the mixed solution consists of 3.75 parts of water, 0.125 parts of concentrated solution 1 and 0.125 parts of concentrated solution 2. Approximately 10% ACS was included in solution 1.

Test injections with Rhoca Gil were carried out periodically in the north tunnel ridge from the end of March 1997 to the end of June 1997 and test injections were carried out in the south tunnel ridge at the end of April. In August more widespread injection began in both the north ridge and intermediate ridge which continued until 29 September when work stopped. For a few days at the end of September injection also took place in the south ridge. A total of 360 tonnes was needed at first but by the end of construction 1,400 tonnes had been injected on a stretch totaling approximately 550 m.

The mixture was pressed into forty injection bore holes, 9 metres long, located at a 200' angle radially out from the front of the tunnel and with 50 cm space between. The solution was injected to a predetermined counter-pressure by machine through a steel nozzle with rubber sleeves, which were placed in the borehole openings.

The tunnel driving work came to a halt on 29-30 September 1997, when it became clear that the grouting agent had leaked out into the adjoining watercourse. Acrylamide and NMA, which are integral parts of the product, had not hardened completely into a polyacrylamide gel, but instead high levels were found in the leakage water, which was pumped up from the intermediate ridge (work site in the middle of the tunnel) at Severtorp and then conveyed into the Vadbäcken Creek.

Exposure control

Workers who would be handling the grouting agent, were given training in 1997 by supplier representatives on how to use the grout, and the risks that would be involved. The manufacturer, Rhône-Poulenc, indicated that protective gloves, protective goggles and thick clothing was required when injecting and “self contained breathing protection” should be used.

According to the Board of Occupational Safety and Health Regulations the limit in air for acrylamide of 0.03 mg/m³ applied. In the product information sheet the limit was given wrongly as 3 mg/m³. Because of its acrylamide content one of the components, Solution 1, was classed as poisonous.

The Labour Inspectorate inspected the work environment conditions on two occasions. A systems inspection was planned to check that the employer’s preventive environmental measures were working properly. This inspection had still not been done in September 1997. Later in October the Labour Inspectorate undertook several inspections. Included in its demands was that Skanska should draw up handling and safety instructions for the work which would continue with thermosetting plastic components during the clearing up period, give adequate training to the staff who would be working with these types of components and record the measures which were taken or would be taken before work in the tunnels resumed.

The work involved risk of skin exposure to solutions 1 and 2, the ready-mixed substance, the discharge from the tunnel mouth (injected substance which sprayed back from cracks in the face of the tunnel) and leakage water. In addition there was a risk of inhalation of acrylamide/NMA and formaldehyde. People working in the tunnels displayed symptoms such as a pricking sensation or numbness in various parts of the body, as well as skin irritation and breathing difficulties.

Sampling and analysis methods

Personal and static (fixed position) air monitoring was carried out. For static sampling, the sampling pump was located in the injection vehicle approximately 2 m above the floor of the tunnel, near to the roof.

All samples were analysed using gas chromatographic or high performance liquid chromagraphic techniques. Acrylamide and NMA were analysed by the Analysis Laboratory in Lund and formaldehyde was analysed by the Clinic for Industrial and Work Environmental Medicine in Orebo. The analysis methods at the time when early samples were taken were not technically capable of differentiating between and acrylamide and NMA.

Inhalation Exposure

Tables 4.10 and 4.11 show inhalation exposure during grouting. **Tables 4.12 and 4.13** give inhalation exposure when grouting had ceased.

Table 4.10 Exposure during grouting (period when analytical ability could not differentiate between acrylamide and N-methylolacrylamide)

Sample Type	Site Details	Sampling Time (min)	Acrylamide and NMA ($mg \cdot m^{-3}$)	Formaldehyde ($mg \cdot m^{-3}$)	Comments
North entrance – East tunnel point 191/656 –Approximately 6 m^3 injected during measurement period					
Personal	M1	165	0.27	-	90% of sampling period close to mineral specimen, remainder by injection vehicle
Static	M2	160	0.24	0.48	In injection vehicle approximately 2m above floor. Cap missing on blender
Middle entrance – East tunnel point 1/125 –Approximately 2 m^3 injected during measurement period					
Personal	M1	165	0.05	-	50% of sampling period close to mineral specimen, remaining time close to injection vehicle. Large water leak during sampling period
Static	M2	170	0.05	0.25	In injection vehicle approximately 2m above floor. Cap on blender
North entrance – West tunnel point 191/770-Approximately 5.5 m^3 injected during measurement period					
Personal	M1	165	0.34	-	90% of sampling period close to mineral specimen, remainder by injection vehicle
Static	M2	170	0.12	-	In injection vehicle approximately 2m above floor. Cap on blender

Table 4.11 Exposure during grouting (period when analytical ability could differentiate between acrylamide and N-methylolacrylamide)

Sample Type	Site Details	Sampling Time (min)	Acrylamide ($mg \cdot m^{-3}$)	NMA ($mg \cdot m^{-3}$)	Comments
North entrance – West tunnel point 191/770 - Approximately 5.5 m^3 injected during measurement period					
Personal	M1	165	0.076	0.064	70% of sampling period close to mineral specimen, remainder by injection vehicle
Static	M2	170	0.061	0.051	Injection vehicle approximately 2 m above floor
Middle entrance – East tunnel point 1/125 194/680 – Approximately 4 m^3 injected during measurement period					
Personal	M1	135	0.046	0.043	20% of sampling period close to mineral specimen and remainder by injection vehicle.
Static	M2	140	0.05	0.036	In injection vehicle approximately 2 m above floor. Cap on mixer, 4 injection hoses in operation
Middle entrance 194/680 - Unloading of rock mass after blasting containing agent Rhoca Gil					
Personal	M1	215	0.008	0	In bucket loader CAT 980c. Tube broken in opening

Table 4.12 Exposure during period when grouting had ceased (period when analytical ability could differentiate between acrylamide and N-methylolacrylamide

Sample Type	Details	Sampling Time (min)	Acrylamide ($mg \cdot m^{-3}$)	NMA ($mg \cdot m^{-3}$)
Static sampling site after work with injection agent Rhoca Gil stopped				
Static	South entrance W, 197/350	160	0.0001	0.0001
Static	South entrance E, 197/490	158	0.0002	0.0002
Static	Middle entrance, 365	180	0.0006	0.0002
Static	Middle entrance, 365	172	0.0045	0.0002
Static	North entrance E, 191/744	186	0.0017	0.0002
Static	North entrance E, 191/452	179	0.0054	0.0004
Static	South entrance W, 198/800	175	0.0006	<0.0001
Static	South entrance E, 198/185	170	0.0006	<0.0001
Static	Middle entrance, 750	190	0.0002	0.0001
Static	Middle entrance, 1090	190	0.001	0.0001
Static	North entrance E, 191/770	170	0.0039	0.0002
Static	North entrance W, 191/454	170	0.0024	0.0001

Table 4.13 Exposure during period when grouting had ceased (period when analytical ability could differentiate between acrylamide and N-methylolacrylamide

Sample Type	Site Details	Sampling Time (min)	Acrylamide ($mg \cdot m^{-3}$)	NMA ($mg \cdot m^{-3}$)	Comments
North entrance – East tunnel point 191/680 720					
Personal	M1	180	0.005	0.004	Assistant during drilling. Break through to the control/check layer taken place before the test/sample 10% of acrylamide and 35% of NMA found in control layer/strata. The sample has higher NMA content, might have been caused by water splashing on to equipment
Personal	M2	240	0.005	0.0003	Water sample tester
Static	M2	235	0.006	0.001	In drilling rig approximately 2 m above floor
North entrance – East tunnel point 191/680 720					
Personal	M1	180	0.012	0.011	Assistant during drilling. Higher NMA content - possibly caused by water splashing on sampling equipment
Personal	M2	180	0.007	0.001	Water sampler tester
Static	M3	195	0.01	0.002	In drilling rig approximately 2 m above floor
North Entrance – West tunnel point 191/680					
Static	M4	230	0.018	0.004	By tunnel wall approximately 1.5 m above ground
North entrance – West tunnel point 191/520 540					
Personal	M1	140	0.005	0.001	Assistant during drilling. Out of tunnel 10 minutes
Personal	M2	130	0.005	0.001	Tester of water samples. Out of tunnel 10 minutes
Static	M3	130	0.007	0.001	In drilling rig approximately 2 m above floor.
Middle entrance - no work being undertaken in the tunnel					
Static	M1	135	0.0014	0.0001	By tunnel wall - point 980
Static	M1	140	0.0016	0.0003	By tunnel wall - point 1070
Static	M1	130	0.0047	0.0001	By tunnel wall - point 1128
North entrance – West tunnel point – 191/600 590					
Static	M1	165	0.0036	0.0004	In drilling rig approximately 2 m above floor
Middle entrance point 1120-1130					
Static	M1	165	0.0028	0.0002	In drilling rig approximately 2 m above floor
Static	M2	155	0.0035	0.0002	Passenger cage. Assistant during drilling
North entrance					
Static	M1	120	0.0042	0.0003	West tunnel point 191/600
Static	M2	120	0.0045	0.0004	Test. Passenger cage. West tunnel point 191/765/630

The work in the tunnel was undertaken in two shifts: 05:00 to 15:45 and 15:15 to 02:00. Grouters would have been potentially exposed for up to 9 hours per day and the sampling periods were between 120 and 240 minutes. If exposure results are taken as 8-hour TWA exposures then it can be seen that all personal sampling results, except the sample taken at the middle entrance point, exceeded the Swedish occupational exposure limit of 0.03 mg/m^3. This is likely to be an overestimate for the first three personal samples since in **Table 4.10** the results are quoted for both acrylamide and NMA. The actual results in question were 0.27, 0.05, 0.34 mg/m^3 for both acrylamide and NMA (since the analytical method used in these early tests could not differentiate between acrylamide and NMA) and 0.076, 0.046 and 0.008 mg/m^3 for acrylamide itself (determined using a refined method; **Table 4.11**). The first and third samples were of the order of 0.3 mg/m^3 although the fan was switched off for 60 minutes during the collection of the third sample.

It is understood that the bulk of the injection work stopped soon after the last samples were taken on the 30 September 1997. Six static samples were collected on the 10 October 1997 in the north, middle and south parts of the tunnel (**Table 4.12**) and this was followed by six more samples one week later (**Table 4.13**). The highest of these was 0.0054 mg/m^3, taken at the north entrance.

Exposures will vary depending on a number of factors: the location of the grouters with respect to the injection operation, the posture adopted, whether the grouters wear appropriate personal protective equipment and if they do, whether they wear it correctly and the personal hygiene of the grouters, etc.

Owing to the relatively few numbers of personal samples and the obvious variation in the exposure of the grouters, it is not possible to conduct a statistically valid assessment of exposures during the grouting operations. The available data suggest that inhalation exposure even after injection ceased had the ability to exceed 0.012 mg/m^3 acrylamide (8-hour TWA).

It is not clear whether the sampling procedures included collection of acrylamide and NMA vapour and mist. It is thought probable that there would have been large amounts of liquid and possibly mist generated in the tunnel. The air sampling used two filters, suggesting that both vapour and mist were collected. There is no further information available on how the sampling was carried out.

Dermal exposure

No quantitative data were available on dermal exposure to workers in the tunnel. Reports by workers during subsequent medical examination and information in questionnaire responses indicate that high dermal exposure occurred, with gloves and overalls becoming wet to the extent that some workers used rain suits to protect themselves against the leakage water. However, they did not change wet gloves or coveralls until they came off shift and those using rain suits considered that this did not fully protect against dermal exposure. It is likely that the dermal exposure would not have been so high if the grout had behaved as intended when applied. The acrylamide would have polymerised and not been released into the leakage water.

Occupational exposure during the small-scale use of acrylamide grout

Acrylamide grouts can be used on a small scale in sewer rehabilitation of lateral and main lines. Leaking pipes and joints can be sealed remotely using equipment that incorporates a closed-circuit television system, an inflatable packer, and a grout delivery system. The camera provides

pictures of the inside of the sewer line to a worker who controls the packer and the grout delivery system from a control board inside a service truck. Exposure to grouts from this use typically occurs during grout mixing and equipment disassembly and clean-up operations.

In manhole sealing, a worker enters the manhole and uses a hand-held device to inject the grout into holes drilled in the side of the manhole. The grout flows into the soil surrounding the manhole, sealing cracks and preventing water infiltration. Worker exposure occurs in all phases of this operation (grout mixing, injection, equipment disassembly and clean up).

There is no information on exposure during the small-scale use of acrylamide grout in the EU. However, there have been three surveys on the use of acrylamide in the USA in the sewer industry and information from these is presented below.

Hills survey

An in-depth industrial hygiene survey was conducted at a sewer line repair site in Springdale, Ohio to assess exposure to acrylamide during a grouting process (Hills, 1986).

Two workers performed the repair. Closed circuit television was used in the actual grouting process to position the packer but the mixing of the acrylamide-based grout was done from a van containing the grouting equipment.

First the site of a leak was found using the video camera and then both ends of the packer were inflated, isolating the leaking joint. Above ground grouting material was poured into a mixing tank containing water and injected under pressure from the service truck via hoses to the centre of the packer and forced into the surrounding soil. Once the leak was sealed, the packer was deflated and moved to the next joint.

It was observed that during the mixing process some of the acrylamide powder spilled on the side of the tank and the floor. This spillage was not cleaned up and after the bags of solid acrylamide were empty, they were rolled up and placed in a corner of the van. These actions clearly gave rise to potential exposures to acrylamide. It was also noted that the employees did not wear the appropriate personal protective equipment to prevent exposure to acrylamide.

Personal air monitoring was conducted for the full day (9 hours). Exposures for each of the two workers were 0.002 and 0.009 mg/m^3. Personal air sampling was also undertaken during the mixing of the two batches of acrylamide that were used on the day of the survey (mixing lasted 18 minutes) and both of the results were below the limit of detection for the method that was used (limit of detection not provided). Static (fixed position) samples were collected in the garage and van and these gave results of 0.001 and 0.009 mg/m^3 respectively.

With respect to dermal exposure, five “wipe” samples were collected. Four of these contained 0.6 µg of acrylamide whilst the fifth (from the outside of the acrylamide mixing tank) contained 44 µg of acrylamide. The insides of the protective gloves that were used were washed out with water and these were found to contain 65 µg of acrylamide in total.

Given that there was little or no vapour/mist generation then the opportunity for inhaling acrylamide was relatively limited. However, there was a significant potential for dermal exposure because it was difficult to avoid skin contact with acrylamide.

McHugh survey

The objective of this study was to measure occupational inhalation and dermal exposure to acrylamide for manhole, mainline and house lateral line sealing operations (McHugh, 1987).

The manhole sealing operations were performed using an electric drill and injection gun. The worker entered the manhole and performed a visual inspection of the sidewalls. When a groundwater leak was found, holes were drilled into the wall of the manhole in the proximity of the leak. Acrylamide monomer and a catalyst were pumped through separate lines to the injection gun. The two components mixed at the injection nozzle and were pumped into the drilled holes. The acrylamide grout combined with the surrounding soil, forming a gel-like seal to stop the leak.

Mainline sealing operations were performed by remote control using a sleeve packer and a video camera unit, which were pulled through the sewer line by a power winch. This procedure permitted visual inspection, pressure testing, and sealing of the sewer pipe in one pass. Lateral line sealing operations are similar to mainline sealing and use the same equipment except for a specially designed sleeve packer. The three line maintenance operations used similar equipment, which was carried on self-contained mobile vans, trucks or trailers. Typical grouting equipment common to all line maintenance procedures included chemical mixing tanks; water storage tank; electric generator; air compressor; chemical pump; operator control panel; and quad-line chemical hoses on a power reel assembly. In addition to this equipment, mainline and lateral line sealing operations utilised a sleeve packer, camera assembly and remote-controlled power winch.

The results shown in **Table 4.14** demonstrate a potential for airborne exposure to acrylamide during sewer line maintenance operations. Three of six air samples collected in the breathing zone of the line maintenance employees exceeded the Threshold Limit Value (TLV) of 0.03 mg/m³ published by the American Conference of Government Industrial Hygienists (ACGIH, 1999 edition). The airborne exposures however, did not exceed the OSHA Permissible Exposure Limit, which at the time of the survey was 0.3 mg/m³.

The maintenance supervisor involved in manhole sealing operations at site No 1 was found to have the highest exposure to airborne acrylamide. The utility worker performing a similar job at site No 2 experienced a tenfold lower exposure. This difference may be attributed to differences in the manhole configurations at the two sites and the orientation of the ventilation duct outlet relative to the worker's breathing zone. The grout foreman's higher exposure relative to the labourer's exposure at site No 3 may be attributed to the chemical mixing operation. The lowest acrylamide exposure was found during the lateral line sealing operation at site No 4. Grout chemicals were not mixed during the survey at site No 4. The utility worker spent most of his time outside of the grouting vehicle.

Table 4.14 Occupational inhalation exposure to acrylamide for manhole, mainline and house lateral line sealing operations (McHugh survey, 1987)

Sample Type	Sample Time (min)	Acrylamide Exposure ($mg \cdot m^{-3}$)	Comments
Personal Site No. 1	162	0.12	Manhole sealing operations. Manhole 9 m deep. Air forced into the confined space at the top of the manhole at a flow rate of 17 m^3/min. Worker stood on ladder inside the manhole. Worker's breathing zone was approximately 0.3 m from the injection point at shoulder level. Forced air ventilation duct was approximately 4 m from the worker
Personal Site No. 2	163	0.01	Utility worker sealing a manhole. Manhole 3 m deep. Air forced into confined space at the top of the manhole at a flow rate of 17 m^3/min. Injection holes were approximately 0.3 – 0.6 m from the bottom of the 3 m manhole. Injection holes were at waist level when the worker stooped down to inject the grout. The outlet of the ventilation duct was at a distance of approximately 1.3 m from the worker
Personal Site No. 3	480	0.06	Mainline sealing operations. Involved in the chemical mixing operation. Dust was observed when acrylamide monomer was poured into the mixing tank
Personal Site No.3	480	0.04	Mainline sealing operations.
Site No.3 (area samples)	480	0.05	Mainline sealing operations. Inside grouting rig
Personal Site No. 4	483	0.008	Lateral line sealing
Site No. 4 (area samples)	460	0.08	Lateral line sealing. Inside the grouting vehicle near the mixing tanks

Table 4.15 Summary data for dermal exposure

Type of sample	Time or place of sampling	Site	Range of acrylamide exposure mg/100cm^2 (unless otherwise indicated)
Hand rinses	On arrival	-	n.d.-1.75 mg
Wipe samples	Outside of mixing tank	-	0.007-7.10
Wipe samples	Catalyst mixing tanks	-	0-0.23
Wipe samples	Handles of mixing paddles	-	0.014-1.07
Wipe samples	Injection guns	1	0.216
Wipe samples	Injection guns	2	0.577
Wipe samples	Chemical hoses	1	0.023
Wipe samples	Chemical hoses	2	0.143
Wipe samples	Packer/camera assembly before insertion	3 & 4	0.002-0.021
Wipe samples	Packer/camera assembly after removal	3 & 4	0-0.280
Wipe samples	RPE: inside of face piece	-	0-0.016
Wipe samples	RPE: outside of face piece	-	0.002-0.008
Wipe samples	Outside of rubber work gloves	-	0.037-0.809
Glove rinse (1)	Inside of rubber glove	2	1.37 mg
Glove rinse (1)	Inside of rubber glove	4	2.49 mg

Dermal contact to acrylamide was estimated using direct and indirect methods. The rate of exposure was measured using pads placed directly on the skin on the upper back, forearms, thighs and knees. These were worn throughout the day.

Field survey teams indicate that manhole-sealing operations have the highest potential for dermal contact relative to mainline and lateral line sealing operations. The manhole sealing operations observed presented a higher potential for dermal contact because the grout was injected manually by the worker with an injection gun in a confined workspace. These conditions exposed the worker to chemical runoff and splashes during the injection process and to skin contact with contaminated equipment.

For mainline and lateral line sealing operations, which were performed using remote-controlled equipment, field observation indicated that dermal contact was caused mainly by contact with contaminated equipment. The potential dermal contact caused by chemical runoff or splashes was minimal with the exception of the chemical mixing operation.

The results of the hand rinses indicated that hand contact to acrylamide began very early in the work shift, even before the equipment was set up. This hand contamination most likely occurred during the drive to the field site in the grouting vehicle. Protective gloves were not used by any of the workers until they arrived at the site and began to assemble the equipment.

At sites Nos 3 and 4, wipe samples of the packer/camera assembly were taken before insertion into the sewer line and after it was removed. The amount of water running in the sewer line may influence the amount of acrylamide contamination remaining on the packer/camera assembly after removal from the sewer line. The water in the sewer line is likely to rinse off some portion of the acrylamide monomer from the exterior of the packer/camera assembly.

Glove rinses were conducted at two field sites to determine the amount of acrylamide found on the inside of rubber gloves worn during the line maintenance operation (**Table 4.14**). It was not determined whether the acrylamide contamination was due to wearing the gloves over previously contaminated hands or due to permeation of acrylamide through the glove material. The comparative values inside and outside of the respirators and gloves should not be construed to represent protection factors associated with the respective personal protection equipment.

It should be noted that for these operations, most of the personnel wore the personal protective equipment provided, which consisted of: uniform clothes (slacks and short sleeve shirts), safety shoes and additional personal protective equipment that was worn for specific operations. The crew members entering the manhole to inject the chemical grout wore a hard hat, eye goggles, disposable Tyvek overalls, MSA Comfo II half mask respirator fitted with dual organic vapour filter cartridge, rubber boots worn over safety shoes, and water resistant protective gloves worn over surgical gloves.

Cummins et al. survey

Exposure monitoring consisted of area and personal air sampling, wipe sampling and hand rinses for in line chemical sewer grouting using acrylamide at three locations (Cummins et al., 1987).

There are inadequate exposure data available from this survey and so it has not been included in the risk characterisation.

4.1.1.1.8 Inhalation exposure (general discussion)

Table 4.16 summarises all the available inhalation exposure data. In the acrylamide industry the majority of exposures are confined to one manufacturer of acrylamide, two manufacturers of both acrylamide and polyacrylamides and a further six sites manufacturing only polymers. There are also a number of smaller companies in the EU manufacturing polymers, generally for specific uses. It is understood that the only other applications where workers may be handling acrylamide is the use of electrophoresis gels and the use of acrylamide grouts.

Acrylamide manufacture

At two of the EU manufacturing plants approximately 10% (ten results) of exposures were reported to be greater than 0.3 mg/m^3 8-hour TWA; at the third plant all results were less than this figure. Nine of these results, including the highest of 1.3 mg/m^3 8-hour TWA, were from the only EU manufacturer producing acrylamide powder. At this plant, powder is bagged using an enclosed automated bagging machine, although operators occasionally enter the area. A mandatory respirator programme is in place at the plant for this area, therefore actual exposures are likely to be lower. Geometric and arithmetic means show exposure at this plant (disregarding attenuation from respiratory protection) to be about 0.1 mg/m^3 and 0.2 mg/m^3 8-hour TWA, respectively during manufacture.

All EU acrylamide manufacturing plants operate RPE programmes to reduce exposure during tasks giving rise to the higher exposures. There are tasks which give rise to high short-term exposure, such as cleaning and maintenance. The degree to which the respirator will reduce exposure will depend on factors such as the type, its condition and how it is worn. Actual exposures are therefore likely to be lower than the results in **Table 4.16**. It was not clear from the submitted data as to why occupational exposures at the German plant were lower, although the stricter exposure limits may be an influence.

Table 4.16 Summary of occupational exposure data detailed in the exposure assessments

Industry	Source	No. of samples	Arithmetic mean ($mg \cdot m^{-3}$)	Geometric mean ($mg \cdot m^{-3}$)	Range ($mg \cdot m^{-3}$)	% > 0.3 $mg \cdot m^{-3}$
Acrylamide manufacture	UK	11	0.18	0.09	0.05-0.34	9
	Germany *	44	0.01	**	<0.001-0.022	0
	Netherlands *	87	0.17	0.13	<0.05-1.3	10
Polyacrylamide manufacture	UK*	422	0.05	**	0.01-0.77	2
	UK*	10	0.03	0.02	0.001-0.08	0
	UK*	4	0.01	0.01	0.01	0
	Germany*	**	**	**	all <0.03	0
	Germany*	23	0.03	0.02	<0.001-0.099	0
Electrophoresis gels	UK (man.) ++	4	0.03	0.006	0.002-0.012	0
	UK (use)	2	0.04	NA	<0.005/0.067	0
Users of polyacrylamide	EASE	NA	NA	NA	0.0001-0.003	0
	UK *	**	**	**	all < 0.015	0
	Netherlands	NA	NA	NA	<0.001-0.012	0
Large-scale use of acrylamide grouts (tunnels)	Sweden ***	9	0.018	0.01	0.005 – 0.076	0
Small-scale use of acrylamide grouts (sewers)	US	5	0.047	0.029	0.008-0.12	0

* Personal samples
** Information not available.
*** Excluding results which are acrylamide/NMA
\+ These may not be 8-hour TWAs.
++ Only results from inside the air fed suits presented.
NA = Not applicable.

Polyacrylamide manufacture

In general, exposures during the manufacture of polyacrylamides appear to be lower than during monomer production. Five EU polymer plants submitted data, only one of which showed exposure in excess of 0.3 mg/m^3 8-hour TWA. The data from this plant showed 2% of the 422 results to be in excess of the above figure. These were mainly historical from a plant before the installation of extraction ventilation to vessels. One further result was due to a leaking vessel. Geometric and arithmetic means were generally in the range 0.01 mg/m^3 to 0.05 mg/m^3 8-hour TWA. Exposures are again likely to be lower than this as most polymer plants supply RPE.

The highest exposures were during maintenance and cleaning activities. Results obtained from one UK site showed exposures of up to 1.44 mg/m^3 8-hour TWA, although in these situations respiratory protection is always worn. This is understood often to be full-face airline breathing apparatus.

Polyacrylamide use

Although measurements for acrylamide during the use of the polymers are limited, these measurements and the EASE model predict the highest exposures to be about 0.001-0.003 mg/m^3 8-hour TWA. These are for exposure to mists and are likely to be over estimations. Further exposure from the de-polymerisation of the polymer, as explained earlier, is extremely unlikely.

Preparation and use of electrophoresis gels

Airborne concentrations at the one producer of electrophoresis gels that supplied data showed relatively high levels of up to 4.22 mg/m^3. However, the wearing of air-fed suits is mandatory. Actual exposures will therefore be substantially lower; results of measurements inside the suits were on average about 0.03 mg/m^3. It is not known whether other plants use similar production methods, however, airborne levels at other plants are unlikely to be higher. If plants handle acrylamide in a similar way it is likely that similar breathing apparatus schemes are in place. The actual exposures (inside an air-fed suit) are therefore likely to be representative of this industry. Exposures during the preparation of the gels were also relatively low, owing to the short duration of the task and small quantities involved.

Large-scale use of acrylamide grouts and acrylamide grouting agents

Using acrylamide grout for tunnelling is a large diffuse use which is therefore difficult to control so exposures are likely to be high. Exposure data are available for the use of acrylamide/NMA grouts in tunnels in Sweden and Norway. At these locations environmental exposure to acrylamide was detected and in Sweden, worker exposure led to symptoms consistent with acrylamide-induced effects on health. It is understood that in both instances the product was applied by the correct means and therefore the exposure data are interpreted as representative for normal use. From measurements taken in Sweden, all personal samples, except one, exceeded the Swedish occupational exposure limit of 0.03 mg/m³ but this is likely to be an over estimate as the first samples were quoted for both acrylamide and N-methyloacrylamide. This is because the analytical technique at the time did not allow for separate determination of acrylamide concentrations. The available data suggest that inhalation exposure even after injection ceased had the ability to exceed 0.012 mg/m^3 (8-hour TWA) acrylamide.

Small-scale use of acrylamide grouts and acrylamide grouting agents

Data are available from two surveys. The highest exposure value of 0.12 mg/m^3 (not 8-hour TWA) will be used in the risk characterisation.

4.1.1.1.9 Dermal exposure (general discussion)

Dermal exposure to acrylamide may occur where workers come into contact with surfaces contaminated by splashes or condensed vapour or as a result of direct splashes on to the skin. Workers at acrylamide manufacturing plants and polyacrylamide manufacturing plants are supplied with chemical gloves to reduce this exposure.

A UK manufacturer of both the monomer and polymer carried out dermal exposure measurements by requesting operators to wear cotton liners inside their chemical gloves. However, this was not done to assess an individual's dermal exposure. The company reported

that it had not yet validated the procedure and that the results should be used with caution because the cotton gloves may absorb acrylamide and it is possible that this is an overestimate of exposure. The results were, however, still used in this risk assessment as they provide a reasonable indication of dermal exposure. Results were reported as mg per glove. Dermal exposures were calculated from this, which showed exposure to be generally higher at the polymer plants. The results, however, showed a high degree of variability, therefore it is unlikely that there is a true difference between the plants. The highest results for the acrylamide and polymer production plants were 0.01 mg/cm^2/day and 0.08 mg/cm^2/day respectively.

The geometric and arithmetic means for the acrylamide plant were 0.002 mg/cm^2/day and 0.004 mg/cm^2/day respectively. The geometric and arithmetic means for the polymer plants were 0.0001-0.004 mg/cm^2/day and 0.0004-0.01 mg/cm^2/day respectively. These results are likely to represent exposure resulting from reasonably good practices. The highest results may result from situations such as operators wearing damaged or old gloves, or removing them before leaving the plant and therefore receiving exposure from contaminated hand rails, walls, doors etc.

The degree to which glove permeation occurs and the suitability of the gloves was not established. The type of glove worn at each EU plant was not established, although it appears that most companies do provide dermal protection. At the UK plant the operators are supplied with PVC gloves. The above results are considered to provide a reasonable estimate of dermal exposure during the manufacture of acrylamide and polyacrylamides in the EU.

Dermal exposure during the handling of acrylamide polymers is significantly lower than the above and is likely to be highest where operators handle the neat polymer. The EASE model and industry measurements predict this to be $1 \cdot 10^{-4}$-$1 \cdot 10^{-5}$ mg$\cdot$cm^{-2}/day. In most situations operators only handle diluted polymer and thus exposures are likely to be significantly lower than these values.

During the use of electrophoresis gels technicians wear gloves (usually disposable) to avoid contaminating the acrylamide gel. This is also a short-duration task, therefore exposure is likely to be minimal.

During large-scale use of acrylamide grouts in tunnels it is possible that excessive dermal exposure may occur when the product does not react as it should following application and acrylamide solution leaks out of the tunnel walls. Also, the excessive water in the tunnel and the awkward working positions that the workers have to deal with would mean there would be a greater potential for dermal exposure to occur as the water would run down workers' arms. There is no measured or quantitative information regarding dermal exposure and so the only information comes from reports given by workers.

For small-scale use of acrylamide grouts in sewers, dermal exposure of 5 mg/hour will be used in the risk characterisation. Additional dermal exposure from inside gloves was measured at 2.49 mg/glove, which would equate to 4.98 mg per working shift.

4.1.1.2 Consumer exposure

4.1.1.2.1 Introduction

Within the EU, 99% of the monomer produced is converted to the polymer before use. The small amount of monomer used directly is not relevant for consumer exposure. Although most monomer is converted to the polymer, it is the monomer itself and in particular the levels of

residual monomer in the polymer which are the important issue. Degradation of the polymer to produce acrylamide monomer is very unlikely (see Section 4.1.1.1.5). The only point of concern is therefore the level of the free monomer already present in the product. The residual level of monomer in the polyacrylamide is kept below 0.1% w/w in the EU and most values are much lower than this, due to the fact that within the EU any preparation containing more than 0.1% w/w acrylamide has to be classified as a Category 2 carcinogen.

The polymers are used in a variety of processes, many of which have no consumer application. There is a variation in usage across the continents. Although denture fixatives have been suspected of containing polyacrylamide, the UK manufacturers, who also export to other countries within the EU, have confirmed that none of these products are currently manufactured with polyacrylamide as an ingredient.

The uses of the polymer in Europe, where there may be relevance to consumer exposure, are detailed below.

4.1.1.2.2 Use of polyacrylamides in cosmetics

There are no measured data available on levels of polyacrylamide used in cosmetics and therefore calculated data have been used. The Cosmetic, Toiletry & Perfumery Association of the United Kingdom has surveyed its members and report that polyacrylamide is used in cosmetic preparations (rinse-off and non rinse-off skin products) at a level of up to 2%. This survey also showed that there is use for polyacrylamides in suntan lotions. A level of 2% polyacrylamide can be taken as being maximal for cosmetics. The usual specification calls for a maximum monomer level in the polymer of below 0.01% (Phillipson, 1996, personal communication). It can be assumed that the dermal route is the only route that needs to be addressed as inhalation is likely to be negligible and oral ingestion should not be relevant.

Model scenario for dermal exposure

Non-rinse skin products

The use of non-rinse products (these could include general purpose cream, body lotion, setting products and nail products) and rinse-off skin products will add to the total body burden of polyacrylamide monomer as calculated using use levels suggested by the TGD (1996). For general purpose creams it is assumed that a concentration of 1 mg/cm^2 could be used twice daily over the total body area of 19,400 cm^2 (TGD, 1996). This 19.4 g of cream, used twice and containing 2% of polymer which contains 0.01% monomer, would lead to a monomer exposure of 78 μg daily. In practice, it is assumed by some authorities that even extensive use will only produce an exposure to 2 g of cream daily because of the reduced area likely to be used (ECETOC, 1994). Clearly total skin cover, twice daily, is likely to be excessive but half of this quantity, resulting in exposure to 39 μg of monomer, can be taken as a reasonable worst-case scenario. In the same way the daily body lotion use could be 15 g (TGD, 1996) which converts to exposure to 30 μg of monomer. It is unlikely that both of these products would be used simultaneously so the value of 39 μg of acrylamide monomer will be used as the total daily exposure.

The setting product use (12 g) could mean exposure to 24 μg of monomer on the day of use and nail products (0.25 g) may add a further 0.5 μg of monomer exposure.

Total daily exposure to non-rinse skin products is thus calculated to be 65 μg.

Rinse-off skin products

Rinse-off skin products containing polyacrylamide could include shampoo (12 g daily) but it is assumed that only 10% of these products are left on the skin (TGD, 1996) so that the exposure to monomer, calculated as in the other scenarios described above, equates to 2.4 μg.

Totalling up these potential daily exposure produces 67 μg of the acrylamide monomer from non-rinse off and rinse off cosmetics. This figure represents a reasonable worst-case scenario for daily deposition of acrylamide on the skin of a frequent user of cosmetics.

The above exposure estimates are based on maximum monomer level in the polymer of 0.01%. However, very recently, the Scientific Committee on Cosmetic products and Non-Food Products intended for Consumers (SCCNFP) recommended revised levels of acrylamide in cosmetics. The recommended tolerable level for non-rinse products is <0.1ppm and for rinse-off products, <0.5ppm. These levels will result in exposures which are 1,000 and 200 fold lower, respectively than those calculated above for non-rinse and rinse-off products.

4.1.1.2.3 Use of polyacrylamide in gardening

Polyacrylamide gels are used in soil conditioners for consumer use. These packs contain 33% polyacrylamide gel and the instructions on the pack suggest a vigorous mixing of the pack contents with the soil or compost. Pack sizes for amateur use vary from 10 g to 150 g and the recommended concentrations are 1 to 1.75 g per litre of compost or 100 g per m^2 of soil. A few varieties of compost already contain low levels of added polyacrylamide but these are the exceptions. As there are no measured data on dermal exposure to acrylamide monomer in these procedures a calculation has been made to obtain this information.

If the residual acrylamide monomer level is 0.1% then, when a consumer mixes together 17.5 g of conditioner with 10 litres of compost, they are handling a maximum of 5.8 mg ($17.5 \cdot 0.33 \cdot 0.001$) of acrylamide monomer. Once wet this compost will contain a concentration of 5.8 mg/litre of acrylamide in the water (assuming that 10% of the compost is water, that it is perfectly mixed and that all of the monomer has dissolved in the water). Assuming a surface area of 820 cm^2 (area of the hands) and a liquid film thickness of 0.01 cm (EPA default) in contact with the hands there will be an exposure to about 0.8 ml of solution which will contain about 5 μg of acrylamide.

4.1.1.2.4 Paper and pulp products

Polyacrylamide is used in the pulp and paper production industry as a binder and as a retention aid for fibres. The USA, Germany and the Netherlands approve the use of polyacrylamides for indirect food additives as components of paper and paperboard. No measurements are available on the levels of residual acrylamide left on the paper but it is possible to calculate the maximum levels.

A 0.2% polymer working solution is typically used by the paper industry (Section 4.1.1.1.4.) and typical values for the polyacrylamide/dry paper ratio are 1.5 kg/tonne. As the maximum free acrylamide in the polyacrylamide is 0.1% w/w this means that there is a maximum of 1.5 g/tonne (1.5 ppm) of acrylamide in the dry paper. However polyacrylamide is added at the wet end of the paper making where solid pulp is present in water at about 1% w/w. Acrylamide is extremely

water-soluble (Section 1.3.6.) and any acrylamide present would remain in the aqueous phase which is largely removed, producing a paper with about 10% moisture content. This is a 100-fold dilution of any free acrylamide so that the absolute maximum level of acrylamide in paper is assumed to be 15 mg/tonne (15 ppb) - below the limit of detection.

This level of acrylamide assumes that there has been no degradation or reaction, which is unlikely given the reactivity of free acrylamide. Hence there would appear to be a negligible exposure to consumers in this application.

4.1.1.2.5 Coating applications

Polyacrylamides have been used as dispersants and bindings in coatings. Water-based paints containing 0.1-0.5% polyacrylamides have improved pigment suspension and flow. Since 1952 many patents have been granted describing the use of polyacrylamide resins in surface coatings and thermosetting acrylics. These resins are used as coatings in home appliances, building materials and car parts. However when polyacrylamides are used in coating resins they are in a reacted form, usually copolymerised with acrylates. Any free acrylamide in this reactive medium is likely to be at a very low level. Analysis has indicated that the residual monomer is present in a concentration below the detection limit of 0.01% (Wright, 1995). Hence there is likely to be negligible exposure to consumers.

4.1.1.2.6 Textiles

Polyacrylamides have been used in the past as sizing agents for wool. They have also been used to bind textile fibres and as water repellents. A survey carried out by The International Wool Secretariat has disclosed that polyacrylamide is not used as a sizing agent anywhere within the EU. Hence there is no known consumer exposure for these applications.

Overall consumer exposure to acrylamide

The relevant consumer exposures to acrylamide are from the use of cosmetics and as a conditioner for soils. The use of polyacrylamide in soil conditioning could produce a maximum sporadic exposure of 5 μg of acrylamide monomer every time the conditioner is used. The use of polyacrylamide in cosmetics may result in a heavy user of the cosmetics having a potential daily exposure to 67 μg of acrylamide monomer.

These values will be taken forward the risk characterisation.

4.1.1.3 Humans exposed via the environment

In calculating the indirect exposure of humans via the environment drinking water is assumed to be the only significant intake of acrylamide (exposure via food is expected to be negligible – see Section 3.1.7). The daily dose through intake of drinking water can be calculated from the maximum expected concentration of acrylamide in drinking water (0.125 μg/l), the daily intake of drinking water (2 l/day) and the average bodyweight of humans (70 kg) according to the equation:

$$DOSE = \frac{Concentration \cdot Intake}{Bodyweight}$$

For acrylamide this gives a human dose of 0.0036 μg/kg bodyweight/day.

In addition, the use of grouts for sewer repairs and construction may lead to levels of acrylamide in drinking water. It should be noted that these are local exposure scenarios.

- In Section 3.1.4.1.1 a $PEC_{local,water}$ of 3.9 μg/l is predicted for the use of acrylamide grouts for sewer repair applications. **Table 3.6** in Section 3.1.4.2 indicates a measured level of 400 mg/l acrylamide in drinking water arising from this use. However the data are old (1975) and probably do not reflect the current situation. If it were assumed that this water is used directly for drinking then the human dose of acrylamide would be 0.11 μg/kg bw/day, based on the calculated concentration.

- A number of measurements of acrylamide in surface water and groundwater have been made following recent tunnelling incidents in Scandinavia. A worst-case assumption would be that water from these sources is used for drinking water. The highest concentration of acrylamide in surface water following the tunnelling incident in Sweden was 92 mg/l in the Vadbäken creek. This would give a predicted exposure of 2.62 mg/kg bw/day. Ninety days after the incident the highest concentration measured was 0.1 mg/l, which would give a predicted exposure of 2.86 μg/kg bw/day.

  The highest groundwater concentration following the tunnelling incident in Sweden was 5.1 mg/l. This would give a predicted exposure of 0.15 mg/kg bw/day.

4.1.1.4 Combined exposure

The worst-case combined exposure would be to a person who works with acrylamide grouts in a small-scale operation, who uses consumer products containing acrylamide and is exposed via the drinking water. The total combined exposure to a person under these circumstances is dominated by the occupational exposure of 0.45 mg/kg/day. Therefore this will be the total combined exposure carried forward to the risk characterisation.

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

Inhalation

There are no data available.

Oral

Groups of male mice received a single oral dose of 116-121 mg/kg [^{14}C] acrylamide and were sacrificed 0.33, 1, 3, and 9 hours, and 1, 3, and 9 days post-administration (Marlowe et al., 1986). In addition, pregnant females received acrylamide on days 13.5 and 17.5 of gestation and were sacrificed at 3 and 24 hours post-administration. Sections for whole-body autoradiography were taken at each of the sacrifice times; quantitation of radioactivity intensity was by visual inspection.

As expected, radioactivity in males was highest in stomach and intestinal contents 0.33 hours post-administration. In addition, the epithelia of oral cavity and oesophagus, and liver and gall bladder exhibited radiolabel. To a lesser extent, labelling also occurred in bronchial epithelium, testis, and brain. At 1 hour, all of these tissues showed higher levels of radioactivity, with the exception of stomach and intestinal tract. At 3 hours, very little radioactivity was seen in the stomach. At this stage, radioactivity was still seen in intestinal contents. Also, high concentrations were still present in kidneys, testis, pancreas, and the lens of the eye. Within the brain, radioactivity appeared to be highest in the region of the cerebellar cortex.

At 9 hours, the pattern of distribution was essentially similar, except that some radioactivity was apparent once more in the stomach. The authors suggest that this may have indicated coprophagy. At 24hours, radioactivity levels had declined substantially in the liver, kidneys, and pancreas and most other organs with the exception of testis, and intestinal contents. Some portions of hair appeared to be labelled suggestive of contamination from urine and/or faeces during grooming activity.

Three days post-administration, distribution of radioactivity was uniform and low with the exception of the epididymis (lumen and wall of epididymal ducts). At 9 days, the only significant levels of radioactivity were seen in the reproductive tract (epithelium of glans penis). There was apparently no accumulation of radiolabel in the urinary bladder.

On the 13.5-day pregnant female mice radioactivity was uniformly distributed in both dams and foetuses at 3 and 24 hours. For 17.5-day pregnant mice, radioactivity in the foetus was concentrated in the kidney, bladder, liver, and intestinal contents. In addition, high levels of radioactivity were seen in foetal skin. In this study, no notable accumulation of radioactivity was seen in peripheral nerves of adults of foetuses at any time-point.

As part of a study examining potential strain differences in the tissue distribution and macromolecular binding of acrylamide using two different routes of exposure (oral and dermal), groups of 5 male SENCAR and BALB/c mice received a single oral dose of 100 mg/kg aqueous [2,3-^{14}C] acrylamide (Carlson and Weaver, 1985; Carlson et al., 1986). For the work on

distribution, animals were sacrificed 15 minutes, 30 minutes, 1, 6, 12, 24, and 48 hours after administration. For work on macromolecule binding (RNA, DNA, and protein), tissue samples were taken at 6 and 48 hours. Tissue samples were taken from the stomach, liver, testes, and skin. The types of adduct formed were not identified.

Following oral administration, radioactivity was found in all tissues that were examined from 15 minutes onwards, although the lowest levels were found in skin, and peak concentration in testes was not achieved until around 1-6 hours post-administration. Generally, peak values occurred at around 30 minutes to 1 hour in the other tissues that were examined and declined gradually over the 48-hour sampling period. There were no clear differences between strains.

In the macromolecule binding investigation, at 6 hours and 48 hours, the radiolabel was found bound to DNA, RNA, and protein in both strains and in all tissues examined. Similar amounts of radiolabel were bound to DNA, RNA, and protein for each of the tissues examined except skin where values were slightly lower and there were no obvious strain differences.

In an extensive study of metabolism, groups of 4 male F344 rats and 3 male B6C3F$_1$ mice received a single oral administration of 0 or 50 mg/kg aqueous [1, 2, 3-^{13}C] acrylamide (>99% pure) (Sumner et al., 1992; abstracted by Fennell et al., 1990). Urine samples were collected over a 24-hour period and analysed by nuclear magnetic resonance (NMR). Approximately 50% of the acrylamide administered was found in rat and mouse urine as metabolites or parent compound on completion of the 24-hour collection period. This result is in good agreement with other studies (Miller et al., 1982; Ramsey et al., 1984).

Urinary metabolites amongst acrylamide-exposed animals were identified as N-acetyl-S-(3-amino-3-oxopropyl) cysteine (the N-acetyl-cysteine conjugate of acrylamide, following glutathione conjugation accounting for 67% of the total urinary metabolites found in rats, 41% of the total found in mice), N-acetyl-S- (3-amino-2-hydroxy-3-oxopropyl) cysteine (16% in rats, 21% in mice), N-acetyl-S- (1-carbamoyl-2-hydroxyethyl) cysteine (9% in rats, 12% in mice), glycidamide (6% in rats, 17% in mice), 2,3-dihydroxy-propionamide (2% in rats, 5% in mice), and a small amount of the parent compound (which was not possible to quantify) (see **Figure 1** for proposed metabolic pathway).

In a briefly-reported study of metabolism and excretion, groups of male rats received single oral doses of 0, 25, 50, or 100 mg/kg acrylamide (Dixit et al., 1982). Urine samples were collected over a 24-hour period. Two major metabolites were identified in the urine of acrylamide-exposed animals - N-acetylcysteine-S-propionamide methyl ester and cysteine-S-propionamide methyl ester. The presence of these metabolites indicates that glutathione conjugation occurred and is in agreement with the results obtained by Sumner et al. (1992). In this study, the total amount of acrylamide excreted as thioethers, in the urine that was collected was about 7% of the administered dose. This value was somewhat lower than that reported by Ramsey et al. (1984) and Ikeda et al. (1987) although in the studies described below the collection period in this study was shorter. Also, it was unclear what precautions were taken to ensure complete collection.

Groups of 3 male rats received a single oral application of 1, 10, or 100 mg/kg aqueous [2,3-^{14}C] acrylamide (Miller et al., 1982). Urine and faeces were collected daily for up to 7 days. At each dose level 53-67% of the radiolabel administered was excreted within 24 hours, and by 7 days 65-82% had been eliminated. Approximately 74% was recovered in urine samples and about 8% in faeces. In addition, an intravenous study showed the major metabolite to be N-acetyl-S-(3-amino-3-oxypropyl) cysteine. This accounted for approximately 50% of the administered dose, approximately 2% was eliminated as the parent compound and the identity of other potential metabolites was not determined.

Groups of 4 male rats received repeated oral applications of 0.05 or 30 mg/kg aqueous [1,3-^{14}C] acrylamide once per day for up to 13 days (Ramsey et al., 1984). Levels of radioactivity were determined in total blood, erythrocytes (RBC), urine, brain, liver, kidneys, testes, epididymis, sciatic nerve, skin, and the remaining carcass at the end of the study.

Radiolabel was detected in all of the tissues sampled, although the highest levels were measured in the erythrocytes, liver, kidneys, testes and epididymides with particularly high values being recorded in erythrocytes. Apparently, there was no accumulation in the brain or sciatic nerve at either dose level. The concentration of ^{14}C in RBC reached peak levels on approximately day 4 or 5 and remained at a plateau for the rest of the administration period.

At both dose levels, approximately 60% of the radiolabel that was administered on each day was excreted on that same day. The major metabolite (accounting for ~70% of the total urinary ^{14}C activity) was identified as N-acetyl-S- (3-amino-3-oxopropyl) cysteine. Unchanged acrylamide accounted for less than 5% of the total urinary radioactivity. Other potential metabolites were not identified.

These results suggest persistence of acrylamide or its metabolites in RBC following repeated-exposure and contrast with the lower levels of erythrocyte radioactivity seen in the single-exposure study using dogs and pigs by Ikeda et al. (1987), summarised below.

Groups of 3-8 male dogs and 3-6 male miniature pigs received 1 mg/kg/day acrylamide administered in the diet for a period of 3-4 weeks followed by a single oral dose of 1 mg/kg aqueous [1-^{14}C] acrylamide (Ikeda et al., 1987). Dietary administration of non-radiolabelled acrylamide continued until sacrifice. Animals were sacrificed 6 hours, 1, 2, 4, and 14 days after administration of radiolabelled acrylamide. Urine and faeces samples were collected and a wide range of tissues analysed for the presence of radioactive material. The tissues analysed were blood, heart, lung, liver, spleen, GI tract, kidney, testes, skeletal muscle, bile and gall-bladder, brain, and fat. At the 6-hour time point only, the amount of radiolabel in a series of brain and spinal cord sections was determined in both pigs and dogs. In addition, evolved CO_2 was collected over a 2-day period from just one dog. In this study, no attempt was made to identify potential metabolites.

For both species, and in all tissues examined, the recovery of radiolabel was greatest 6 hours after administration and declined gradually over the 14-day observation period. In dogs, the greatest amount of radiolabel was recovered from skeletal muscle; approximately 35% of the administered dose was found in this tissue at 6 hours. Smaller amounts were found mainly in the liver, blood and GI tract (14%, 5%, and 5% respectively) and the total amount accounted for after 6 hours was about 64% of the administered dose. On day 2, 17% of the administered dose was found in muscle, with little (approximately 1%) being found in the GI tract. This result indicates that, for dogs, acrylamide is rapidly absorbed following oral administration. Acrylamide was found in all tissues sampled at all observation points (indicating wide distribution) except at 14 days where none was detected in the bile and gall bladder. Only small amounts (<1%) were found in brain or fat (measured at 6 hours only). The low amounts (<1%) of radiolabel found in the bile and gall bladder at all of the time-points investigated suggest that biliary excretion is not a major route of excretion of acrylamide or metabolites. On completion of 14 days, <1% of the administered dose was found in individual tissues, except muscle which still contained about 5%.

In pigs, again the greatest amount of radiolabel was recovered from skeletal muscle; approximately 32% of the administered dose was found in this tissue at 6 hours. At the same time-point, recovery was 20% from GI tract, and 5% from each of liver, fat, and blood, with

smaller amounts being found in other tissues. The total amount accounted for at 6 hours was about 71% of the administered dose. On day 2, a large amount of radiolabel (17%) was still found in the GI tract indicating that, in pigs, absorption was slower than that seen in dogs. acrylamide was found in all tissues sampled at all observation points (indicating wide distribution) except at 4 and 14 days where none was detected in the bile and gall bladder. Only small amounts (<1%) were found in brain, although for pigs, fat was one of the major sites of distribution. The low amounts (<1%) of radiolabel found in the bile and gall bladder suggest that biliary excretion is not a major route of excretion of acrylamide or metabolites. On completion of 14 days, <1% of the administered dose was found in individual tissues, except muscle which still contained approximately 7%.

The brain and spinal cord sections from both species taken at 6 hours did not indicate any areas in these tissues to which acrylamide may have been particularly distributed, although the incorporation in dogs was greater than that of pigs.

In terms of excretion products, determination of exhaled $^{14}CO_2$ from a single dog accounted for only 5% of the administered dose over 2 days. The urine was the major route of excretion of radiolabel accounting for approximately 60% of the administered dose for both species on completion of 14 days and the faeces accounted for a further 7-27%. Most of the radiolabel was in fact recovered from the urine during the first 2 days, with only very little extra being excreted over the next 12 days. The pattern of excretion from faeces was somewhat different: approximately 7% of the administered dose was accounted for after 14 days in beagles, about 27% after 14 days in pigs (although for pigs, most of this was accounted for in the first 4 days). The larger recovery of radiolabel from faeces and the GI tract in pigs again indicates that acrylamide is less well absorbed by the oral route than in dogs.

Summary of oral studies

Overall, the oral studies indicate that acrylamide is rapidly absorbed and widely distributed in all species that have been investigated (rats, mice, dogs, miniature pigs). Some accumulation in erythrocytes was noted following repeated administration. An autoradiography study in mice also showed accumulation of the acrylamide or its metabolites in the reproductive organs of males and rapid and extensive distribution to the developing foetus in pregnant females. Binding of acrylamide or metabolites to RNA, DNA and protein was seen to occur in a range of tissues. Studies in rats have shown that direct conjugation of acrylamide with glutathione is the major route of metabolism, with formation of the epoxide glycidamide (with the potential for subsequent conjugation with glutathione) also being apparent. Excretion of the parent compound and/or metabolites was rapid and extensive and mostly via the urine, with smaller amounts eliminated via the faeces and exhaled CO_2.

Dermal

As part of a study described earlier (see Oral section) groups of 5 male SENCAR and BALB/c mice received a single dermal application of 100 mg/kg [2,3-^{14}C] acrylamide in ethanol, presumably with an exposure period of up to 48 hours (Carlson and Weaver, 1985; Carlson et al., 1986). It was unclear whether or not acrylamide was applied under an occlusive dressing and whether or not any precautions were taken to prevent oral exposure. To investigate the distribution, animals were sacrificed 15 minutes, 30 minutes, 1, 6, 12, 24, and 48 hours after administration. For work on macromolecule binding (RNA, DNA, and protein), tissue samples were taken at 6 and 48 hours. Tissue samples were taken from the stomach, liver, testes, and skin

from the application site. Binding of radiolabel to DNA, RNA, and protein was assessed in each of these tissues.

High levels of radioactivity were found in all tissues including skin samples during the first hour post-administration. In liver, lung, and testes the levels reached a peak at 30-60 minutes. Levels of radioactivity amongst all tissues declined rapidly from 6 hours onwards. High levels were noted in the stomach of both strains with SENCAR mice showing higher values during the first hour only for all tissues except skin where BALB/c values were higher. After 48 hours, there were no clear differences between strains in each of the tissues examined.

At 6 hours and 48 hours, radiolabel was found bound to DNA, RNA, and protein in both strains in all tissues examined. Six and 48 hours after application, the liver showed the highest level of binding to DNA and RNA. At both time-points, the amount of label bound to protein was highest in skin samples. Six hours after administration, the amount of protein binding was less in BALB/c mice than SENCAR mice. There were no other clear differences between strains at this time-point or at 48 hours.

When comparing the oral and dermal routes of administration, higher levels of radioactivity were seen in each tissue and at each time point when using the oral route. The obvious exception was that higher levels of radioactivity were found in DNA, RNA and protein taken from skin following dermal application.

Groups of 3 rats received a single dermal application of 2 or 50 mg/kg aqueous [1,3-^{14}C] acrylamide, presumably with an exposure period of up to 48 hours (Ramsey et al., 1984). It was unclear whether or not acrylamide was applied under an occlusive dressing and whether or not any precautions were taken to prevent oral exposure. Levels of radioactivity were determined in total blood only. Approximately 25% of the dose applied was absorbed during the first 24 hours. The clearance of radiolabel from blood was apparently biphasic, with a half-life of about 2 hours for the first phase. Gas-chromatography analysis of blood plasma samples demonstrated that elimination was predominantly of the parent compound. The second phase appeared to be due to the clearance of radiolabelled metabolites and had a half-life of about 10 hours.

Groups of male mice received a single dermal application of 0 or 100 mg/kg acrylamide in acetone, presumably in contact with the skin for up to 4 hours (Mukhtar et al., 1981). It was unclear whether or not acrylamide was applied under an occlusive dressing and whether or not any precautions were taken to prevent oral exposure. Skin and liver samples were taken at 2 and 4 hours post-administration and assayed for levels of glutathione (GSH), and the activity of GSH-transferase, and aryl hydroxylase enzymes. Decreases in all these parameters compared to control values were noted in skin and liver at both time points, with the effect being more pronounced at 4 hours. For example, 60% and 80% decreases in glutathione levels were noted at 4 hours in skin and liver respectively. The one exception to this trend was liver GSH-transferase activity, which was apparently unaffected 2 hours post-administration, but was decreased at 4 hours.

The *in vitro* dermal absorption of ^{14}C-radiolabelled residual acrylamide monomer within three different 1% aqueous polyacrylamide solutions (estimated to contain 410-1,333 ppm monomer) was assessed using skin samples from 5 male F344 rats (Frantz et al., 1986; 1985). The study demonstrated that residual monomer from polyacrylamide solutions was well absorbed across rat skin *in vitro.*

Overall, the dermal studies that were available indicated that acrylamide was rapidly and extensively absorbed in rats and mice. Acrylamide or its metabolites bind to RNA, DNA, and protein in a range of tissues. Evidence was again acquired that glutathione conjugation has a

major role in acrylamide metabolism. The pattern of excretion of acrylamide and metabolites following dermal exposure has not been assessed.

Other

Although the following studies involved exposure routes that are not directly relevant to normal human exposure, they provide some further information on the metabolism and excretion of acrylamide:

Analysis of blood samples from rats treated intraperitoneally with acrylamide indicated that the glycidamide metabolite is able to form an adduct with cysteine residues in haemoglobin (Calleman et al., 1990; Bergmark et al., 1991).

Nine mice received a single intraperitoneal dose of 0.2 mg/kg [1,2-^{14}C] acrylamide (Carrington et al., 1991). Cytoskeletal, cytosolic and Triton X-100 soluble proteins were extracted from brain and spinal cord and radiolabelled proteins were analysed by SDS-PAGE/autoradiography. Radiolabel was associated with a large number of proteins from both tissue preparations, although particularly with medium weight (130 kD) and high molecular weight (180 kD) neurofilament proteins and lower molecular weight microtubule associated proteins (MAPs). Samples were taken at 1, 3 and 7 days post-administration, and over this time period there was a marked decrease in radiolabel binding particularly at day 3, although for the lower molecular weight MAPs binding was slightly more persistent.

This study demonstrates that at a relatively low dose of acrylamide, administered to mice by the intraperitoneal route, acrylamide or metabolites were bound to a wide range of proteins, and that there appeared to be a particular affinity for microtubule-associated proteins.

Groups of 6 male mice received a single intraperitoneal injection of [1-^{14}C] acrylamide (Sega et al., 1989). Animals were sacrificed at 4 hours, and then daily for 23 days post-administration and the epididymides and vasa deferentia were removed. DNA binding by acrylamide was measured by DNA purification and radioactivity quantification, and acrylamide-protamine binding was measured in terms of s-carboxyethyl cysteine adduct formation. The amount of radiolabel bound to sperm reached a peak at around day 9 in vasa deferentia and was a day or two earlier in the caudal epididymides.

It was estimated that the alkylation (measured in terms of formation of S-carboxyethylcysteine from cysteine groups) of DNA in sperm accounted for only about 0.5% of the total sperm head alkylation. Most of the sperm head binding of acrylamide appeared to be associated with cysteine residues in the protein, protamine.

4.1.2.1.2 DNA alkylation

Studies in extracellular systems

Acrylamide (>99% pure) was incubated in a phosphate-buffered solution at pH 7 with calf thymus DNA for 40 days at 37°C (Solomon et al., 1985). The reaction products obtained indicated that alkylation of DNA had occurred leading to the production of small quantities of a variety of different adducts with deoxyguanosine, deoxyadenosine (the major site of alkylation in this system), and deoxycytidine. Adducts with deoxythymidine were not detected.

Studies *in vivo*

Information was available indicating that DNA alkylation occurs in the liver and, to a much lesser extent, in testes following a single ip. injection of 46 mg/kg acrylamide (see Sega and Generosol, 1990, Section 4.1.2.7 and Sega et al., 1989, Toxicokinetics Section).

4.1.2.1.3 Studies in humans

Very little information is available on the toxicokinetics of acrylamide in humans.

Blood samples were obtained from a group of 41 workers occupationally exposed to acrylamide at a factory in China (Bergmark et al., 1993) and haemoglobin was extracted for analysis of acrylamide and glycidamide adducts. Workers were potentially exposed to acrylamide by inhalation and dermal routes - air concentrations ranged from 0.11-8.8 mg/m^3 (8-hour TWA) with an occasional peak value of up to 153 mg/m^3; skin peeling was observed on the hands indicative of significant dermal exposure.

The following valine adducts were released by acid hydrolysis of haemoglobin: N-(2-carboxyethyl)valine and N-(2-carboxy-2-hydroxyethyl)valine. The latter being indicative of epoxide formation and is consistent with earlier work in rats which also indicated the formation of glycidamide following acrylamide exposure (see Calleman et al., 1990 and Bergmark et al., 1991). A group of 10 control workers was also used in this study; a very low level of N-(2-carboxyethyl)valine was found in the blood sample of one of these individuals, who was a smoker (0.01 nmol/g haemoglobin, compared to 0.3-34 nmol/g in acrylamide-exposed workers).

An abstract of a case report (Donovan and Pearson, 1987 - see Acute Toxicity Section) reported severe signs of systemic toxicity despite attempts to empty the stomach contents within approximately 3 hours of deliberate oral ingestion of acrylamide. This would indicate the potential for rapid and extensive absorption of acrylamide by the oral route.

4.1.2.1.4 Summary of toxicokinetics

There are few data available on the toxicokinetics of acrylamide in humans. From one limited study that is available, inhalation and/or dermal absorption of acrylamide was indicated by the presence of acrylamide-associated haemoglobin adducts. Another case report indicated rapid and extensive absorption of acrylamide by the oral route. Furthermore, the neurotoxic effects of acrylamide seen in humans demonstrate uptake of the substance and distribution of acrylamide or its metabolites occurred to either skeletal muscle or nerves associated with the affected muscles.

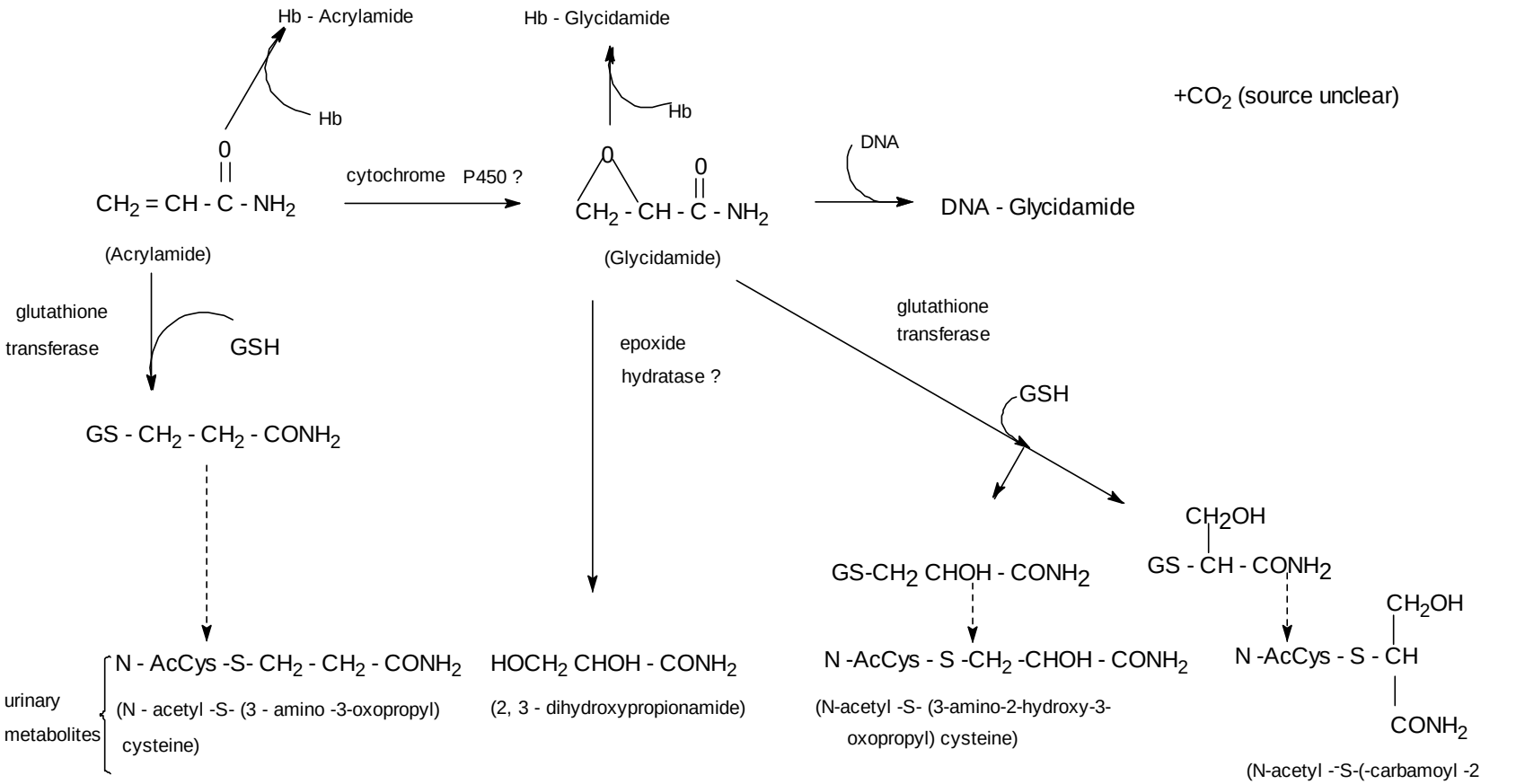
These limited observations in humans are supported by the information obtained in animal studies. Acrylamide is rapidly and extensively absorbed by the oral and dermal routes in a number of animal species, and either the parent molecule or metabolites appear to be widely distributed. Although no data are directly available, similar consequences following inhalation exposure would be expected. Whole-body autoradiography has demonstrated that distribution to male reproductive organs occurs and that acrylamide and/or metabolites readily cross the placenta with subsequent widespread distribution within the developing foetus. Acrylamide or metabolites have been shown to bind to DNA, RNA, and protein and has been shown to alkylate

a DNA-associated protein in developing spermatids, although there was a low level of binding to DNA itself.

Metabolites of acrylamide identified in rats and mice suggest that major routes of metabolism are via direct conjugation with glutathione, and the formation, presumably via cytochrome P450 oxidation, of glycidamide, an epoxide intermediate. Evidence for epoxide formation was also obtained from samples of haemoglobin taken from acrylamide-exposed humans. This epoxide can also undergo subsequent conjugation with glutathione.

Excretion in rats and mice is rapid and occurs mainly via the urine; smaller amounts are excreted via the faeces, and some acrylamide is metabolised to form CO_2, which is exhaled.

Figure 1 Proposed metabolic pathway



Adapted from IARC (1994)

Hb = Haemoglobin
AcCys = Acetyl cysteine

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

Inhalation

A study was described in which 6 male rats were exposed (nose only) to aerosolised 51% aqueous acrylamide solution for 1 hour (Keeler et al., 1975). A group of 5 males were used as controls. The exposure concentration achieved was 12.1 mg/l (equivalent to approximately 6 mg/l acrylamide), the mean particle size was 1.95μm and 99% of droplets were <6μm. Animals were observed for 14 days post exposure.

One acrylamide-exposed animal died 24 hours post exposure and was examined for macroscopic pathological abnormalities. Although full details were not available, it was reported that there were no abnormalities seen at this examination. There were no exposure-related mortalities. Amongst surviving animals, there were no adverse effects on bodyweight gain, and no clinical signs of toxicity were observed during exposure or in the 2-week observation period.

Acrylamide exhibited little toxicity in this study. However, significant absorption of acrylamide would be expected via the inhalation route (as with oral and dermal routes). On this basis, approximately comparable toxicity from acrylamide could be anticipated via all three routes of exposure (oral, dermal, and inhalation).

Oral

Groups of 10 male F344 rats received single doses of 50, 100, 125, 200, or 250 mg/kg aqueous acrylamide (Tilson and Cabe, 1979). An additional 20 male rats were used as controls. The rats receiving 0, 50, 100, and 200 mg/kg were also tested at 12 hours and 7 days post-dose to assess changes in forelimb grip strength, hindlimb motor function, and overall muscular strength and coordination.

There were no mortalities at exposures up to and including 125 mg/kg. At 24 hours post-dose there were 3/10 and 7/10 mortalities at 200 and 250 mg/kg respectively. On completion of 7 days there were 8/10 mortalities and 10/10 at these 2 exposure levels. The median lethal dose (LD_{50}) was calculated to be 175 mg/kg at 7 days.

Clinical signs of toxicity at 12 hours included postural and motor incoordination, hindlimb muscular dysfunction, hyperreflexia, recurrent episodes of tonic-clonic convulsions, and tremor particularly at 250 mg/kg. Details were not provided of the incidence and severity of these findings at any other exposure level. In addition, amongst high dose animals only, diarrhoea and increased urination were reported. At 12 hours post-dose the inclined screen test demonstrated increased motor dysfunction at 100 and 200 mg/kg. Decreased forelimb and hindlimb strength was noted at 200 mg/kg 12 hours after dosing, although only the hindlimb effects attained statistical significance. At 7 days, there was no significant difference in the performance of animals at 0, 50, and 100 mg/kg. The consequences of the 200 mg/kg dose could not be interpreted as there were insufficient survivors.

In a briefly reported investigation, the LD_{50} for female Porton rats was calculated to be 203 mg/kg (Fullerton and Barnes, 1966). The number of rats used was not stated. Macroscopic pathological examination was performed although the extent was unclear. Microscopic examination included kidney, spleen, pancreas, adrenals, lungs, liver, brain and bladder.

At exposure concentrations at or around 203 mg/kg, rats were reported to have a fine tremor, lasting approximately 48 hours. Animals either recovered completely or died within 3 days. There were no macroscopic lesions reported at necropsy. The only microscopic abnormality noted was fine fatty accumulation in the liver. No other abnormalities were reported.

As part a study investigating the effects of repeated-exposure, groups of 4 mice received a single dose of acrylamide in a saline solution by the oral route (Hashimoto et al., 1981). An LD_{50} value of 107 mg/kg was obtained. No further details were available.

Groups of 5 rats, 4 guinea pigs, and 4 rabbits received single oral doses of 126 or 252 mg/kg ACR (>99% purity) (McCollister et al., 1964). In addition, one group of 4 rabbits received a single oral dose of 63 mg/kg.

All animals receiving 252 mg/kg died within 24 hours and at 126 mg/kg one rabbit died. Other signs of toxicity at this exposure level were weight loss (not quantified) amongst the rats and guinea pigs and lethargy amongst rats only. Tremors and pupil dilation were observed in the rabbits. For the rabbits receiving 63 mg/kg an unquantified weight loss was again reported. It was concluded that, for each species, the LD_{50} was in the range 150-180 mg/kg. No further information was provided.

Groups of 30-day old (prepubertal) and 60-day old ("adult") male mice received a single oral dose of 0, 100, or 150 mg/kg acrylamide (Sakamoto et al., 1988). The dosing vehicle and the number of mice used was not described. From 1-10 days post-administration, surviving mice were sacrificed and a testis and epididymides removed for macroscopic and microscopic examination.

All animals at 100 mg/kg survived, but 50% of prepubertal mice, and 65% of adult mice at 150 mg/kg died during the 10-day observation period. Testicular weight was apparently unaffected. There were no histopathological abnormalities noted in the various stages of spermatogenesis in controls. However, for acrylamide-exposed mice, severe lesions were reported 1 day after administration of 100 or 150 mg/kg. In adult and prepubertal mice receiving 150 mg/kg acrylamide, the nuclei of most spermatids were vacuolated on day 1 post-administration although spermatogonia and early pachytene spermatocytes were unaffected. On day 2, damage was more pronounced, with pyknotic spermatids seen. After this time-point the damage in spermatids gradually declined. It was apparent that the early and mid-phase spermatids appeared to be more sensitive than later spermatids. Spermatogonia, spermatocytes, Sertoli, and Leydig cells appeared to be unaffected. Recovery was noted from day 7-10 post-administration. Similar, but less severe findings were noted in animals receiving 100 mg/kg.

Dermal

Groups of 2 male and 2 female rabbits received 102, 405, 806, and 1,612 mg/kg aqueous acrylamide (purity not stated) applied to the shaven skin of for 24 hours under an occlusive dressing (Keeler et al., 1975).

Three out of 4 animals at 1,612 mg/kg died, and 1/4 at 806 mg/kg died. There were no mortalities in other treated groups. The median lethal dose (LD_{50}) was calculated to be equivalent to 1,148 mg/kg. At the two highest exposure levels tremors and incoordination of hindlimbs were noted, and, in addition, the surviving female at 1,612 mg/kg was in poor condition and lost weight. All other animals showed some bodyweight gain at the end of the observation period. No signs of systemic toxicity were seen at 102 and 405 mg/kg. At the site of application, slight to moderate swelling, and slight to moderate erythema was seen in all exposed animals. At 806 and 1,612 mg/kg the erythema developed into white skin or white skin with red patches.

In a study of dermal absorption, 12.5% aqueous solution of acrylamide (>99% purity) was applied using an occlusive technique to the shaven skin of two rabbits at 63, 126, 500, and 1,000 mg/kg (McCollister et al., 1964). In addition, four rabbits received 252 mg/kg. One rabbit at 1,000 mg/kg died within 2 days. Weight loss (unquantified) and erythema (severity unknown) were reported at 500 mg/kg. No other information was available.

In a brief report, an unknown number of rats were administered 300, 400, 500, 600, or 700 mg/kg acrylamide (purity not stated) in a mixture of sunflower oil and water applied to skin for a 4-hour period (Novikova, 1979). The lowest lethal dose was stated to be 400 mg/kg, which the authors also reported as a "provisional median lethal dose". No further details were available for animals treated by this route although additional information was presented using tail immersion as a route of exposure; these other studies are not considered to be providing any useful information due to the unconventional exposure regime.

4.1.2.2.2 Studies in humans

An abstract is available of a case report relating to the deliberate ingestion of ~18g acrylamide crystals by a 48 kg woman (a single dose of approximately 375 mg/kg) (Donovan and Pearson, 1987). There were no clinical signs of toxicity on arrival at hospital or in the next 2½ hours. However, despite attempts to empty the contents of the stomach, approximately 5 hours after the ingestion of acrylamide hallucinations and hypotension were reported. Subsequently, 9 hours after ingestion, seizures were reported. This was followed by gastrointestinal bleeding, respiratory distress, and undefined symptoms of peripheral neuropathy and "hepatotoxicity" three days after ingestion. Peripheral neuropathy was still present 2 months later.

Although it is difficult to draw firm conclusions from an isolated case report, the observations and the approximate dose stated to have been involved, are consistent with the results obtained in animal studies.

4.1.2.2.3 Summary of acute toxicity

For humans, only limited data are available. However there is an abstract of a case report relating to the deliberate ingestion of acrylamide. The findings and approximate dose level thought to be involved are consistent with information from animal studies. Acrylamide is toxic by the oral route of administration (with LD_{50} values in the range of 107-203 mg/kg in rats), harmful by the dermal route (LD_{50} of about 1,150 mg/kg in rabbits), and, with the presumption that it would also be well absorbed by the inhalation route, could be presumed to be harmful by the inhalation route. The principal effect prior to death is neurotoxicity (manifested by severe clinical effects) although severe effects on spermatid development were also noted in mice. For classification, see Chapter 1.

4.1.2.3 Irritation

4.1.2.3.1 Skin

Studies in animals

In a recent unpublished study conducted according to modern protocol standards 0.5g acrylamide moistened with water was applied to the shaved, intact skin of a group of 3 New Zealand White rabbits under a semi-occlusive dressing for 4 hours (Mercier, 1997a). Skin reactions were scored at 24, 48, and 72 hours post-application and graded according to the EU scheme.

There were no mortalities, and there was no indication of whether or not there were any clinical signs of toxicity. There was no erythema or oedema at any of the time-points when reactions were scored.

Similarly, in a study conducted to the same standards, there were no signs of skin irritation using 0.5ml 50% aqueous acrylamide (Mercier, 1997b).

A study was described in which an unknown volume of 51% aqueous acrylamide was applied to the shaven skin of 6 rabbits under a semi-occlusive dressing for 4 hours (Keeler et al., 1975). Skin reactions were scored at 24 and 72 hours post-application. Slight reactions were reported, although individual scores were not presented.

The same report also briefly describes a study in which 0.5 ml of 51% aqueous acrylamide was applied once per day for 10 days under a semi-occlusive dressing to the shaven abdominal skin of 3 rabbits (Keeler et al., 1975). Local skin reactions were scored at 24, 48, and 72 hours. The mean score for erythema was 1.4 (maximum, 2); for oedema the mean (and maximum) score was 1.0.

A 10% aqueous solution of acrylamide was applied 5 days/week for 2 weeks under an occlusive dressing to the shaved skin of one rabbit (McCollister et al., 1964). No significant responses were recorded.

In another dermal study by the same authors (see Acute toxicity, Section 4.2.2) the application of approximately 17 ml of a 12.5% solution to the shaved skin of a rabbit caused erythema of unknown severity.

Studies in humans

Case reports and workplace surveys have demonstrated skin effects usually attributable to occupational exposure to aqueous solutions of acryalmide. It is unclear whether or not the effects seen were due to a primary irritant inflammatory response, to local cytotoxicity, and/or sensitisation. However, for convenience, the effects are reported in this section.

A recent study of workers occupationally exposed to solutions containing approximately 30% aqueous acrylamide monomer in the manufacture of monomeric and polymeric acrylamide reported skin effects as well as some potential signs of neurotoxicity (He et al., 1989, see also Repeated dose toxicity Section, 4.1.2.6). Results of a questionnaire indicated skin peeling from the hands in 38/71 (54%) workers, and a clinical examination showed this finding in 16/71 (23%). This compared with a control group in which the incidence was 2/51 (4%). Erythema was recorded in 16/71 (23%) of workers compared with none in the control group.

A study of occupational exposure to acrylamide was available (McHugh, 1987). Three out of the five workers reported some skin irritation effects that were attributed to acrylamide exposure during sewer grouting operations (the physical form of acrylamide was unclear). Two reported peeling skin on the palms of his hands and another noted an "acne-like" dermatitis on the face and hands following occupational use of acrylamide.

For all of the following case reports it is unclear to what extent findings may be due to dermal exposure to acrylamide. Some reported the use of gloves, although it is not clear if these adequately protected the individual.

A case report described skin rash on the forearms (in addition to a number of other health effects) in a worker occupationally exposed to monomeric acrylamide (Auld and Bedwell, 1967). The individual was regularly exposed to 10% aqueous acrylamide solution, and a polymerising catalyst, ß-dimethyl-amino-proprionitrile. The skin effects were attributed to acrylamide.

Another case report described (as well as a number of other health effects) moist, red raised ulcerations on the palms and soles of an individual occupationally exposed to monomeric acrylamide powder (Davenport et al., 1976). It is unclear if the effects on feet were as a result of direct contact with acrylamide.

A report described 6 individuals with neurological findings attributed to occupational exposure to acrylamide (Garland and Patterson, 1967 - see also Repeated dose toxicity, Section 4.1.2.6). Three out of the six individuals reported some skin effects. The first person was reported to have hands that were excessively moist, and layers of skin peeling off within one month of first starting work with acrylamide. The second person had layers of skin peeling off within 3 months of first starting work with acrylamide. After a recovery period of 2 weeks under hospital supervision, the individual started back at work. Some months later there were no abnormalities reported. It is unclear whether or not suitable dermal protection was adopted following the return to work. The third individual also reported layers of skin peeling off and excessively moist hands. No further details were available. Another report described 6 individuals with neurological findings attributed to occupational exposure to acrylamide monomer (Kesson et al., 1977). All 6 were also observed to have peeling skin.

4.1.2.3.2 Eye

Studies in animals

In a recent unpublished study conducted according to modern protocol standards 82 mg powdered acrylamide (equivalent to a volume of about 0.1ml) was applied to one eye of each of 3 New Zealand White rabbits (Mercier, 1997c). Reactions were scored at 24, 48, and 72 hours and up to 21 days post-application with grading according to the EU scheme.

There were no mortalities, and there was no indication of whether or not there were any clinical signs of toxicity. For iridial reactions the mean score for each animal over 24, 48 and 72 hours was 1.0; corneal opacity means ranged from 2.0-2.3; conjunctival redness 2.0 and chemosis 1.3-2.0. Iridial reactions were still apparent at day 14 post-application although there were no abnormalities noted at day 21. Overall, these results indicate that acrylamide is an eye irritant.

A similar study was conducted to the same standards using 0.1ml 50% aqueous acrylamide (Mercier, 1997d). Signs of eye irritation were observed, not surprisingly of a lesser intensity than using 100% acrylamide, and were reversible by day 7.

A report briefly describes a study in which 0.1ml 51% aqueous acrylamide was applied to the eyes of each of 3 rabbits (Keeler et al., 1975). Signs of eye irritation were observed that were not inconsistent with the more recent studies by Mercier.

Another brief report indicates some signs of eye irritation due to 'small amounts' of 10% or 40% acrylamide but limited reporting details and the use of a non-standard protocol make it difficult to draw firm conclusions (McCollister et al., 1964).

Studies in humans

No useful data are available on the eye irritation potential of acrylamide in humans.

4.1.2.3.3 Summary of irritation

Based on human experience, it appears that acrylamide is a skin irritant, with skin peeling being a particular problem. There are no adequate data relating to human ocular exposure, but information from animal studies suggests that acrylamide should be considered as an eye irritant. For classification, see Chapter 1.

4.1.2.4 Corrosivity

There are no data available to suggest that acrylamide is corrosive.

4.1.2.5 Sensitisation

4.1.2.5.1 Skin

Studies in animals

Positive results were reported in two recent guinea pig maximisation tests, conducted according to modern protocol standards (Allan, 1995*;* Stockhausen GmbH, 1995). Groups of 20 test and 10 control animals received dermal challenge concentrations of up to 25% aqueous acrylamide following induction of the test animals with up to 50% topically and up to 3.5% or intradermally. In the test by Allan (1995), skin reactions were observed in most control animals, but were less severe than reactions in test animals. Taking the control reactions into consideration, a positive skin response, exceeding the degree of reaction seen amongst the controls, was recorded in 40% of the test animals. In the test by Stockhausen GmbH (1995), there were apparently no skin reactions in control animals but 85% of test animals gave a positive response. On the basis of these results, acrylamide should be considered as a skin sensitiser in animals.

Studies in humans

A case report briefly described an individual who developed “itchy, exudative” lesions on the hands and wrists within 6 months of working with the production of polyacrylamide gels (Lambert et al., 1988). This was in spite of wearing gloves, although it is unclear to what extent these may have prevented dermal exposure to acrylamide. Similar lesions had been observed 10 years previously when working with the same materials.

Patch-testing with the International Contact Dermatitis Research Group (ICDRG) standard series and “acrylic and methacrylic allergens” (not further defined) was negative. Patch-testing with 1% acrylamide in petrolatum produced a positive skin reaction after 48 hours, which remained evident for 6 days. Although of questionable ethics, patch testing of 20 control subjects with 1% acrylamide was negative.

Another case report briefly described an individual who, after 4 months of working with the production of polyacrylamide gels developed eczema (Dooms-Goossens et al., 1991). The subject was also exposed to a number of “irritants” (unidentified), NN'-methylenebis-acrylamide, as well as acrylamide and polyacrylamide. As with the report described above, this individual wore gloves, although it was apparent from this report that the latex gloves used did not provide a barrier against acrylamide. Again, there was an improvement following a change of work.

Patch-testing with the “European standard series”, and a series of undefined acrylic and methacrylic allergens was negative. An open test with a solution containing 30% acrylamide, and 0.8% NN'-methylenebis-acrylamide was positive at 2 and 4 days. Patch testing with 5% acrylamide in petrolatum was positive at 2 and 4 days in this individual, but was negative in 20 control subjects.

4.1.2.5.2 Respiratory sensitisation

There are no data available. However, there is no evidence for respiratory sensitisation in humans, despite the widespread use of acrylamide. Therefore it is considered that respiratory sensitisation is of low concern.

4.1.2.5.3 Summary of sensitisation

Animal data provide clear evidence for the skin sensitisation potential of acrylamide; these data are supported by evidence of skin sensitisation from two individual case reports of workers occupationally exposed to materials containing acrylamide. There are no data available regarding respiratory sensitisation. For classification, see Chapter 1.

4.1.2.6 Repeated-dose toxicity

4.1.2.6.1 Studies in animals

Inhalation

There are no data available.

Oral

A large number of animal studies are available, with investigations performed in a variety of different species. Because of the well-recognised toxic properties of acrylamide, each one of these has paid particular attention to effects associated with neurotoxicological parameters. The following studies that have been reviewed are those with routes of administration corresponding to those to which humans are exposed.

Studies in rodents

- Rats

In a comprehensive study, groups of 10 male and 10 female F344 rats received 0, 0.05, 0.2, 1, 5, and 20 mg/kg/day acrylamide (>99% purity) in drinking water for 90 days (Burek et al., 1980). There were an additional 10 males per group used for a 144-day recovery period and a further 6-9 males for interim sacrifices and electron microscopy during the 90-day exposure period. Investigations conducted weekly included: recording of bodyweight, clinical signs of toxicity, tests for peripheral neuropathy (measuring foot-splay after being dropped onto a horizontal surface from a low height), water consumption. In addition, haematology was performed on day 76, at termination, and on day 60 of the recovery period for those not sacrificed after 90 days of acrylamide exposure. Urinalysis was performed on day 76 and on completion of the exposure period. Extensive macro- and microscopic pathology examinations were performed on 59 males and 60 females after 92-93 days of acrylamide exposure and on 4 males per exposure group after 144 days of recovery. Electron microscopy was performed on males during the 90 days of acrylamide exposure (on days 7, 33, and 90) and during the 144-day recovery period (days 25, 111, and 144 of recovery).

One male at 20 mg/kg/day was found dead on day 87. There were no other mortalities and the results of pathology examinations for this animal were incorporated with those sacrificed after 90 days. Reduced bodyweight gain was noted only at 20 mg/kg/day (21% reduction in males and 24% reduction in females at 90 days). Amongst those animals that were examined (males only), bodyweight was restored by day 141 of the recovery period. Significantly reduced water consumption was noted amongst females at 20 mg/kg/day (up to 40% reduction).

Statistically significant increases in landing foot-spread measurements were observed amongst males and females at 20 mg/kg/day on day 22 and effects were more pronounced at day 29 such that this test was discontinued to prevent injury. Other clinical signs of toxicity included curling of toes, splayed hindlimbs, incoordination, and hindlimb weakness. At the end of 90 days there was a loss of use of hindlimbs. The landing foot spread test was performed on males and females at 5 mg/kg/day on day 29 revealed no abnormalities.

A landing foot spread test was also conducted on day 12 of the recovery period for control males and males that had received 5 mg/kg/day. There were no abnormalities seen in this test and there were no clinical signs of toxicity at this or lower exposure levels. Males that had received 20 mg/kg/day were not subject to the landing foot spread test during the recovery period, but by day 7 of the recovery period some were able to use their hind limbs. At day 111 of the recovery period, curling of the toes was still apparent, and there were still some signs of posterior weakness. By day 144 there were no behavioural abnormalities.

Given the nature of other effects noted in this study, there were no remarkable results obtained from blood biochemistry examinations. There were no changes observed in the urinalysis parameters recorded.

Haematology examinations on day 76 and at termination showed decreased PCV, RBC, and haemoglobin (Hgb) values amongst males and females at 20 mg/kg/day. Significant decreases in these parameters were also observed at termination amongst females at 5 mg/kg/day. Haematology performed on day 4 of the recovery period still showed a reduction in PCV, RBC, and Hgb values amongst males that had received 20 mg/kg/day. By day 60 of the recovery period a slight, but statistically significant, reduction in RBC was still observed; other values had returned to normal. There were no further haematology examinations. As with the blood biochemistry examinations, the magnitude of effects was not reported.

Increased organ weights (relative to bodyweight) were observed in males and females at 20 mg/kg/day for brain, heart, liver, and kidneys (magnitude not given). Also at this exposure level decreased relative thymus weight was observed in females, and reduced testes weights were observed in males. Increased relative liver weight was observed amongst males at 5 mg/kg/day. On day 144 of the recovery period, brain, kidney, and liver weights were still increased amongst males that had received 20 mg/kg/day.

Macroscopic examination after 90 days showed the following changes amongst males and females at 20 mg/kg/day: urogenital fur staining, decreased adipose tissue, small liver (due to reduced bodyweight), dark kidneys, foci or areas of mottled appearance in the lungs, small or flaccid testes, and small accessory genitalia in males, small uterus in females, dull appearance or loss of striated appearance of peripheral nerves, atrophy of skeletal muscle in the posterior portion of the body, distension of the bladder, and diffuse mural thickening of the stomach. There were no significant macroscopic pathology observations at lower dose levels.

Macroscopic pathology examinations performed on 4 male rats from each group after 144 days of recovery showed lesions only amongst those rats that had received 20 mg/kg/day: these were dark testes in 3/4 males and slightly distended urinary bladder in all four.

Histopathology of peripheral nerves after 90 days showed axon and myelin degeneration: both enlarged and unusually small axons were observed, others were fragmented or broken, or absent. Myelin degeneration was prominent and observed as clumping of myelin, myelin debris, vacuolization, or absence of myelin. There also appeared to be increased interstitial space between individual nerve fibres. These peripheral nerve lesions were seen to a marked extent in all animals at 20 mg/kg/day. Peripheral nerve lesions were also observed in most animals at 5 mg/kg/day but varied in severity from equivocal to very slight (focal or multifocal changes in individual nerves) in 9/10 males and 6/10 females. Spinal cord sections were taken from the cervical, thoracic, and lumbrosacral regions. Equivocal to slight degenerative myelopathy (demyelinization, swollen astrocytes, and swollen axons) was seen in the dorsomedial funiculi of one or all spinal cord sections in 5/10 males and 9/10 females at 20 mg/kg/day only. Transverse sections through the cerebrum, cerebellum, and midbrain did not reveal any abnormalities amongst those animals examined (control and high dose levels).

The other major pathology findings, after 90 days of treatment at 20 mg/kg/day, were atrophy of skeletal muscle in 2/10 males and 8/10 females; ulcerative gastritis or hyperkeratosis of the non-glandular stomach (4/10 males); testicular atrophy (10/10 males); mineralisation of focal or multifocal seminiferous tubules of the testes (5/10 males); increased cellular debris and/or decreased spermatogenic elements in the tubular lumina of epididymides (9/10 males);

vacuolisation of the smooth muscle of the bladder (1/10 males and 2/9 females); and suppurative, chronic-active or granulomatous inflammation in the lungs (3/10 males and 5/10 females).

Portions of perfused sciatic and brachial nerves from males after 25, 111, and 144 days of recovery were also examined. Nerve damage similar to that seen during the treatment phase was seen in males that had received 20 and 5 mg/kg/day only. Findings after 25 days of recovery were apparently more severe than those observed during the 90-day exposure period but subsequently gradual recovery of the nerve damage was observed such that at 144 days of recovery only very slight to slight alterations were seen in sciatic nerves of males that had received 20 mg/kg/day. However, peripheral nerve lesions (altered tinctorial properties and/or vacuolization of fibres) were still present at this dose in sciatic and brachial nerves although findings were less severe than after 90 days of treatment. There was evidence that some regeneration had occurred. There were no signs of nerve damage at this time point in other groups.

At the end of the recovery period, all four males that had received 20 mg/kg/day still had testicular lesions (slight focal or multifocal atrophy of seminiferous tubules and mineralisation and cellular debris in focal or multifocal tubules). Lesions in the urinary bladder had essentially recovered by this time. Some inflammatory lesions were observed in the liver and lungs of males that had received 20 mg/kg/day although the significance of these was uncertain.

Electron microscopy of nerve tissue provided additional evidence of substantial neuropathy, with some post-exposure recovery, at 20 and 5 mg/kg/day. There were also some axolemmal invaginations at 1 mg/kg/day at 90 days. No ultrastructural changes were observed at lower doses.

In summary, this study demonstrated that oral administration of acrylamide to rats for 90 days principally resulted in severe lesions of peripheral nerves and spinal cord at 20 mg/kg/day (with associated clinical signs of toxicity); atrophy of skeletal muscle; testicular atrophy (although all of the stages of spermatogenesis were still apparent); decreased red blood cell parameters. Peripheral nerve lesions were also observed at 5 mg/kg/day, and slight changes in nerve tissue (visualised only by electron microscopy) were seen at 1 mg/kg/day. No effects were seen at 0.2 mg/kg/day or less. Where nerve damage was produced there was some, but not complete, recovery after a 144-day post-exposure recovery period.

In a 2-year combined chronic toxicity/carcinogenicity study reported more fully later (see Johnson et al., 1986, Carcinogenicity Section 4.1.2.8.) groups of 60 male and 60 female F344 rats received 0, 0.01, 0.1, 0.5, or 2 mg/kg/day acrylamide in drinking water. Mortality was significantly increased amongst males and females receiving 2 mg/kg/day. Histopathologically, degenerative lesions of the tibial nerve were seen at 2 mg/kg/day, but not at lower doses although investigations with respect to potential neurotoxicity were not as extensive as those reported by Burek et al. (1980). Information was also available from another carcinogenicity study (American Cyanamid Co., 1989; Friedman et al., 1995) for which further details are given in Section 4.1.2.8.

In another study, groups of 10 F344 male rats received 0, 5, 10, or 20 mg/kg aqueous acrylamide by gavage 3 days/week for 13 weeks (Tilson et al., 1979). A range of behavioural tests (hindlimb extensor response, spontaneous motor activity, forelimb grip strength) were performed predose and in weeks 1, 4, 7, 10, and 13 of acrylamide exposure. After 13 weeks, neuropathological examination (medulla oblongata, sciatic nerve at mid-thigh, branches of tibial nerve supplying calf muscles) was performed on 5 controls, all animals at 10 mg/kg and 5 out of 10 animals at 20 mg/kg. The remaining animals in the control and top-dose groups were retained for further behavioural tests at weeks 1 and 5 of a recovery period followed by a neuropathological examination.

There was no mention of any observations (positive or negative) at 5 mg/kg. Reduced bodyweight gain (approximately 15% reduction) was noted amongst animals receiving 10 (only up to week 7) or 20 mg/kg. Hindlimb extensor response was reduced only at 20 mg/kg in weeks 7, 10, and 13 and in week 1 of recovery. No abnormality in hindlimb response was seen after 5 weeks of recovery. Reduced spontaneous locomotor activity was noted only at 20 mg/kg in weeks 10 and 13. Recovery was complete after 5 weeks post-exposure. Forelimb grip strength was reduced at 20 mg/kg on weeks 4 and 7 and in week 1 of recovery but not at any other time point.

After 13 weeks of exposure slight neuropathology (distal nerve fibre degeneration) was seen in 9/10 animals and moderate neuropathology (formation of Schwann cell columns) in 1/10 at 10 mg/kg. All 5 animals at 20 mg/kg that were examined showed moderate damage (fibre degeneration and Schwann cell column formation with regenerating or remyelinating fibres). After 5 weeks of recovery animals at 20 mg/kg still showed moderate neuropathology (distal degeneration of large diameter myelinated fibres with clusters of small regenerating myelinated fibres in peripheral nerves).

In summary, peripheral neuropathy (seen histopathologically and by behavioural tests) was observed at 10 and 20 mg/kg acrylamide when administered 3 days/week for 13 weeks by the oral route. Recovery was seen by 5 weeks at 10 mg/kg but not at 20 mg/kg. Groups of 4 male Wistar rats received 0, 52, 80, 125, or 200 mg/l acrylamide in drinking water for 90 days (Tanii and Hashimoto, 1983). The published report did not state actual daily dosages but assuming a mean bodyweight of 200 g and daily water consumption of 30ml; these concentrations would approximate to 0, 7.5, 12, 19, and 30 mg/kg/day.

A slight reduction in bodyweight gain was noted amongst all treated animals (4% reduction at the lowest exposure level, 10% at the highest). Rotarod performance was recorded weekly; the results at day 90 showed impairment only at the two highest exposure levels (3/4 animals at approximately 19 mg/kg/day and 4/4 animals at approximately 30 mg/kg/day). No other rotarod results were available. Other clinical signs of toxicity apparently included weakness, tendency towards spreading and dragging hind limbs and occasionally, amongst more severely affected animals, urinary incontinence; however it was not clear which groups these findings were seen in.

Light microscopy examination was performed on posterior tibial nerves and sural nerves from the lower calf muscle region and showed moderate to severe changes: shrinkage and loss of myelinated fibres, myelin retraction, and corrugation of myelin sheaths at about 30 mg/kg/day. The incidence and severity of findings at other exposure levels was not reported and hence a NOAEL was not identifiable from this study.

Similar findings were observed in an older study (Fullerton and Barnes, 1966) in which male and female Porton rats received 0, 100, 200, 300, 400 ppm acrylamide by dietary administration for 48 weeks. Assuming 300g bodyweight and 30g/day food consumption this levels correspond to approximately 0, 10, 20, 30, 40 mg/kg/day. This study also demonstrated reduced maximal nerve conduction velocity (measured in the hind paw) amongst animals at 200 ppm or more for 6 months or 400 ppm for about 2 months (approximately 20 and 40 mg/kg/day, respectively). Recovery of this parameter was noted after 5-9 months without exposure to acrylamide.

Following on from a single-exposure study reported earlier (see Acute toxicity Section 4.1.2.2.), groups of 10 F344 rats were administered 0, 10, or 20 mg/kg aqueous acrylamide by oral gavage 5 days/week for 4 weeks followed by a 2-week recovery period (Tilson and Cabe, 1979). Investigations included recording of bodyweight, forelimb grip strength once per week, hindlimb extensor response once per week, and an inclined screen test.

Group mean bodyweight loss and reduced weight gain were seen amongst treated animals during the 4-week treatment period (overall weight gain was reduced by 10% at 20 mg/kg and 4% at 10 mg/kg). Bodyweight gain resumed during the recovery phase and slightly exceeded that of controls. For the inclined screen test there was a dose-related increase in the prevalence of animals that were severely impaired during the treatment period. There was no indication of the time of onset of changes or if there was a clear progression in severity over the treatment period. In the 2-week recovery period there was a decrease in the prevalence of severe findings although some animals at 10 mg/kg were still moderately affected and others at 20 mg/kg were still severely affected. Hindlimb extensor response was decreased amongst both groups of treated animals during the treatment period. Progressive recovery was seen in the 2-week recovery period, although animals at 20 mg/kg were still significantly affected. Forelimb grip strength was apparently unaffected by acrylamide exposure.

In a study to validate a functional observational battery (FOB) conducted according to US EPA guidelines (1985) and to assess motor activity, groups of 10 male and 10 female Sprague-Dawley rats received 0, 10, or 30 mg/kg/day aqueous acrylamide (99% pure) by oral gavage 7 days/week for 3 weeks (Schulze and Boysen, 1991). The 3-week exposure period was followed by 10 days recovery before readministration of 0, 10, or 20 mg/kg/day acrylamide for one week (the high dose level was reduced due to 4 male and 2 female mortalities). The FOB was conducted pre-exposure, 1, 6, and 24 hours after the first administration and once per week thereafter. Parameters recorded in the FOB are extensive, including physical appearance, assessment of movement, response to stimuli such as sound or tactile response, and grip strength. Other observations included measurement of food consumption and bodyweight gain, and terminal histopathology on all major organs including eyes (with optic nerve), sciatic, tibial, and sural nerves, lumbar and cervical dorsal and ventral roots, dorsal root ganglion, trigeminal ganglion, and sections from different regions of the brain and spinal cord.

Bodyweight gain and food consumption was statistically significantly reduced amongst animals at 30/20 mg/kg/day (22% reduction in female bodyweight and 26% in males). The onset of alterations in FOB parameters was about 2 weeks after commencement of acrylamide exposure. At 30/20 mg/kg/day the following changes were noted in the FOB: an increased incidence of rigid/difficult handling, slight ptosis, slight to moderately impaired respiration, soiled fur, increased incidence of vocalisation, increased urination, hunched posture/prostration, slight to severely impaired gait, abnormal behaviour, reduced tactile response, impaired righting reflex (also seen at 10 mg/kg/day), decreased rearing counts (also seen at 10 mg/kg/day), reduced forelimb and hind limb grip strength, reduced response to bright light, and reduced activity.

Histopathologically, examination of white matter from cervical and lumbar spinal cord sections, trigeminal and dorsal root ganglia, sciatic, tibial, and sural nerves showed altered diameter of axons (increased or decreased diameter), disruption, fragmentation and distortion of axons, and/or dilation and fragmentation of myelin sheaths, and occasionally an increased number of macrophages. The findings were more prevalent and more severe in animals at 30/20 mg/kg/day than at 10 mg/kg/day. Brain regions were not significantly affected. There was also an increased incidence of splenic pigment was observed in males and females at 30/20 mg/kg/day and also in females at 10 mg/kg/day, increased incidence of granulomatous inflammation in the lungs of animals at 30/20 mg/kg/day, and haemorrhage of the urinary bladder in several males at 30/20 mg/kg/day.

In a study to validate a neurotoxicity screening battery, groups of 10 male and 10 female Sprague-Dawley rats received 0, 12.5, 25, or 50 mg/kg/day aqueous acrylamide by gavage for 7 days followed by a 7-day observation period (Newton et al., 1992; Hughes et al., 1994). Examinations were limited to a functional observational battery (FOB) and histopathological examination of nervous tissue. The parameters recorded in the FOB were scored pre-exposure, and on days 7 and 14. The FOB was conducted according to EPA Guidelines (1991). Histopathological examination was performed on 5 males and 5 females on day 15 and included; forebrain, mid-brain, cerebellum and pons, medulla oblongata, spinal cord, trigeminal ganglia, dorsal root ganglia and fibres, ventral root fibres, and sciatic, sural, and tibial nerves.

Reduced activity was noted in all acrylamide-exposed groups, with a higher prevalence being seen at 50 mg/kg/day on day 7. Bodyweight gain was reduced amongst all acrylamide-exposed groups on days 7 and 14 but only achieved statistical significance at 50 mg/kg/day (14% and 11% reduction in males and females respectively on day 14). At 50 mg/kg/day (but not at the lower doses) hindlimbs were splayed with a corresponding impairment of mobility, a reduced number of rearing counts were observed. Mean forelimb and hindlimb grip strength was reduced amongst males and females at 50 mg/kg/day on days 7 and 14. Landing foot splay was increased amongst all acrylamide-exposed animals on days 7 and 14, although to a lesser degree amongst animals at 12.5 and 25 mg/kg/day.

Histopathologically, axonal degeneration (minimal to marked) was seen in all animals at 50 mg/kg/day particularly in the sural and tibial nerves and to a lesser degree (trace) in a small number of animals at 25 mg/kg/day. No effects were seen in the nerves of animals examined at 12.5 mg/kg/day.

Studies were available investigating changes in brain biogenic amine levels and the possible relationship with acrylamide neurotoxicity (Aldous et al., 1983; Dixit et al., 1981; Husain et al., 1987). Although some changes were observed (which were inconsistent between the studies), it seems unlikely that biogenic amine levels are directly related to the neurotoxic effects induced by acrylamide. There is some evidence to suggest that the neurotoxicity may arise as a result of changes in microtubule formation in the nerve fibres themselves; the apparent alterations in brain biogenic amines may be a secondary consequence of systemic toxicity.

- Mice

In a study focusing on potential neurotoxic and testicular effects, groups of 6 male mice received 0 or approximately 36 mg/kg acrylamide (>95% purity) in saline by oral gavage twice weekly for 8 weeks (Hashimoto et al., 1981). Clinical signs of toxicity in the test group included weakness and ataxia of the hind limbs, and in some cases aggressiveness and increased “alertness”. Rotarod performance was assessed twice weekly and showed a clear and progressive decrease from week 3 onwards in the length of time that acrylamide-exposed animals were able to stay on the rod. Relative testicular weight was reduced (83% of control value). There were no abnormalities seen in the terminal haematology examination (red and white cell counts, haemoglobin concentration, and haematocrit). Light microscopy of the testes showed “degeneration of epithelia in spermatids and spermatocytes” (presumably meaning that a reduced number of spermatids and spermatocytes were observed in the epithelium when compared with controls), reduction in spermatozoa, the presence of multinucleate giant cells. Sertoli cells and interstitial cells were apparently unaffected. In addition, the epididymides were apparently unaffected. No further histopathological investigations were performed.

In a comparative study of a small number of chemicals, groups of 5 female BALB/c mice received 0 or 26 mg/kg/day acrylamide (99% pure) in drinking water for 12 days (Gilbert and Maurissen, 1982). Following a recovery period of 44 days treated animals then received 20 mg/kg/day acrylamide for 19 days. An additional control group received 4-6% saccharin in order to mimic the reduction in water consumption in acrylamide-exposed animals, which may have affected performance in some of the tests conducted. Another "control" group was given a restricted amount of food each day for similar reasons. Rotarod tests were conducted twice per week and were repeated 3 times on each of those occasions and a landing foot-spread test was conducted once per week and was repeated 5 times.

Hind limb foot-splay was increased from 6 days, and rotarod retention time decreased from 8 days after initial acrylamide exposure. Water consumption was reduced from day 1, although this was probably related to unpalatibility. Actual bodyweight loss was noted from day 2 giving an overall loss by day 12 of 19% compared to pre-exposure weight, which could be partially attributed to reduced water consumption. After the 44th day recovery period, bodyweight values, water consumption, hind limb foot-splay, and rotarod retention were all apparently restored to control values. A similar pattern of effects and time taken to the onset of effects was noted for this second exposure period. Bodyweight, water consumption, and rotarod retention values were restored by day 31 of the recovery period following the second acrylamide exposure. The hind limb foot-splay effects were still unresolved after the 31st day recovery period.

Animals receiving distilled water, saccharin, or restricted food intake group showed no obvious changes in rotarod performance or hind limb foot-splay demonstrating that the impairment in performance in test animals was due to acrylamide.

Summary of studies in rodents

In rats, repeated oral administration of acrylamide at doses of 20 mg/kg/day and above produced severe lesions in the peripheral nerves with associated clinical signs of peripheral neuropathy. At these dose levels, marked toxicity was also produced at other sites, particularly atrophy of skeletal muscle, testicular atrophy, and decreased erythrocyte parameters. Histopathological examination of tissues in 2-year rat carcinogenicity studies showed peripheral nerve lesions at 2 mg/kg/day, and no effects at 0.5 mg/kg/day. Peripheral nerve lesions occurred at 5 mg/kg/day in a 90-day study, and slight changes visualised only by electron microscopy were seen in peripheral nerve tissue at 1 mg/kg/day. No effects were seen at 0.2 mg/kg/day.

In mice clinical signs of peripheral neuropathy were seen at 20 and 26 mg/kg/day for 19 or 12 days respectively. In addition, there was a specific examination of germ cells showing loss of spermatids and spermatocytes amongst animals exposed to approximately 36 mg/kg/day twice per week for 8 weeks. These mice studies were not designed to investigate NOAELs.

Studies in cats

Groups of 17-23 cats of unknown breed received 0 or 15 mg/kg/day acrylamide (>98% purity) by dietary administration 7 days/week for up to 16 weeks (Post and McLeod, 1977).

Abnormal gait (hind limbs affected only) was noted within 4-6 weeks and from 12-16 weeks animals were unable to walk, showed weight loss (not quantified), and diarrhoea. Motor conduction velocity in the posterior tibial nerve and greater splanchnic nerve was significantly reduced from week 12. The amplitude of externally recorded muscle action potential (from muscles in the foot) and action potential in the greater splanchnic nerve were reduced from week 4-6, and markedly so from week 12. Fibre density of large diameter nerve fibres in the region of

the left gastrocnemius muscle (NMG) and small fibres in the vagus nerve and greater splanchnic nerve were reduced from week 4-6 onwards and were slightly more reduced from week 12.

Histopathology of nerve fibres supplying the NMG, greater splanchnic nerve (a branch of the sciatic nerve) and left cervical vagus nerve only showed a reduced number of myelinated fibres which was more pronounced in NMG. Electron microscopy showed an increased density of neurofilaments, and abnormal membranous configurations between the axolemma and Schwann cell membrane. Degenerating fibres of the NMG and splanchnic nerve showed loss of myelin and unmyelinated fibres also showed signs of degeneration.

Groups of 1-3 cats of unknown origin received 0, 0.03, 0.1, 0.3, 1, 3, or 10 mg/kg/day acrylamide by dietary administration 5 days/week for up to 1 year (McCollister et al., 1964). Two control animals died and one was killed due to intercurrent infection after less than 6 months.

Signs of peripheral neuropathy (loss of use of hind limbs, abnormal gait) were observed at 1 mg/kg/day and above. However, all animals at 0.03, 0.1, and 1 out of 2 cats at 0.3 mg/kg/day died apparently from intercurrent infection. It was reported that there were no pathological abnormalities attributable to acrylamide at any exposure level. However, the extent of examination was unclear and firm conclusions are hard to draw due to the general poor condition of animals used in this study.

Studies in dogs

Fourteen dogs received 7 mg/kg/day acrylamide by dietary admixture for about 10 weeks (Satchell and McLeod, 1981). There were no control animals used. Clinical signs of toxicity included severe impairment of hindlimb function: 'toe-folding' being observed from about day 30, ataxia from about day 40, clear signs of muscle weakness from around day 50, and regurgitation from around day 60. Expansion of the oesophagus (megaoesophagus) was noted radiologically in 3 out of 14 dogs. However, the significance of this finding is uncertain as only 3 animals were examined, there were no controls for this part of the study, and megaoesophagus was reported by the authors to occur spontaneously in the dog with the aetiology unknown.

In another study focusing on respiratory effects, 4 dogs received 6 mg/kg/day acrylamide (99% pure) in gelatin capsules for 6-7 weeks, with up to 8 weeks recovery (Hersch et al., 1989). Resting respiration was measured using an intratracheal technique, and electrocardiography, electroencephalography, and heart rates were recorded. In 2 animals blood levels of CO_2 and transcutaneous oxyhaemoglobin were also recorded. The Hering-Breuer lung inflation reflex was quantified by measuring the duration of apnoea produced during lung inflation and was used as an indicator of the function of the vagus nerve. Parameters for each animal were recorded pre-exposure and served as controls for this study.

One animal was killed due to pneumonia at around week 10. Loss of use of hind limbs and “toe-folding” were observed from about week 3 and resolved during the 5th week of recovery. Decreased respiratory frequency and slightly increased tidal volume were observed during the ACR exposure period but were restored during the recovery period. The Hering-Breuer lung inflation reflex was impaired (as indicated by increased tidal volume and decreased respiratory frequency). Other parameters were not adversely affected. The toxicological significance of these respiratory effects is unclear, although the altered Hering-Breuer reflex could be indicative of damage to the vagus nerve.

Studies in non-human primates

In an extensive study, four feral-born Macaque monkeys received 10 mg/kg/day acrylamide (>99% purity) in fruit juice for 5 days/week for 44-61 days, until the time of onset of clinical signs of toxicity (Maurissen et al., 1983). For animal welfare reasons, treatment with acrylamide was not continued beyond this point and animals were allowed to recover, with examinations still performed, for a period of up to 146 days. Two control animals received tap water only, for about 13 weeks using a similar dosing regime. Investigations included recording bodyweight, clinical signs of toxicity, a visuomotor task (time taken to pick up a food reward) performed twice per week, sensitivity to an electrical or a vibration stimulus also performed twice per week, and sural nerve histopathology performed first when vibration thresholds were elevated (about day 51-58 of acrylamide exposure) and then during the recovery phase (up to 146 days after the last acrylamide exposure).

Amongst treated animals, clinical signs of toxicity included loss of balance, decreased activity, hindlimb weakness, and forelimb tremor in the final week of acrylamide treatment for one particular animal. With the exception of forelimb tremor, which persisted for up to 4 weeks, these clinical signs of toxicity resolved within 2 weeks post-treatment. During the treatment period actual bodyweight loss (up to 30% reduction) was noted in 3/4 animals. However, one of the 2 control animals also showed bodyweight loss (approximately 25% reduction).

Amongst treated animals, response to a 60Hz electrical stimulus was not apparently affected during or after treatment. However, there was a decreased sensitivity (as assessed by an increased time to key-pressing) towards a vibration stimulus (40 Hz and 150 Hz) during the treatment phase with effects being even more pronounced in the first 10 weeks post-treatment. An increased time taken to pick up a food reward was noted in test animals towards the end of the treatment period and was more pronounced in the first 3 weeks post-treatment.

Sural nerve biopsies were prepared from 2 acrylamide-exposed animals, firstly when vibration thresholds were elevated (day 51 and 58 of treatment) and then during the recovery phase (day 146 and 136). At the first examination, in some areas there were no axons visible and myelin had formed balls or whorls although most nerve fibres appeared to be normal under light microscopy. Electron microscopy also showed that most myelinated nerve fibres were apparently normal but others showed axolemma invagination, disruption of myelin, other “severe axonal alterations” (not further described) or a loss of axons. Some Schwann cells lacked an axon and contained contorted and apparently disintegrating myelin - several Schwann cells contained lipid vacuoles. In one animal about 25% of nerve fibres were affected, but in the other, only “occasional” fibres were affected. No abnormalities were observed in unmyelinated nerve fibres.

The second biopsy (performed during the recovery phase when no abnormalities were seen in vibration sensitivity) showed that degenerative changes were less frequent than during the treatment period and regenerative fibres were also seen. Loss of vibration sensitivity did not appear to be associated with the neuropathological findings.

Three adult Macaque monkeys received 10 mg/kg acrylamide in fruit juice 5 days/week for 6-9 weeks (Maurissen et al., 1990). In addition, 2 monkeys were used as controls. This study had a second treatment period after a 30-week recovery. The study focused on recording bodyweight, time taken to pick up a food reward, response to electrical stimulus, and response to a vibration stimulus (40 and 150 Hz). Results were essentially similar to those obtained by Maurissen et al. (1983).

In a study investigating potential effects on the visual system, a group of 7 Macaque monkeys received 10 mg/kg/day acrylamide in fruit juice for 5 days/week for up to 13 weeks with approximately 20-30 weeks recovery (Eskin et al., 1985). In addition, there were 2 control animals. Brain, optic nerve and eyes were removed for histopathological (light and electron microscopy) examination.

For acrylamide-treated animals sacrificed immediately after 9-13 weeks, distal axonal swelling was most prominent in distal optic tract fibres, particularly within the lateral geniculate nucleus. Myelin sheaths were disproportionately thin and degenerating myelin and occasional shrunken axons were observed. Degenerating myelin and degenerating/atrophic axons were seen in the optic nerve and the proximal optic tract. In the lateral geniculate nucleus of the brain, axonal swellings were again seen and occasional alterations in the retinal axon terminals and synapses were observed by light and electron microscopy. Dilatation of the axonal terminals, degeneration of myelin, degenerating/atrophic axons, and an increased number of astroglial processes were also seen in the lateral geniculate nucleus. No abnormalities were seen in controls.

The optic nerves of acrylamide-exposed animals showed a loss of axons, and diminished numbers of fibres in the optic nerve. Electron microscopy showed disproportionately thin myelin sheaths, densely packed astroglial processes, lipid vacuolation, and degenerating myelin fragments in the phagocytes and astrocytes.

To summarise, this study shows marked effects, including loss of ganglion cells (particularly near the fovea), and axonal loss in the nerve fibres of the optic tract and in the lateral geniculate nucleus of the brain of non-human primates exposed to 10 mg/kg acrylamide, 5 days/week for up to 13 weeks.

Three adult Macaque monkeys received 10 mg/kg acrylamide in fruit juice 5 days/week for 33-47 doses (6-10 weeks - marked ataxia was observed at this point) (Merigan et al., 1982). One animal was sacrificed immediately after this administration period for histopathological examination, and the other two were observed for a further 90 days. A fourth animal was used as a control. Observations for visual acuity and flicker-fusion were performed 5 mornings/week and cortical evoked potentials were recorded in 2-3 afternoons/week. Visuomotor coordination was monitored daily by measuring the time taken to pick up a food reward. Bodyweight values were apparently measured but no results were presented. No histopathological information was available in this report.

A marked increase in cortical evoked potential was observed after about 4 weeks of acrylamide exposure. This change preceded a decrease in visual acuity and flicker-fusion frequency apparent 2 weeks later. A marked increase in the time taken for a pick-up test was also apparent towards the end of the treatment-period and was still markedly increased for about 2 weeks after cessation of dosing. Flicker-fusion frequency was restored within 3 weeks, and cortical evoked potential values were restored within 7 weeks. Visual acuity stabilised within 3 weeks but remained at a level below that recorded pre-exposure for the rest of the 90-day post-exposure observation period. In addition, weight loss, hind limb weakness, gait disturbances, and tremors were observed amongst acrylamide-exposed animals. There were no abnormalities seen in pupillary or eye movement, and no other ophthalmological changes noted. There were no significant changes observed in the control animal.

In the companion study of two investigations of potential effects to the visual system (see Eskin et al., 1985 for histopathological details), a group of 3 Macaque monkeys received 10 mg/kg/day acrylamide in fruit juice 5 days/week for about 6-10 weeks (Merigan et al., 1985). One animal was used a control. One acrylamide-exposed monkey was sacrificed on animal welfare grounds

after about 10 weeks, and the other 2 had a 140-day recovery period. A number of tests for visual capacity were conducted.

Reduced contrast sensitivity was noted at the end of the exposure period. Visual acuity was also impaired in all acrylamide-exposed animals with a slight recovery within 5 weeks post-administration. Flicker-fusion frequency was reduced from about week 2 onwards and recovered within 5 weeks post-administration. Visual-evoked potentials were impaired amongst acrylamide-exposed animals. The authors suggested that the changes in latency and increase in amplitude correlate with a conduction block in large diameter optic nerve fibres.

In a briefly-reported study (although the only non-human primate study investigating a range of different exposure levels), one female monkey (unspecified species) per dose level received either 0, 0.03, 0.1, 0.3 (2 animals at this exposure level), 1, 3, or 10 mg/kg/day aqueous acrylamide by oral gavage or dietary administration 5 days/week for up to 1 year (McCollister et al., 1964). After acrylamide exposure haematology, blood cholinesterase measurements, and macro- and microscopic pathology were conducted. There were no details available regarding these examinations.

Clear and severe clinical signs of neuropathy were apparent at 10 mg/kg/day. At 3 mg/kg/day occasional abnormalities were observed; reduced knee jerk reaction, reduced pupillary reflexes (response to bright light), and lethargic behaviour. At 0.1, 0.3 and 1 mg/kg/day acrylamide exposure for 1 year there were no apparent effects on bodyweight, no clinical signs of toxicity, no changes in haematology (although it is unclear which parameters were recorded), liver and kidney weights, and no macroscopic or microscopic pathology abnormalities (extent of examination unclear, but probably included at least the brain and spinal cord).

It is difficult to draw firm conclusions from this study due to limited reporting, and the use of only one animal per dose level.

Summary of studies in non-human primates

Recent repeated oral exposure studies using 10 mg/kg/day for up to 12 weeks were associated with clinical signs of peripheral neuropathy, and neuropathological effects and neurological dysfunction particularly in relation to the use of limbs. Most of the changes that were observed were reversible on completion of approximately 30 weeks without acrylamide exposure. Similar exposure levels produced marked effects on the visual system assessed by changes in functional parameters and supported by histopathological effects (loss of ganglion cells, loss of axonal cells in the optic tract and brain). Unfortunately the one study using lower dose levels was of insufficient scope and quality to yield clear results, and a NOAEL for primates is not available.

Dermal

Very limited information is available regarding animal studies conducted using the dermal route of exposure:

In a study reported only in abstract form, groups of rabbits (unknown number) received 0, 0.5, 5, or 50 mg/kg/day acrylamide by dermal application (vehicle unknown) for up to 12 weeks (Drees et al., 1976). At 5 weeks one half of the rabbits in each group were killed - it is unclear what investigations were performed on these. Treatment at 50 mg/kg/day was stopped at this time-point and animals were observed for the remaining 7 weeks. Animals in other treatment groups continued to receive acrylamide. Clinical signs of neurotoxicity (no details available) were observed in most rabbits at 50 mg/kg/day. During the 7th week recovery period the severity

decreased such that almost all animals showed no clinical signs of toxicity. No clinical signs of toxicity were observed in any other exposure group. Haematology, blood biochemistry, macroscopic and microscopic pathology examinations showed no abnormalities although no details were given on the extent of examinations or of when they were conducted.

In a dominant lethal assay (see Gutierrez-Espeleta et al., 1992, Mutagenicity Section, 4.1.2.7), no signs of neurotoxicity were observed amongst groups of 24-30 male mice were exposed by the dermal route to 0, 25, 50, 75, 100, or 125 mg/kg/day acrylamide in 70% aqueous methanol for 5 days. The full extent of examinations was not clear, so no firm conclusions can be drawn.

4.1.2.6.2 Studies in humans

Case reports

A case report described earlier (see Skin Irritation, Section 4.1.2.3) involves an individual occupationally exposed for at least 6 months to monomeric acrylamide powder (Davenport et al., 1976). Signs of skin irritation and excessive perspiration on hands and feet were observed although it was unclear whether or not effects on the feet were as a result of direct contact with acrylamide. There was no information available regarding exposure levels or routes of exposure. The individual was reported to have worn protective clothing (including gloves and face mask) although it is unclear if these were adequate in preventing acrylamide exposure.

Within 9 months of commencement of working with acrylamide, marked weight loss, fatigue, loss of appetite, and unsteady gait were reported. A tingling sensation and loss of use of the hands, and impaired speech were subsequently observed. In addition, muscle weakness (relating to the use of wrists and ankles) reduced muscle tone, incoordination of upper limbs, and the tremor of the hands were noted. There was also a partial loss of pain, temperature sensation, and response to touch below the forearm and below the mid-calf areas, loss of balance coordination (i.e. positive Romberg test) and a marked reduction in tendon and plantar reflexes. Fine nystagmus was also observed on lateral gaze (but not on central gaze, indicating cerebellar impairment).

Motor nerve conduction velocity in the left and right peronal nerves was apparently normal although reduced muscle action potential was recorded in the gastrocnemius and anterior tibial muscles in both legs. Sural nerve biopsy showed diffuse fibrosis and loss of nerve fibres and enlarged axons occasionally without myelin sheaths. Electron microscopy showed a number of axons with fine bundles of filaments, which were randomly orientated (rather than the usual regular longitudinal orientation).

Two months after cessation of work with acrylamide, the clinical signs of toxicity were still apparent; one year after, there was a near-complete recovery (although weakness of ankles was still observed).

Similar clinical signs of toxicity with reversibility of effects were observed by Auld and Bedwell (1967), Garland and Patterson (1967), Kesson et al. (1977), Mapp et al. (1977), Morvillier (1969), Satoyoshi et al. (1971), Takahashi et al. (1971), but in most cases these authors did not perform histopathological examinations. Although the findings can be associated with acrylamide exposure, the level of exposure and route(s) of exposure in each case remains unclear.

Three of the individuals previously investigated by Garland and Patterson, 1,967 were examined electrophysiologically and histopathologically (Fullerton, 1969). No information was available on the nature or extent of past exposure to acrylamide. Motor nerve conduction velocity and muscle action potential was recorded in the muscles of the hand or foot. Sensory nerve action potentials were recorded at the median or ulnar nerves at the wrist. Sural nerve biopsies were taken from the ankle region for histopathology.

Motor nerve conduction velocities in median, ulnar, lateral, and popliteal nerves were mostly within control ranges except in one individual where the right lateral popliteal maximal conduction velocity was below the control range. Muscle action potentials were dispersed (responses were prolonged). Motor nerve conduction velocity showed little change in the upper arm, but was prolonged in the forearm and elbow down to the wrist. For sensory conduction, there was no or only slightly recordable ascending action potential from the median nerve at the wrist suggesting functional impairment of sensory fibres.

Histopathologically, single fibres from the sural nerve were examined from two of the acrylamide-exposed individuals and three age-matched control subjects. There were no degenerative fibres observed in the controls and in one of the acrylamide-exposed individuals. This acrylamide-exposed person had not worked with acrylamide for approximately 8 months. However, the second individual from which samples were taken had been exposed up until 10 weeks before biopsy. Some fibres were seen showing signs of Wallerian degeneration (fatty degeneration of the myelin sheath). In addition, there was a tendency towards decreased internodal length in some large diameter fibres in the two acrylamide-exposed people suggesting degeneration with subsequent regeneration. Large diameter fibres with short internodal length were not seen in controls. In one exposed individual, there was a slight decrease in fibre density (expressed as fibres/mm^2) of large diameter fibres and a corresponding increase in density of small diameter fibres.

A family of 5 individuals was accidentally exposed to acrylamide in water used for drinking and washing following local grouting activities (Igisu et al., 1975). The duration of exposure was approximately 1 month, and the concentration of acrylamide in water was reported to be 400 ppm although this was measured only at one point in time one month after the initial grouting operation. It is unclear if levels would have uniform been throughout this one month exposure period. Also with no clear information as regards water consumption and use for washing and other domestic purposes, the actual exposure is difficult to quantify. The health effects reported were similar to those noted in other case studies, with recovery essentially complete after 4 months.

Reports published by the US EPA and NIOSH (Hills and Griefe, 1986; McHugh, 1987) indicate skin reactions (such as peeling of the palms of hands) associated with the use of acrylamide in sewer grouts. In addition, the report by Hills briefly summarises the number of cases of ill health (to the extent that the information was available) reported over some years from around the world (**Table 4.17**).

Table 4.17 Case reports of ill health following the use of acrylamide grouts (cited Hills and Griefe, 1986)

Year	No of Cases	Exposure Location
1967	1	Canada
1971	1	France
1972	1	France
1977	6	England

Although it is reasonable to attribute some effects to acrylamide, it is difficult to draw conclusions from these reports given that little is known of any concurrent exposures (for example, other substances are likely to be used as well as acrylamide). Also little is known about the extent of exposure (particularly dermal exposure). Overall there is insufficient information to use this information for risk assessment purposes.

Workplace surveys

HSE have summarised reports presented in confidence by Sweden that contain occupational hygiene and health effects information in relation to sewer grouting.

The Rhoca Gil grouting agent was used in tunnel construction at Hallandsås during 1997 for approximately 6months (Nordander et al., 1998). About one week after the end of the 6 months use of this grouting agent, there was a cross-sectional study of adverse health effects using a self-administered questionnaire, medical examination including sensation of vibration (in the toes only), and an assessment of haemoglobin adducts (to acrylamide and/or NMA). No control group was used for the questionnaire. Although a group of 12 controls were used for comparison of haemoglobin adduct information, no details were provided about this group.

A total of 242 workers who were thought by management to have been in contact with Rhoca Gil were identified for study. Of these, 223 (92%) made themselves available for investigations, the other 19 would not participate or had moved away from the area. Following the questionnaire and medical interviews 50 workers were selected for further investigation of vibration sensitivity. It was noted that there were no background data for vibration sensation in the toes as the instrument is more routinely used on the fingers. The questionnaire indicated that 29/223 (13%) workers reported prickling or numbness in the feet or lower legs and 34/223 (15%) reported prickling or numbness in the hands after starting work with Rhoca Gil. Flaking of skin on the hands was noted by 13/223 (6%), increased sweating in the hands and/or feet by 9/223 (4%), and irritation of the skin by 47/223 (21%); none of these effects were characterised in more detail. Irritation of the eyes was reported by 71/223 (34%), irritation of the nose by 56/223 (25%) and irritation of the throat by 69/223 (31%). Other subjectively reported symptoms included cough, breathlessness, headache, nausea, and dizziness. Some of the symptoms (e.g. increased sweating on hands/feet and flaking skin, particularly if it was on the palms, although this is not clear from the report) are suggestive of the involvement of acrylamide. However, it is difficult to determine the true significance of many of these effects (such as “irritation”) given that they are self-reported, there was a lack of definition of what was experienced, and that no control group was used; it is difficult to attribute these entirely to acrylamide.

Blood samples for analysis of haemoglobin adducts to acrylamide and/or NMA were taken from 77 of the 223 workers. From this group the range of acrylamide-haemoglobin adducts was 0.04-4.31 nmol/g globin (mean 0.24 nmol/g) and in controls was 0.02-0.07 nmol/g (mean

0.04 nmol/g). An attempt was made to correlate the levels of acrylamide-haemoglobin adducts with the estimates of exposure; in general terms, the group of people that were stated to be most heavily exposed appeared to have the highest adduct levels. This would probably be expected, but given the lack of clear information on the extent of exposure via all routes it is difficult to derive anything quantitatively from the data. Attempts were also made to correlate the subjectively recorded symptoms of ill health with adduct levels. Again given the inadequacies of the information from the questionnaire and the fact that many findings were local in nature (e.g. irritant effects) rather than systemic, it is difficult to draw any meaningful conclusions from such an analysis.

Mean vibration thresholds at 8 Hz and 125 Hz recorded in the toe of one foot did not show any biologically significant difference between those apparently not exposed and workers who were allegedly to be exposed to Rhoca Gil. The vibration threshold at 8 Hz; controls, range 98-135 dB, mean 112 dB, and in the 'most heavily exposed' group had a range of 98-13 5 dB, mean 111 dB. At 125 Hz, the range in controls was 119-160 dB (mean 140) and in the most heavily exposed was 111-160 dB (mean 137). It is also noted that no useful background data were available for the use of this technique on toes. An attempt was made to correlate the vibration threshold with the self-reported symptoms. Again no meaningful conclusions can be drawn due to the inadequacies of information from the self-reported questionnaire, and also that, in the first instance, there was no measurable difference in vibration threshold between those workers who were exposed to Rhoca Gil and those apparently unexposed. The correlation coefficient, r^2 was 0.13 indicating a very poor correlation.

Overall, this report indicated a high prevalence of some self-reported symptoms particularly in the group of people that appeared to be most heavily exposed. However, for some of these (such as “eye irritation” and “respiratory tract irritation”), it is difficult to attribute entirely to acrylamide. However, there were clearly some findings suggestive of acrylamide exposure (such as skin flaking). There was a lack of clear quantitative exposure information and it is difficult to be sure to what extent other substances in the working environment may have contributed to any effects experienced. It could be seen that an increased level of haemoglobin adducts was measured in those workers that were apparently most heavily exposed. Measures of sensation to vibration also did not show any meaningful changes amongst those workers apparently exposed to Rhoca Gil.

Following the above survey of 50 selected workers, the same 50 workers (plus an additional one) were re-examined for vibration sensitivity and by using a self-administered questionnaire 6 months after the original investigation (Hagmar et al., 1998). In addition, the “rate of motor nerve control” and the “rate of sensory nerve control” were determined in the right arm, and lower leg, and temperature threshold was determined in the left foot. Apparently, these were also performed as part of the investigations 6 months earlier but no experimental data were presented in relation to these endpoints. Of these 51 workers identified, 45 made themselves available for this follow-up analysis.

Apparently, prickling sensation, pain, and numbness were reported in the hands, feet and lower legs but no details were provided on the results of the questionnaire. Blood samples for analysis of haemoglobin adducts to acrylamide and/or NMA were taken from 20 of the 45 workers that were selected. From this group the range of acrylamide haemoglobin adducts was 0.4-17.7 nmol/g globin (mean 2.0 nmol/g). No control data were presented and it is unclear why the haemoglobin adduct levels should appear to be higher than those in the earlier after several months free of exposure to Rhoca Gil (there is no evidence that acrylamide has a long half-life or bioaccumulates).

Overall, few firm conclusions can be drawn from this follow-up examination due to limited reporting details and the apparently anomalous response in the measurement of haemoglobin adducts some months after the cessation of exposure.

A group of 71 workers at a factory in China producing acrylamide monomer and polymers were examined by questionnaire, physical and neurological examinations, tests for visual acuity and a visual field test, skin temperature, electrocardiography, electroencephalography, haematology, blood biochemistry and urinalysis investigations (He et al., 1989). These workers had been potentially exposed to acrylamide for 1 to 18 months. A group of 51 age-matched control subjects was selected from the local area. It was not clear from the report whether or not all of the acrylamide-exposed workforce was involved in this investigation. Approximately one year prior to investigations the atmospheric concentration of acrylamide were stated to have reached 5-9 mg/m^3 during the polymerisation process. Shortly before examinations, following a renovation of processes, values were reported to be about 0.03 mg/m^3. It is unclear whether or not these values represented 8-hour time-weighted averages (TWA) and if they were from personal monitoring or static samples. In addition, dermal exposure was reported to be extensive, with workers washing their hands in water contaminated with up to 410 mg/l acrylamide.

The questionnaire showed statistically significant increases in the prevalence of skin peeling from hands (54%, 4% in controls), numbness of hands and feet (21%, 4% in controls), "lassitude" (20%, 2% in controls), sleepiness (17%, 0 in controls), muscle weakness (15%, 0 in controls), clumsiness of hands (11%, 0 in controls), anorexia (11%, 2% in controls), unsteady gait (8%, 0 in controls), coldness of hands and feet (8%, 0 in controls), difficulty in grasping (7%, 0 in controls), and stumbling and falling (7%, 0 in controls). In addition, a non-significant increase in the prevalence of sweating (38% in acrylamide-exposed compared with 27% in controls) and dizziness (10%, 4% in controls) was noted.

Initial effects were characterised by peeling of the skin and excessive sweating of the hands. These effects were probably mainly due to dermal exposure. Muscle weakness of the legs, and numbness and tingling of hands and feet was reported by 20% of workers after approximately 3-10 months exposure. Shortly before improvements were made, nine workers developed lassitude, sleepiness, anorexia, loss of bodyweight, progressing to an inability to hold objects, unsteady gait, and loss of balance. Three of these 9 also showed horizontal nystagmus, and loss of sensation to vibration and loss of tendon reflexes. After removal from exposure to acrylamide for a period of 3-5 months a considerable, but not complete, recovery was reported.

Sensory impairment, including sensation of vibration, pain, touch, and position was recorded in up to 17% of workers compared with none in the control group. Distal skin temperature was also apparently lower in the acrylamide-exposed workers but no values were presented in the report. Muscular atrophy in the hands was reported in 4 (6%) workers compared with none in controls, and an increased prevalence of diminished or a loss of reflexes was reported in biceps, triceps, knee, and ankle. A positive Romberg test was reported in 15/71 (21%) of acrylamide-exposed workers compared with 3/51 (6%) in the control group.

In the electroneuromyographic studies 3/69 acrylamide workers showed spontaneous denervation potentials (2 with fibrillation and 1 with a positive wave). Other effects were recorded: prolonged duration of motor unit potentials in 40/69 acrylamide workers compared to 4/48 controls, increased polyphasic potential in 29/69 acrylamide workers and a "discrete pattern of recruitment" in 9/69 compared to none in the control group. These electroneuromyographic changes are suggestive of partial denervation and axonal degeneration but the changes were also

seen in 25 workers who did not have any apparent clinical signs of neurotoxicity. Thus, the partial denervation and axonal degeneration could be subclinical effects.

Amongst acrylamide-exposed workers, the H-reflex (recorded in the soleus muscle of the lower leg) and ankle tendon reflex were non-responsive in 23/69 (33%) and 21/69 (30%) respectively compared to normal responses in all of the control group. The changes in H-reflex were only seen in workers with clinical signs of neurotoxicity, whereas the changes in ankle tendon reflex were seen in the presence and absence of clinically observable effects.

Action potentials were recorded from the median and ulnar nerves at the wrist and elbow and also in the sural and peroneal nerves in the lower leg. The nerve conduction velocity was only marginally affected in the peroneal nerve. However, the mean nerve action potential amplitude from the sural, median and ulnar nerves was significantly decreased compared to the control group.

There were no other effects that were considered to be related to acrylamide exposure amongst the following measurements: serum β-glucuronidase, serum IgG, IgA, or IgM, urinalysis (although it was unclear which parameters were recorded), serum enzyme levels, electroencephalography, and electrocardiography.

To summarise this study, although there is very limited information regarding exposure, levels of up to 5-9 mg/m³ airborne acrylamide were associated with clear clinical signs of peripheral neuropathy as well as effects on balance and nystagmus. There were also measurable effects on nerve action amplitude indicative of neuropathy. Dermal exposure to acrylamide is also likely to have contributed to effects such as skin erythema and skin peeling on the hands and is also likely to have contributed to other systemic effects, although this could not be quantified from this study. The effect(s) on human health in relation to the reduction in exposure to about 0.03 mg/m³ just prior to the conduct of this study were unclear from this report.

The vibration sensitivity threshold (measured at 120 Hz) of a group of 41 workers exposed to acrylamide for 0.5-8 years was compared with that of a group of 105 age-matched, apparently healthy, control subjects (Deng et al., 1993). Vibration sensitivity threshold was measured using a forced-choice procedure (detecting which of 2 posts is vibrating) and a method-of-limits procedure (determining the point or intensity at which a vibration can be sensed) at the fingers and toes. Airborne exposure to acrylamide ranged from 0.2-1.58 mg/m³ although it was unclear whether or not this was an 8-hour TWA and if values were from personal monitoring or static sampling. The extent of dermal exposure to acrylamide was unclear.

In the control group there was no significant gender differences and no significant difference between right and left side; however, there was an age-dependent increase in vibration threshold. As a group mean, acrylamide-exposed workers showed a significant increase compared to controls in vibration threshold for both age ranges assessed (<31 years and 31-40 years) in the index finger and great toe. Individually, 24/41 acrylamide-exposed workers showed a vibration threshold higher than the upper limit of the control values.

This study demonstrated an impairment of sensitivity towards vibration amongst workers exposed to acrylamide indicative of peripheral neuropathy. The airborne exposure range was given as 0.2-1.58 mg/m³ but there were some uncertainties regarding the meaning of this description.

A brief report of a polyacrylamide manufacturing plant identified 5 workers as showing some clinical signs of peripheral neuropathy from a total workforce of 71 at a factory in South Africa. The one with the most severe peripheral neuropathy was reported to also have cerebellar and

ocular impairment (details not given) (Myers and Macun, 1991). After 5 years without exposure, recovery was reported to be incomplete in all 5 cases. Following this initial observation, the remaining 66 individuals were evaluated for neuropathological effects; three individuals were excluded from the analysis of results due to alcoholism or use of neurotoxic drugs. Personal exposures of each worker were not recorded but 8-hour TWAs were estimated for each task by one individual walking through the factory carrying personal monitoring equipment and from these readings each task was categorised. Twenty-two workers were thought to be exposed to airborne levels of <0.3 mg/m³, and 41 above this level (4 workers were apparently exposed to approximately 0.75 mg/m³). Assessment was by questionnaire and physical examination. At this factory, there were no "unexposed" individuals, no engineering measures to reduce exposure, and no respiratory protection. Some protective clothing was available, but it was unclear whether or not this actually prevented dermal exposure.

A higher prevalence (although not always statistically significant) of a number of effects was reported amongst workers exposed to >0.3 mg/m³ acrylamide compared to workers exposed to <0.3 mg/m³; weakness (5/21 vs. 3/42), effects on "sensation" (7/21 vs. 4/42), effects on fingertip skin, including skin peeling and sweating, changes in skin colour (6/21 Vs 1/42). There were no demonstrable effects on vibration sense, tactile or pain responses, reflex actions measured at the ankle, knee, biceps and triceps, arm or leg coordination, or Romberg test. However, as comparisons were not made with a control group that was not exposed to acrylamide, firm conclusions are hard to draw.

In a follow-up study of the same workplace using a questionnaire, physical examination and tests for vibration sensitivity, a group of 75 workers were studied (Bachmann et al., 1992). Personal exposures ranged from 0.02-2.39 mg/m³ with an overall mean of 0.16 mg/m³ (presumably 8-hour TWA values). Some measures were taken to reduce dermal exposure (those exposed to >0.3 mg/m³ wore gloves). No control group was used for this study but comparisons were made between those exposed to mean levels <0.3 mg/m³ and to those exposed to mean levels greater than this value. Dermal exposure could not be quantified.

There was no clinical evidence of muscle wasting, loss of position sense, or any positive Romberg tests. However, slightly impaired tactile response was noted in a small number of individuals from both groups. Increased prevalences of a number of symptoms typical of acrylamide exposure (such as skin peeling, sweating hands, numbness of hands and feet) were observed amongst those exposed to >0.3 mg/m³ in comparison with those exposed to a lower mean value. There were no significant differences in vibration sensitivity (forced choice and method-of-limits) between the two groups being compared. Overall, as with the previous study at this factory, an increased prevalence of some symptoms related to peripheral neuropathy was observed in workers exposed to >0.3 mg/m³. These results are difficult to interpret particularly with respect to individuals exposed to mean values <0.3 mg/m³ in the absence of an unexposed control group.

Some further information is described within the cohort mortality studies in Section 4.1.2.8.

4.1.2.6.3 Summary of repeated-dose toxicity

Human evidence from case reports and workplace surveys demonstrates neuropathological effects (principally peripheral neuropathy) following exposure to acrylamide. In most of the case reports the route and extent of exposure was unclear, although a combination of inhalation and dermal exposure was likely. One case report of accidental oral ingestion, which was likely to have involved repeated rather than single exposure to acrylamide, also demonstrated similar neuropathic effects by this route.

The workplace studies indicate that workers reported to be exposed to airborne levels of >0.3 mg/m³ (8-hour TWA) showed an increased prevalence of symptoms related to peripheral neuropathy compared with those exposed to <0.3 mg/m³. The significance of effects at <0.3 mg/m³ was unclear because control groups were not included for comparative purposes. In the workplace surveys that were available, it was impossible to quantify the contribution to dose due to dermal exposure, and hence the airborne levels cited, may not be representative of total acrylamide exposure and thus the dose received. Overall, there is no adequate human information to establish a dose-response relationship.

There were no animal studies relating to inhalation exposure and no firm conclusions could be drawn from the studies that were available using the dermal route of exposure due to insufficient reporting details. However, for oral exposure, much information was available, most of which related to neurotoxicity.

The effects that have been observed in these studies provide supporting evidence for the effects that have been observed in humans:

Signs of neurotoxicity such as loss of use of limbs, tremor, loss of balance and loss of axons and ganglion cells, as well as other degenerative changes in peripheral and optic nerves, and degeneration of the lateral geniculate nucleus were observed amongst the species studied (primates, dogs, cats, rodents). Additionally, in mice degeneration of spermatids and spermatocytes was observed in one repeated-exposure study. This observation was consistent with a single-exposure study in mice and toxicokinetic studies demonstrating distribution of acrylamide to male reproductive organs.

Many of the animal studies available were deliberately constructed to observe neuropathic effects without attempting to identify a no-adverse effect level (NOAEL). The clearest information that is available is from rodent studies. Histopathological examination of tissues in 2-year rat carcinogenicity studies showed peripheral nerve lesions at 2 mg/kg/day, and no effects at 0.5 mg/kg/day. These observations were consistent with 90-day rat studies and studies in mice of shorter duration, which demonstrated similar, effects at slightly higher exposure levels. In addition, a specific examination of germ cells from Acrylamide-exposed mice showed a loss of spermatids and spermatocytes amongst animals receiving approximately 36 mg/kg/day for 8 weeks. This study was not designed to identify a NOAEL. Primate studies were also not designed to identify a NOAEL but, in addition to peripheral neuropathy, also showed degenerative changes in the optic nerve and lateral geniculate nucleus following oral exposure to approximately 10 mg/kg/day for up to 13 weeks. For classification, see Chapter 1.

4.1.2.7 Mutagenicity

4.1.2.7.1 Studies *in vitro*

Studies in bacteria

A number of well-conducted published and unpublished liquid preincubation and plate incorporation bacterial mutagenicity tests exist in which 100-50,000 µg/plate acrylamide was assayed; results were consistently negative using *Salmonella typhimurium* tester strains TA 98, TA 100, TA 102, TA 1535, TA 1537, and TA 1538, and *E.coli WP2 uvrA*- in the presence and absence of metabolic activation (Bull et al., 1984a; Godek et al., 1982a; Hashimoto and Tanii, 1985; Jung et al., 1992; Knaap et al., 1988; Lijinsky and Andrews, 1980; Muller et al., 1993; Tsuda et al., 1993; Zeiger et al., 1987).

In a fluctuation test (to determine mutations in genes conferring resistance to streptomycin) in *Klebsiella pneumoniae* the mutation frequency was not significantly altered by 100-10,000 µg/ml acrylamide (Knaap et al., 1988).

In a bacterial transfection assay using *E.coli* CR63 cells, a linear increase in percentage inhibition of transfection (apparently indicative of mutagenic potential in this assay system) was noted using up to 10 µg acrylamide (Vasavada and Padayatty, 1981). The significance of this finding is uncertain, particularly in view of the negative results in standard bacterial assay systems.

Studies in mammalian cells

Cytogenetics assays

A well-conducted *in vitro* cytogenetics assay was available using V79 Chinese hamster cells exposed to 0-3,000 µg/ml acrylamide (>98% purity) with and without metabolic activation (Knaap et al., 1988). The exposure period was for 3 hours with fixation following a further 15 hours. A dose-related significant increase in the number of metaphases with chromosome aberrations was observed with and without metabolic activation. There was no clear information available regarding cytotoxicity.

Another *in vitro* cytogenetics assay used V79H3 Chinese hamster cells exposed to 0-5 mM (0-355 µg/ml) acrylamide (>99% purity) without metabolic activation (Tsuda et al., 1993). The exposure period was 24 hours and fixation followed a further 20 or 40 hours later. Again, there was a dose-related, statistically significant increase in the number of metaphases with chromosome aberrations. There was also a dose-related, statistically significant increase in polyploid cells at 20 and 40 hours (29% and 24% respectively at 4mM, 284 µg/ml). The incidence of chromosome aberrations and polyploid cells was less than 2% in negative controls, and the frequencies amongst acrylamide-exposed cells exceeded those of the positive control, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). There was no clear information available regarding cytotoxicity.

Acrylamide is clearly a direct-acting clastogen in mammalian cells *in vitro*.

Gene mutation assays

A number of published and unpublished *in vitro* gene mutation assays using mouse lymphoma L5178Y and Chinese hamster ovary cells (TK and HPRT deficiency) were available (Godek et al., 1982b; 1984; Knaap et al., 1988; Moore et al., 1987; Tsuda et al., 1993).

In an assay using Chinese hamster V79H3 cells (HPRT locus), cells were exposed without metabolic activation to 0-7 mM (0-500 µg/ml) acrylamide for 24 hours (Tsuda et al., 1993). The number of mutants per 10_6 survivors was 9, 8, 6, 5, 1, 2 at 0, 71, 213, 355, 416, 500 µg/ml, respectively. At 213 µg/ml survival was 59%, at 355 µg/ml or more, survival was 21% or less. Acrylamide was not mutagenic in this assay.

The following two assays reported either an equivocal or negative result but cell survival was noted to be high (about 70%). The current practice for *in vitro* mammalian cell gene mutation assays is to produce assay conditions in which survival is reduced to 20-50% and hence there are some doubts regarding the stringency of the test conditions employed.

Chinese hamster ovary cells (HPRT locus) were exposed to 37.5-900 µg/ml acrylamide (unknown purity) with and without S9 for 5 hours (Godek et al., 1982b). A slight dose-related increase in mutant frequency (less than 50% above the solvent control value of about 10 mutants/10^6 survivors) with S9 was observed at 300 µg/ml and above and at all exposure levels without S9.

In a follow-up study, a negative result was obtained using up to 1,500 µg/ml acrylamide with and without S9 (Godek et al., 1984). The frequency of mutants amongst acrylamide-exposed cells was not significantly greater than the negative controls. Survival was approximately 70%, cytotoxicity was observed at 1,500 µg/ml with S9, and mutant frequency was 285-330 mutants/10^6 survivors in the positive controls EMS and DMN.

In a mouse lymphoma L5178Y TK +/- assay, cells were exposed to 0-7,500 µg/ml acrylamide with and without metabolic activation for 2, 4, or 20 hours (Knaap et al., 1988). At each of the time-points, with and without metabolic activation (+ or - S9), there was a slight dose-related increase in mutant frequency: 19-36 mutants/10_6 survivors in controls; with 2 hours exposure $39/10^6$ at 2,500 µg/ml -S9 and $119/10^6$ at 7,500 µg/ml +S9, with 4 hours exposure $160/10^6$ at 2,000 µg/ml -S9 and $189/10^6$ at 2,500 µg/ml +S9; with 20 hours exposure $89/10^6$ at 300 µg/ml -S9 and $57\text{-}95/10^6$ at 300 µg/ml in the presence of metabolic activation (from primary rat liver or Syrian hamster ovary cells). These increases were associated with low survival (usually less than 30% survival). A positive result was obtained in this assay.

Similar increases associated with low survival were noted by Moore et al. (1987) in an assay using 0-850 µg/ml acrylamide (>99% pure) for 4 hours without metabolic activation studying large and small colonies. An increased mutant frequency (70-400 mutants/10^6 survivors, approximately $20/10^6$ in controls) was observed at 500 µg/ml acrylamide and above, and percentage survival was 40% at 500 µg/ml, 10% at 850 µg/ml. There was a dose-related increase in the frequency of small colonies and at 750 µg/ml and above mutants were mainly small colonies. The increase in small colony formation is suggestive of clastogenicity, and a separate assay for chromosome aberrations confirmed the increase (27% cells had chromosome and chromatid breaks or rearrangements).

The overall pattern from mammalian cell gene mutation assays is that acrylamide is a direct-acting mutagen, probably causing clastogenic effects rather than gene mutations.

DNA synthesis and repair

In an *in vitro* assay to measure unscheduled DNA synthesis (UDS) 0-100 mg/ml acrylamide was incubated with freshly isolated rat hepatocytes for 18-20 hours (Naismith and Matthews, 1982). Viability of the harvested cells was 88%. The positive control was 2-acetamidofluorene (2-AAF). Net nuclear grain counts were statistically significantly increased above negative controls (26-45 at 1-33 mg/ml, compared to about 1 in the negative controls) and in the first assay were close to the count achieved by 2-AAF. There was no clear dose-response for UDS. These results were confirmed by a repeat experiment.

An *in vitro* UDS assay was available in which 0-50 mM (0-3.55 mg/ml) acrylamide was incubated with rat hepatocytes for 18 hours (Miller and McQueen, 1986). Cytotoxicity (cell death) was observed at the highest concentration. A negative result was obtained in this and in a repeat experiment in that there was not an increase of 5 net nuclear grain counts over negative control values and there was no clear dose-response in the counts observed. The positive control 2-aminofluorene gave a clear increase in UDS.

In another *in vitro* rat hepatocyte UDS assay, 0-10 mM (0-710 µg/ml) acrylamide was incubated with the cells for 17-19 hours without metabolic activation (Butterworth et al., 1992). Amongst acrylamide-exposed cells there was no significant increase in the number of net nuclear grains when compared with the negative control. Toxicity (assessed by the morphological appearance of cultures) was observed at the highest concentration level. The sensitivity of the assay was confirmed using DMN, which gave a clear increase in net nuclear grains. Acrylamide did not induce UDS in this assay.

Two abstracts were available which briefly described rat hepatocyte UDS assays using acrylamide concentrations of up to 30 mM (about 2 mg/ml) (Barftnecht et al., 1987; Barftnecht et al., 1988). Acrylamide reportedly gave positive results in the presence and absence of metabolic activation at the higher exposure levels used in these experiments. In the absence of any other experimental details it is difficult to draw any firm conclusions about the validity and reliability of these results.

Rat hepatocytes previously irradiated with ultraviolet (UV) light to induce DNA damage were incubated with 10^{-3}-10 mM (0.7-710 µg/ml) acrylamide and tritiated thymidine (Miller and McQueen, 1986). The incorporation of tritiated thymidine was then assessed autoradiographically. A dose-dependent increase in net nuclear grain counts was observed following UV irradiation, and 10 mM (710 µg/ml) acrylamide only slightly increased the amount of DNA repair (from about 60 net nuclear grains to about 75). Lower levels of acrylamide than this did not affect DNA repair, and higher levels were reported to be cytotoxic. In addition, density gradient centrifugation of hepatocyte DNA on a caesium chloride gradient following incubation with BrdU and 10 mM (710 µg/ml) acrylamide showed that acrylamide did not induce DNA repair under the conditions of that system.

Overall, there are inconsistent findings in the available *in vitro* UDS studies and it is difficult to draw a definite conclusion.

Sister chromatid exchange

In an assay for sister chromatid exchange (SCE), V79H3 Chinese hamster cells were exposed to 0-3 mM (0-213 µg/ml) acrylamide (>99% purity) without metabolic activation for 24 hours followed by an additional 28 hours for bromodeoxyuridine (BrdU) incorporation; 50 cells per exposure level were counted (Tsuda et al., 1993). A slight, but dose-related and statistically

significant increase in SCE was seen (50% increase compared to an 800% increase by the positive control, mitomycin C). Similar findings were observed by Knaap et al., 1988 testing V79 cells in the range 0-3,000 µg/ml with and without metabolic activation with a 3-hour exposure period followed by 21-25 hours BrdU incorporation.

Chinese hamster ovary cells were exposed to 0-500 µg/ml acrylamide (purity not stated) with and without metabolic activation (Sorg et al., 1982b). The cells were treated for 5 hours followed by a 27-hour BrdU incorporation time and 30 cells per exposure level were counted for SCE. The results did not show statistically significant or dose-related increases in SCE compared to the solvent control. The positive controls ethyl methanesulphonate (EMS) and dimethylnitrosamine (DMN) doubled the number of SCE seen per cell in comparison with control. Comparing the results of positive controls in the previous experiment (Tsuda et al., 1993) it is possible that, under the conditions used, this assay may not have been sensitive enough to detect clear dose-responses in an increase in SCE. Therefore, the validity of the negative result obtained in this report is unclear.

Cell transformation

Cell transformation is not a reliable indicator of genotoxicity. Nevertheless, the studies have been summarised in this section for convenience. A number of published and unpublished mammalian cell transformation assays are available using BALB/3T3, C3H/10T½, and NIH/3T3 cell lines with and without metabolic activation (Banerjee and Segal, 1986; Microbiological Associates, 1984a, 1982a, 1982b; Tsuda et al., 1993). Positive results were obtained in all assays except those reported in Microbiological Associates (1982b). Overall, acrylamide increased cell transformation frequency in the *in vitro* cell lines tested, generally in the presence or absence of metabolic activation.

Other in vitro studies

A dose-related increase in the percentage of cells with spindle disturbances (increases in c-mitoses and fragmented or bridged ana-telophase figures) was observed by Adler et al. (1993) using Chinese hamster V79 cells exposed to up to 1 mg/ml acrylamide for 6 hours without metabolic activation.

Amongst controls, the incidence of c-mitoses was 1% compared to 91% at 0.5 mg/ml. The incidence of fragmented or bridged ana-telophase figures was 0.2% in controls compared with 1.1% at 0.1 mg/ml with no such figures being observed at higher concentrations due to the blocking of metaphase. For example, at 0.5 mg/ml more than 90% of metaphase figures were damaged, with a large number of figures scattered in the cytoplasm. In addition, the polyploidy index (the number of polyploid cells as a percentage of total mitoses observed) increased in a dose-related manner (from 1.4% in controls to 4.4% at 0.5 mg/ml and 9.8% at 1 mg/ml. At concentrations up to 0.5 mg/ml there were no clear signs of cytotoxicity, and at 1 mg/ml pyknosis was observed indicating the onset of a cytotoxic effect. The results of this study indicate that acrylamide is a potent spindle poison.

Human fibrosarcoma cells were exposed to 0-10 mM (0-710 µg/ml) acrylamide for 4 hours (Sickles et al., 1995). Cells were processed by Giemsa staining of chromosomes and then immunofluorescence staining to visualise microtubules. Colchicine was used as a positive control.

In negative controls 2% of cells were observed to be in mitosis. Colchicine dispersed the chromosomes throughout the cytoplasm and increased the percentage of cells in mitosis to 10% as expected from a substance that arrests cells in mitosis by disassembly of microtubules.

Acrylamide also caused a concentration-dependent increase in the number of cells in mitosis, but unlike with colchicine the chromosomes in cells exposed to acrylamide appeared to be condensed at the metaphase plate at all exposure levels used (>1mM, 71 µg/ml). This effect was more pronounced at higher concentrations. Hence with acrylamide, the formation of the mitotic spindle and chromosome alignment on the spindle was not apparently adversely affected, although subsequent chromsome segregation and migration were affected.

In an SV40-DNA amplification study CO60 Chinese hamster cells were exposed to 0-150 µg/ml acrylamide (Vanhorick and Moens, 1983). Cells were incubated with acrylamide for 24 hours before hybridization with ^{32}P-labelled SV40-DNA. Increased synthesis of SV40 DNA (DNA amplification) was seen at acrylamide concentrations of >50 µg/ml, concentrations that were associated with cell survival of 57% or less. The authors considered this result to indicate that acrylamide had little or no ability to induce SV40-DNA amplification (i.e. little or no potential to directly damage DNA in this system). The true significance of the findings in this invalidated system is unclear.

Summary of *in vitro* studies

Acrylamide is not mutagenic in standard bacterial assays when tested in the presence or absence of metabolic activation systems. However, acrylamide was clearly clastogenic (direct-acting) in mammalian cells *in vitro,* producing chromosome aberrations and polyploidy in two different cell systems investigated. Supporting evidence for *in vitro* clastogenicity was also evident in mammalian cell gene mutation assays.

4.1.2.7.2 Studies *in vivo*

Studies in Drosophila

Published and unpublished reports were available using different assay systems (investigating somatic and germ cells), with a mixture of positive and negative results (Batiste-Alentorn et al., 1991; Foureman et al., 1994; Knaap et al., 1988; Microbiological Associates, 1984b; Tripathy et al., 1991). The overall significance of these results for human health is unclear and much greater weight can be put on the mammalian cell system results, both *in vitro* and *in vivo*.

Somatic cells

Cytogenetics

Groups of 5 male mice received a single intraperitoneal (ip.) injection of 0 or 100 mg/kg aqueous acrylamide and 50 bone marrow cells per mouse were analysed for chromosome aberrations at 6, 18, 24, and 48 hours post-administration (Cihak and Vontorkova, 1988). No positive control was used. A clear, statistically significant increase in the number of metaphases with chromosome and chromatid breaks (3-11% excluding gaps compared to 1% in controls) was noted at the three later time-points with a maximum value at 24 hours.

Groups of 5 male and 5 female mice received single ip. injections of 0 or 100 mg/kg acrylamide in saline (Adler et al., 1988). Samples of bone marrow were taken at 12, 18, 24, 30, and 36 hours for analysis of chromosome aberrations. Statistically significant increases in the number of metaphases with chromosome and chromatid breaks were observed (2.6-4.4% excluding gaps compared to 0.7% in controls). The maximal response was at 18 hours in this assay. An additional dose-response assay was performed with mice receiving 0, 50, 100, and 150 mg/kg

acrylamide with a sampling time of 18 hours. A positive control group received cisplatin. Statistically significant increases in the frequency of aberrant cells were seen at all exposure levels (2.1%-4.1% excluding gaps compared to 0.3% in the negative control and 3.6% for the positive controls). Mitotic index was reduced by up to 27% compared with negative controls.

In another study, groups of 5 male mice received 500 ppm acrylamide (approximately 60 mg/kg/day assuming 25g bodyweight and food consumption of 3g/day) by dietary administration for 1, 2 or 3 weeks, or a single ip. injection of 0 or 100 mg/kg (Shiraishi, 1978). At least 100 bone marrow cells were scored for chromosome breaks per animal at 1/2, 1, 11 and 12 days post-administration in the case of ip. treated mice, and immediately after sacrifice at weeks 1, 2, and 3 for animals receiving dietary acrylamide (animals were pretreated with colchicine). There was no mention of the use of positive controls in this study. Following single-exposure there was an increase in metaphases with chromosome breaks (2.7% in negative controls, 3.5-7% in treated animals). There was an increase in the frequency of cells with aneuploidy or polyploidy (3.7% in negative controls, 5-10.5% in treated animals). For animals treated by the dietary route there were similar slight increases. In addition, there was also a slight, but not statistically significant increase in SCE/cell (2.9 in controls and 3.5-3.7 in treated animals). It was not clear from the results that were presented whether or not results at each time point were compared with control groups or if any statistical comparisons had been performed. The author concluded these changes to be negative responses. However, there are some doubts about the validity of this assertion due to the limitations in the presentation of information. The values presented for chromosome breaks would, by current standards, indicate a positive result.

In a further study using cells from an unconventional source, groups of 4 male mice received single ip. injections of 0, 50, 100, or 125 mg/kg acrylamide (Backer et al., 1989). Positive controls received cyclophosphamide. Mitoses were analysed from 100 spleen lymphocytes at each exposure level 24 hours post-administration only. There was no clear increase in the frequency of metaphases with chromosome aberrations but there was a non-statistically significant increase in chromatid aberrations: 5% at 125 mg/kg compared to 2% in negative controls at 125 mg/kg. There was also a significant dose-related increase in the frequency of SCE/cell. In view of the non-validated nature of this assay system no firm conclusions can be drawn from the results.

Overall, the results of these studies indicate that acrylamide produces chromosome aberrations in somatic cells *in vivo*.

Micronucleus assays

In addition to the chromosome aberration analysis performed on bone marrow cells taken from acrylamide-exposed mice a micronucleus assay was conducted (Adler et al., 1988, see Cytogenetics section above for details). There was no significant change in the ratio of polychromatic to normochromatic erythrocytes (P/N ratio). However, clear, statistically significant increases in micronucleus frequency were observed following single intraperitoneal administration of 100 mg/kg acrylamide at 18, 24, and 30 hours with maximum values at 24 hours (0.66% compared to 0.13% in negative controls). Subsequently, groups of 5 male and 5 female mice received 0, 50, 75, and 125 mg/kg acrylamide with samples taken at 24 hours. Clear, statistically significant, and dose-related increases in micronucleus frequency were seen at all exposure levels. A frequency of 1% was observed with the positive control, cisplatin, and at 125 mg/kg acrylamide the frequency was 0.9%.

A number of other *in vivo* micronucleus assays in male and female mice were available all giving positive results using cells taken from bone marrow, spleen or peripheral blood with up to 150 mg/kg aqueous acrylamide administered by the intraperitoneal route singly or repeatedly (Backer et al., 1989, Cao et al., 1993, Cihak and Vontorkova, 1988, Cihak and Vontorkova, 1990, Knaap et al., 1988, Russo et al., 1994). Sampling times ranged from 6-72 hours with the peak effects generally observed at 24 hours.

A negative result was obtained in an unpublished *in vivo* bone marrow mouse micronucleus assay using males and females receiving 75 mg/kg aqueous acrylamide by oral gavage singly or repeatedly (Sorg et al., 1982a). Clinical signs of toxicity were observed following single and repeated (2x) administration of 75 mg/kg. Sampling times were 30, 48, and 72 hours. The positive control used was triethylenemelamine, given intraperitoneally. Overall, there are some doubts about the validity of the negative result obtained as no sampling times less than 30 hours were used.

Liver UDS assay

In an *in vivo* liver UDS test groups of rats received single or repeated (5x) ip. injections of 0, 30, or 100 mg/kg acrylamide (Butterworth et al., 1992). In the case of single exposure, animals were sacrificed 2 or 12 hours post-administration and for repeated-exposure animals sacrifice was 4 hours after the last injection. Hepatocytes were isolated and incubated with tritiated thymidine for 4 hours. There were no increases in net nuclear grain counts. The positive control, DMN, produced a clear response. Thus, ACR did not cause UDS in liver cells *in vivo*.

Mammalian spot test

As part of a study investigating potential mutagenic and developmental effects (see also Section 4.1.2.9 Toxicity for reproduction), groups of 31-93 pregnant female mice received single or 3 daily ip. injections of 0, 50 or 75 mg/kg aqueous ACR on day 12 or days 10, 11, and 12 (Neuhauser-Klaus and Schmahl, 1989). Approximately 220-300 offspring per dose level were available for examination. A positive result (doubling in the number of spots of genetic relevance compared to negative controls) was reported following single exposure to either 50 or 75 mg/kg and to repeated exposure to 50 mg/kg/day. Repeated exposure to 75 mg/kg resulted in increased embryotoxicity and cytotoxicity.

Studies in transgenic mice

As part of a validation for a new test method, groups of mice received 5 daily ip. injections of 0 or 50 mg/kg acrylamide - 3, 7, and 10 days later the *LacZ* mutation system was used to determine mutant frequency (MF) in bone marrow only (Hoorn et al., 1993 and Myhr 1991). An increase in MF was noted ($62\text{-}89 \cdot 10^6$ compared to $15\text{-}26 \cdot 10^6$ in controls), although with no clear pattern with respect to sampling time. Procarbazine and ethyl nitrososurea gave more substantial increases. Although demonstrating positive results, the full significance of the results in this as yet unvalidated assay is unclear but the result does provide support for the view that acrylamide is an *in vivo* genotoxicant.

Summary of *in vivo* mammalian somatic cell assays

Acrylamide is clearly mutagenic *in vivo*, producing positive results particularly in the bone marrow micronucleus assay. The pattern of results indicates clastogenicity or interference with mitosis rather than gene mutation activity.

Germ cells

Cytogenetics

Groups of 5-16 male mice received single ip. injections of 0 or 75 mg/kg acrylamide and were mated with untreated females 7 days later, or received 125 mg/kg with mating 7 or 28 days later, or 5 daily injections of 50 mg/kg with mating 7 days post-administration (Pacchierotti et al., 1994). Chromosome aberrations from one-cell zygotes were scored at 5 hours with at least 100 metaphases scored per group except for animals receiving repeated exposure to acrylamide where chromosome effects were very common and fewer metaphases were analysed. In addition, flow cytometry was performed for cells taken from testicular preparations at 3 and 35 days after treatment with up to 150 mg/kg acrylamide (elongated spermatids, round spermatids, diploid cells, S-phase cells, tetraploid cells, and elongated/elongating diploid spermatids were counted).

From one-cell zygotes a statistically significant, dose-related increase in the frequency of aberrations was noted following mating at 7 days and to a lesser degree at 28 days after mating with chromosome fragments, dicentrics and translocations being prominent. For repeated exposure 85% of zygotes were reported to contain chromosome aberrations.

From testicular cell populations there was a marked decrease (~74% of control values) in tetraploid cells 3 days after treatment amongst animals receiving a single exposure. The total cell number was not apparently affected. At 35 days there was a statistically significant, dose-related decrease in the percentage of elongated spermatids (70% of control values at 150 mg/kg) and a statistically significant increase in elongated/elongating diploid spermatids suggesting impaired segregation during mitosis. A decrease in diploid spermatids was noted 3 days post-exposure following single exposure but not after repeated exposure; the authors suggest that this effect was due to spermatocytes being affected during meiosis by the initial injections.

Following on from the assay reported in the section on somatic cell effects, groups of 5 male mice received 500 ppm acrylamide (approximately 60 mg/kg/day assuming 25g body weight and food consumption of 3g/day) by dietary administration for 1, 2 or 3 weeks or a single ip. injection of 0 or 100 mg/kg (Shiraishi, 1978). At least 100 metaphases from spermatogonia were scored for chromosome aberrations per animal at 12 and 24 hours, and 11 and 12 days post-administration in the case of ip. treated mice and immediately after sacrifice for animals receiving dietary acrylamide. In addition, 50-100 spermatocytes in the diakinesis-metaphase *I* stage were examined from each animal.

An increased incidence of spermatogonia with aneuploidy, chromosome breaks, and sister chromatid exchanges was seen using both exposure regimes. Similarly, amongst primary spermatocytes there was a marked increase in sex-chromosome and autosomal univalents, fragments and rearrangements observed in both exposure regimes.

Also following work in somatic cells, groups of 4 male mice received single ip. injections of 0, 50, 100, or 125 mg/kg acrylamide (Backer et al., 1989). Chromosome and chromatid aberrations were scored in spermatogonia and spermatocytes 24 hours post-administration only. There were no significant changes in the number of chromosome/chromatid aberrations or hyperploidy compared to negative controls. This study is limited by the use of only one sampling time.

As part of a dominant lethal assay summarised below (Smith et al., 1986) cytogenetic examination was performed on rat spermatocytes taken from 10-11 males exposed to 0, 1.5, 3, or 6 mg/kg/day acrylamide in drinking water for 80 days and after a 12-week recovery period. No

increase in structural aberrations was observed on completion of 80 days although a slight increase in reciprocal translocations was noted amongst treated animals (0, 1, 1, and 2 in each of the groups respectively). The significant increase in pre-implantation loss would suggest that an adequate exposure level was used. However, no further details were available regarding the conduct of this investigation hence it is difficult to draw any firm conclusions regarding the potential to form chromosome aberrations in rat spermatocytes from this report.

Germ cell micronucleus assays

Micronucleus formation in spermatids was studied using groups of 5 male rats receiving single ip. injections of 0, 50, or 100 mg/kg acrylamide (Lahdetie et al., 1994). Animals were sacrificed on days 1, 3, 18 and 19 post-administration and 2000 spermatids/animal were analysed. The positive control used was mitomycin C. following this, groups of 5 male rats received 4 daily ip. injections of 50 mg/kg acrylamide and were sacrificed on days 1, 3, 18 and 19 post-administration. Analysis of spermatids at these sacrifice times correspond to cells that would have been exposed to acrylamide as spermatocytes in diplotene-diakinesis (day 1), late pachytene (day 3), and preleptotene stages of meiosis (day 18) and as intermediate and type B spermatogonia (days 18 and 19). A statistically significant increase in the number of micronuclei was observed from cells sampled on day 18 following exposure to 4·50 mg/kg (2.0/1000 early spermatids compared to 0.55/1000 in negative controls).

Groups of 4-5 male rats received 0, 50, or 100 mg/kg acrylamide by single ip. exposure or 4 daily injections of 50 mg/kg (Xiao and Tates, 1994). Spermatocytes were isolated and analysed for micronucleus formation on days 1, 3, 15, 18, 19, and 20 after acrylamide administration.

Statistically significant increases in micronucleus formation were observed 18-20 days after single ip. exposure to 0, 50 or 100 mg/kg acrylamide and the effect was more marked following 4 daily ip. injections of 50 mg/kg. For single exposures, the frequency of micronucleus formation amongst acrylamide-treated animals was 3.3-4.2/1000 early spermatids compared to 1.5/1000 in the negative controls 18-20 days after acrylamide-administration. Slight, but not statistically significant, increases were also observed 1-3 days after administration (up to 2/1000 at 100 mg/kg). The most significant increases (day 18-20) would have corresponded to spermatids initially exposed in the preleptotene stage - a result consistent with a number of other studies. Amongst repeated-exposure animals, the frequency of micronucleus formation reached 6.4/1000 on day 19.

A similar increase in micronucleus formation was reported by Russo et al. (1994) using Golgi-phase and Cap-phase spermatids (post-meiotic developmental stages) from acrylamide-exposed mice. In addition, differentiating spermatogonia were assessed for SCE – a statistically significant, exposure-related increase in SCE was noted.

DNA synthesis and repair

Groups of 4-6 male mice received single ip. injections of 0, 8, 16, 31, 63, or 125 mg/kg acrylamide (Sega, 1990). Tritiated thymidine was injected into the testes 0-48 hours after acrylamide administration and sperm from the caudal epididymides were recovered 16 days post-administration for UDS analysis. In addition, groups of 4-6 male mice received a single ip. injection of 0 or 125 mg/kg acrylamide with tritiated thymidine injected into the testes 6 hours later and sperm from caudal epididymides (spermatozoal to early spermatocytes at the time of treatment) was recovered at 2-3 day intervals for up to 30 days post-administration for UDS analysis. Also, groups of 4 male mice received ip. injections of 46 mg/kg [^{14}C]-acrylamide. DNA

was extracted from liver and testes samples 1-24 hours post-administration and analysed for radioactivity.

In the first experiment, there was a clear increase in UDS, the maximum response of one order of magnitude greater than that of controls occurring 6 hours after tritiated thymidine injection. This peak response related to sperm, which would have been in the early spermatid stage at the time of acrylamide exposure. For the second experiment, no significant amounts of tritiated thymidine were incorporated during the first 10 days following exposure to 125 mg/kg acrylamide but from days 12-27 a positive UDS response was seen. In the third experiment DNA alkylation was observed, which reached maximum levels 4-6 hours post-administration in the testes and 1-2 hours post-administration in the liver, with levels being substantially (10-fold) lower in testes than liver.

In an *in vivo* spermatocyte UDS test groups of F344 rats received single or repeated (5x) ip. injections of 0, 30, or 100 mg/kg acrylamide (Butterworth et al., 1992). In the case of single exposure, animals were sacrificed 2 or 12 hours post-administration and for repeated-exposure animals sacrifice was 4 hours after the last injection. Following repeated administration of 30 mg/kg there was a statistically significant increase in the number of net nuclear grain counts (5.4 and 5.6 compared to 0 in controls). The positive controls MMS and cyclophosphamide gave counts of 4.9 and 6.5 respectively. For single administration, the increase was not significant (1.5 and 1.6). Overall, the results indicate that acrylamide caused an increase in UDS in rat spermatocytes following repeated exposure.

Dominant lethal assays

- Studies in mice

Groups of 24-30 male mice received 0, 25, 50, 75, 100, or 125 mg/kg/day acrylamide in 70% aqueous methanol by the dermal route for 5 consecutive days (Gutierrez-Espeleta et al., 1992). Males were then mated with untreated females from day 7-10 after the last exposure. There was a slight, but treatment-related decrease in the mean number of implantations per pregnant female (21 in controls, 16 at 125 mg/kg/day). The percentage of dead implants (3%, 11%, 20%, 46%, 61%, and 76% respectively at 25, 50, 75, 100, and 125 mg/kg/day) and number of pregnant females with one or more dead implant was statistically significantly increased amongst all treated animals when compared with controls (20/78 in controls, 29/36, 40/43, 51/57, 40/42, and 27/27 respectively). The number of live embryos per pregnant female was approximately 10 in controls and decreased in a treatment-related manner to approximately 2 at 125 mg/kg/day. There was a corresponding treatment-related increase in dominant lethality (up to 91% at 125 mg/kg/day). Overall, these results indicate that repeated dermal exposure of male mice to 25 mg/kg/day or more acrylamide for 5 days resulted in dominant lethal effects in the progeny, and the reduced number of pregnant females is suggestive of reduced male fertility.

Male mice received a single ip. injection of 125 mg/kg or 5 daily injections of 50 mg/kg acrylamide (>99% pure) prior to mating with females (Shelby et al., 1986). In addition there was an assay performed with mating over a limited period (days 6-10 after acrylamide-treatment) such that the available sperm would derive from cells exposed as late spermatids or epididymal sperm. Dominant lethality, observed as an increased frequency of dead implants particularly between days 4-12 post-administration, was noted following single and repeated exposure. The early increase was suggestive of an effect on late spermatids and early spermatozoa, a result consistent with DNA alkylation studies reported by Sega and Generoso (1990a), effects noted in testicular cell

populations by Sakamoto et al. (1988) (see Section 4.1.2.2 Acute toxicity), and the distribution studies by Sega et al. (1989), and Marlowe et al. (1986) (Section 4.1.2.1 Toxicokinetics).

In a study reported as a brief abstract, groups of male mice received approximately 0, 0.7, 2.1, or 6 mg/kg/day acrylamide in drinking water for 140 days (20 weeks) (Bishop et al., 1991). Males were then mated with untreated females which, 16 days later, were examined for implantations, live/dead foetuses, and resorptions. A significantly higher percentage of resorptions was found amongst animals receiving 6 mg/kg/day (13% compared to 7% in controls). This report should be treated with a little caution due to insufficient reporting detail, but the results provide further support that acrylamide induces dominant lethal mutations in the germ cells of male mice.

Other positive results for dominant lethality in mice were obtained as part of studies to investigate heritable translocations (Shelby et al., 1987; Adler et al., 1994; Ehling and Neuhauser-Klaus, 1992 - see below for details). In addition positive results for dominant lethality were also obtained in a combined dominant lethal/two-generation reproduction study (NTP, 1993; Chapin et al., 1995) and in another crossover breeding study (Sakamoto and Hashimoto, 1986) - see Toxicity to reproduction, Section 4.1.2.9.

- Studies in rats

Groups of 10-11 male rats were exposed to 0, 1.5, 3, or 6 mg/kg/day acrylamide in drinking water for a total of 80 days (Smith et al., 1986). After 72 days males were mated with untreated females. Significantly increased pre-implantation loss was noted amongst females mated with high dose males. Post-implantation loss was increased at 3 and 6 mg/kg/day. There were no clinical or histopathological signs of neurotoxicity which may have affected male fertility.

Other positive results for dominant lethality were reported in studies in which rats received up to 100 mg/kg/day for 5 days by oral gavage as part of investigations into potential reproductive effects (Sublet et al., 1989; Tyl, 1998a; see also Section 4.1.2.9) and also as part of a combined two-generation/dominant lethal assay in which rats received up to 5 mg/kg/day acrylamide in drinking water for 10 weeks (Tyl, 1987; see Section 4.1.2.9).

Heritable translocation assays

Groups of 120 male mice received 5 daily ip. injections of 0, 40 or 50 mg/kg acrylamide (99% pure) and were mated with untreated females 7-10 days after the last injection (Shelby et al., 1987). Male progeny were weaned and females discarded. The males were then mated with additional untreated females. If reduced fertility was observed, the males were again mated with additional females, which were sacrificed on day 14 for examination of the uterine contents. In addition, spermatocytes from males with reduced fertility underwent cytogenetic analysis. The repeated administration of 50 mg/kg/day acrylamide resulted in a "high level" (percentage not stated) of dominant lethal mutations, and 40 mg/kg/day produced approximately 70% dominant lethality. For the heritable translocation studies, 49/125 (39%) males at 50 mg/kg/day and 39/162 (24%) at 40 mg/kg/day were either sterile or semisterile. This compared with a historical control incidence of 17/8095 (0.2%). All 10 males selected for cytogenetic analysis were confirmed as translocation carriers. In addition, 31-85% of females mated with semisterile males carried dead implants, compared with 0-9% in females mated with apparently non-sterile males.

A single ip. injection of 0, 50 or 100 mg/kg or 5 daily injections of 50 mg/kg acrylamide in male mice (Adler et al., 1994). There was a similar selection of sterile/semisterile animals to the previous study. Dominant lethal effects were observed at the highest exposure level and there

was an exposure-related increase in heritable translocations; 3/8700 (0.04%) in controls, 2/362 (0.6%) at 50 mg/kg, 10/367 (2.7%) at 100 mg/kg, 23/105 (22%) at 5·50 mg/kg.

The results of these two studies demonstrate that acrylamide caused heritable translocations in mice.

Specific-locus assays

Groups of male mice received ip. injections of 0, 100, or 125 mg/kg aqueous acrylamide (Ehling and Neuhauser-Klaus, 1992) and were serially mated with untreated females (homozygous for a number of key physical features). A high frequency of specific-locus mutations was noted for males mated with females 5-8 days and 9-12 days after injection (6-14 mutations per locus per 10^5 gametes for males receiving 100 and 125 mg/kg Vs 1.3 per 10^5 gametes in controls). This would indicate that specific-locus mutations occurred in male spermatozoa and spermatids.

In another assay for specific-locus mutations, male mice received 5 repeated ip. doses of 50 mg/kg (Russell et al., 1991). Increased frequencies of specific-locus mutations were observed for males mated with females 8-14 and 15-21 after injection suggestive of specific-locus mutations amongst the late spermatid and spermatozoal stages.

Studies in transgenic mice

As part of study to validate a new test method, groups of male mice received 5 daily ip. injections of 0 or 50 mg/kg acrylamide with assays performed on testicular cell preparations examining the *LacZ* mutation system 21-23 days later (Murti et al., 1994). There was no clear increase in mutations noted in this system. Microscopic examination showed an increase in the number of "unusually large cells" which were speculated, but not proven by the authors, to be due to interkinetic delay during meiosis caused by acrylamide. Overall, no firm conclusions can be drawn on the nature and significance of effects seen.

Other germ cell studies

Groups of male mice received a single ip. injection of 0 or 100 mg/kg acrylamide (99.9% pure) following testicular injection of tritiated thymidine, and mature spermatozoa were removed daily for 21 days post-administration (Sega and Generoso, 1990). DNA was removed by alkaline elution and a significant increase in single-stranded breaks was observed in treated animals, the greatest elution rate being observed in the second week post-exposure. Further analysis showed that DNA breakage following acrylamide exposure was occurring mainly in early and mid-late spermatids as well as pachytene spermatocytes.

Other information regarding DNA binding, distribution to male reproductive organs, and effects on testicular cell populations was available in the studies by Sega et al. (1989) and Marlowe et al. (1986) (see Toxicokinetics, Section 4.1.2.1), Sakamoto et al. (1988) (see Acute toxicity, Section 4.1.2.2).

Summary of *in vivo* mammalian germ cell assays

Acrylamide is clearly positive in a number of different germ cell assays (chromosome aberrations, micronucleus assays, UDS, dominant lethal assays, heritable translocation, and specific locus assays) indicating that it is a germ cell mutagen.

4.1.2.7.3 Summary of mutagenicity

A substantial body of information is available covering many genotoxicity end points. Although acrylamide is not mutagenic in bacteria, its mutagenic potential is clearly shown in mammalian systems *in vitro.* It is a direct-acting mutagen and there is also a large body of evidence clearly demonstrating that acrylamide is genotoxic *in vivo* to both somatic cells and germ cells. In the case of germ cells, acrylamide has been demonstrated to induce heritable mutations. For classification, see Chapter 1.

4.1.2.8 Carcinogenicity

4.1.2.8.1 Studies in animals

Inhalation

No carcinogenicity studies were available using the inhalation route of exposure.

Oral

In a combined chronic toxicity/carcinogenicity study conducted according to modern protocol standards, groups of 90 male and 90 female F344 rats received 0, 0.01, 0.1, 0.5, or 2.0 mg/kg/day aqueous acrylamide (>96% pure) in drinking water for up to 2 years (Johnson et al., 1984; 1986). Routine observations included clinical signs of toxicity, bodyweight, food and water consumption, haematology, urinalysis, blood biochemistry, macroscopic and microscopic pathology. Groups of 10 animals were selected at 6, 12, and 18 months for interim sacrifice.

A statistically significant increase in mortality was noted from 21 months onwards amongst males and females receiving 2 mg/kg/day. A slight decrease in bodyweight (up to 4%) was noted amongst males at 2 mg/kg/day and there were no significant effects on food and water consumption and no clinical signs of toxicity. There were no significant adverse effects on haematology, blood biochemistry, or urinalysis examinations, organ weights, or macroscopic pathology at 6, 12 and 18 months. However, at 24 months, there was an increase in the number of subcutaneous and mammary gland masses amongst females at 2 mg/kg/day. Histopathologically, there were no abnormalities at 6 months. At examinations performed from 12 months onwards there was an increase in the incidence and severity of tibial nerve degeneration amongst males at 2 mg/kg/day and from 18 months onwards in females at 2 mg/kg/day (focal swelling of nerve fibres with fragmentation of myelin and axon, and the formation of vacuoles containing small round eosinophilic globules and macrophages). There were no clear changes amongst animals at lower exposure levels or amongst other peripheral nerve samples (saphenous branch of the femoral nerve and brachial plexus).

In males, there was a statistically significantly increased incidence of benign follicular cell adenomas of the thyroid at the highest dose level (1/60, 0/58, 2/59, 1/59, 7/59). In females there was a non-significant increase in the incidence of benign follicular cell adenomas of the thyroid (0/58, 0/59, 1/59, 1/58, 3/60) and malignant adenocarcinomas (1/58, 0/59, 0/59, 0/58, 3/60). In females there was a statistically significant increase in the incidence of malignant adenocarcinomas in the uterus (1/60, 2/60, 1/60, 0/59, 5/60, or 1.7%, 3.3%, 1.7%, 0, 8.3%). The historical control range was stated to be 0-2.3%. In males there was a statistically significant increase in the incidence of malignant testicular mesothelioma at 0.5 and 2 mg/kg/day (3/60,

0/60, 7/60, 11/60, 10/60 or 5%, 0, 12%, 18%, 17%). The historical control incidence was 3.1% with a range of 2-6%.

In males there was a non-significant increase in the incidence of malignant astrocytomas in the spinal cord (1/60, 0/60, 0/60, 0/60, 3/60). There were also non-significant increases in malignant astrocytotomas in the brain of females (0/60, 1/60, 0/60, 0/60, 3/60), glial proliferation in the brain suggestive of an early tumour (0/60, 0/60, 0/60, 1/60, 3/60), and malignant astrocytomas in the spinal cord (1/60, 0/59, 0/60, 0/60, 3/61). In addition, malignant astrocytomas were also observed in the brain (3/60, 0/60, 0/60, 2/60, 2/60), and glial proliferation (suggestive of an early tumour) in 0/60, 0/60, 0/60, 1/60, 1/60. The effects in astrocytomas for brain and spinal cord in males and females do not show any clear dose-response but there are some concerns as these tumours are occurring in potential target tissues and, according to the authors, the concurrent control values may have been abnormally high so trends would not have been clear. Also, the group sizes used in this study may not have been sufficiently large enough to detect clear increases. Overall, because of these limitations, the toxicological significance of the presence of these astrocytomas in this study is unclear.

For females, there was a statistically significant increase in the incidence of benign papillomas in the oral cavity at 2 mg/kg/day (0/60, 3/60, 2/60, 1/60, 7/61) and a non-significant increase in focal hyperplasia (1/60, 2/60, 1/60, 0/60, 4/61). The incidence of malignant carcinomas did not show any clear dose-response (0/60, 0/60, 0/60, 2/60, 1/61). For males, the incidence of tumour formation in the oral cavity did not show any clear exposure relationship (carcinomas 2/60, 0/60, 1/60, 0/60, 2/60, and papillomas 4/60, 7/60, 0/60, 5/60, 4/60) although there was a statistically significant increase in focal hyperplasia of the hard palate (0/60, 1/60, 1/60, 1/60, 4/60, 5/60). Again, although effects are not clear, there are some concerns as there is a possibility that hyperplasia and subsequent, but unclear, tumour formation may have arisen as a result of local effects due to the route of exposure employed.

In females there were increases in benign and malignant tumours of mammary glands (10/60, 11/60, 9/60, 19/58, 23/61 and 2/60, 1/60, 1/60, 2/58, 6/61 respectively or 17%, 18%, 15%, 33%, 38% and 3%, 2%, 2%, 3%, 10%), benign pituitary gland adenomas (25/59, 30/60, 32/60, 27/60, 32/60 or 42%, 50%, 53%, 45%, 53%), and benign tumours of the clitoral gland (0/2, 1/3, 3/4, 2/4, 5/5). In males there were increased incidences of benign tumours in the adrenal glands (pheochromocytoma) (3/60, 7/59, 7/60, 5/60, 10/60 or 5%, 12%, 12%, 8%, 17%). The increased incidences of mammary tumours, benign pituitary adenomas and adrenal pheochromocytomas are of doubtful toxicological significance due to the poor dose-response and high historical control incidence (18% for benign mammary tumours, 2% for malignant mammary tumours - NTP data only, 28-47% for pituitary adenomas, 1-14% for pheoachromocytomas). For clitoral adenomas the total number of tissues examined was too small to draw any firm conclusions.

A second, carcinogenicity study was conducted according to modern protocol standards using larger group sizes to clarify the tumour profile:

Groups of 75-204 male F344 rats received 0, 0.1, 0.5, and 2 mg/kg/day acrylamide (99.9% pure) in drinking water for up to 2 years, and groups of 50-100 females received 0, 1, and 3 mg/kg/day (American Cyanamid Co., 1989; Friedman et al., 1995). Two control groups were used for each sex. Routine observations included clinical signs of toxicity, food and water consumption, bodyweight, macroscopic- and microscopic pathology.

Increased mortality was noted amongst males receiving 2 mg/kg/day from 17 months onwards and in females at 3 mg/kg/day in the final month (75% Vs 44-53% in males, 49% Vs 28-40% in females at termination). There was an increased incidence of palpable subcutaneous masses in

males and females during the last 6 months. There were no clinical signs of toxicity and no adverse effects on food and water consumption. Bodyweight was statistically significantly reduced by up to 9% amongst all treated males and by up to 7% in females (although not attaining statistical significance at 1 mg/kg/day) compared to the pooled control values at termination.

An increase (about 20-30%) in testicular weight was noted in males at 0.5 and 2 mg/kg/day. However this was considered to be of uncertain importance due to the high background incidence of interstitial cell tumours in this strain of rat - testes with large tumours which would have distorted testicular weight values were not excluded from organ weight analysis.

Histopathologically, an increased incidence of minimal to mild degeneration of the sciatic nerve (vacuolation of nerve fibres) was observed amongst females at 3 mg/kg/day and in males at 2 mg/kg/day.

There were increases in thyroid follicular adenomas (attaining statistical significance at 2 mg/kg/day) and a non-significant increase in carcinomas amongst males (3/204, 9/203, 5/101, 12/75 and 3/204, 3/204, 0/102, 3/75 respectively). Similarly, in females, there were increases in thyroid follicular adenomas and carcinomas (0/100, 7/100, 16/100 and 2/100, 3/100, 7/100 respectively). In males, there was a statistically significant increase in malignant scrotal mesothelioma at 2 mg/kg/day (8/204, 9/204, 8/102, 13/75).

In the brain the following increased incidences of benign and malignant tumours were noted: astrocytoma (1/204, 0/98, 0/50, 2/75 or 0.5%, 0, 0, 3% at 0, 0.1, 0.5, and 2 mg/kg/day respectively amongst the males and 0/100, 2/100, and 2/100 or 0, 2%, 2% at 0, 1, and 3 mg/kg/day respectively in females), meningioma (0/100, 2/100, and 3/100 in females, no significant increase in males), malignant reticulosis (0/100, 2/100, and 3/100 in females, no significant increase in males). These tumours may not be related to acrylamide exposure; combined historical control data from NTP studies exist with a range of glial cell tumours in the brain of up to 4%. Individual occurences astrocytomas in the spinal cord were observed in males and females but at very low incidence (0/172, 1/68, 0/102, 1/51 in males and 1/100 at 3 mg/kg/day, none in other groups for females). These findings do not show clear dose responses, and do not attain statistical significance. However, some concerns do remain, as there is a suggestion, although not convincing, of some changes at the highest dose levels and because the brain and spinal cord represent possible target tissues for acrylamide.

In females, there were increased incidences of mammary gland fibroadenomas (9/96, 20/94, 26/95 respectively) and adenocarcinomas (2/96, 2/94, 4/95). As with the previous study, these tumours are of doubtful toxicological importance as mammary tumours occur at a high spontaneous incidence in rats. There were no significant increases in the incidence of neoplastic findings in the uterus, clitoral gland, pituitary gland and oral cavity. Unfortunately, sections did not appear to be taken from the oral cavity of all available animals making it difficult to draw firm conclusions regarding potential tumour formation at this site.

Overall, for acrylamide-exposed rats there are clear increases in tumours in several organs. Some of the tumour types observed in these two rat studies show a possible relationship with disturbed endocrine function (for example thyroid, testicular mesothelioma, adrenals) and raise the possibility of a hormonal mechanism. However, acrylamide is clearly genotoxic and it is possible that such tumours could have arisen following direct damage to the hormone-producing organ. There is also a suggestion of tumours in the brain and spinal cord and, although the picture is not clear, these are possibly acrylamide-induced.

Dermal

No carcinogenicity studies were available using the dermal route of exposure.

Other animal studies

The following 2 studies are not true carcinogenicity bioassays but provide some, albeit limited, information:

In a skin initiation/promotion assay, groups of 16-40 female SENCAR and ICR mice received 0 or up to 50 mg/kg aqueous acrylamide (>99% pure) or acrylamide in ethanol (for dermal studies) 3 days/week for 2 weeks by oral gavage, ip. injection or topically (Bull et al., 1984a; 1984b). Tetradecanoyl-phorbol acetate (TPA) was then administered dermally 3 days/week for 20 weeks to most groups (there were some non-TPA controls) and animals were sacrificed on completion of 52 weeks. An acrylamide dose-related increase in tumour formation was noted for all routes of acrylamide administration when TPA was administered subsequently but there was no increase in tumour incidence in mice treated with acrylamide but not subsequently with TPA. These results suggest that acrylamide was "initiating" tumour formation.

In addition, in a lung adenoma bioassay (Bull et al., 1984a), groups of 16-40 male and female A/J mice received up to 25 mg/kg aqueous acrylamide by oral gavage or up to 60 mg/kg by the ip. route 3 days/week for 8 weeks. These animals were sacrificed after 8-9 months. In the adenoma bioassay, there was also an exposure-related increase in the formation of lung tumours. The enhancement of benign lung tumour incidence by acrylamide in a mouse strain showing a high background incidence of such tumours is of doubtful significance in relation to human health.

4.1.2.8.2 Studies in humans

A cohort mortality study was available investigating populations of workers exposed to acrylamide at 3 factories in the United States and one in the Netherlands (Collins et al., 1989). The cohort was defined as all workers hired between January 1, 1925 and January 31, 1973 with data collected up to December 31, 1983. From the US factories 8,508 men were identified, with complete follow-up for 94% - of this group 5,847 were still alive at the end of the study, 2,148 were dead (with the cause of death not determined in 111), and 513 were lost to the follow-up. In the Netherlands 346 men were identified, 315 were alive at the end of the study, 11 were dead (with the cause of death not determined in 2), and 20 lost to follow-up.

Occupational exposure information was available from 1977 (8-hour TWA values from personal monitoring). Estimates were made of exposure before this time on the basis of these values and from the knowledge of processes involved. The extent of dermal exposure was unclear. Comparisons were made against an internal population where cumulative airborne exposure was less than 0.001 mg/m3-years (approximately equivalent to one day exposure to 0.3 mg/m^3). There were 2,293 in the "acrylamide-exposed" group (those exposed to >0.001 mg/m^3-years) and 8,094 people in the group of "unexposed" workers (those exposed to <0.001 mg/m^3-years). Amongst the 2,293 "acrylamide-exposed" workers, there was no clear breakdown of the numbers in each of the sub-groups used (0.001-0.03, 0.03-0.3, >0.3 mg/m^3-years). For the "acrylamide-exposed" group, approximately half of the person years of exposure were at 0.3 mg/m^3-years or more (Collins et al., 1990). In this group the median exposure was around 5 mg/m^3-years (equivalent to about 15 years exposure to 0.3 mg/m^3).

Overall, taking into account the entire cohort membership, the standardised mortality ratio (SMR) for all causes of death was not increased at any of the four plants. There were no statistically significant increases in the SMR for cause-specific mortality amongst the workers exposed to >0.001 mg/m^3-years ("acrylamide-exposed"). When this group was further subdivided there were no statistically significant increases in deaths resulting from any cause in any of the three exposure categories (overall 33 deaths observed against 44 expected at 0.001-0.03 mg/m^3, 97 observed vs. 97 expected at 0.03-0.3 mg/m^3, and 169 observed vs. 158 expected at >0.3 mg/m^3). Amongst "acrylamide-exposed" workers, there was a slight, but not statistically significant increase, in cancer of the pancreas (8 observed, SMR=2.03, 95% confidence intervals, CI = 87-400). This was further broken down to show the following observed/expected (O/E) ratios: 19/21 in the "non-exposed" group, 1/0.7 in the 0.001-0.03 mg/m^3-year group, 2/1.8 in the 0.03-0.3 mg/m^3-year group, and 5/3.8 in the >0.3 mg/m^3-year group. No clear exposure-response emerges from this data.

It was stated that this study would have been able to detect a 25% increase in total cancer, 50% increase in respiratory cancers, and a 3-fold increase in cancer of the brain and central nervous system with a power of 80%.

Overall, this study did not reveal any significant increase in mortality from any given cause, including site-specific cancer, amongst the workers potentially exposed to acrylamide at these plants.

A second, but much smaller, cohort mortality study was available (Sobel et al., 1986) amongst 371 workers potentially exposed to acrylamide from 1955 in the manufacture of acrylamide monomer and polyacrylamide. The cohort included all those employed up until and including December 31, 1982. Of the total population at this site, 357 had no exposure to organic dyes, and from these, a total of 20 had died. Personal 8-hour TWA airborne exposure levels were available - before 1957 these ranged from 0.1-1 mg/m^3, from 1957-1970 from 0.1-0.6 mg/m^3 and from 1970 were <0.1 mg/m^3. Again, the extent of dermal exposure was unclear. Exposure also potentially involved acrylonitrile and organic dyes - data were presented separately for those exposed to organic dyes for more than 5 years.

For the total cohort (including those exposed to organic dyes) 29 deaths were observed against 38 expected. However, there was an increase in the number of deaths from all cancers (O/E=11/7.9) related to increases in death due to cancer of the digestive tract (O/E = 4/1.9, SMR=2.02, CI=57-539) and also cancer of the respiratory system (O/E = 4/2.9, SMR=1.38, CI=38=353). When workers who had been exposed to organic dyes for more than 5 years were excluded (14 workers were excluded, leaving a group size of 357 workers) no statistically significant increases in the number of observed deaths due to any cause were observed. The largest value was death due to cancer of the digestive tract in which there were 2 mortalities against 1.6 expected, SMR = 1.24, CI=15-452.

The authors stated that a 2-fold increase in total cancer could have been observed with 80% power. The power of this study in respect of detecting cancer at any specific site was severely limited by small numbers.

4.1.2.8.3 Summary of carcinogenicity

Acrylamide is carcinogenic in animals producing increased incidences in a number of benign and malignant tumours identified in a variety of organs (for example thyroid, adrenals, testicular mesothelioma). The tumour types observed show a possible relationship with disturbed endocrine function and raise the possibility of a hormonal mechanism. There is also a suggestion of tumours in brain and spinal cord and, although the picture is not clear, these are possibly acrylamide-induced. Given the genotoxicity profile of acrylamide, genotoxic activity cannot be discounted from contributing to tumour formation. There are no mechanistic arguments to indicate that these findings would be restricted to animals and not humans.

The potential carcinogenicity of acrylamide has not been thoroughly investigated in humans. Two human cohort mortality studies did not show any clear increase in cause-specific mortality as a result of acrylamide exposure although there were clear inadequacies in one of the two studies available. No firm conclusions can be drawn from these studies. For classification, see Chapter 1.

4.1.2.9 Toxicity to reproduction

4.1.2.9.1 Studies in animals

Effects on fertility

Rats

In a one-generation reproduction study, groups of 15 male Long-Evans rats received 0, 50, 100, or 200 ppm acrylamide in drinking water for up to 10 weeks, and females received 0, 25, 50, and 100 ppm for 2 weeks prior to mating, during gestation, and also during lactation (Zenick et al., 1986). For males and females, the mean exposure levels, based on bodyweight and water consumption values presented were approximately 0, 4, 8 and 10 mg/kg/day and 0, 5, 10, and 15 mg/kg/day respectively, although for pregnant and lactating females values would have been somewhat higher.

During the 10-week exposure period copulatory activity and sperm parameters were assessed in males. In week 10, males from the control- and high-exposure groups were mated with untreated females. Males were subsequently sacrificed and their organs were examined macroscopically, with histopathological examination of testes and counts made of spermatids and epididymal sperm. The untreated females were sacrificed on day 17 of pregnancy and the number of foetuses and implantation sites were recorded.

To assess female reproductive performance, in the third week of treatment acrylamide-exposed females were paired with untreated males for up to 7 days. Females were allowed to deliver and pups were eventually sacrificed on day 42.

Signs of toxicity amongst males receiving 200 ppm (approximately 10 mg/kg/day) were so severe (including one mortality, loss of use of hind limbs, marked bodyweight loss, and reduced water consumption) that these were sacrificed for humane reasons after about 5-6 weeks of exposure. Mortality was not observed amongst other groups although loss of use of hind limbs was observed from week 8 amongst males at 100 ppm (approximately 8 mg/kg/day).

In the copulatory activity assessment, a marked decrease in the number of intromissions was seen during week 6 for males at 200 ppm, which was presumably related to their poor clinical condition. The mount latency was apparently not affected by acrylamide, although in their final week only 4/12 males at 200 ppm and 11/15 at 100 ppm managed to ejaculate within a 30 minute allotted time span.

Evaluation of ejaculated semen was conducted in week 9 amongst males receiving 0, 50, or 100 ppm. There was a statistically significant reduction in sperm count at 100 ppm (67% reduction). Sperm motility and morphology could not be adequately assessed at 100 ppm because semen was recovered from the uterus of only one female despite the fact that 11/15 males at 100 ppm ejaculated. For the other females, semen was found only in the vagina. Sperm count, motility and morphology, and seminal plug weight were not affected for males in other groups. Only 33% of females that were paired with males receiving 100 ppm were pregnant compared to 79% impregnation of females mated with control males. There was also a statistically significant increase in the incidence of post-implantation loss amongst females impregnated by males receiving 100 ppm (8% in controls, 32% at 100 ppm). This parameter was not assessed for males that received 50 or 200 ppm.

For males at 200 ppm that were sacrificed after 6 weeks there were apparently no significant effects on organ weight, or sperm parameters. At the terminal kill for the other groups (after 11 weeks) there were no significant changes in organ weights (liver, brain, kidney, adrenals, spleen, heart, testes, prostate, vas deferens, epididymides) or sperm counts. Overall, copulatory ability (reduced intromission and impaired ejaculation) was altered at 100 and 200 ppm although it is likely to have been influenced by the impaired hind limb function.

There were no mortalities amongst females, although there was a loss of use of hind limbs during weeks 1 and 2 of the gestation period for females receiving 100 ppm. There was also a significant reduction in bodyweight (approximately a 10% reduction compared to control) at this exposure level at the end of the second week prior to gestation, which remained throughout the rest of the study. At 50 ppm, bodyweight gain was reduced (approximately a 10% reduction) throughout lactation.

Reduced birth weight and subsequently reduced bodyweight gain was noted amongst male and female pups born to females that received 50 or 100 ppm acrylamide. The reduced birth weight of pups was indicative of a slight retardation in pup development which was also suggested by a delay in vaginal opening (about 36 days at 100 ppm, about 33 days in all other groups. As there was no cross-fostering in this study it was difficult to determine whether or not the subsequent effects on pup weight were as a result of direct exposure to acrylmide *in utero*, from maternal milk or indirectly related to poor milk production and impaired nursing ability of acrylmide-affected mothers.

Overall, this study indicates that male copulatory activity was impaired at 100 ppm (approximately 8 mg/kg/day) or more. However, in this study, it is likely that impaired mating ability was been secondary to neurotoxic effects (such as hind limb splaying). There was a marked reduction in sperm count at 100 ppm although sperm motility and morphology could not be adequately assessed. This effect does not provide conclusive evidence but suggests that male fertility might be impaired. In addition, reduced birth weights and delayed vaginal opening were suggestive of retarded development of offspring. The increased incidence of post-implantation loss may have been connected with dominant-lethal mutations, which is in agreement with studies reported in Section 4.1.2.7 and with other developmental studies reported later in this section. There was evidence of generalised toxicity at 50 and 100 ppm, seen as impaired

bodyweight gain during pregnancy and lactation. The retardation in pup development may be a secondary effect related to maternal toxicity.

In a combined two-generation/dominant lethal assay groups of 30 male and female F344 rats received 0, 0.5, 2, or 5 mg/kg/day acrylmide (>99% pure) in drinking water for 10 weeks (Tyl, 1987). This was followed by a 2-week mating period, with acrylamide exposure continuing through gestation, parturition and lactation. F_0 males were then mated over a 3-week period with untreated females to study dominant lethal effects (see also Section 4.1.2.7). After weaning of the F_1 offspring the male and female parents and 10-20 offspring were sacrificed for extensive histological examination. Thirty F_1 males and females selected for mating to produce the F_2 generation were exposed to acrylamide for 11 weeks with histopathology examination on F_2 animals.

There were no treatment-related mortalities. Loss of use of hind limbs was observed amongst F_0 males and females at 5 mg/kg/day and head tilting was also seen in males. A slight, but statistically significant, and exposure-related reduction in bodyweight gain was noted amongst all acrylamide-exposed F_0 animals during the first 11 weeks of exposure (10% lower than control for males and females at 5 mg/kg/day). Maternal weight gain at 5 mg/kg/day was reduced during gestation and lactation (29% lower than controls) although this was possibly related to the reduced number of developing foetuses. Food and water consumption was unaffected. Fertility index and gestation length were not adversely affected although the number of implantations per dam and the number of live pups per litter were reduced at 5 mg/kg/day (7 implantations/dam compared to 10/dam in controls, 5 live pups/litter vs. 10 in controls). During the later stages of lactation, bodyweight gain of male pups from mothers receiving 5 mg/kg/day was reduced (9% lower than controls). There were no obvious intergroup differences in bodyweight amongst female F_1 pups. Necropsy of F_0 males and females and F_1 animals did not reveal any abnormalities.

In the dominant lethal assay, there were no effects on the mating index but the number of viable implantations was reduced at 5 mg/kg/day (7.5/litter compared to 9.4 in controls), and there was an increase in resorptions at 5 mg/kg/day (14% compared to 6% in controls) indicating a positive result.

The actual bodyweight and bodyweight gain of F_1 parents were slightly reduced at 2 and 5 mg/kg/day during the 11-week pre-breeding period (about 5-10% lower than controls) with maternal weight gain still reduced during gestation (about 14% and 35% lower than controls at 2 and 5 mg/kg/day respectively). Head tilting was noted in F_1 males at 5 mg/kg/day. Food consumption was not affected. Water consumption amongst females at 5 mg/kg/day was reduced in the pre-breeding period, by about 10%.

As with the F_0 breeding generation, fertility indices and gestation length amongst F_1 animals were not affected by acrylamide exposure, although the number of implantations and live pups was decreased at 5 mg/kg/day (7 implantations/dam at 5 mg/kg/day compared to 11/dam in controls, 5 live pups/litter at 5 mg/kg/day versus 11/litter in controls). F_2 Pup bodyweight was reduced by up to 7% at 5 mg/kg/day. Histopathologically, minimal to mild axonal fragmentation and swelling were observed in sciatic and tibial nerve sections from F_1 males at 5 mg/kg/day. There were no other histopathological abnormalities amongst F_1 parents or the F_2 offspring.

Overall, this study showed that there were no clear effects on the fertility of male and female rats at the exposure levels tested (up to about 5 mg/kg/day for 10-11 weeks) in either the F_0 or F_1 breeding populations. In accordance with results reported in Section 4.1.2.7, dominant lethal effects were apparent, and would probably have been responsible for the reduction in the

numbers of live pups at each mating stage. The exposure levels used were associated with signs of toxicity in the parents (reduced bodyweight gain, signs of peripheral neuropathy) indicating that appropriate doses were used.

As part of a dominant lethal assay in which groups of 15 male Long-Evans rats received 0, 5, 15, 30, 45, or 60 mg/kg/day acrylamide by oral gavage for 5 days, there was a marked, statistically significant, reduction in male fertility at 15 mg/kg/day or more (Sublet et al., 1989). At 15 and 30 mg/kg/day the fertility index (number of pregnant/number of sperm positive females) was reduced, only in week 1 post-administration, to 46% and 17%, respectively. At 45 mg/kg/day, reductions were only significant in weeks 1 and 3 (15% and 67 % respectively), and at 60 mg/kg/day reductions were observed in the first 4 weeks (7% in weeks 1 and 3, 53-60% in weeks 2 and 4).

Following this dominant lethal assay, groups of 10 males received up to 45 mg/kg/day acrylamide (>99% pure) by oral gavage for 5 days. Mating behaviour and evaluation of sperm were assessed in weeks 1, 2, 3, and 4 using ovariectomised, hormonally primed females which were sacrificed 15 minutes after ejaculation. In addition, further groups of 15 males were mated with proestrus females at 4 one-weekly intervals after acrylamide exposure for examination of ovaries, oviducts, and uteri.

Copulatory behaviour of males (mount and ejaculation latency, number of mounts and intromissions) was unaffected by acrylamide exposure. In week 1 after exposure of males to 15 and 45 mg/kg/day an increased number of females did not have sperm in the uterus (percentages with sperm were 100%, 60%, and 20 % at 0, 15, and 45 mg/kg/day respectively). In subsequent weeks, there were no clear effects on uterine sperm.

Sperm samples from males receiving 45 mg/kg/day were not located for analysis in week 1 - the reason for this was not clear. Examinations were further limited by the use of only two exposure levels, 0 and 45 mg/kg/day. The only statistically significant effects on sperm were observed in week 3 post-administration. There were slight reductions in sperm count ($61 \cdot 10^6$ at 45 mg/kg/day compared with $82 \cdot 10^6$ in controls), percentage motility (58% at 45 mg/kg/day, 75% in controls), curvilinear velocity (122 µm/s versus 132 µm/s), linearity and straight line velocity. These changes, in themselves, are of limited significance, but support the observation of reduced male fertility in the dominant lethal assay. In addition, there was a statistically significant reduction in the percentage of ova fertilised by males exposed to 15 and 45 mg/kg/day in week 1 (29% and 41% compared to 84% in controls), and at 45 mg/kg/day in week 3 (12% compared to 65% in control). Overall, this study demonstrated reduced fertility in male rats in an exposure regime, which did not apparently affect mating performance through neurotoxicity; male fertility was impaired at exposure levels of 15 mg/kg/day or more for 5 days.

In a study repeating the conditions of part of the study by Sublet et al. (1989), groups of 25 male Long-Evans rats received 0, 5, 15, 30, 45, or 60 mg/kg/day aqueous acrylmide by oral gavage for 5 days (Tyl, 1998a). On the third day after completion of the treatment period these males were mated overnight 1:1 with untreated females in pro-oestrus/oestrus. Subsequently, females were assessed for the presence of vaginal sperm and the fore- and hind limb grip strength of males was measured on the day after mating. The males were then killed; evaluations included epididymal sperm counts and motility, and a histopathological assessment of the sciatic nerve in 5 perfusion-fixed males per group. On day 15 of gestation, the females were sacrificed; assessments included counts of corpora lutea and uterine implantation sites (total, resorbed, live and dead). Mating, fertility and pregnancy indices were calculated respectively by: the ratios of the number of males that mated/number of males paired, number of males siring litters/number

of males impregnating females, and the number of pregnant females/number of males impregnating females.

There were no mortalities amongst parental animals. Clinical signs of toxicity amongst males included pilo-erection, poor grooming, lethargy and unsteady movement at 60 mg/kg/day, and pilo-erection and poor grooming at 45 mg/kg/day. There were no observable changes in forelimb grip strength, although hindlimb grip strength was impaired at 60 mg/kg/day. Bodyweight gain was reduced by 21-81% amongst males at 15, 30, 45 mg/kg/day compared with controls, and at 60 mg/kg/day there was a reduction in actual bodyweight compared with the mean weight at the start of the study. There were no lesions observed histopathologically in the sciatic nerve of those males examined.

Mating, fertility and pregnancy indices were all adversely affected following treatment of males with 15-60 mg/kg/day. The mating indices were 64% (16/25), 60% (15/25), 48% (12/25), 52% (13/25), 56% (14/25) and 38% (9/24) for males that had received 0, 5, 15, 30, 45 or 60 mg/kg/day respectively. Fertility and pregnancy indices were 81%, 80%, 67%, 69%, 64%, and 22% respectively, although these values were not statistically significant compared to the control. There were no significant differences in the numbers of corpora lutea amongst females indicating that females from each of those groups were equally fertile. Amongst males, there were no decreases in sperm count or motility. However, although not attaining statistical significance, sperm count was slightly increased in males receiving 60 mg/kg/day ($60 \cdot 10^7$ compared to $48 \cdot 10^7$ in controls). This was speculated, by the authors of the report, to be possibly as a result of reduced ejaculatory behaviour or impaired sperm transport. There was also a marginal increase in sperm beat cross frequency (a measure of side-to-side movement) at 60 mg/kg/day (29.7 Hz compared to 21.9 Hz in controls) which may be indicative of impaired swimming ability.

Although exhibiting considerable variation, pre-implantation losses were not affected by acrylamide. There were increased resorptions seen at 15 mg/kg/day or more both in terms of litters affected and the mean proportion of resorptions within litters; 31% of litters (0.38 resorptions/litter) had resorptions in controls, 33% (10.6 resorptions/litter) at 5 mg/kg/day and 75-100% litters with resorptions (16.6-45.6 resorptions/litter) from animals receiving 15-60 mg/kg/day acrylamide. Similarly, because figures relate to the increase in resorptions, post-implantation loss was increased in animals receiving 45 mg/kg/day or more; 3% loss per litter in controls, 11-19% at 5-30 mg/kg/day, 27-46% in females at 45 or 60 mg/kg/day.

Overall, this study indicates that signs of systemic toxicity (a marked reduction in bodyweight gain or bodyweight loss, and general clinical signs of poor condition) were observed at 15 mg/kg/day or more. The only overt effect on peripheral neuropathy was impaired hindlimb function in males at the highest dose level only. Sperm counts and motility parameters were not adversely affected. However, at 15 mg/kg/day or more male fertility was impaired. From these data, therefore, it is unclear if the impaired male fertility is directly due to acrylamide effects on sperm development or as a secondary consequence of a more generalised poor condition. It is probable that at least at the highest dose level general toxicity may have contributed to poor mating performance. Even so, fertility was seen to be reduced at this dose level in those males, which mated successfully.

Mice

In a continuous breeding study, groups of 18-39 male and female Swiss mice received 0, 3, 10, or 30 ppm acrylamide (>98% pure) in drinking water (approximately 0, 0.7, 3, 9 mg/kg/day) (NTP, 1993; Chapin et al., 1995). F_0 animals were allowed to breed and produce several litters over a 28-week period. Females were allowed to deliver the F1 pups and continued to receive acrylamide during lactation and 20 offspring from each group were then selected for a second generation of breeding, producing F2 pups. A dominant lethal assay (see Section 4.1.2.7) was performed after about 20 weeks of acrylamide exposure using 10 F_0 males mated with untreated females. A crossover breeding trial (including extensive histopathological investigations) was performed after 27 weeks of acrylamide treatment using 10 F_0 animals per group. Hind- and fore-limb grip strength of F_0 animals was assessed at weeks 0, 3, 6, 9, 12, 17, and 26 of the study.

Amongst parental animals, there were no exposure-related mortalities. There were no effects on the bodyweight of F_0 animals or water consumption. In addition, there were no effects on hind- and fore-limb grip strength of F_0 animals. A positive result was obtained in the dominant lethal assay from males exposed to 30 ppm. For F_0 animals, there were no effects on the number of litters produced, and no effects on gestational length, F_1 pup weight or the sex-ratio of F_1 animals obtained. The number of live pups per litter was slightly, but statistically significantly, decreased at 30 ppm with 12 pups/litter versus 14 in controls. In the cross over breeding trial, there were no apparent effects on fertility index although fewer pups per litter were born from males exposed to 30 ppm acrylamide mated with unexposed females (9 pups/litter versus 11/litter in controls). There was no difference from control in the number of pups per litter produced by mating treated females with untreated males. For F_0 males there were no acrylamide-related changes in sperm and spermatid count and no apparent changes in the number of motile or abnormal sperm. For females receiving acrylamide for 27 weeks, there were no effects on oestrus cycle length or the time spent in each stage of the cycle. On completion of the 27-week dosing period there were no effects on organ weights including testes, prostate and ovaries. There were no histopathological abnormalities observed including examination of sural and gastrocnemius nerves, testes or epididymides in males and the uterus, and ovaries from females.

There were no exposure-related effects on F_1 post-natal survival or on bodyweight gain post-weaning. After weaning, when F_1 pups were directly exposed to acrylamide in drinking water, a slight but statistically significant reduction in bodyweight gain was noted amongst females at 30 ppm - 8% lower than controls. Fore- and hind-limb grip strength tests in weeks 3, 5, 7, 10, and 16 from animals in the F_1 generation showed that forelimb grip strength was clearly reduced amongst males at 30 ppm in week 16 although this marginal change was not considered to be related to acrylamide exposure. Food consumption in the F_1 generation males and females was unaffected.

Organ weights, including the testes, epididymides, prostate and ovaries were obtained from 20 F_1 males and females at necropsy about 10 weeks post-partum. Decreased prostate weight (about 14% lower than controls) was noted amongst males receiving 30 ppm. There were no other effects on weights of reproductive organs. Amongst F_1 females there was a decrease in combined kidney and adrenal weight at 10 and 30 ppm (5% reduction at 30 ppm and 8% at 10 ppm). There were no statistically significant differences in epididymal sperm concentration between control and 30 ppm groups of F_1 males, or spermatid concentration, and no significant differences in sperm motility. There were no effects on oestrus cycle parameters amongst F_1 females at 30 ppm. No histopathological abnormalities were noted amongst any of the tissues examined.

Assessment of reproductive performance of F_1 breeding pairs was limited by the unusually low mating (approximately 50%) and pregnancy indices (approximately 60%) in controls. However, there was a reduction in the number of live pups per litter born from F_1 breeding pairs at 30 ppm (15 pups/litter in controls versus 8/litter at 30 ppm). The sex-ratio and pup weight (F_2) were not affected.

Overall, this study showed that there were no clear effects on the fertility of males and females at the exposure levels tested (up to about 9 mg/kg/day for 27 weeks) in either the F_0 or F_1 breeding populations. In accordance with results reported in Section 4.1.2.7, dominant lethal mutations in male germ cells would probably have been responsible for the reduction in the numbers of live pups at each mating stage. The exposure levels in this study were not associated with any clear signs of neurotoxicity, or any histopathological effects on major organs but there were other signs of toxicity (such as prenatal mortality, and reduced bodyweight gain). These effects indicate that sufficiently high doses were used in the attempt to express effects on fertility.

In another cross-over breeding study, groups of 9-24 male and female ddY mice received 0, 21, 43, 64, or 85 µg/ml acrylamide in drinking water for 4 weeks (Sakamoto and Hashimoto, 1986). Assuming a bodyweight of 35g and 5 ml/day water consumption this would result in dose levels of about 0, 3, 6, 9, and 12 mg/kg/day. On completion of the dosing period, half of the treated males and all of the females were mated with untreated controls of the opposite sex. Uterine contents were examined on day 13 of gestation for implants and resorptions except for half of the females at the highest exposure level, which were allowed to complete their gestation period and deliver pups which were examined for a further 4 weeks for any abnormalities. Immediately after the 4-week exposure period, the males not being used for mating were killed and organ weights were determined (liver, testes and seminal vesicles) with further examinations for sperm count and sperm cell morphology.

The highest exposure level (about 12 mg/kg/day) was associated with loss of use of hind limbs amongst treated males and females on completion of 4 weeks exposure. Bodyweight, food and water consumption was unaffected.

The fertility rate, assessed on day 13 and also on the day of delivery was clearly affected only at the highest exposure level when treated males were mated with control females (2/9 pregnant compared with 8/9 in controls on day 13, and 3/15 versus 12/15 on the day of delivery). In addition, there were statistically significant reductions in the number of foetuses per dam at the highest exposure level and also at the next highest treatment level (~9 mg/kg/day).

At week 4, there was a slight, but not statistically significant, reduction in testes weight of males receiving approximately 12 mg/kg/day (about a 10% reduction). Also, epididymal sperm count was significantly reduced from males receiving the highest exposure of acrylamide ($23 \cdot 10^5$/mg epididymis compared to $36 \cdot 10^5$/mg in controls) and there was an increase in head and tail abnormalities in sperm (8% vs. 4%). Overall, this study showed clear effects on epididymal sperm count following exposure of males to approximately 12 mg/kg/day for 4 weeks and reduced fertility after 2 or 4 weeks. However, the effects on fertility were observed at a level that was associated with impaired hind limb function. It is unclear from this study whether or not the impaired fertility was secondary to neurotoxicity.

Effects on development

Rats

In a study conducted according to modern protocol standards, groups of 29-30 mated Sprague-Dawley rats received 0, 2.5, 7.5, or 15 mg/kg/day aqueous acrylamide (about 98% pure) by oral gavage on gestation days 6-20 (Sleet et al., 1988; Field et al., 1990). On day 20, pregnant females were sacrificed, uteri examined (number of implantation sites, resorptions, live and dead foetuses). Foetuses were thoroughly examined macroscopically for visceral and skeletal abnormalities (including the head).

There were no maternal mortalities and no clear clinical signs of toxicity. When corrected for gravid uterine weight, maternal bodyweight gain was decreased amongst animals receiving 7.5 and 15 mg/kg/day (12% and 18% reductions respectively). There were no apparent effects on embryo/foetal viability, growth or malformations. There was a slight, but not statistically significant, increase in the incidence of skeletal variations (percentage of litters with variations - 61% in controls, 92% at 15 mg/kg/day, and percentage of foetuses with variations per litter - 14% in controls, 24% at 15 mg/kg/day). The most frequently observed variation was the presence of a rudimentary extra lumbar rib. This finding is considered likely to be an indirect consequence of maternal toxicity or stress and is of limited toxicological importance.

Overall, the results of this study indicate that, in rats, there were no significant effects on embryo/foetal development following exposure of pregnant females during the major period of organogenesis, at exposures resulting in some signs of maternal toxicity (up to 15 mg/kg/day).

In a recent study focusing on neonatal development, groups of 12 pregnant female Sprague-Dawley rats received 0, 5, 10, 15, or 20 mg/kg/day aqueous acrylamide (>99% pure) by oral gavage on gestation day 6 to lactation day 10 (Wise et al., 1995; abstracted by Wise et al., 1992). Females with pups were sacrificed on lactation day 24-29. On the day of delivery all pups were examined for external abnormalities and litter sizes were adjusted to 5 pups per lactating female. On day 11 post-partum, one male and one female were sacrificed for assessment of brain, spinal cord and peripheral nerves. In week 4 post-partum the pups were isolated from the lactating females for behavioural testing. The male and female offspring that were selected for the passive avoidance test on days 24 and 31 post-partum were subsequently sacrificed in week 11 for examination of brain and nervous tissue. Animals used for other tests (open field motor activity on days 13, 17, and 21, auditory startle habituation on day 22, passive avoidance test on days 24 and 59) were killed without further examination of tissues.

No pregnant females died during the study, however animals at 20 mg/kg/day were sacrificed in the late gestation/early post-natal period due to excessive pup mortality (33% of pups died in the first 3 days). Loss of use of hind limbs was observed post-partum amongst mothers receiving 20 and 15 mg/kg/day although there was some reversibility at 15 mg/kg/day after cessation of treatment. Maternal bodyweight gain was statistically significantly reduced amongst females at 15 and 20 mg/kg/day during gestation (14% and 26% lower than controls). For animals that were dosed during the lactation period (up to day 10 post-partum) maternal bodyweight gain was significantly reduced at 10 and 15 mg/kg/day (45% and 90% lower than controls) although there was recovery after cessation of dosing.

At birth, there was a decrease in the number of live pups at 15 and 20 mg/kg/day (although only attaining statistical significance at 20 mg/kg/day) - 14 and 10 live pups/litter compared to 16 in controls. During the first 3 days 33% of pups at 20 mg/kg/day died leading to the early termination of this group. Between days 4-21 post-partum 13% of pups at 15 mg/kg/day died

compared to none in controls. The neonatal mortality was almost certainly due to the poor condition of females receiving 15 and 20 mg/kg/day. No external abnormalities were observed in the pups, no clinical signs of toxicity observed during lactation, and there were no apparent effects on the ability of mothers to nurse pups.

Bodyweight gain was reduced amongst all acrylamide-exposed pups during lactation (up to day 21); effects at 5 mg/kg/day were marginal and reversible by day 14 post-partum; at 10 mg/kg/day values were 9-23%, and at 15 mg/kg/day 8-47% lower than control values. Subsequently, in the post-weaning period, weight gain at 5 mg/kg/day was unaffected, at 10 mg/kg/day was reduced in males (6% lower than controls), and at 15 mg/kg/day was reduced in both sexes (by 15-23%).

There were no apparent intergroup differences amongst adults in the open field tests conducted on days 13, 17 and 21 post-partum. No clear treatment-related findings were seen in pups. In the auditory startle habituation test on day 22 post-partum there were decreases in average peak amplitude amongst males and female pups at 15 mg/kg/day and in adult females at 15 mg/kg/day. There were no apparent effects amongst any of the groups in the passive avoidance test.

Absolute brain weight of pups at 10 and 15 mg/kg/day was decreased when compared with controls (7-14% lower) from animals examined on day 11 and in week 11 post-partum. These effects likely to have been secondary to the large reduction in bodyweight gain (up to 47% at 15 mg/kg/day) because there were slight increases in brain weight relative to bodyweight. There were no histopathological changes in the brain, spinal cord, and peripheral nerves of pups examined on day 11 or in week 11 post-partum.

To summarise this study, clear effects in neonates were only seen at levels where there was evident maternal systemic toxicity (15 mg/kg/day) and there were no histopathological effects observed on peripheral and central nervous tissue samples in pups. Marginal and transient effects on post-natal bodyweight gain were seen at 5 mg/kg/day, but this effect was considered of doubtful importance.

In an earlier study, groups of 8 pregnant Porton rats received 0, 200, or 400 ppm acrylamide by dietary admixture on days 1-20 of gestation (Edwards, 1976). Assuming a bodyweight of 300 g and food consumption of 20g/day, these dietary concentrations would result in dose levels of about 0, 15, and 30 mg/kg/day. The endometria were examined for evidence of resorption, and foetuses weighed and examined for visceral and skeletal abnormalities. At 200 and 400 ppm, there were signs of loss of use of hind limbs amongst the pregnant females and reduced food consumption at 400 ppm (approximately 48% reduction). Amongst progeny, bodyweight was reduced (by about 25%) although this could be attributed to reduced maternal food consumption and was therefore of minimal significance with respect to developmental effects. There were no other effects on development.

Overall, in this study continuous exposure of pregnant female rats to dietary concentrations of 400 ppm acrylamide (approximately 30 mg/kg/day) was associated with minor effects on development seen only at maternally toxic concentrations.

Mice

In a study conducted according to modern protocol standards, groups of 30 pregnant Swiss mice received 0, 3, 15, or 45 mg/kg/day aqueous acrylamide (~98% pure) by oral gavage on gestation days 6-17 (Price et al., 1988; Field et al., 1990). On day 17, pregnant females were sacrificed, uteri examined (number of implantation sites, resorptions, live and dead foetuses). Foetuses were thoroughly examined macroscopically for visceral and skeletal abnormalities (including the head).

There were no exposure-related maternal mortalities, although clinical signs of toxicity (loss of use of hind limbs) were observed amongst females at 45 mg/kg/day. Maternal bodyweight was statistically significantly reduced at 15 and 45 mg/kg/day (9% and 16% reduction respectively), which was directly related to the statistically significant reduction in gravid uterine weight.

Mean foetal bodyweight was reduced amongst male and female offspring from mice that had received 45 mg/kg/day (15% reduction for both sexes). As with the accompanying rat developmental study, there was also a slight, but not statistically significant, increase in the incidence of skeletal variations amongst all acrylamide-exposed mice (percentage of litters with variations - 44% in controls, 64% at 15 mg/kg/day, and percentage of foetuses with variations per litter - 7% in controls, 15% at 15 mg/kg/day). The most frequently observed variations were the presence of an extra lumbar rib or rudimentary extra rib; findings considered being of limited toxicological importance. No other evidence of developmental toxicity was apparent.

Overall, the results of this study indicate that, in mice, there was reduced foetal bodyweight only at a dose level (45 mg/kg/day) resulting in significant maternal toxicity. There was no evidence of significant developmental toxicity at 15 mg/kg/day and below.

Lactation studies

Groups of 6 female Wistar rats received 0 or 25 mg/kg/day acrylamide in saline by oral gavage during lactation for 21 days (Husain et al., 1987). Females were killed and discarded without further examination after weaning on day 21. Levels of noradrenaline (NA), dopamine (DA), 5-hydroxytryptamine (5-HT), and monoamine oxidase (MAO) and acetylcholinesterase (AChE) from the whole brain of 1-3 rats on days 2, 4, 8, 15, 30, 60, and 90 post-partum. It was not clear if studies on rats before day 21 included females.

Clinical signs of toxicity were poorly described in the study report, however loss of use of hindlimbs was reported although it was unclear whether or not this was seen in dams only. There were no clear indications of other effects in pups or dams. In the first experiment there were no significant changes in bodyweight or brain weight amongst the male pups (data not reported). During the first 15-30 days post-partum, statistically significant reductions in NA, DA, and 5-HT were observed amongst whole brain samples from pups whose mothers had received acrylamide when compared with controls. Effects were more pronounced amongst younger animals.

The changes in biogenic amines were reported to be accompanied by functional changes (such as loss of use of hindlimbs). The limited details available make this association rather difficult to assess adequately. Subsequently, there has been a comment from one of the original authors to indicate that the loss of use of hindlimbs was only seen in dams hence the changes in biogenic amines may not be directly associated with acrylamide exposure but may be a secondary result of parental toxicity. The study does show that acrylamide exposure results in changes in the levels of biogenic amines and enzymes associated with neurotransmitter metabolism. These changes appeared to be more marked amongst younger animals. However, the toxicological

significance of these biochemical changes is unclear and it is impossible to determine whether or not changes in these parameters were directly related to any clinical signs of toxicity observed. The apparent alterations in brain biogenic amines may be a secondary consequence of systemic toxicity in the dams.

In a well-reported, but limited study repeating the conditions of the study by Husain et al. (1987), groups of 15 timed-mated pregnant female Wistar rats received 0 or 25 mg/kg/day acrylamide in saline by oral gavage throughout the lactation period for 21 days (Tyl, 1998b). On completion of the lactation period, surviving male pups were retained without acrylamide-treatment; bodyweights were obtained at weekly intervals,

Two dams died during the dosing period (one due to a dosing error) and food and water consumption were reduced amongst surviving females. Bodyweight gain was significantly affected and at the end of the lactation period the treated dams had lost bodyweight (a mean reduction of about 8%). Clinical signs of toxicity included many indicators of an overall poor condition, as well as signs of neurotoxicity including loss of use of hind limbs (although histopathology examinations revealed no lesions in the sciatic nerve). Many pups died during the lactation period with little or no observable milk in their stomachs. At the start of the post-lactational observation period, the bodyweight of surviving pups from dams that had received acrylamide was significantly lower than controls (40% at day 7 of the post-lactational period) but progressively decreased as time went by (the mean weight of pups of acrylamide-treated dams was only 16% lower than that of controls by day 70 of the post-lactational period). Clinical signs of toxicity immediately on completion of the lactational period related to the general poor condition of pups, but steadily improved over time. Forelimb and hind limb grip strength of the pups from acrylamide-exposed dams was reduced compared to controls on day 7 of the post-lactational period. However, this would appear to be as a result of generalised poor condition due to near-starvation. Again, with the passage of time, there was recovery of the grip strength.

These results contradict those of Husain et al. (1987), and indicate that the treatment levels received by lactating females produced marked toxicity to the point where milk production was severely compromised or even ceased. The inconsistency raises concerns about the conclusions that can be drawn from the earlier less well-reported work. On balance, it is likely that effects seen in the pups in these studies were due to poor nutrition because of the effects of acrylamide on lactation. However, these results do not help to resolve the issue of whether or not acrylamide can be transmitted via breast milk and if so whether neurotoxicity could be induced in lactating pups.

Overall, no conclusions can be drawn from these studies about whether or not acrylamide can be transmitted through maternal milk.

4.1.2.9.2 Studies in humans

There are no data available.

4.1.2.9.3 Summary of toxicity to reproduction

There are no data available in humans. In animals, impaired fertility was demonstrated in male rats exposed to 15 mg/kg/day or more for 5 days. The impaired fertility may have been associated with effects on sperm count and sperm motility parameters; at higher doses, general systemic toxicity may have contributed to this impairment. In other rat studies effects on fertility

were less clear, with impaired copulatory ability possibly arising as a secondary result of neurotoxic effects (such as impaired hind limb function). Studies did indicate marked reductions in sperm count, which also suggests that male fertility could be impaired. In mice impaired fertility was also observed in one study (with exposure levels of up to 12 mg/kg/day for 4 weeks) with marked effects on sperm parameters. As with the rat studies, it was unclear whether or not impaired fertility was secondary to neurotoxicity. In some studies it was possible to identify NOAELs; no effects on fertility in rats were observed in a 2-generation reproduction study in which males and females of each generation received 5 mg/kg/day for 10-11 weeks. No clear effects on fertility were seen in a continuous breeding study in mice exposed to about 9 mg/kg/day acrylamide for up to 27 weeks.

Studies in rats and mice demonstrated some minor signs of developmental toxicity (increased incidence of skeletal variations and slightly impaired bodyweight gain) at exposure levels that were associated with maternal toxicity during the major period of organogenesis (about 15 mg/kg/day or more for rats and about 45 mg/kg/day for mice). Such effects are considered likely to be secondary to maternal toxicity and are therefore of limited toxicological significance. There was no evidence of selective developmental toxicity at exposure levels in rats or mice that were not associated with maternal toxicity. Studies have attempted to investigate whether or not acrylamide could induce toxicity in rat pups during lactation. However, the dose level used induced significant effects in dams and on lactation such that no conclusions could be drawn with respect to acrylamide-specific effects mediated via breast milk. For classification, see Chapter 1.

4.1.3 Risk characterisation

4.1.3.1 General aspects

Limited data are available relating to human exposure to acrylamide. However acrylamide is toxic by the oral route of administration, harmful by the dermal route, and predicted to be harmful by the inhalation route. Acrylamide is a skin and eye irritant.

Animal data provide clear evidence for the skin sensitisation potential of acrylamide. There are no data available regarding respiratory sensitisation. However, there is no evidence for respiratory sensitisation in humans, despite widespread use of acrylamide. Therefore it is considered that respiratory sensitisation is of low concern.

Human evidence demonstrates neuropathological effects, principally peripheral neuropathy, following exposure to acrylamide. In most reports the routes and extent of exposure were unclear, although a combination of inhalation and dermal exposure was likely. One case report of accidental oral ingestion, which was likely to have involved repeated rather than single exposure to acrylamide, also demonstrated similar neuropathic effects by this route. In the workplace surveys that were available, it was impossible to quantify the contribution to dose due to dermal exposure, and hence the airborne levels cited may not be representative of total acrylamide exposure and thus the dose received. Overall, there is no adequate human information to establish dose-response relationships.

Most of the repeated exposure animal data available relate to oral exposure. The effects that have been observed in these studies provide supporting evidence for the effects that have been observed in humans. There were no animal studies relating to repeated inhalation exposure and no firm conclusions could be drawn from the studies that were available using the dermal route of exposure; however the effects are predicted to be similar via these routes.

In animals, signs of neurotoxicity such as loss of use of limbs, tremor, loss of balance and, histopathologically, loss of axons and ganglion cells, as well as other degenerative changes in peripheral and optic nerves, and degeneration of the lateral geniculate nucleus were observed. Primate studies also showed degenerative changes in the optic nerve and lateral geniculate nucleus following oral exposure to approximately 10 mg/kg/day for up to 13 weeks. The clearest information available is from rodent studies. Histopathological examination of tissues in 2-year rat carcinogenicity studies showed slight peripheral nerve lesions in the absence of any clinical signs of toxicity at 2 mg/kg/day, and no effects at 0.5 mg/kg/day.

A substantial body of information is available covering many genotoxicity endpoints. Acrylamide is not mutagenic in bacteria. However, studies in mammalian cells *in vitro* demonstrate that it is a direct-acting mutagen. There is also a large body of evidence clearly demonstrating that acrylamide is genotoxic *in vivo* to both somatic and germ cells. In the case of germ cells, acrylamide has been demonstrated to induce heritable mutations.

Acrylamide is carcinogenic in animals, producing increased incidences in a number of benign and malignant tumours identified in a variety of organs (for example the thyroid, adrenals, testes). The tumour types observed show a possible relationship with disturbed endocrine function and raise the possibility of a hormonal mechanism. There is also some inconclusive evidence that acrylamide may induce neoplastic neural lesions (tumours in brain and spinal cord). Given the genotoxicity profile of acrylamide, genotoxic activity cannot be discounted

from contributing to tumour formation. There are no mechanistic arguments to indicate that these findings would be restricted to animals and not relevant to humans.

The potential carcinogenicity of acrylamide has not been thoroughly investigated in humans, and no firm conclusions can be drawn from the available human cohort mortality studies.

In relation to reproductive toxicity, impaired fertility was demonstrated in male rats exposed to 15 mg/kg/day or more for 5 days and in mice exposed to up to 12 mg/kg/day for 4 weeks. The impaired fertility may have been associated with effects on sperm count and sperm motility parameters. Degeneration of spermatids and spermatocytes was observed in one repeated-exposure study in which animals received approximately 36 mg/kg/day by the oral route for 8 weeks. In other studies effects on fertility were less clear, with impaired copulatory ability possibly arising as a secondary result of neurotoxic effects (such as impaired hind limb function). Overall, there is sufficient evidence to conclude that acrylamide impairs male fertility. In some studies it was possible to identify NOAELs; no effects on fertility in rats were observed in a 2-generation reproduction study in which males and females of each generation received 5 mg/kg/day for 10-11 weeks. No clear effects on fertility were seen in a continuous breeding study in mice exposed to about 9 mg/kg/day acrylamide for up to 27 weeks.

Studies in rats and mice demonstrated some minor signs of developmental toxicity (increased incidence of skeletal variations and slightly impaired bodyweight gain) at exposure levels that were associated with maternal toxicity during the major period of organogenesis (about 15 mg/kg/day or more for rats and about 45 mg/kg/day for mice). Such effects are considered likely to be secondary to maternal toxicity. There was no evidence of selective developmental toxicity at exposure levels in rats or mice that were not associated with maternal toxicity. In a lactation study, loss of use of hindlimbs was noted amongst pups indicating that the pups were receiving acrylamide via maternal milk. For reproductive toxicity, there are no data available in humans.

Overall, the toxicological properties of acrylamide have not been thoroughly investigated in humans and there are only limited quantitative human exposure data. The toxicological properties of acrylamide have been comprehensively investigated in animal studies. The key health effects are neurotoxicity, genotoxicity, carcinogenicity, and also reproductive toxicity. For neurotoxicity and reproductive toxicity it is possible to identify NOAELs from animal data. A clear NOAEL for neurotoxicity was identified as 0.5 mg/kg/day and only slight effects (some histopathological changes in the absence of clinical signs of toxicity) were seen at 2 mg/kg/day. The NOAEL for neurotoxicity is 10-fold lower than the NOAEL for reproductive effects, hence controlling for neurotoxicity should adequately control for reproductive effects. However, acrylamide is a direct-acting genotoxicant *in vivo* to both somatic and germ cells. In the case of germ cells, acrylamide has been demonstrated to induce heritable mutations. For such effects it is not possible to identify reliable thresholds. Acrylamide is carcinogenic in animals producing increased incidences in a number of benign and malignant tumours identified in a variety of organs. Although there is a possible relationship with disturbed endocrine function and hence the possibility of tumour induction being related to endocrine imbalance, genotoxic activity cannot be discounted from contributing to tumour formation. There are no mechanistic arguments to indicate that these findings would be restricted to animals and not relevant to humans. The potential carcinogenicity of acrylamide has not been thoroughly investigated in humans and no firm conclusions can be drawn from the available human cohort mortality studies.

The lead effects of acrylamide are genotoxicity and the potential for carcinogenicity. For both mutagenicity and carcinogenicity it is not possible to identify a threshold level of exposure below which there would be no risk to human health and it is not possible to derive a

toxicologically valid margin of safety. Margins of Safety (MOS) can be calculated for neurotoxic and reproductive effects to determine the adequacy of current control measures with respect to these endpoints. These effects are potentially applicable to humans and NOAELS (and LOAELS) have been identified. The calculations are made on the basis of systemic dose, that is inhalation and dermal uptake combined in these calculations. No calculations are made for local or site specific effects (e.g. irritation), as it is not possible to evaluate specific MOS values in any quantitative way.

4.1.3.2 Workers

4.1.3.2.1 Introduction

In the UK and Germany acrylamide is only produced as an aqueous solution, whereas in the Netherlands acrylamide is produced as both powder and aqueous solution. Small amounts of acrylamide powder are imported into the EU for production of polyacrylamides. The total EU production figure is reported to be 80,000-100,000 tonnes/annum, 30,000-40,000 tonnes of which is produced in the UK.

Approximately 99.9% of acrylamide in the EU is used in the production of polyacrylamides which have a residual monomer content of <0.1% w/w. About 80-90% of polyacrylamide is used in wastewater treatment, paper and pulp processing and mineral processing where the polymer is generally diluted to 0.05-0.5% w/w before use. Other uses include crude oil production, cosmetic additives (such as body lotion and shampoo), soil and sand stabilisation, coatings and paints, textile processing, and polyacrylamide gel electrophoresis. For monomer manufacture, it is estimated that up to about 650 workers in the UK and up to about 5,300 in the EU are exposed during manufacture and use. It is not possible to estimate with any accuracy the total number of people exposed to residual free acrylamide in polyacrylamide: it is likely to be tens of thousands. The degradation of polyacrylamide to release free monomeric acrylamide is reported to be unlikely.

The majority of exposures will be during the manufacture and use of the 50% aqueous solution, due to its large market share. During the use of aqueous acrylamide, exposure will be to vapour from the solution. In addition, dermal exposure occurs from direct contact of the solid or solutions with the skin or where workers come into contact with contaminated surfaces. It is unlikely that the generation of aerosol droplets will occur during any of its uses. During manufacture of solid monomeric acrylamide and in manufacture of solid grade polymers, inhalation and dermal exposure will be to dust and to vapour from sublimation of the solid. Exposure to acrylamide may occur during tasks such as bagging, bag opening, cleaning filters, spillages, and maintenance. Exposure to acrylamide monomer may occur from residual acrylamide monomer during the use of polyacrylamide in industries such as wastewater treatment, paper and pulp processing, and mineral processing, and also during the preparation and use of polyacrylamide gels particularly in research establishments, hospitals and universities.

The large-scale use of acrylamide grouts occurs for structural water control and geotechnical grouting operations and the small-scale use occurs for sewer line sealing and manhole sealing.

Both structural water control and geotechnical grouting operations involve manual injection techniques. Worker exposures may occur during grout mixing, injection equipment disassembly and clean up.

Dermal exposure in large-scale use can result from injecting grout in the tunnels and from leakage water and where workers come into contact with surfaces contaminated directly by the solid or solutions or by condensed vapour; or as a result of direct contact on to the skin.

4.1.3.2.2 Monomer manufacture

Inhalation exposure

In the UK, exposure to airborne acrylamide monomer during manufacture is approximately 0.2 mg/m^3 (8-hour TWA) from personal monitoring data. The highest reported value was 0.34 mg/m^3. Respiratory protective equipment is generally worn by operators during tasks where they come into contact with acrylamide, therefore these exposures were attenuated. The automatic bagging process in the Netherlands occasionally exceeds 0.3 mg/m^3 (8-hour TWA). Therefore workers entering this bagging area are required to wear respiratory protective equipment.

Assuming that a 70-kg worker inhales 10 m^3 air per day and assuming 100% absorption, the mean body burden of acrylamide monomer is calculated to be $0.2 \cdot 10/70 = 0.03$ mg/kg/day.

Dermal exposure

During monomer manufacture the maximum extent of dermal exposure was estimated from sampling performed by manufacturers to be 0.01 mg/cm^2/day. However, this high value was not considered to be representative of routine exposure. The mean value was approximaetly 0.004 mg/cm^2/day. In the absence of clear, reliable quantitative data, it would be reasonable to assume that 75% of the acrylamide on skin would be absorbed (rather than 100% which seems unlikely). Assuming an area of skin of 820 cm^2 (two hands), and a bodyweight of 70 kg, then these values represent $0.004 \cdot 820/70 \cdot 0.75 = 0.04$ mg/kg/day.

Combined exposure

The total daily body burden of acrylamide resulting from dermal and inhalation exposure would be 0.03 + 0.04 = 0.07 mg/kg/day (approximate mean value). This value is approximately 7 times lower than the NOAEL of 0.5 mg/kg/day for neuropathological effects and about 30 times lower than the LOAEL of 2 mg/kg/day for the slight neuropathological effects that were observed in an animal study (see **Table 4.18**).

Table 4.18 Monomer manufacture

Effect *	Estimated total exposure # (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Estimated MOS based on NOAEL	Estimated MOS based on LOAEL	Conclusion
Neurotoxicity	0.07	0.5 a)	2 b)	7	30	ii
Reproductive toxicity	0.07	5 c)	12 d)	70	170	ii

* The major systemic effects, other than genotoxicity and carcinogenicity are presented here
\# Worst-case scenario estimates, total from all routes
a) Data from 2-year rat carcinogenicity study
b) Data from 2-year rat carcinogenicity study - slight changes in nerves only seen histologically
c) Data from 2-generation rat reproduction study
d) Data from 5-day dominant lethal study

4.1.3.2.3 Polyacrylamide manufacture

Inhalation exposure

In the UK, the mean exposure to airborne monomeric acrylamide during polymer manufacture is generally about 0.05 mg/m^3 (8-hour TWA) with 98% of values less than 0.3 mg/m^3 (8-hour TWA). The highest value was 0.77 mg/m^3. Workers in the UK are reported to wear respiratory protective equipment in situations where exposure to acrylamide monomer is likely. Data from a German manufacturer showed that exposure to acrylamide during polyacrylamide manufacture was less than 0.03 mg/m^3 (8-hour TWA).

The mean body burden from inhalation predicted to arise during manufacture is approximately $0.05 \cdot 10/70 = 0.007$ mg/kg/day.

Dermal exposure

Dermal exposure to acrylamide during polymer manufacture in the UK results from contact with surfaces contaminated with the solid, aqueous solutions or condensed vapour, or direct contact with the skin. During polymer manufacture dermal exposure was estimated from sampling performed by manufacturers to be 0.0002-0.08 mg/cm^2/day. However, there was considable diversity in the figures obtained and the higher end of this range was an outlying value and not considered to be indicative of routine exposure. The arithmetic mean value was approximately 0.01 mg/cm^2/day.

Assuming that 75% of the acrylamide on skin would be absorbed, the mean body burden during polymer manufacture is approximately $0.01 \cdot 820/70 \cdot 0.75 = 0.09$ mg/kg/day.

Combined exposure

The total daily body burden of acrylamide resulting from dermal and inhalation exposure would be 0.007 + 0.09 = 0.1 mg/kg/day for polyacrylamide manufacture (approximate mean value). This value is approximately 5 times lower than the NOAEL of 0.5 mg/kg/day for neuropathological effects and 20 times lower than the LOAEL of 2 mg/kg/day for the slight neuropathological effects that were observed in an animal study (see **Table 4.19**).

Table 4.19 Polymer manufacture

Effect *	Estimated total exposure # (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Estimated MOS based on NOAEL	Estimated MOS based on LOAEL	Conclusion
Neurotoxicity	0.1	0.5 a)	2 b)	5	20	ii
Reproductive toxicity	0.1	5 c)	12 d)	50	120	ii

* The major systemic effects, other than genotoxicity and carcinogenicity are presented here
\# Worst-case scenario estimates, total from all routes
a) Data from 2-year rat carcinogenicity study
b) Data from 2-year rat carcinogenicity study - slight changes in nerves only seen histologically
c) Data from 2-generation rat reproduction study
d) Data from 5-day dominant lethal study

4.1.3.2.4 Polyacrylamide use

Inhalation exposure

The main uses of polyacrylamide are in wastewater treatment, paper and pulp processing and mineral processing. The level of residual acrylamide monomer is kept below 0.1%. In the paper industry, exposure to monomeric acrylamide from the polyacrylamide solutions used was estimated using the EASE model (assuming 0.1% acrylamide in polyacrylamide) to be up to 0.003 mg/m^3 (8-hour TWA). During packaging of polyacrylamide at production plants exposure to acrylamide was estimated (from workplace measurements) to be up to 0.015 mg/m^3 (8-hour TWA).

The mean body burden resulting from inhalation exposure in paper manufacture is up to approximately $0.003 \cdot 10/70 = 0.0004$ mg/kg/day.

For the packaging of polyacrylamide, the mean body burden is approximately $0.015 \cdot 10/70 = 0.002$ mg/kg/day.

Dermal exposure

When handling undiluted polymer (which may occur in any of the occupational settings in which polyacrylamide is used), the EASE model estimates exposure up to 0.0004 mg/cm^2/day and if a 1:500 dilution of a polyacrylamide solution is used then exposure is estimated to be $2 \cdot 10^{-6}$-$2 \cdot 10^{-7}$ mg/cm^2/day.

Assuming that 75% of the acrylamide on skin would be absorbed, the mean body burden when handling undiluted polymer is approximately $0.0004 \cdot 820/70 \cdot 0.75 = 0.004$ mg/kg/day.

The mean body burden when handling 1:500 diluted polymer is approximately $2 \cdot 10^{-6} \cdot 820/70 \cdot 0.75 = 2 \cdot 10^{-5}$ mg/kg/day.

Combined exposure

The total daily body burden of acrylamide resulting from dermal and inhalation exposure of undiluted polyacrylamide would be up to 0.002 + 0.004 = 0.006 mg/kg/day. This value is approximately 83 times lower than the NOAEL of 0.5 mg/kg/day for neuropathological effects and 333 times lower than the LOAEL of 2 mg/kg/day for the slight neuropathological effects that were observed in an animal study. Therefore, the estimated total body burden of acrylamide during the use of polyacrylamide does not give significant cause for concern for neurotoxicity and **conclusion (ii)** is reached.

4.1.3.2.5 Polyacrylamide gel electrophoresis

Inhalation exposure

Polyacrylamide gels are prepared either from acrylamide powder, aqueous solutions, or can be purchased as ready-made plates. The highest exposures to acrylamide will occur when a user prepares gels from raw materials. Two measurements were available - the highest of which was 0.067 mg/m^3. This exposure value was not an 8-hour TWA as this procedure is usually performed once during a day to prepare a stock solution from which plates are poured. Shift exposure is therefore likely to be less than this. However, a reasonable worst-case scenario

where this procedure is performed repeatedly throughout a day would result in a body burden of 0.067 10/70 = 0.01 mg/kg/day.

Dermal exposure

Dermal exposure may occur during the handling of polyacrylamide gels although the nature of investigations performed using polyacrylamide gel electrophoresis tends to limit the extent to which gels are handled directly. Gloves would generally be used for these procedures. Although the gloves used may not prove to be a completely effective barrier to acrylamide, the duration of exposure is such that significant permeation is unlikely.

Combined exposure

If the assumption that dermal exposure is negligible is valid then the combined exposure to monomeric acrylamide during the use or preparation of polyacrylamide gels is approximately 0.01 mg/kg/day. As calculated previously, this value does not give significant cause for concern for neurotoxicity and **conclusion (ii)** is reached.

4.1.3.2.6 Large-scale use of grouts

Only inhalation exposure data were available from the measurements taken at Hallandsås. However, it is understood from reports by workers that high levels of dermal exposure occurred because of the contaminated water, which fell into the tunnel from the walls.

Inhalation exposure

The inhalation exposure assessment indicates that the highest airborne exposures during injection of the grout were 0.076 mg/m^3. Although there was clear evidence for extensive dermal exposure, no clear information is available regarding the extent of such exposure. Assuming that a worker weighs 70 kg, inhales 10 m^3 air per working day and that there is 100% absorption via the inhalation route, then the estimated body burden from inhalation exposure is 0.01 mg/kg/day.

In the period following grout injection when other work continued in the tunnel the highest measurement of acrylamide alone from a personal sampler was 0.012 mg/m^3 (measured at least 5 weeks after injection of the grout). It is relevant to carry out a risk characterisation for exposure at this time since this will affect workers other than those who actually applied the product and forms an ongoing working environment. If it is assumed that a worker exposed to this concentration has a respiratory rate of 10 m^3/day, 100% respiratory absorption and weighs 70 kg, the estimated body burden is 0.0017 mg/kg/day (or 1.7 µg/kg/day).

Dermal exposure

The lack of reliable inhalation exposure data and the total lack of dermal exposure data make it impossible to estimate the total systemic body burden during use of the products.

Combined exposure

In conclusion, the level of risk to workers using acrylamide-containing grouts in large-scale use is uncertain. The main uncertainties lie in the exposure assessment. The potential of the substance for genotoxic and carcinogenic effects gives cause for concern to human health for this kind of open system activity. Furthermore, tunnel workers were exposed to levels associated

with neurotoxic effects consistent with those caused by acrylamide. Hence, these exposures may also give rise to concern for other adverse effects, such as reproductive toxicity. Therefore, **conclusion (iii)** is reached for all above-mentioned endpoints.

4.1.3.2.7 Small-scale use of grouts

The highest air level of acrylamide measured during small-scale grouting applications is 0.12 mg/m^3 (McHugh, 1987). Assuming a working day of 7 hours/day, a respiratory rate of 10 m^3/day, 100% absorption and a body weight of 70 kg, this results in a daily inhalation exposure of 0.017 mg/kg/day.

The highest rate of dermal exposure (measured by pads on the body) was 5 mg/h. Assuming a working day of 7 hours, a dermal absorption rate of 75% and a body weight of 70 kg, this results in a daily exposure of 0.375 mg/kg/day. The highest contamination from wipe samples/rinses was obtained within one glove, which was 2.49 mg. Although it is not known whether this exposure occurred over one use or more, it is known that this was the exposure just prior to the measurement; it might have occurred all day if workers do not change their PPE during the day. Using the same assumptions, this would result in a daily exposure of 0.054 mg/kg/day for two gloves.

Using these maximum levels, the estimated total combined exposure would be 0.45 mg/kg/day. This is likely to be an overestimate in that it uses all maximum exposures and a single worker may not conduct all the high-exposure tasks all day. Exposure to acrylamide for small-scale work would not be likely to last all day, even if the job did, since much of the time is spent preparing for the grouting and clearing up afterwards. The grout itself does not last long before spoiling or setting so exposure is limited by necessity for the work. It is not known how often acrylamide grouts would be used (e.g. once a week or every day).

Using these calculated estimates of exposure and body burdens, MOSs are derived as shown in **Table 4.20**.

Table 4.20 MOS values for small-scale uses

Effect *	Estimated total Exposure (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Estimated MOS based on NOAEL	Estimated MOS based on LOAEL	Conclusion
Neurotoxicity	0.45	0.5 a)	2 b)	~1	~4	iii
Reproductive Toxicity	0.45	5 c)	12 d)	~11	~27	iii

* The major systemic effects, other than genotoxicity and carcinogenicity are presented here

a) Data from 2-year rat study

b) Data from 2-year rat study - slight changes in nerves only seen histologically

c) Data from 2-generation rat reproduction study

d) Data from 5-day dominant lethal study

4.1.3.2.8 Summary of the risk characterisation for workers

The key toxicological endpoints for acrylamide are neurotoxicity, genotoxicity, carcinogenicity and reproductive toxicity.

For neurotoxicity and reproductive toxicity following acrylamide exposure, NOAELs can be identified from the animal studies. For neurological damage, a reliable NOAEL in rats of 0.5 mg/kg/day has been identified, with a LOAEL of 2 mg/kg/day for slight neurological effects (histopathological changes in the absence of any clinical signs of neurotoxicity). The NOAEL for neurotoxicity is 10-fold lower than the NOAEL for reproductive effects hence controlling for neurotoxicity should adequately control for reproductive effects. The exposure assessment indicates that the highest levels of exposure are likely to be associated with monomer manufacture, polymer manufacture and use of acrylamide grouts in large- and small-scale applications.

Exposure during monomer manufacture and polymer manufacture gives rise to estimated total body burdens (from inhalation and dermal exposure) of 0.07 mg/kg/day and 0.1 mg/kg/day, respectively. A 5- to 7-fold difference is obtained between these estimated exposures and the NOAEL of 0.5 mg/kg/day for neurotoxic effects and the exposures are 20- to 30-fold lower than the LOAEL of 2 mg/kg/day for neurotoxic effects where only slight effects were observed histopathologically. These margins offer reasonable reassurance that the risk of neurotoxicity arising from occupational exposure to acrylamide is low and **conclusion (ii)** is reached.

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

It is not possible to estimate reliably the exposure during the use of acrylamide grouts in large-scale applications, and therefore the level of risk is uncertain. However, workers were exposed to levels giving rise to neurotoxic effects, consistent with those caused by acrylamide, and therefore these levels give cause for concern, for neurotoxicity and for other adverse effects (**conclusion (iii)**). Exposures during the use of acrylamide grouts in small-scale applications result in an estimated body burden of 0.45 mg/kg. The MOS for neurotoxicity is low and indicates a cause for concern for human health (**conclusion (iii)**). The MOS for reproductive toxicity is higher but it is also judged to be a cause for concern given uncertainties in the dose-response relationship for effects on fertility and in exposure estimates (**conclusion (iii**)).

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

For mutagenicity and carcinogenicity (which might involve a genotoxic mechanism) it is not possible to reliably identify a threshold level of exposure below which there is no increased risk and the magnitude of the risk of cancer at occupationally relevant exposure levels is not clear. Therefore, **conclusion (iii)** is reached for these endpoints for all occupational exposure scenarios. There should be a requirement to reduce exposure to acrylamide as far as is reasonably practicable.

4.1.3.3 Consumers

Within the EU there is no exposure of consumers directly to acrylamide monomer; there is the potential for indirect exposure due to the presence of residual monomer from polyacrylamides that may be used in consumer products.

4.1.3.3.1 Use of polyacrylamides in cosmetics

Non-rinse products

The total daily exposure to acrylamide monomer via non-rinse products is estimated to be 65 µg/day, based on a maximum monomer level in the polymer of 0.01%.

Rinse-off products

The total daily expoure to acrylamide monomer via rinse-off products is estimated to be 2.4 µg/day, again based on a monomer level of 0.01%.

Total exposure to acrylamide in cosmetic additives

Totalling up these potential daily exposure produces 67 µg of the acrylamide monomer. This figure represents a reasonable worst-case scenario.

Assuming 75% absorption by the dermal route, a mean bodyweight of 70kg, the body burden arising from skin exposure is calculated to be $67/70 \cdot 0.75 = 0.7$ µg/kg/day (0.0007 mg/kg/day).

However, the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) has recently considered the levels of acrylamide in cosmetic products.

The SCCNFP opinion on cosmetics was adopted by the plenary session of the SCCNFP on 30 September 1999. The SCCNFP recommended that a tolerable level of acrylamide in cosmetics is:

1. Body-care leave on products (non rinse products) = <0.1ppm
2. Any other cosmetic product (rinse-off products) = <0.5ppm

These levels will result in exposures that are 1,000- and 200- fold lower for non-rinse and rinse-off products respectively than those calculated based on a level of 0.01% acrylamide. The SCCNFP considers that these concentrations arising from the low residual level of acrylamide in polyacrylamide do not pose a significant cancer risk.

4.1.3.3.2 Use of polyacrylamide in gardening

Model calculations indicate that, for this scenario, a person may be exposed to approximately 5 µg acrylamide monomer by the dermal route. Assuming 75% absorption and 70 kg bodyweight this represents a body burden of $5/70 \cdot 0.75 = 0.05$ µg/kg/day ($5 \cdot 10^{-5}$ mg/kg/day).

4.1.3.3.3 Combined exposure to consumers

The total daily body burden arising as a result of skin exposure to acrylamide for consumers is estimated to be $0.0007 + 5 \cdot 10^{-5} = 0.001$ mg/kg/day. The major contribution comes from dermal exposure via the use of cosmetics, based on a level of monomer in the polymer of 0.01% (**Table 4.21**). As indicated, following the opinion of the SCCNFP, exposures resulting from the use of new cosmetic products, complying with the SCCNFP recommended levels, will result in exposures which are 2-3 orders of magnitude lower than previous exposures.

Table 4.21 Margins of safety for consumers

Effect *	Estimated total exposure # ** (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Estimated MOS based on NOAEL	Estimated MOS based on LOAEL	Conclusion
Neurotoxicity	0.001	0.5 a)	2 b)	500	2,000	ii
Reproductive toxicity	0.001	5 c)	12 d)	5,000	12,000	ii

* The major systemic effects, other than genotoxicity and carcinogenicity are presented here
\# Worst-case scenario estimates, total from all routes
** Combined exposure assuming a limit of 0.01% acrylamide in cosmetic products
a) Data from 2-year rat carcinogenicity study
b) Data from 2-year rat carcinogenicity study - slight changes in nerves only seen histologically
c) Data from 2-generation rat reproduction study
d) Data from 5-day dominant lethal study

4.1.3.3.4 Summary of the risk characterisation for consumers

The exposure assessment indicates that the estimated consumer exposure gives a total body burden of 0.001 mg/kg/day. This is 500-fold lower than the NOAEL of 0.5 mg/kg/day for neurotoxic effects and 2000-fold lower than the LOAEL of 2 mg/kg/day where only slight effects were observed histopathologically. These margins offer good reassurance that the risk of neurotoxicity arising from consumer exposure to acrylamide is low.

In contrast, for mutagenicity and carcinogenicity (which might involve a genotoxic mechanism) it is not possible to reliably identify a threshold level of exposure below which there is no increased risk. It is evident that the levels of exposure involved are very small and therefore the risk of cancer and mutagenicity to consumers is probably very small.

The issue of tolerable levels of acrylamide in cosmetics has recently been addressed by the SCCNFP, and maximum levels for the concentration of residual acrylamide in polyacrylamide contained in cosmetic products have now been recommended. The SCCNFP opinion is that these concentrations arising from the low residual level of acrylamide in polyacrylamide do not pose a significant cancer risk.

Thus overall, **conclusion** (**ii**) is reached for the risk characterisation for consumers in relation to neurotoxicity and reproductive toxicity.

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

In relation to mutagenicity and carcinogenicity, although thresholds cannot be reliably identified, the risks are considered to be very low, **conclusion** (**iiia**) is reached.

Conclusion (iiia) Risks cannot be excluded for all other 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.

4.1.3.4 Humans exposed via the environment

4.1.3.4.1 Drinking water

In relation to scenarios except where grouts have been used, the maximum concentration of acrylamide in drinking water is 0.125 μg/l (Section 3.1.2.2). This concentration is the maximum possible concentration of acrylamide in drinking water from water treatment works using polyelectrolyte flocculants. Assuming 2 litres consumption per day, an average bodyweight of 70 kg and a worst-case situation where 100% of the available acrylamide enters the drinking water, then it is estimated that exposure would be up to 0.0036 μg/kg/day.

For neurotoxicity and reproductive toxicity, the difference between exposure and NOAELS/LOAELS are very large, and thus it is concluded that there is no cause for concern for these endpoints (**Table 4.22**) and **conclusion** (**ii**) is reached.

Although it is not possible to identify a threshold level of exposure below which there is no increased risk of genotoxicity and carcinogenicity, given the very small exposure predicted, it is concluded that there would be a negligible residual risk, **conclusion** (**iiia**) is reached.

Table 4.22 Margins of safety for exposure via the environment (scenarios other than use of grouts)

Effect *	Estimated total exposure # (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Estimated margin of safety (MOS) based on NOAEL	Estimated margin of safety (MOS) based on LOAEL	Conclusion
Neurotoxicity	$3.6 \cdot 10^{-6}$	0.5 a)	2 b)	140,000	550,000	ii
Reproductive toxicity	$3.6 \cdot 10^{-6}$	5 c)	12 d)	$1.4 \cdot 10^{6}$	$3 \cdot 10^{6}$	ii

* The major systemic effects, other than genotoxicity and carcinogenicity are presented here
\# Worst-case scenario estimates, total from all routes
a) Data from 2-year rat carcinogenicity study
b) Data from 2-year rat carcinogenicity study - slight changes in nerves only seen histologically
c) Data from 2-generation rat reproduction study
d) Data from 5-day dominant lethal study

In relation to the use of acrylamide grouts in small-scale operations, the estimated local exposure via contaminated drinking water is 0.11 μg/kg/day (Section 3.1.7). The differences between exposures and the NOAEL of 0.5 mg/kg/day for neurotoxic effects and the LOAEL of 2 mg/kg/day are very large. This is also the case for the differences between exposure and the NOAEL and LOAEL for reproductive toxicity (**Table 4.23**). The risk characterisations indicate that there is no cause for concern for human health for these endpoints from exposure to acrylamide following the use of grouts on a small scale and thus **conclusion** (**ii**) is reached.

Although it is not possible to identify a threshold level of exposure below which there is no increased risk of genotoxicity and carcinogenicity, given that exposure following this use is very small, it is concluded that there would be a negligible residual risk, **conclusion** (**iiia**) is reached.

Table 4.23 Margins of safety for exposure via the environment (small-scale use of acrylamide grouts)

Effect	Estimated total exposure (µg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Estimated MOS based on NOAEL	Estimated MOS based on LOAEL	Conclusion
Neurotoxicity	0.11	0.5 a)	2 b)	>4,500	>18,000	ii
Reproductive toxicity	0.11	5 c)	12 d)	>45,000	>100,000	ii

a) Data from 2-year rat study
b) Data from 2-year rat study - slight changes in nerves only seen histologically
c) Data from 2-generation rat reproduction study
d) Data from 5-day dominant lethal study

In relation to the use of acrylamide grouts in large-scale operations, the exposure assessment indicates that the worst-case estimated local exposure would be 2.62 mg/kg/day (Section 3.1.7). This is based on the use of surface water as the source of drinking water immediately following an incident of high environmental contamination such as that which occurred at Hallandsås in Sweden.

The differences between the estimated worst-case exposure and the NOAEL of 0.5 mg/kg/day for neurotoxic effects and the LOAEL of 2 mg/kg/day are very small. The margins of safety are also too small for reproductive toxicity (**Table 4.24**). These risk characterisations indicate a cause for concern for human health and **conclusion (iii)** is reached for these endpoints.

For genotoxicity and carcinogenicity it is not possible to identify a threshold level of exposure below which there is no increased risk. It is evident that the levels of exposure involved are excessive for these endpoints and therefore the risk of carcinogenicity and mutagenicity is a cause for concern leading to a **conclusion** (**iii**).

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

Table 4.24 Margins of safety for exposure via the environment (large-scale use of acrylamide grouts).

Effect	Estimated total exposure (mg/kg/day)	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Estimated MOS based on NOAEL	Estimated MOS based on LOAEL	Conclusion
Neurotoxicity	2.62	0.5 a)	2 b)	<0.2	<1	iii
Reproductive toxicity	2.62	5 c)	12 d)	2	<5	iii

4.1.3.4.2 Plants and food products

There are no data available on acrylamide concentrations in biota or food products. In aquatic species, exposure may occur via acrylamide in water although the potential for accumulation in aquatic organisms is very low (see Section 3.1.3.3 and 3.1.7) hence the concentrations of acrylamide in biota would be expected to be negligible.

For plants and food products, exposure may be via air or contaminated water during growth or manufacture. Atmospheric concentrations of acrylamide are very low (Section 3.1.6) and it is unlikely that plants and food products are contaminated to any significant degree via this route.

Based on log K_{ow} values, the absorption of acrylamide by plants from contaminated water is likely to be negligible and contamination of food products during manufacture is only likely to occur as a result of accidental contamination of water supplies.

4.1.3.4.3 Summary of the risk characterisation for humans exposed via the environment

For scenarios excluding the use of grouts in large-scale operations, the exposure assessment indicates that the estimated indirect exposure via the environment from drinking water is very small. A difference of approximately 4 or more orders of magnitude is obtained between these estimated exposures and the NOAEL of 0.5 mg/kg/day for neurotoxic effects and a difference of 5 or more orders of magnitude between exposures and the LOAEL of 2 mg/kg/day where only slight effects were observed histopathologically. These margins offer good reassurance that the risk of neurotoxicity arising from indirect exposure via the environment from these drinking water sources is low (**conclusion (ii)**).

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

For mutagenicity and carcinogenicity (which might involve a genotoxic mechanism), although it is plausible a threshold may exist, it is not possible to reliably identify a threshold level of exposure below which there is no increased risk. It is evident that the levels of exposure involved are very small and therefore it is concluded that there would be a negligible, **conclusion** (**iiia**) is reached.

Conclusion (iiia) Risks cannot be excluded for all other 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.

In relation to the use of acrylamide grouts in large-scale operations, the exposure assessment indicates that the worst-case estimated local exposure would be 2.62 mg/kg/day. The differences between this estimated worst-case exposure and the NOAEL and LOAEL for neurotoxic effects are very small. The margins of safety are also too small for reproductive toxicity (**Table 4.24**). These risk characterisations indicate a cause for concern for human health and **conclusion** (**iii**) is reached.

For mutagenicity and carcinogenicity (which might involve a genotoxic mechanism) it is not possible to identify a threshold level of exposure below which there is no increased risk. It is evident that the levels of exposure involved are excessive for these endpoints and therefore the risk of carcinogenicity and mutagenicity is a cause for concern leading to a **conclusion** (**iii**).

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

4.1.3.5 Combined exposure via all routes

Human exposure to acrylamide indirectly via the environment from sources other than using grouts in large-scale operations is clearly negligible. In addition, exposure via consumer products is also very small. The most significant route of exposure is in occupational settings, the contribution from the environment and from consumer products is negligible in comparison and does not add significantly to the overall body burden.

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

There is no classification with regard to the following, flammability (autoignition 394°C), explosive properties and oxidising properties; these properties are not considered to pose a hazard. It is noted that acrylamide should be stored, transported and handled under the correct conditions. The recommended conditions for acrylamide dry crystals are to avoid direct sunlight; crystal temperatures above 50°C; and initiators such as bisulphites, peroxides, reducing agents, oxidising agents and redox systems. For aqueous solutions of acrylamide the recommended conditions are to store below 32°C and above the crystallisation point. Avoid contamination with iron or rust, initiators such as bisulphites, peroxides, reducing agents, oxidising agents and redox systems and prevent the loss of dissolved oxygen. A general warning to this effect is recommended, and is currently in practice.

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

5 RESULTS

5.1 INTRODUCTION

Acrylamide is usually made as a 30-50% aqueous solution although a crystalline form of acrylamide is produced at one European production plant. Small amounts of acrylamide powder are imported into the EU for production of polyacrylamides. The total EU production figure is reported to be 80,000-100,000 tonnes/annum.

Approximately 99.9% of acrylamide in the EU is used in the production of polyacrylamides which have a residual monomer content of <0.1% w/w. About 80-90% of polyacrylamide is used in wastewater treatment, paper and pulp processing where the polymer is generally diluted to 0.05-0.5% w/w before use. Other uses include crude oil production, cosmetic additives (such as body lotion and shampoo), soil and sand stabilisation, and polyacrylamide gel electrophoresis. For monomer manufacture, it is estimated that up to about 650 workers in the UK and up to about 5,300 in the EU are exposed during manufacture and use. It is not possible to estimate with any accuracy the total number of people exposed to residual free acrylamide in polyacrylamide: it is likely to be tens of thousands. The degradation of polyacrylamide to release free monomeric acrylamide is reported to be unlikely. Acrylamide can also be used in the formulation of grouting agents. Acrylamide grouts are no longer thought to be produced within the EU but are imported from outside the EU. The one known EU producer of an acrylamide grout stopped production at the end of 1997 and has no plans to restart production.

5.2 ENVIRONMENT

Local releases of acrylamide to the environment may occur during production and use as an intermediate in the production of polyacrylamides. The use of polyacrylamides may lead to the release of residual monomer to the environment. The use of acrylamide and N-methyolacrylamide grouts may also give rise to environmental releases of acrylamide. These releases have been quantified in the assessment and used to calculate PECs for various environmental compartments.

For the aquatic compartment the PEC/PNEC ratio is <1 for water and sediment from local sources for production, polyacrylamide production and use of acrylamide-based grouts in pipeline and sewer repairs and manhole sealing operations and background levels. No risks are expected for sewage treatment plant from any use. No further information and/or testing or risk reduction measures are required beyond those which are already being applied. For the use of acrylamide-based grouts in construction applications a PEC/PNEC ratio > 1 is calculated based upon measured levels and there is a need for limiting the risk to aquatic organisms.

For the terrestrial compartment the PEC/PNEC ratio is <1 for local sources and background levels for production of acrylamide, production of polyacrylamides, use of polyacrylamide and use of acrylamide-based grouts in pipeline and sewer repairs and manhole sealing operations. A PEC for soil from release from use in tunnelling cannot be calculated from the information available, but it is possible that high levels could be found in pore water under some circumstances. The effects data available for the terrestrial compartment are not sufficient to allow a PNEC to be derived, so the current PNEC is based on the aquatic PNEC - this could be refined by testing on terrestrial species.

No effects are expected in the atmospheric compartment and so no further information and/or testing or risk reduction measures are required beyond those which are already being applied.

The available information suggests that there is no risk from secondary poisoning. No further information and/or testing or risk reduction measures are required beyond those which are already being applied. However, there is a risk to organisms due to direct exposure to contaminated water from the use of acrylamide-based grouts in construction applications.

Results

Conclusion (i) There is need for further information concerning the toxicity of the substance to terrestrial organisms.

This conclusion applies to the terrestrial compartment for use of acrylamide-based grouts in construction applications. Both the PEC and the PNEC for this use could be refined. However, the control strategy for the aquatic compartment is also expected to remove any risk to the terrestrial compartment, and hence no specific activity is considered necessary at this time. Any further information and/or testing requirements should await the outcome of the risk reduction measures on releases to the environment.

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

This conclusion applies to the aquatic and terrestrial ecosystems (production of acrylamide, production of polyacrylamides, use of polyacrylamides and use of acrylamide based grouts in pipeline and sewer repairs and manhole sealing operations), microorganisms in the sewage treatment plant, atmosphere and accumulation via the food chain (secondary poisoning).

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 use of acrylamide based grouts in construction applications, and to indirect exposure of other organisms through contaminated water from the same use.

5.3 HUMAN HEALTH

5.3.1 Human health (toxicity)

The extent of information available in humans is limited. However, acrylamide has been comprehensively studied in animals.

In animals, acrylamide is well absorbed by oral and dermal routes and presumably also by the inhalation route. Whilst a wide range of health hazards are associated with acrylamide, it is felt that the main issues are those of neurotoxicity, genotoxicity, carcinogenicity, and reproductive toxicity. For neurotoxicity and reproductive toxicity NOAELs have been identified. The NOAEL for neurotoxicity is 10-fold lower than that for reproductive effects hence controlling for neurotoxicity should control for reproductive effects. For genotoxic effects, acrylamide is a direct-acting mutagen and is an *in vivo* mutagen in somatic cells and germ cells. In the case of germ cells, acrylamide has been demonstrated to induce heritable mutations. It is not possible to identify a reliable threshold level for these genotoxic effects.

In addition, acrylamide is carcinogenic in animals producing increased incidences in a number of benign and malignant tumours identified in a variety of organs. The tumour types observed show a possible relationship with disturbed endocrine function and raise the possibility of a hormonal mechanism. There is also a suggestion of tumours in brain and spinal cord and, although the picture is not clear, these are possibly acrylamide-induced. However, given the genotoxicity profile of acrylamide, genotoxic activity cannot be discounted from contributing to tumour formation. For genotoxicity and carcinogenicity (which may involve a genotoxic mechanism) it is not possible to reliably identify a threshold level of exposure below which there is no risk. There are no mechanistic arguments to indicate that these findings would be restricted to animals and not relevant to humans. The potential carcinogenicity of acrylamide has not been thoroughly investigated in humans, and no firm conclusions can be drawn from the available cohort mortality studies.

5.3.1.1 Workers

In view of the carcinogenic and mutagenic nature of acrylamide and in view of the low MOS values obtained for neurotoxicity and reproductive toxicity in some exposure scenarios **conclusion (iii)** is 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 applies to

- concerns for mutagenicity and carcinogenicity as a consequence of exposure arising from production of the substance, use as an intermediate in the chemical industry for the production of polyacrylamide, use of polyacrylamide, use of polyacrylamide gels for electrophoresis and use of acrylamide based grouts (small and large scale applications),
- concerns for neurotoxicity and reproductive toxicity as a consequence of exposure arising from the small- and large-scale use of acrylamide based grouts.

For large-scale uses of acrylamide grouts the risk characterisation indicates that the exposures that took place following the use in the Hallandsås tunnel were very high. It is understood that the use pattern was normal in the sense of how the product was applied although what happened afterwards may not always occur with the use of these products. However, part of the risk to be considered is how probable it is that unpredictable and excessive exposure will occur during large-scale applications, and that adverse effects to human health will occur. It is considered that this risk is very high for a substance such as acrylamide because of its hazardous properties and because workers are usually applying the product in enclosed or poorly ventilated areas.

No exposure data for small-scale use of acrylamide grouts could be obtained from Europe and the only such data available were from the USA. However, it is likely that working patterns and practices are similar in Europe, for these kinds of tasks. It therefore seems reasonable to assume that exposures would also be similar, and possibly higher where working practices were below the standard reported from these surveys. Given the carcinogenic and genotoxic properties of acrylamide, exposures need to be kept as low as possible. The calculated risk characterisation for neurotoxicity and reproductive toxicity also indicates a possible cause for concern. The information presented in this report indicates that dermal exposure, in particular, can be considerable.

5.3.1.2 Consumers

Polyacrylamide enters a range of consumer products such as soap, shaving foam and hair gels, and gardening products. There are no measurements available, but estimations of consumer exposure lead to values approximately 50 times lower than those encountered occupationally with the major contribution thought to arise from the use of polyacrylamide in cosmetics. Although thresholds cannot be reliably identified, the risk of mutagenicity and carcinogenicity is considered to be very low, therefore **conclusion (iiia)** applies.

Conclusion (iiia) Risks cannot be excluded for all other 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.

5.3.1.3 Humans exposed via the environment

In relation to scenarios except where grouts have been used, acrylamide enters the environment directly via industrial emissions but also indirectly through the addition of polyacrylamide as a flocculating agent to drinking water. Although some measured data are available, these are not considered to be entirely adequate and the risk characterisation with respect to human health is based on the use of modelling techniques. The estimated exposures, for reasonable worst-case scenarios are low.

In relation to the use of acrylamide grouts in large-scale operations, the estimated exposures for reasonable worst-case scenarios are high. Thresholds for genotoxic and carcinogenic effects cannot be reliably identified, and these exposure levels give rise for concern. There is further concern for the threshold effects of neurotoxicity and reproductive toxicity. The result of the assessment of indirect exposure via the environment for the large-scale use of grouts is that **conclusion** (**iii**) applies.

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

In relation to the use of acrylamide grouts in small-scale operations, the estimated exposures for reasonable worst-case scenarios are low. Although there may be some residual risk of mutagenicity and/or carcinogenicity this is likely to be very low. The result of the assessment of indirect exposure via the environment for scenarios except the large-scale use grouts is that **conclusion (iiia)** applies.

Conclusion (iiia) Risks cannot be excluded for all other 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.

Estimations indicate that consumer exposure is very low, with the major contribution thought to arise from the use of polyacrylamide in cosmetics. Although thresholds cannot be reliably identified, the risk of mutagenicity and carcinogenicity is considered to be very low.

5.3.1.4 Combined exposure

Human exposure to acrylamide indirectly via the environment from sources other than using grouts in large-scale operations is clearly negligible. In addition, exposure via consumer products is also very small. The most significant route of exposure is in occupational settings, the contribution from the environment and from consumer products is negligible in comparison and does not add significantly to the overall body burden.

5.3.2 Human health (risks from physico-chemical properties)

Conclusion (**ii**) is reached because there are no risks from physicochemical properties arising from the use of acrylamide.

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

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
bw	body weight / *Bw*, *bw*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
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 50 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)

EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
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 III of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling

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

US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
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 III of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)

Appendix A Contribution to an existing substances review of acrylamide toxicity: the contribution of biological monitoring

Summary

Biological monitoring of exposure to acrylamide is possible by measurement of acrylamide-valine adducts in haemoglobin or by measurement of mercapturic acids in urine. However, there is insufficient data to set guidance values.

Exposure and Uptake

An estimate of total body load of haemoglobin-acrylamide adducts was compared to an assessment of total airborne acrylamide exposure in workers (Bergmark et al., 1993). The observed concentration of acrylamide-haemoglobin adducts in workers was 10-30 times higher than would be expected from the measured concentrations of airborne acrylamide and this was taken as confirmatory evidence that acrylamide is dermally absorbed in humans.

Biological exposure levels

The little occupational exposure data available is based on two papers (Bergmark et al., 1993, Calleman et al., 1994). A comparison of these papers suggests that they are probably looking at the same population and the papers should be viewed as complimentary. Biological monitoring values were correlated with an index of neurological symptoms. The index was derived from the accumulated data of clinical examination, results from a vibration sensitivity test apparatus and an electroneuromyographic apparatus (Calleman et al., 1994). In the same paper values were reported for airborne acrylamide. No attempt was made to correlate these measurements with biological monitoring values because skin absorption was considered the primary route of exposure. Blood samples were taken 1-hour post shift and urine was collected for 24 hours beginning at the start of the workers shift (Calleman et al., 1994).

Free acrylamide in plasma

Free acrylamide was measured in the plasma of 41 workers (Bergmark et al., 1993). Free acrylamide was only detected in 17 samples and the maximum concentration measured was 3.5µmol/l. However, these results did not correlate well with an index of neurological symptoms (corr coeff=0.15, p=0.31). This suggests that measurements of free acrylamide in plasma may not be useful for biological monitoring.

Haemoglobin adducts of acrylamide and glycidamide.

Glycidamide-valine adducts were measured in the haemoglobin of 6 out of 41 workers exposed to acrylamide and acrylonitrile (Bergmark et al., 1993). The results were in the range 1.6-32 nmol/g of haemoglobin and correlated well with acrylamide-valine adduct measurements. Calleman et al. (1994) measured acrylamide-valine adducts in haemoglobin. These results, for a work force of 41 who were exposed to acrylamide, were in the range 0.3-34 nmol/g of haemoglobin. There was a close correlation between acrylamide-valine adduct concentrations and an index of neurological symptoms for each worker (Corr coeff=0.67, $p<0.001$). This suggests that measurement of acrylamide-valine adducts are suitable for biological monitoring.

Mercapturic acids

Mercapturic acids were measured in the urine of 41 workers involved in the production of acrylamide (Calleman et al., 1994). The values for each job category are shown in **Table A.1**. A correlation with indices of neurological symptoms was intermediate between those of free acrylamide and valine adducts (Corr coeff=0.42, $p<0.01$). This indicated that measurement of mercapturic acids may be suitable for biological monitoring in instances where measurement of haemoglobin acrylamide-valine adducts is inappropriate.

Table A.1 Biological monitoring results for different job categories
(From Calleman et al., 1994)

Job Category	Free Acrylamide µmol/litre	Mercapturic Acid µmol/24 hrs	Acrylamide-Valine adducts nmol/g Hb	Airborne acrylamide concentrations mg/m^3 *
Controls n=10	0.92	3.0 ± 1.8	0	-
Packaging n=5	2.2	93 ± 72	3.9 ± 2.5	-
Polymerisation n=12	1.3	58 ± 75	7.7 ± 3.4	0.19-8.8
Ambulatory n=7	2	53 ± 35	9.5 ± 7.3	-
Synthesis n=13	1.8 ± 0.8	64 ± 46	13.4 ± 9.8	0.11-3.01

* A 10-minute sampling period was used. It was unclear whether the results were expressed as time weighted averages.

Four workers were excluded from this analysis because they had not been exposed to acrylamide for the last four months or had less than 6 months exposure in the work place.

Job categories

Synthesis: Conversion of acrylonitrile to acrylamide, Concentration of the solution and its aliquoting into smaller quantities.
Polymerisation: treatment of acrylamide solution to give a polyacrylamide-starch co-polymer.
Packaging: Drying, grinding and bagging of polyacrylamide
Controls: Unexposed subjects
Ambulatory: Not adequately explained in text

Measurement for Biological Monitoring

The papers by Bergmark et al. (1993) and Calleman et al. (1994) are primarily population studies and provide limited data concerning the limits of detection and precision of the methods used.

Free acrylamide in plasma

Free acrylamide in human plasma was measured using Gas Chromatography with electron capture detection (Calleman et al., 1994). The lower limit of detectability for this assay was 0.56 µmol/l. Several non-occupationally exposed control subjects had values slightly above the lower limit of detection. However, these results can probably be regarded as artefactual because the same subjects did not exhibit detectable acrylamide-haemoglobin adducts.

Haemoglobin adducts

Acrylamide-valine adducts in haemoglobin were measured using a modified Edman degradation technique (Tornquist et al., 1988) with subsequent derivatisation using pentafluoro phenyl isothiocyanate prior to analysis by Gas Chromatography/Mass Spectroscopy (Bergmark et al., 1993; Calleman et al., 1994). The Mass Spectrometer was used in negative ion chemical ionisation mode with an internal standard of deuterated hydroxyvaline. This method was used to measure adduct levels in human haemoglobin samples from workers exposed to acrylamide and values as low as 0.01 nmol/g of haemoglobin were detected.

Glycidamide/haemoglobin adducts were measured by hydrolysing samples in acid together with a deuterated internal standard, adducts were partially purified using anion exchange chromatography and derivatized using methanol/HCl together with heptafluorobutanoic anhydride.

Glycidine/cysteine adducts were then identified and measured by Gas Chromatography/Mass Spectroscopy (Bergmark et al., 1993). The Mass Spectrometer was used in positive ion chemical ionisation mode. Values as low as 1.6 nmol/g of haemoglobin were detected. However, only a limited number of samples were measured using this technique due to the labour intensive methodology.

Mercapturic acids

Mercapturic acids were measured in urine by hydrolysing the mercapturic acids to S-(2-carboxyethyl) cysteine which was derivatized with a fluoraldehyde reagent prior to analysis by HPLC with fluorescence detection (Calleman et al., 1994). This method detected mercapturic acids formed by acrylamide and acrylonitirile. No lower limit of detection was quoted for this method but the background level of S-(2-carboxyethyl) cysteine in the urine of unexposed subjects were observed.

Acrylamide concentrations in the aquatic environment may be measured by a variety of techniques. The major techniques that may be used are bromination, refractive index, polarography, and gas chromatography. For aquatic systems the most sensitive method is electron capture gas chromatography (sensitivity 0.1 ppb). There are some problems in analysing acrylamide in water because it hydolyses under acidic or alkaline conditions.

European Commission

EUR 19835 EN **European Union Risk Assessment Report**
Acrylamide, Volume 24

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

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The report provides the comprehensive risk assessment of the substance acrylamide. It has been prepared by the United Kingdom 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 ecosystem has been determined. For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The human health risk assessment for acrylamide concludes that there is at present concern for workers and humans exposed via the environment. For consumers the risk assessment concludes that a risk cannot be excluded as the substance is identified as a non-threshold carcinogen. The risks though are low and this should be taken into account when considering the feasibility and practicability of further specific risk reduction measures. The risk assessment for the environment concludes that there is at present concern for aquatic ecosystem, while no concerns were identified for atmosphere, terrestrial ecosystem and for microorganisms in the sewage treatment plant from sources of acrylamide covered by Regulation 793/93. However, more information is needed about the toxicity of acrylamide to terrestrial organisms for use of acrylamide-based grouts in construction applications.

The conclusions of this report will lead to risk reduction measures to be proposed by the Commission's Committee on risk reduction strategies set up in support of Council Regulation (EEC) 793/93.

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