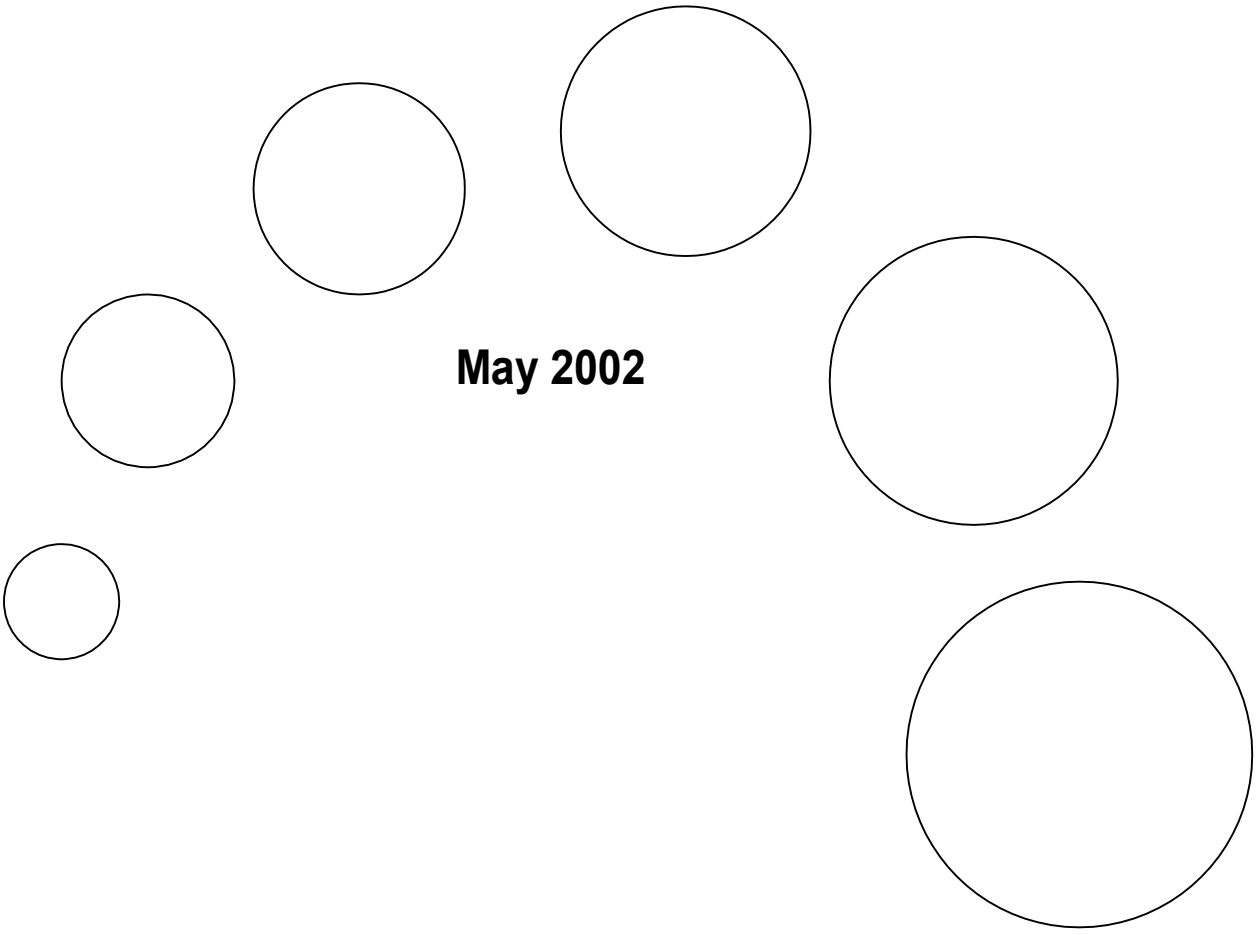
NICNAS

National Industrial Chemicals Notification and Assessment Scheme

Acrylamide

Priority Existing Chemical Assessment Report No. 23



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Preface

This assessment was carried out under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). This Scheme was established by the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act), which came into operation on 17 July 1990.

The principal aim of NICNAS is to aid in the protection of people at work, the public and the environment from the harmful effects of industrial chemicals.

NICNAS assessments are carried out in conjunction with Environment Australia and the Therapeutic Goods Administration, which carry out the environmental and public health assessments, respectively.

NICNAS has two major programs: the assessment of the health and environmental effects of new industrial chemicals prior to importation or manufacture; and the other focussing on the assessment of chemicals already in use in Australia in response to specific concerns about their health and/or environmental effects.

There is an established mechanism within NICNAS for prioritising and assessing the many thousands of existing chemicals in use in Australia. Chemicals selected for assessment are referred to as priority existing chemicals.

This priority existing chemical report has been prepared by the Director of NICNAS, in accordance with the Act. Under the Act, manufacturers and importers of priority existing chemicals are required to apply for assessment. Applicants for assessment are given a draft copy of the report and 28 days to advise the Director of any errors. Following the correction of any errors, the Director provides applicants and other interested parties with a copy of the draft assessment report for consideration. This is a period of public comment lasting for 28 days during which requests for variation of the report may be made. Where variations are requested, the Director's decision concerning each request is made available to each respondent and to other interested parties (for a further period of 28 days). Notices in relation to public comment and decisions made appear in the *Commonwealth Chemical Gazette.*

In accordance with the Act, publication of this report revokes the declaration of this chemical as a priority existing chemical; therefore manufacturers and importers wishing to introduce this chemical in the future need not apply for assessment. However, manufacturers and importers need to be aware of their duty to provide any new information to NICNAS, as required under section 64 of the Act.

For the purposes of section 78(1) of the Act, copies of assessment reports for new and existing chemical assessments may be inspected by the public at the library of the National Occupational Health and Safety Commission (NOHSC). Summary Reports are published in the *Commonwealth Chemical Gazette*, which are also available to the public at the NOHSC library.

Copies of this and other priority existing chemical reports are available on the NICNAS web site. Hardcopies are available from NICNAS either by using the order form at the back of this report, or directly from the following address:

GPO Box 58

Sydney

NSW 2001

AUSTRALIA

Tel: 1800 638 528

Fax: +61 (02) 8577 8888

Other information about NICNAS (also available on request and on the NICNAS web site) includes:

- NICNAS Service Charter;
- information sheets on NICNAS Company Registration;
- information sheets on the Priority Existing Chemicals and New Chemical assessment programs;
- safety information sheets on chemicals that have been assessed as priority existing chemicals;
- details for the NICNAS Handbook for Notifiers; and
- details for the *Commonwealth Chemical Gazette*.

More information on NICNAS can be found at the NICNAS web site:

http://www.nicnas.gov.au

Other information on the management of workplace chemicals can be found at the web site of the National Occupational Health and Safety Commission:

http://www.nohsc.gov.au

Overview

The chemical acrylamide (CAS No 79-06-1) was declared a priority existing chemical for full risk assessment on 1 August 2000, in response to concerns relating to possible human health and environmental hazards associated with the high-volume use of the monomer and widespread use of polyacrylamide products, particularly in effluent and water treatment. Concern over the use of acrylamide-containing grouts was also identified in the declaration notice.

Acrylamide is not manufactured in Australia, but approximately 5000 tonnes are imported and used annually. Acrylamide-derived monomers and polymers containing small amounts of residual acrylamide are also imported.

The major use of acrylamide in Australia is in the production of polyacrylamides and co-polymers, which are used for a variety of purposes. The only other reported use of acrylamide in Australia is in the laboratory preparation of polyacrylamide gels used for electrophoresis. Overseas, acrylamide and acrylamide related monomers have been used in grout products for in situ polymerisation in operations such as pipeline/manhole repair (small scale) and in tunnel construction (large scale). No recent importers or users of grouts in Australia were identified.

The main uses of polyacrylamides are as flocculants for treating industrial effluent/wastewater e.g., from mining and paper/pulp manufacture, for sewage treatment, and to a lesser extent, treatment of drinking water. Other uses of polyacrylamides are in surface coatings and adhesives, textile dyeing, leather processing, paper and cardboard manufacture and in cosmetics. Residual acrylamide monomer in polyacrylamide products is generally below 0.1%, although up to 2% monomer levels have been reported in polyacrylamides used in some surface coatings.

Environmental exposure to acrylamide may occur from polymer manufacture and from polymer use. Although some release to air is possible, the vast majority (>99%) of acrylamide released to the environment is likely to partition to water. Although both biotic and abiotic degradation occurs, polyacrylamide is unlikely to degrade to acrylamide in the environment. In the atmosphere, acrylamide is highly reactive with hydroxyl radicals and therefore the concentrations will be low and very short lived.

The available ecotoxicological data, indicates that acrylamide is slightly toxic to aquatic plants (lowest EC50=33.8 mg/L) and organisms (lowest Daphnia EC50=98 mg/L and lowest fish EC50=85 mg/L). While studies have shown that it does repel colonisation by marine bacteria and mussels, its direct toxicity to these organisms cannot be concluded from the studies. It appears that acrylamide does have some toxic effect on micro-organisms and terrestrial plants but only to a slight degree. Based on current patterns (excluding grouting) of monomer and polymer use, risks to the environment are expected to be low.

In Australia, occupational exposure to acrylamide is reported to be limited to workers engaged in polymer production and laboratory workers using acrylamide in the preparation of polyacrylamide gels for electrophoresis. Exposure to residual

acrylamide monomer may also occur from use of polyacrylamides. Exposure of the general public to acrylamide may also occur from use of polyacrylamides in drinking water treatment and their use in cosmetics and food packaging. Occupational exposures are likely to result from inhalation and dermal exposure, whereas public exposures will be almost exclusively via dermal and oral routes.

Acrylamide is absorbed by oral, inhalation and dermal routes and following absorption, is widely distributed in the body. Acrylamide is extensively metabolised mainly via glutathione conjugation of parent compound and the epoxide metabolite, glycidamide. Both acrylamide and glycidamide bind to RNA, DNA and proteins and form adducts with haemoglobin in blood.

Acrylamide has been shown to be a skin irritant in humans, with skin sensitisation potential and eye irritant properties demonstrated in animals. The critical effect from both acute and repeated exposure to acrylamide is neurotoxicity. Peripheral neuropathy followed by central nervous system effects result from prolonged exposure to acrylamide. Other consequences which have been demonstrated in animals following repeated exposure, include carcinogenicity and reproductive effects. No firm conclusions regarding carcinogenicity in humans could be drawn from available epidemiological studies. Similarly, no conclusions could be drawn regarding the mechanism of action for tumourigenicity in animals and its relevance to humans, as evidence indicates both genotoxic and epigenetic modes of action.

Based on acrylamide-induced neurotoxicity, it was concluded that all occupational scenarios associated with the use of acrylamide present a moderate to high degree of risk to workers, particularly where solid (crystal) acrylamide is used. Although these risks are significantly reduced by the wearing of personal protective equipment and by reducing airborne levels with engineering controls, a considerable amount of uncertainty is associated with dermal exposure, particularly as limited evidence from overseas monitoring data indicates significant skin absorption even when wearing protective gloves. Occupational risks from use of polyacrylamide products (solid or liquid) were considered to be low.

Public heath risks from ingestion of acrylamide in water and food and from dermal absorption from cosmetic products were considered to be negligible.

A number of recommendations are made for reducing occupational health and safety risks from acrylamide. These include the establishment of NOHSC health surveillance guidelines and development of a biological exposure index (BEI), following completion of overseas work on method validation. Such a standard would provide a more accurate measure of exposure than air monitoring, as skin absorption would also be taken into account. A number of engineering controls are also recommended for potential high exposure work scenarios. Provision of adequate hazard information is considered central to occupational risk reduction and it is recommended that MSDS and labels be amended in line with relevant NOHSC Codes of Practice and to reflect the updated hazard classification recommended in this report.

Although the public health assessment concludes that the risk associated with residual acrylamide in cosmetics is negligible, it is recommended that the National Drugs and Poisons Schedule Committee (NDPSC) consider regulating the level of polyacrylamide permissible in such products in Australia. NHMRC regulations already exist for use of polyacrylamides in drinking water treatment.

The secondary notification requirements of this report specify that importers/suppliers of acrylamide-containing grouts provide information to NICNAS for further assessment.

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Acronyms and Abbreviations

α	alpha
Å	angstrom
ACGIH	American Conference of Governmental Industrial Hygienists
ACS	Australian Customs Service
ADG Code	Australian Dangerous Goods Code
AMPA	Acrylamide Monomer Producers Association
ANZFA	Australian and New Zealand Food Authority
β	beta
BEI	Biological Exposure Index
bw	bodyweight
C	centrigrade
cAMP	Cyclic adenosine monophosphate
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
cm	centimetre
CNS	central nervous system
d	day
DNA	deoxyribonucleic acid
EASE	estimation and assessment of substance exposure
EC	European Commission
EC50	median effective concentration
EC100	concentration effective for 100% of organisms tested
ED10	dose effective for 10% of recipients
EU	European Union
EUSES	European Union System for the Evaluation of Substances
F0	parental generation
F1, F2	first filial generation, second filial generation
g	gram
h	hour

HPLC	high-performance liquid chromatography
HSE	Health and Safety Executive
IARC	International Agency for Research on Cancer
IBC	intermediate bulk container
IPCS	International Programme on Chemical Safety
ip	intraperitoneal
ITER	International Toxicity Estimates of Risk
IUCLID	International Uniform Chemical Information Database
kg	kilogram
Koc	organic carbon partition coefficient
Kow	octanol/water partition coefficient
L	litre
LC50	median lethal concentration
LC100	Concentration lethal to 100% of organisms tested
LCT	Leydig cell tumours
LED10	the lower 95% confidence limit of dose associated with the ED_{10}
LU	luteinizing hormone
LD50	median lethal dose
LOAEL	lowest-observed-adverse-effect level
M	metre
MAPK	mitogen activated protein kinase
MBA	methylene-bis-acrylamide
MFO	mixed function oxidase
min	minute
ML	megalitre
mL	millilitre
MLE	maximum likelihood for effect
mm	millimetre
MOA	mode of action
MOE	margin of exposure
mol	mole
mRNA	messenger ribonucleic acid

MSDS	Material Safety Data Sheet
NDPSC	National Drugs and Poisons Schedule Committee
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NIOSH	National Institute of Occupational Safety and Health
NMA	N-methylolacrylamide
NOAEL	no-observed-adverse-effect level
NOHSC	National Occupational Health and Safety Commission
NTP	National Toxicology Program
NOEC	no-observed-effect concentration
OECD	Organisation for Economic Cooperation and Development
OSHA	Occupational Safety and Health Administration (USA)
Pa	pascal
PCNA	proliferating cell nuclear antigen
PDGF	platelet derived growth factor
PEC	predicted environmental concentration
PNEC	predicted-no-effect concentration
PNS	peripheral nervous system
ppb	parts per billion
PPE	personal protective equipment
PPG	Polyelectrolyte Producers Group
PVC	polyvinyl chloride
RNA	ribonucleic acid
s	second
SCBE	self contained breathing equipment
SCE	sister chromatid exchange
SIAR	SIDS Initial Assessment Report
SIDS	Screening Information Data Set
SMR	standardised mortality ratio
STEL	short-term exposure limit
STP	standard temperature and pressure

SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
TGA	Therapeutic Goods Administration
TPA	tetradeconylphorbol acetate
TSH	thyroid stimulating hormone
TVA	tunica vaginalis mesotheliomas
TWA	time-weighted average (NOHSC)
µg	microgram
UK	United Kingdom
µmol	micromole
UCL	upper confidence limits
UDS	unscheduled DNA synthesis
UN	United Nations
US	United States
US EPA	United States Environmental Protection Agency
UV	ultraviolet
v/v	volume per volume
WHO	World Health Organization
w/v	weight per volume

1. Introduction

1.1 Declaration

Acrylamide (CAS No 79-06-1) was declared a priority existing chemical under the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act) by the Minister for Employment, Workplace Relations and Small Business by notice in the *Commonwealth Chemical Gazette* of August 2000.

The grounds for declaring acrylamide a priority existing chemical were:

- the high volumes imported into Australia;
- use in a number of industrial processes with occupational and public exposure to a range of products containing the chemical;
- the extent of its use particularly in polymer form in effluent, sewage and water treatment;
- possible use in grouts in Australia; and
- exposure may give rise to adverse health effects.

1.2 Purpose and scope of the priority existing chemical assessment

This is a full risk assessment, covering all industrial uses of acrylamide in Australia.

The objectives of this assessment are to:

- characterise current and potential environmental, occupational, and public exposure to acrylamide;
- characterise the human health hazards and environmental effects/impact;
- assess current risk management measures for acrylamide including occupational exposure standards and other current standards and guidelines;
- make recommendations on control measures for the management of the risks to occupational/public health and appropriate hazard communication measures;
- make recommendations on control measures for the management of environmental hazards along with information on disposal and waste management.

1.3 Sources of information

In accordance with the Act, manufacturers and importers of acrylamide were required to apply for assessment and supply relevant data. No manufacture of acrylamide takes place in Australia. Applications were received from importers, formulators and end users of acrylamide. Following declaration of acrylamide as a priority existing chemical, 11 companies and one non-government organisation, applied for assessment. These comprised companies importing/supplying acrylamide and acrylamide co-

monomers and companies using acrylamide for manufacture of polymers. At a meeting of applicants/notifiers in September 2000, it was explained that the assessment would concentrate on uses of acrylamide and that the data requirements for polyacrylamides would be restricted to use, production volumes and levels of residual monomer in these products.

To enhance efficiency and avoid duplication of effort, an important strategy for this assessment was to utilise overseas assessments recently carried out by other organisations, namely, the European Union (EU) Risk Assessment of acrylamide prepared by the Health & Safety Executive of the United Kingdom (UK HSE) and the recently finalised Screening Information Data Set (SIDS) report on acrylamide by the Organisation for Economic Cooperation and Development (OECD). These reports were utilised mainly for sections on health and environmental hazards, but were also a useful source of data for sections on environmental and human (occupational and public health) exposure, where data were not available for Australian scenarios.

Other national and international assessments/reviews on acrylamide were utilised as additional sources of information. These included: EU Scientific Committee on Toxicology, Ecotoxicology and the Environment review of the EU report (CSTEE 2001); Acrylamide Monomer Producers Association (AMPA) & Polyelectrolyte Producers Group (PPG) reviews on mechanisms of acrylamide-induced cancers (Crump 1999a, 1999b, 2000a, 2000b); International Agency for Research on Cancer (IARC 1986; 1994); International Toxicity Estimates of Risk (ITER) review (TERA 1998) and the International Program on Chemical Safety (IPCS) Monograph 49 (IPCS 1985).

A comprehensive literature search was also carried out for data published since the finalisation of those reports. All studies that were not sighted, including some references from the EU & OECD reports, are indicated with an asterisk (*) in this report.

The import of acrylamide into Australia was monitored through information provided by the Australian Customs Service (ACS) and overseas export notifications (i.e. US EPA).

A survey was conducted of capital cities and some regional water supply and sewage treatment authorities to obtain information on the use of acrylamide and n-methylolacrylamide or its polymers in water and sewage treatment, and infrastructure construction and repair.

NICNAS staff also carried out site visits to workplaces involved in polyacrylamide manufacture.

1.3.1 Data supplied by applicants

The following data was received from applicants:

- quantities of acrylamide imported;
- quantities of acrylamide polymer products containing acrylamide imported;
- quantities of acrylamide co-monomer products containing acrylamide imported;

- quantities of acrylamide polymer products containing acrylamide manufactured;
- uses of the chemical and products containing the chemical;
- lists of end-users;
- information on production processes using acrylamide;
- risk management initiatives;
- MSDS and labels;
- results of workplace atmospheric monitoring; and
- unpublished reports and data on health effects/toxicokinetics of acrylamide.

1.4 Peer review

During all stages of preparation, the report has been subject to internal peer review by NICNAS, Therapeutic Goods Administration (TGA) and Environment Australia (EA).

External peer review was not undertaken, because the primary source of the hazard information has already been the subject of significant international peer review via EU and OECD processes.

2. Background

2.1 International perspective

Acrylamide was first produced in Germany in 1893 and commercial production began in 1954. Annual production in the US and Japan in the early 1980's was around 40,000 tonnes in each country. Plant capacity for the 3 major US producers of acrylamide was reported at around 75,000 tonnes in 1993 (IPCS 1985, Mannsville 1993). Total EU production in 1995 was 80,000 to 100,000 tonnes (OECD 2001).

Approximately 99.9% of acrylamide in the EU is used in the manufacture of polyacrylamides. Acrylamide is also used in grouting agents and for in situ preparation of polyacrylamide electrophoresis gels. About 80-90% of polyacrylamide produced is used in wastewater treatment (major use), and paper, pulp and minerals processing, with the remainder used in cosmetic additives, soil and sand stabilisation and oil production.

Occupational, public health and environmental problems have been experienced in some countries from the use of acrylamide and N-methylolacrylamide (NMA) grouting agents.

Acrylamide grout production is thought to have ceased in the EU and US in 1997-98 (EU 2000). Grouting agents (products containing separate quantities of acrylamide, a catalyst and an accelerator, mixed together during use to form the grout) are still imported from overseas. A grouting agent known as Spirogel, containing acrylamide and NMA was recently withdrawn from the market by the manufacturer following health and environmental problems associated with its use in the construction of a rail tunnel in southeast Sweden. In the UK, four companies used NMA-based grouts prior to 1998. All four companies have reportedly now switched to alternatives, which do not contain NMA or acrylamide (EU 2000).

The US EPA had proposed to promulgate Rule 2070-AC17 during the second half of the year 2001, banning the manufacture, distribution and use of acrylamide grouts. The proposed rule was withdrawn by that Agency as of 28 June 2000.

A comprehensive risk assessment of acrylamide was recently carried out by the EU (EU 2000). As a result of recommendations made in that report, the European Commission is currently addressing risk management strategies. In this regard, a proposed risk reduction strategy was prepared for discussion by member states at the EU risk reduction strategy meeting in October 2001. The draft document contains both human health risk and environment risk reduction strategies (EC, 2001). A second meeting to further discuss the proposals is scheduled for mid-2002, with final agreement on the recommendations anticipated by the end of 2002 (Dyne, 2001). This strategy includes a proposed prohibition on the use of acrylamide grouts in both small and large-scale applications under the Marketing and Use Directive (76/769/EEC).

Acrylamide is listed as a high production volume chemical by the OECD and has recently been assessed under the Screening Information Data Set (SIDS) Program (OECD, 2001), in which Australia participates. The SIDS Initial Assessment Report (SIAR) was prepared by the UK, who also prepared the EU report, and therefore these

assessments are not completely separate. The SIAR was agreed upon by Member Countries, including Australia, in June 2001. The report recommends that acrylamide is a candidate for further work and based on an existing regional risk assessment for Europe, national exposure information gathering needs to be considered by OECD countries for grouting applications. This priority existing chemical assessment report follows up this recommendation for Australia.

Due to the potential for exposure to residual monomer in polyacrylamides, restrictions have been imposed in a number of countries on certain regulated applications, including cosmetics, the treatment of drinking water and food contact paper and board packaging. The European Scientific Committee on Cosmetic Products and Non-Food Products has recently (1999) recommended tolerable levels of <0.1 and <0.5 ppm acrylamide in non-rinse-off and rinse-off cosmetic preparations respectively. Some countries require registration of polyacrylamide products used for certain applications e.g. water treatment (Netherlands).

A guidance value for acrylamide in drinking water of 0.5μg/L has been set by the World Health Organisation (WHO, 1993). Overseas occupational Threshhold Limit Value (TLV) exposure standards range from 0.03 to 0.3 mg/m^3. Nearly all countries include a skin notation to indicate the potential hazard from dermal absorption.

2.2 Australian perspective

Acrylamide is not manufactured in Australia. Imported acrylamide has been reported for use in polyacrylamide manufacture and gel electrophoresis only.

A NICNAS survey failed to identify importer(s) or users of acrylamide grouts in recent times. It is known that acrylamide grouts were used in the construction of the Geehi Dam in the Snowy Mountains Hydroelectric scheme in 1962-65.

The monomer content of polyacrylamides used in Australia appears to conform to that in polyacrylamide used overseas. No restrictions on the use of polyacrylamides in Australia were identified.

A number of regulatory controls are in place to control the exposure of workers and the general public to acrylamide. Acrylamide is listed in the NOHSC *List of Designated Hazardous Substances* (NOHSC 1999a) and has an atmospheric occupational exposure standard of 0.03 mg/m^3 (NOHSC 1995). Acrylamide is also regulated for transport under the National Road Transport Commission's Dangerous Goods Code (ADG Code) (FORS, 1998).

With regard to public health regulation, the *Australian Drinking Water Guidelines* (NHMRC 1996) stipulate a limit for acrylamide in drinking water of 0.2 μg/L. Acrylamide is not listed in the Australian Health Minister's Advisory Council's *Standard for Uniform Scheduling of Drugs and Poisons* (SUSDP 2000) or the *Joint Australia New Zealand Food Standards Code* (ANZFA 2000).

However, standard 1.4.3 of the *Joint Australia New Zealand Food Standards Code* outlines the conditions under which it is permissible for articles and materials to be in contact with food. Reference is made to standard 1.4.1, which sets out the maximum level for a number of contaminants that may be present in food as a result of contact with the articles and materials regulated in Standard 1.4.3.

Standard 1.4.3 also contains reference to the Australian Standard for Plastics Materials for Food Contact Use, Australian Standard AS2070-1999. This Standard states that new plastics materials used in the manufacture of plastics items for food contact use shall comply with either the relevant US Food and Drugs Administration regulations or the relevant European Commission directives. Compliance with AS2070-1999 is not mandatory. This reference provides information to manufacturers to assist them in ensuring that the packaging used for food is suitable.

The food legislation in the Commonwealth, the States and Territories, and New Zealand regulate the sale and importation of food in Australia and New Zealand, and prohibit the sale of food that is "unsuitable" or contains a substance that is foreign to the nature of the food. Modified polyacrylamide resins may be used as processing aids for packaged water and in water used as an ingredient in other foods. The *Food Standards Code* states that the maximum permitted level of the resins resulting from this use, is the level that would be consistent with good manufacturing practice.

2.3 Assessments by other national or international bodies

International reviews of the health and/or environmental effects and risk assessments for acrylamide have been carried out by IPCS (1985), IARC (1986, 1994); International Toxicity Estimates of Risk (ITER) review (TERA 1998), OECD SIDS (2001) and EU (EU 2000).

3. Applicants

Following the declaration of acrylamide as a priority existing chemical, ten companies and one non-government organisation (NGO) applied for assessment. The companies supplied information on the properties, import quantities and uses of the chemical. In accordance with the *Industrial Chemicals (Notification and Assessment) Act (1989)*, NICNAS provided the applicants with a draft copy of the report for comments during the corrections/variation phase of the assessment. The applicants were:

Amtrade International Pty Limited
570 St Kilda Road
Melbourne VIC 3004

Australian Council of Trade Unions
393 Swanston Street
Melbourne VIC 3000

BASF Australia Limited
Kororoit Creek Road
Altona VIC 3018

Ciba Specialty Chemicals Pty Limited
6-8 Donaldson Street
Wyong NSW 2259

Cytec Australia Holdings Pty Limited
21 Solent Circuit
Baulkham Hills NSW 2153

Merck Australia Limited
207 Colchester Road
Kilsyth VIC 3137

Ondeo-Nalco Australia Pty Limited
2 Anderson Street
Botany NSW 2019

PCA Hodgson Chemicals Pty Limited
19-25 Anne Street
St. Marys NSW 2760

Rohm and Hass Australia Pty Limited
969 Burke Road
Camberwell VIC 3124

SNF Australia Pty Limited
298 Broderick Road
Lara VIC 3212

Yorkshire Australia Pty Limited
1-13 Rooney Street
Burnley VIC 3121

4. Chemical Identity and Composition

4.1 Chemical identity

IUPAC Name:	2-Propenamide
Chemical Name:	Acrylamide
CAS No.:	79-06-1
EINECS No.:	201-173-7
Synonyms:	Acrylic acid amide; Ethylene carboxamide; Propenoic acid amide; Vinyl amide
Trade Names:	Acrylamide; Acrylamide, Dry Crystals; Acrylamide, 30% aqueous, inhibited; Acrylamide, 50% aqueous, inhibited; Acrylamide, 30% LC, inhibited;
Molecular Formula:	C_3H_5NO
Structural Formula:	$CH_2 = CH - CONH_2$
Molecular Weight:	71.09

4.2 Typical polymer composition

Locally produced and imported acrylamide polymers typically have residual monomer levels of 0.1% or less, but for potable water treatment, monomer levels of < 0.05% are required. Polymers used for surface coatings and adhesives were reported to contain monomer levels up 2%.

Some acrylamide polymers are formulated from acrylamide-derived monomers, from related monomers e.g. NMA and some from acrylamide co-monomers. The main co-monomers are acrylic acid/sodium acrylate used in anionic polyacrylamide and dimethylaminoethylacrylate methyl chloride and acrylamidopropyltrimethylammonium chloride in cationic polyacrylamides, and as such contain residual levels of other monomers.

4.3 Impurities and additives

Commercial grades of acrylamide are over 99% pure. Impurities that may be present in trace amounts are:

Acrylic acid

Acetamide

Acetone

Acrylonitrile

Copper

Formaldehyde

Hydroquinone

Methacrylamide

Methyl ether hydroquinone

Peroxide

Propanamide

Sulfate

For acrylamide produced by the copper catalyst process, copper in the form of copper salts is added to aqueous solutions as an inhibitor at concentrations of not less than 2 ppm.

5. Physical and Chemical Properties

5.1 Physical properties

Present as a white crystalline solid, acrylamide sublimes slowly at room temperature. In the absence of light and at temperatures up to its melting point, it does not polymerise significantly.

The chemical is commercially available either as the solid, a 30% or a 50% aqueous solution, the latter requiring the addition of stabilisers to prevent polymerisation.

Table 5.1 - Physical properties of acrylamide

Property	Value	Reference
Boiling point *		
0.27 kPa	87 ^{0}C	Kirk-Othmer, (1991)
0.67 kPa	103 ^{0}C	Kirk-Othmer, (1991)
1.4 kPa	116.5 ^{0}C	Kirk-Othmer, (1991)
3.3 kPa	136 ^{0}C	Kirk-Othmer, (1991)
Melting point	84.5 ^{0}C	Kirk-Othmer, (1991)
Density	1.122 g/mL at 30 ^{0}C	Kirk-Othmer, (1991)
Water solubility	2.16 g/mL at 30 ^{0}C	Kirk-Othmer, (1991)
Vapour pressure	0.9 Pa at 25 ^{0}C	Kirk-Othmer, (1991)
Partition coefficient (*Log Kow)*	Measured values range from – 1.24 to – 0.67	IUCLID
Autoignition temperature	Not applicable	
Flash point	138 ^{0}C	
Explosive limits	Not applicable	

* Acrylamide polymerises rapidly above its melting point (Kirk-Othmer, 1991). Boiling points are given at pressures other than normal atmospheric.

5.2 Chemical properties

Acrylamide undergoes reactions typical of chemicals containing a reactive double bond and an amide group. At temperatures above its melting point, acrylamide polymerises in a rapid, highly exothermic reaction. Exposure to uv light also results in ready polymerisation (Merck, 1983).

Polymers are hydrophilic with molecular weights of between 1 to 30 million and chain lengths between 14,000 to 420,000 monomer units. When reacted with unsaturated

quaternary ammonium compounds, cationic copolymers are produced, whilst reaction with carboxylic or sulphonic acids yields anionic copolymers.

The process of polymerisation is not 100% complete and polymers have varying residual amounts of unreacted monomer.

Acrylamide is incompatible with reducing agents, copper, aluminium, brass and bronze. Iron or rust may trigger rapid exothermic polymerisation of acrylamide solutions.

5.3 Physico-chemical hazards

Acrylamide crystals can generate dusts which are flammable in air and can explode. The chemical is designated as explosive in the ADG Hazchem Code. Minimum ignition energy is 7 millijoule (850 g/m^3 dust concentration in air, moisture 0.1-0.8%, particle 0.1mm).

5.4 Conversion factors

1 ppm (acrylamide in air) = 5 mg/m^3

1 mg/m^3 = 0.2 ppm

6. Methods of Detection and Analysis

6.1 Identification

Contact with three large-scale users of acrylamide monomer in Australia indicated that these companies do not confirm the identity of the chemical on receipt, rather they rely on the analytical certificate accompanying the shipment.

Acrylamide can be identified by infra-red absorption spectrum in the range 3600 cm^{-1} to 600 cm^{-1}, showing little ultra-violet absorption above 2400 Å, with a maximum probably below 2100 Å. (Carpenter and Davis, 1957).

6.2 Methods of analysis

6.2.1 Analysis in water

A variety of methods are given in the literature. Croll and Simkins, (1972), describe an electron-capture gas chromatography technique for determining acrylamide in water that can measure levels down to 0.1µg/L. As acrylamide is soluble in water, the method involves bromination of acrylamide in aqueous solution then extraction by diethyl ether of the resultant α, β-dibromopropionamide. The extracted solution is then concentrated and injected into the gas chromatograph. The authors note that ethyl acetate was a more efficient solvent than diethyl ether but that the solution was more difficult to concentrate for use in the gas-chromatograph.

Method 8032A of the US EPA describes a modified gas chromatographic method based on bromination of the acrylamide double bond followed by extraction in ethyl acetate. The method can detect acrylamide monomer in river water, sewage, effluent and seawater down to a limit of 0.032 µg/L

6.2.2 Analysis of residual acrylamide in acrylamide polymers

Acrylamide readily polymerises and a method is described by Macwilliams et al, (1965) to determine residual acrylamide in polymers using pology or ultraviolet spectrophotometry. The residual acrylamide is extracted from the polymer using a 70/30 (v/v) methanol water solvent. These methods are claimed to allow detection in the ranges 0.01 - 0.5% and 0.005 – 0.10% acrylamide respectively. An enhanced polarographic method is described by Besto and McLean (1976) for detection of residual monomer in polymers and other substances. The authors use Differential Pulse Polography extracting acrylamide with an 80/20 (v/v) methanol-water solvent, and claim a detection limit of less than 1 ppm. Croll (1971) describes a gas chromatographic method again using 80/20 (v/v) methanol-water with a claimed detection limit of 0.0004% acrylamide.

6.2.3 Atmospheric monitoring

To measure acrylamide levels in the atmosphere, an air sample is collected onto suitable media, extracted and then analysed. In a method used by one importer for

personal monitoring of workers' breathing zones, a portable pump is attached to clothing in the region of the worker's face and air is pumped through a silica gel tube. The acrylamide absorbed by the silica is extracted and the amount determined by one of the range of techniques available including pology raphy, gas chromatography or high-performance liquid chromatography (HPLC).

It has been reported that that monitoring of acrylamide using silica gel is unsuitable for the measurement of air concentrations when crystal acrylamide is used since it only measures acrylamide vapour. It was suggested that to accurately measure air concentrations when using crystal acrylamide a separate filter has to be included to collect respirable acrylamide dust, (SNF 2000).

Method number 21 of the United States Occupational Safety and Health Administration (OSHA, 1980) describes a method of atmospheric sampling where air is pumped through a silica gel tube and a tube packed with a glass fibre filter. Once sampling has been completed, the silica and glass filter are extracted in methanol and the extract analysed by gas chromatography with a nitrogen/phosphorous detector. The method is claimed to measure atmospheric concentrations of acrylamide down to 3.8 $\mu g/m^3$ (1.3 ppb). This method is used in Australia.

A polarographic method is described by the U S National Institute for Occupational Safety and Health (NIOSH, 1976) where air is sampled through a tube containing distilled water at a flowrate of 1 L/min. The acrylamide in the sample is reduced at the double bond by methanol, and the sample is then passed through an ion-exchange resin before a fraction is injected into a differential pulse polarograph. The limit of detection is reported to be 1 µg/mL of acrylamide solution. The total volume of air sampled determines the actual concentration of acrylamide in the sampled air.

6.2.4 Biological monitoring

Analysis of acrylamide in biological systems has also been done using the various methods described above. Work by Poole et al, (1981) and Raymer et al, (1993) describe determination of acrylamide in nerve tissue homogenates by electron-capture gas chromatography. A spectrophotomeric method in the visible wavelengths is described by Mattocks (1968) to analyse acrylamide in rat urine by reacting the sample with diazomethane in methanol-ether.

Another HPLC method is described by Barber et al (2001), for measuring acrylamide and its metabolite glycidamide (also considered to be neurotoxic) levels in rat plasma. Samples are deproteinised with acetonitrile and chromatography performed using isoelectric elution and UV absorption detection. The limit of detection for acrylamide is 0.05 µg/mL of plasma.

A gas chromatography/mass spectrometry procedure to measure acrylamide levels in rat blood is described by Bergmark et al, (1991). The method is based on the determination of haemoglobin cysteine adducts of acrylamide. A later paper by Bergmark et al, (1993) applied the technique to determination of acrylamide exposure in a group of factory workers but measured valine adducts. The technique of Bergmark et al, (1993) was used by Hagmar et al, (2001), where a positive correlation was seen between adduct levels and clinical signs of neurotoxicity in a group of workers exposed to acrylamide and N-methylolacrylamide.

An HPLC assay of the urinary metabolite of acrylamide, N-acetyl-s-(propionamide)-cysteine, described by Wu et al, (1993) is the basis of a screening program for exposure

of workers to acrylamide in a polyacrylamide manufacturing plant. This method is currently being validated.

7. Use, Manufacture and Importation

7.1 Manufacture and importation

Acrylamide is not manufactured in Australia.

Approximately 5000 tonnes were imported in the year 2000. Data collected from industry indicates that this import volume has been static since 1998.

Acrylamide is available in a number of forms in Australia, viz. crystals and aqueous solutions. Acrylamide-derived monomers containing varying amounts of acrylamide, and acrylamide polymers containing small amounts of residual acrylamide are also imported.

The chemical is imported in a variety of containers with the crystals packed in 25 kg paper bags with plastic liners. Aqueous solutions arrive in 1000 L intermediate bulk containers or 20 L steel drums.

Various grades of acrylamide are available with the industrial grade typically being 98% to 99% pure. Chemical for laboratory use ranges from a routine grade for use in analysis through to higher purity grades for electrophoresis and for molecular biology applications. Laboratory use is less than 100 kg per year.

7.2 Uses

7.2.1 Uses in Australia

The uses identified for acrylamide monomer in Australia are:

- industrial manufacture of polymers for a variety of uses (Table 7.1);
- in situ production of acrylamide gels in laboratories for use in electrophoresis;
- as a laboratory reagent for analysis and testing of products and/or media for the presence and/or levels of acrylamide.

7.2.2 Uses of monomer

Industrial production of polymers

The principal use of acrylamide is in the production of high relative molecular mass polyacrylamides or of copolymers, particularly with unsaturated quaternary ammonium compounds (cationic copolymers) or carboxylic or sulphonic acids (anionic copolymers).

Electrophoresis gels

Acrylamide is used in this application mainly by research establishments of universities and hospitals for the separation of nucleic acids. Laboratory staff use the monomer to make a polymer gel for use in electrophoresis. The raw material is either acrylamide

powder or aqueous solutions with various concentrations of acrylamide. Pre-poured gels are also available. Acrylamide used for this application is of a higher grade than that used for other industrial applications, with ultra-pure, sometimes aseptic water used in the solutions.

Grouts

Acrylamide-based grouts can be used in geotechnical applications to increase the absolute strength of a rock or soil mass, and in areas where absolute impermeability to water is required such as water and/or sewer pipes, manholes, tunnels, roads, dams etc.

Grouts consist of a three-part product - the acrylamide powder, an accelerator and sodium persulphate. These are placed in a high pressure injection apparatus, mixed with water in the mixing head of the unit and then applied.

In the other applications for grouts, holes are sealed in the structures after leaks develop.

No importers or users of acrylamide grouts in Australia in recent times could be identified. NICNAS conducted a survey of 10 water supply authorities in Australia to determine (amongst other information) if acrylamide-based grouts were used in their pipes or dams. Four replied and those respondents did not indicate use of the grouts.

Information from suppliers in the waterproofing products industry indicates that acrylamide grouts are not used in Australia and that waterproofing contractors avoid it.

7.2.3 Uses of polymers

Acrylamide is highly water soluble and rapidly polymerises in an exothermic reaction to form water soluble polymers. These can be in the form of aqueous solutions, powders or emulsions. Polymers can be either simply polymerised acrylamide or can be further formulated into other specialty chemicals. The polymerisation process is not 100% complete and polymers have varying residual amounts of unreacted monomer. Typically, locally produced and imported polymers have residual monomer levels of 0.1% or less but for potable water treatment, monomer levels of $< 0.05\%$ are required. Polymers used for surface coatings and adhesives contain monomer levels described as less than 0.1% by two manufacturers, but up to 2% by another. The proportion of polymer added to the components making up surface coatings and adhesives ranges from approximately 1.0% up to 10% for some automotive coatings.

Table 7.1 – Uses of acrylamide polymers in Australia

Product type	Use	Approx quantity of acrylamide used per year in tonnes
Aqueous solution	Textile treatment	1250
	Pigment dispersant	
	Flocculant	
Aqueous solution	Surface coatings	27
Aqueous solution	Leather treatment	10
Aqueous dispersion	Surface coatings	730
	Adhesives	
	Paper manufacture	
Crystalline powder	Flocculant	1200
Oil emulsion	Flocculant	2150
Resins	Paint manufacture	2
Resins	Textile lubricant	9
Gel	Textile dying	10

Flocculation

The largest use of industrially produced acrylamide polymers is in the treatment of wastewater arising from mining activities, paper making, treatment of sewerage and other industrial processes. A smaller use is for treatment of potable water.

Flocculation is based on the principles of colloidal suspensions and is used to clean up liquids, particularly aqueous media, either for disposal or human consumption.

Organic and inorganic materials are found in aqueous media either in solution or undissolved form. Dissolved compounds can be inorganic or organic and have differing molecular weights and particle sizes. Undissolved materials are colloids or suspended material of differing particle size. Colloidal particles have a high surface area and are therefore influenced more by surface phenomena than by gravitational forces. It is estimated that the largest colloidal particles will take two years to settle to a depth of one metre in water. Suspensions of colloidal particles can therefore be very stable over time, with hydrophobic and hydrophilic colloids depending on electrostatic and chemical forces respectively for their stability.

Methods of solid-liquid separation such as filtration, sedimentation and centrifugation are not directly applicable to stabilised solutions, as the particles are too fine. Flocculation destabilises these suspensions by either charge neutralisation or absorption allowing solid-liquid separation.

Flocculation uses hydrophilic polymers with molecular weights of 1-30 million and a chain length of 14,000-420,000 monomer units. These polymers may, by homopolymerisation, be non-ionic in nature, or by copolymerisation, cationic or

anionic. The process involves adsorption of the colloid along the polymeric molecular chain forming “flocs”, thereby allowing separation.

Flocculants are available over a wide range of specifications and particular applications might use a combination of different flocculants and other measures to achieve the desired result.

For example, the range of polyacrylamide-based flocculants supplied by one company for use in potable water treatment plants in Australia has the physico-chemical characteristics listed in Table 7.2.

Table 7.2 – Physico-chemical characteristics of some acrylamide polymers used for potable water treatment in Australia

Product	Ionic character	Molecular weight
1	Non-ionic	High
2	Medium/low cationic	High
3	Medium/low cationic	Very high
4	Low cationic	Medium
5	Low anionic	High
6	Low anionic	Very high
7	Medium anionic	High
8	Medium/low cationic	Very high
9	Low anionic	High

Sydney Water uses 4 tonnes of acrylamide polymers per year to treat water and 160 tonnes per year for wastewater treatment.

For the Adelaide metropolitan system, the supply authority uses approximately 40 tonnes of acrylamide polymer flocculants per year in its treatment plants, which cover both potable water and wastewater treatment. Other water authorities surveyed from around Australia did not respond to the survey.

Surface coatings and adhesives

An aqueous 50% solution of acrylamide is used by another company to manufacture acrylic copolymer dispersions for use in surface coatings and adhesives.

In surface coatings, polymers are used as dispersants and binders to provide better pigment separation and flow. Surface coatings are used on home appliances and in the automotive trade.

Textiles

Specialised gels comprised in part of acrylamide polymer are manufactured by another company for use as lubricants in the textile dyeing industry. The gel is dissolved in a water bath containing the dyeing components to which fabric or finished garments are added. The gel lubricates the cloth preventing it from clumping together and aids pigment dispersion during the dyeing process to ensure an even colour.

Leather processing

The finishing and tanning of leather involves a number of processes which vary according to the type of product required, e.g. the use of shoe leather as opposed to hide in a leather jacket. Acrylamide polymers impart a gloss or specific feel and suppleness to leather. The hide is most commonly placed in a drum with the polymer and various other constituents such as dyes, formaldehyde and pigments, then rolled for about two hours. The polymer can also be brushed or sprayed on. There is no set formulation for the components of the mix and the proportion of acrylamide polymer (and the other components) is at the discretion of the operator seeking to obtain the properties required in the tanned product.

Paper manufacture

Polyacrylamides are used in both the paper production process (as retention and drainage aids) and for treatment of mill wastewater. Polyacrylamide emulsions manufactured by a number of companies are used in the manufacture of cardboard cartons to bind white coatings (which are a mixture of calcium carbonate and clay), onto the cardboard. In paper production retention aids bind fibres, fillers, pigments and other components onto the sheet. Drainage aids provide water removal at the paper forming stage of production. In addition, mills use polyacrylamides to treat wastewater.

Cosmetics

Polyacrylamides are used as thickeners in soap and cosmetic preparations, and in skin care and hair grooming products, to impart a smooth after-feel and shine.

No Australian manufacturers/importers providing data indicated use of their products in cosmetics. However, one cosmetics manufacturer provided the following information on the content of acrylamide polymers and co-polymers in their products.

Polymer name	Use	Max % used (finished product)
Acrylamide/ammonium acrylate copolymer	Hair care	1%
Acrylamide/sodium acryloyldimethyltaurate copolymer	Skin care + eye care	2%
Polyacrylamide	All cosmetics	2%

Oil drilling

Liquid or powder partially-hydrolysed polyacrylamides are used as additives to water-based drilling muds to provide a lubricating film and reduce friction at the drill bit, impart stability to shales and clays and increase viscosity.

7.2.4 Overseas uses

Overseas, the monomer is primarily used in the production of polymers and copolymers. Overseas uses for polyacrylamides not identified in Australia include soil stabilisers and binding agents in foundry sand.

8. Exposure

8.1 Environmental exposure

8.1.1 Release

The main use of acrylamide is in the production of acrylamide polymers and copolymers via various polymerisation processes depending upon the product required. Waste may be liquid effluent (including washings) or solid waste (including out-of-specification material) that may be generated from any of these processes. Where possible, waste material is often fed back into the process. Otherwise liquid waste may undergo treatment on site prior to disposal, while the solid material may go to landfill or be incinerated.

Polyacrylamides are used for water clarification, sludge treatment (dewatering) and sewage sludge thickening, primary settlement, 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). They are also used in research laboratories, surface coatings, adhesives and gels in the textile industry. During use, small amounts of the polyacrylamides may be lost to the environment, but it is unlikely that they will degrade to acrylamide. However, residual acrylamide monomer will be present (generally <0.1%). This is likely to end up in liquid effluent.

Applications overseas include cosmetics and acrylamide-based grouts for the construction of pipelines and tunnels. These uses in Australia appear to be minimal. If they occur then acrylamide would be lost to the aquatic compartment, in very small amounts.

Releases of acrylamide to soil result mainly from application of sewage sludge and aerial deposition, although it is possible that some residual monomer could leach from polyacrylamides used in soil treatment applications.

In Australia, there are no available data for the amount of acrylamide actually lost to the environment. As previously indicated, small amounts may be lost during its use, however, once in polyacrylamides, it will be bound in an inert matrix. Levels of residual acrylamide monomer are likely to be present in very small amounts.

Under the National Pollutant Inventory (NPI), companies who use 10 t of acrylamide or more per year are required to gather acrylamide emission information from July 2001. This information will not be available until January 2003.

Taking into account the chemical nature of acrylamide and its behaviour in the environment, the most important environmental compartment is the aquatic one. A Predicted Environmental Concentration (PEC) can be calculated using the recommended emission factor from the EU Technical Guidance Document (EC, 1996):

Total amount of imported acrylamide	5000 tonnes
Times emission factor	0.003
Giving a waste amount of	15 tonnes

Population of Australia	19 million
Amount of water used per person	150 L/day
Therefore minimum volume of effluent handled in treatment plants Australia-wide	2850 ML/day
Number of days	365
Dilution ratio for receiving waters	1:5
PEC	3 μg/L

This PEC is a worst-case scenario, since it is likely that there will be some biodegradation of acrylamide in the sewage treatment plant.

8.1.2 Distribution

Using the FUGMOD (OECD workshop) model, the distribution of acrylamide in the environment is calculated as (EU, 2001):

	Mackay Level I model	Mackay Level III model
Air	< 0.1%	< 0.1%
Water	99.99%	99.95%
Soil	< 0.1%	< 0.1%
Sediment	< 0.1%	< 0.05%
Biota	< 0.1%	-

The level III model calculation uses a release rate of 1000 kg/h and assumes that releases are to water only. The results show that water is the most important compartment for acrylamide.

8.1.3 Degradation

It should be noted that the EU risk assessment report (2000) gives detailed summaries of the following studies. Asterisks indicate that the studies mentioned are as cited in the EU risk assessment report, and were not individually sighted.

Atmosphere

Acrylamide is likely to react with the photochemically produced hydroxyl radicals (*USEPA, 1994; EU, 2000). Atkinson *(1987) calculated a half-life of 8.3 h for the reaction of acrylamide and hydroxyl radicals at room temperature. An estimated half-life of 6.6 h is given in USEPA *(1994).

Due to its high water solubility, it is likely that acrylamide will be washed out of the atmosphere in rain (see Mackay distribution model on page 5).

Water

Degradation in the aquatic compartment will either occur abiotically or biotically.

Abiotic degradation

Acrylamide is reported as being hydrolysed to acrylic acid and ammonia under strong acid or alkaline conditions *(Jung et al 1980). Moens and Smets *(1957) found that the second order rate constants for alkaline and acid hydrolysis increased as the temperature increased (see Table 8.1). The values give half-lives of >1 year at 55°C in the pH range 5-9.

Table 8.1 - Rate constants (L/mole/s) at various temperatures as determined by Moens and Smets (1957)

Alkaline hydrolysis	Acid hydrolysis
$1.47X10^{-4}$ at 55°C	$1.48X10^{-4}$ at 80°C
$13.8X10^{-4}$ at 85°C	$16.6X10^{-4}$ at 110°C

The photodegradation of acrylamide in surface waters is calculated to give a half-life of about 1 year, based on its reaction with hydroxyl radicals at pH 10.7 (EU, 2000).

The behaviour of acrylamide in sterile river water under alkaline and acidic conditions was reported by Brown et al *(1980a). It was found that after 2000 h, there was no degradation of acrylamide at any pH. However, in unsterilised river water, acrylamide was observed to degrade.

From the available data, it appears that abiotic degradation is dependent upon pH and temperature, but is not significant in the environment when compared to biodegradation.

Biotic degradation (Biodegradation)

There are a number of studies that indicate that acrylamide will undergo biodegradation, but generally after there has been a period of acclimation. These studies are summarised in Table 8.2 with full summaries to be found in the EU risk assessment report. From these results, acrylamide is considered to be readily biodegradable as specified in OECD 301D 'Ready Biodegradability: Closed Bottle Test'.

Other studies show sewage bacterium and heterotrophs use acrylamide as a source of carbon and nitrogen (*Arai et al, 1981; *US EPA 1994; *Klump et al 1986), at varying rates and degrees.

Table 8.2 - Summary of degradation studies

Study/Test method	Concen-tration	Result	References
OECD ready biodegradability: Closed Bottle Test	2 mg/L 5 mg/L 1 mg/L	100% at 28 days 53.3% at 28 days 100% at 28 days	*USA Testing Company Inc., 1991
Spiked natural and polluted water	0.5 mg/L 5.1 mg/L	Lag of 1.5-7.3 days, followed by 100% primary degradation after 4.2-15.6 days	*Brown et al, 1980a
a) Aerated sunlit water	8 mg/L	Lag of 220 h, 100% degradation	*Croll et al, 1974
b) Open and closed bottle seeded with settled sewage effluent	5 mg/L	Quick degradation in closed bottle	
Activate sludge bacterium (*Arthrobacter*)	2-5 g	100% in 7 days	*Yamada et al, 1979

Soil

Studies have also indicated that acrylamide will undergo biodegradation in soil to varying degrees depending on soil type, pH and temperature. It appears that the highest degree of acrylamide degradation occurs in soils with alkaline pH and at higher temperatures. Lande et al *(1979) used the biometer flask method to study the degradation of acrylamide in a range of soil types under aerobic and anaerobic conditions. Under aerobic conditions with 25 mg/kg of acrylamide, the half-lives obtained ranged from 18 to 45 h and when the concentration of acrylamide was increased to 500 mg/kg, the half-lives increased (e.g. 95 h). It was observed that the half-lives in anaerobic conditions were longer.

The effects of waterlogging on the conversion of acrylamide to inorganic nitrogen by soil micro-organisms was studied by Abdelamagid et al *(1982). The findings indicated that acrylamide is hydrolysed in soil to give NH_4^+, which is oxidised to NO_2^- and NO_3^- under aerobic conditions but is accumulated under waterlogged conditions.

In the EU risk assessment report (2000), it is estimated that the half-life for the degradation of acrylamide in soil is 30 days.

Adsorption

The removal of acrylamide from the water column was reported by Brown et al *(1980b). They found that that the presence of sediment or sewage sludge did not alter the removal rate significantly. After 168 days, the acrylamide was completely removed from estuarine and river water samples, however 40-75% was removed from seawater and sewage treatment plant effluents.

Lande et al *(1979) found that acrylamide was most mobile in loamy fine sand (Rf values 0.846-0.880), least mobile in silt clay (Rf values 0.637-0.657) and concluded that overall it is relatively mobile in soil.

In the EU risk assessment report (2000), the Koc for acrylamide has been calculated by the EUSES (European Union system for the Evaluation of Substances) model using a log Kow of –1 to be 0.195.

These values indicate that adsorption of acrylamide to soil and sediment is not significant, thus it will be relatively mobile in most soil types. The EU risk assessment report (2000) presents other studies that support this finding.

Volatilisation

With a vapour pressure of 0.9 Pa at 25°C and a calculated value of $2.97X10^{-5}$ Pa m^3 mol^{-1} at 25°C for Henry's Law constant, acrylamide will be moderately volatile (Mensinck et al, 1995). However, due to its high water solubility, it is unlikely that acrylamide will volatilise from water at ambient temperatures.

8.1.4 Bioaccumulation and metabolism

There have been a number of studies conducted on the accumulation, metabolism and excretion of acrylamide in fish. Some of these are summarised in Table 8.3 with detailed summaries to be found in the EU risk assessment report (2000).

From these studies it can be seen that uptake by fish 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 Kow value of ~-1.0 indicates a very low likelihood of accumulation. These finding are likely to be the same for other aquatic organisms.

Table 8.3: Summary of accumulation studies for acrylamide

Condition	Species	Concentration mg/L	Duration (days)	Comments/findings	BCF	Author
Static	Carp (*Cyprinus carpio*)	0, 1 and 10	20-40	Fish in 1 mg/L slowly accumulated for the first 10 days then rapid increase to day 20 (0.26 mg/kg). Fish in 10 mg/L rapidly accumulated for the first 10 days then slow increase until day 30, then rapid to day 40 (7.56 mg/kg).	0.26 and 0.77	Fujiki et al (1982)
Static	Japanese medaka (*Oryzias latipes*)	0, 1 and 10	20	Fish in 1 mg/L slowly accumulated for the first 10 days then rapid increase to day 20 (0.31mg/kg). Fish in 10 mg/L rapidly accumulated until day 15, then slow increase until day 20 (25.3 mg/kg).	0.31 and 2.53	Fujiki et al (1982)
Static steady-state	Rainbow trout (*Oncirhynchus mykiss*)	0.710	72-h exposure and 96 hrs depuration	Rapid uptake for 24 h in carcass and viscera, then slowed to steady conc. by 72 h. Elimination biphasic (initally fast) with $t_{½,phase\ 1}$=10 h and $t_{½,phase\ 2}$=7.7 days for carcass, and $t_{½,phase\ 1}$=16 h and $t_{½,phase\ 2}$=5.7 days for viscera. After 96 hrs 25% decrease in carcass and viscera. 90% of excreted acrylamide in unchanged form.	1.44 carcass and 1.65 viscera	Petersen et al (1985)

8.2 Occupational exposure

Occupational exposure to acrylamide may occur during transport, storage or use of the chemical. Acrylamide is available either as a crystalline solid or in aqueous solutions of varying concentrations. Acrylamide is not manufactured in Australia. Its identified uses are the production of acrylamide polymers either in manufacturing facilities or in situ in laboratories. The major use of the resultant polymers is in water/wastewater treatment, with the production of adhesives surface coatings and textile/leather treatments, also being important.

Occupational exposure may occur via inhalation of acrylamide dust, powder or vapour (solid sublimes slowly at room temperature) or dermal absorption of solid or monomer solutions. If spilled solutions are allowed to dry out, there is also potential for exposure to dust and vapour from the recrystallised solid. Exposure to residual monomer in polymers can occur during manufacture of the polymer, packaging and end-use of polymers, and can be via the inhalation and dermal routes.

Occupational exposure is discussed in detail in the following sections. Where information is not available for reported/suspected use in Australia, information has been included/supplemented from the EU report (EU 2000).

8.2.1 Importation and storage

Acrylamide is imported in a variety of containers. Crystals are packed in 25 kg paper bags with plastic liners whilst aqueous solutions are supplied in 1000 L intermediate bulk containers. These containers are transported by road or rail either to importers, or more commonly, direct to end-users without being opened. Acrylamide is not repackaged after import. In Australia, use of acrylamide solid exceeds use of solutions whilst in the EU, the predominant monomer form is a 50% solution (EU 2000).

Storage varies from site to site depending on production and safety considerations. Because of the large quantities used, storage is often both on and off-site i.e. held at suppliers. Quantities of solid (crystals) acrylamide stored on-site for polymer production range from 500 kg to around 80 tonnes. One user is planning on increasing storage to around 300 tonnes in the future. A user of 30% aqueous acrylamide stores about 3000 L on site, with another user of 30% aqueous solutions storing up to 30 000 L.

Aqueous solutions delivered in IBCs are pumped to storage tanks via a sparge inserted into an opening at the top of the IBC. This was identified by one applicant as a potential source of exposure to acrylamide vapour.

Acrylamide is also imported as co-monomer mixtures and in residual amounts via pre-formed polymers and co-polymers.

Storage is always in designated dangerous goods warehouses. Exposure during importation and storage is unlikely except in cases of accident.

8.2.2 Manufacture of polymers

Process description and sources of exposure

Powder, emulsion and aqueous forms of polymer are manufactured in Australia.

Manufacture of solid/dried polymer

Currently, powdered polymer is being produced at one site in Australia, A typical production batch size was reported as 10 000 L polymer. Batch sizes are calculated to consume full bags (25 kg bags) of acrylamide crystals so that no partly used bags remain.

In an open system under exhaust extraction, operators cut the bags and the contents emptied into the reaction vessel. The operators wear overalls, Wellington boots, PVC gloves, a PVC apron and an enclosed air hood. Empty paper bags are placed into a plastic bag which, when full, is sealed and disposed of to landfill by a licensed hazardous waste contractor.

The required amount of acrylamide is mixed with water and any other additives and transferred by pump through a closed system to a sealed 3500 L reaction vessel maintained at 80^0C. Additional chemicals are added and polymerisation occurs immediately. The reaction vessel is kept at 80^0C for a further 2 to 3 h holding period after polymerisation to help ensure no unreacted monomer remains. The polymerised gel is tipped into an open transfer system, and pushed into cutting machines by hydraulic rams, where it is cut into approximately 10 mm^3 pieces. Once the gel is loaded into the cutting machines, the process is again fully enclosed until the final powdered product emerges. Two cutting machines (known as "extructors") are situated one above the other. The gel is cut into an intermediate size by the primary extructor and then further reduced in the secondary extructor. When subsequently fed into a final third cutting machine, it is again reduced in size for fluid bed drying. At all stages after polymerisation, the resultant gel is treated with catalyst to further consume any unreacted monomer. After cutting, the gel is dried for 2 to 3 h at 60 to 80^0C and tested for residual monomer. If any monomer is detected, the gel is re-treated. The resultant dried gel is milled to form a powder with a particle size of 1000 microns. The powder is tested for residual monomer and any batch with monomer levels greater than or equal to 0.1% is reprocessed.

Exposure to acrylamide monomer can occur when charging mixing vessels and during laboratory sampling and testing. Exposure to residual acrylamide monomer in the polymer can occur during laboratory sampling and testing, pouring the polymer gel and handling the milled polymer powder. Engineering controls and relevant PPE are used at these stages, except when tipping the gel into hoppers and transferring to extructors. Transferring the product from polymerisation vessels to the extructor takes less than 3 minutes per batch.

Manufacture of polymer emulsions & aqueous solutions

Currently, polymer emulsions and solutions are being produced at eight sites in Australia. The following describes a typical process:

Emulsions made using acrylamide crystals are in typically in batch sizes of 3000 L. Water, acrylamide and additives are dissolved in a mixing vessel. Hydrocarbon oil and other additives are dissolved in a reaction vessel. Aqueous acrylamide solution is

pumped via a closed system to the sealed reaction vessel then mixed with the hydrocarbon oil and additives to form an emulsion. The vessel is heated to between 60 and 70^0C for 1 to 2 h and the emulsion polymerises.

Exposure to acrylamide monomer can occur when charging mixing vessels and during laboratory sampling and testing. Exposure to residual acrylamide monomer in the polymer can occur during laboratory sampling and testing and during packaging of the polymer emulsion. Engineering controls and relevant PPE are used at these different stages.

At another site, the manufacture process of aqueous solutions and emulsions with crystalline acrylamide differs. Bags of acrylamide are loaded by an operator onto a conveyor belt and taken to a partly enclosed cutting machine maintained under negative pressure, which is connected to a scrubber. The bags are cut open by rotary saw enclosed within the machine and the contents automatically tipped into the reaction vessel. The empty bags are automatically sealed in a plastic bag and disposed of to landfill. The operator in this process wears PVC gloves, safety glasses, boots and ordinary overalls but not a respirator. In addition, exhaust fans operate outside the debagging machine to ventilate the general area. The reaction vessel is charged with other ingredients to manufacture the polymer emulsion, sealed and heated to a predetermined temperature for a predetermined time, depending on the product being made. Emulsion production is an almost continuous process.

Dermal and inhalation exposure could occur during automated debagging if a bag is dropped by an operator or during sampling where the operator wearing PPE extracts a sample from the top of the reaction vessel under a suction hood. Laboratory personnel are also at risk of exposure when testing intermediate samples. Testing is done in a fumehood.

Aqueous solutions are made less regularly in an area different to that used for production of emulsions. For these products, the operator wears disposable overalls, boots, PVC gloves, a PVC apron and an enclosed air hood. Charging of the reaction vessel occurs in an open system with exhaust extraction, and the bags are cut open by the operator and contents emptied into the reaction vessel. Empty paper bags are placed into a plastic bag which when full, is sealed and disposed of to landfill. The required amount of crystal is mixed with water and any other additives and transferred by pump to a reaction vessel, into which are added other components e.g. acidic or basic salts for the production of cationic/anionic polymers. The reaction vessel is sealed and heated to a predetermined temperature for a predetermined time, depending on the product being made. Exposure could occur should an operator drop a closed or open bag, or when adding components to the reaction vessel, however full PPE is worn and exhaust fans operate in this area. Laboratory personnel are also at risk of exposure when testing intermediate samples. However, as testing is done in a fumehood by personnel wearing relevant PPE, exposure is controlled.

Maintenance and cleaning

During polymer manufacture, maintenance personnel are likely to experience the highest exposures to acrylamide. Maintenance operations include removal (dig out) of solidified polymer from reaction vessels, cleaning pipework and changing filters.

Precautions and PPE for maintenance[1] vary depending on the process. For maintenance of vessels and lines, equipment is cleaned before any work is carried out. Equipment used for handling monomer, polymer gel and finished powders is thoroughly washed before any maintenance work.

Potential exposure and numbers of workers

Table 8.4 shows that Australia-wide at least 90 workers are potentially exposed to acrylamide at various sites during polyacrylamide production. Some companies produce on an almost continuous basis with others producing periodically.

Table 8.4 - Employees potentially exposed to acrylamide during manufacture of polyacrylamides in Australia.

Company	Presentation	Quantity	Employees per batch	Duration of activity
1	Crystals	33 tonnes/week	Up to 17	Up to 6 h/week, continuous
2	Crystals	150-175 kg/week	Up to 6	Up to 5 h/week, batch process
3	Crystals	500 kg/y	2	30 min/batch
4	Crystals	27 000 kg/y	1	Up to 60 min/batch
5	Crystals	1200 kg/y	1	30 min every 3-4 weeks
6	Crystals	2600 tonnes	30	1-2 h/person
7	30% solution	10 000-14 000 L/week	6-8	5-7 h/week
8	30% solution	5000-6000 L/y	Up to 34	30 min/batch

Monitoring

Australian data

Four of the nine companies using acrylamide in manufacturing processes provided air monitoring data. None provided dermal exposure data for assessment. EASE modelling was carried out for dermal exposure to crystals from an automatic debagging operation and a manual debagging operation as used in Australia. In both instances the predicted dermal exposure was “very low”.

For one polymer manufacturer using aqueous 50% acrylamide, air monitoring results “gathered over the past years” in areas used for weighing raw material, around reaction

1. Some plants operate mandatory schemes

vessels during use and during vessel cleaning, ranged from < 0.0015 mg/m^3 to < 0.0035 mg/m^3. This company has now switched to using 30% solutions. Another user of 30% monomer solution provided limited air monitoring data which indicated a 'single day' TWA (personal sample over 2 h sampling period) of 0.008 mg/m^3. A static sample taken 2 metres from reaction tank vent, gave a result of < 0.001 mg/m^3.

Table 8.5 shows results of personal and static monitoring by a user of acrylamide crystals.

Table 8.5 - Summary of acrylamide air monitoring at a plant using acrylamide crystals for manufacture of polyacrylamide emulsions and solutions

Activity	Type of monitoring	Acrylamide range (mg/m^3)
Manual debagging	Personal	Up to 0.16
Automatic debagging	Personal	0.01
Packaging, decontamination, reacting	Personal	ND
General production, storage, laboratory	Static	ND over 4-7 h
Reaction/blending plants	Static	Up to 0.03 over 4-8 h, sporadic values up to 0.07

ND = Non detectable

Another user of acrylamide crystals has provided more extensive air monitoring data. These are presented in Figure 8.1 and Table 8.6. Figure 8.1 shows average TWA levels over a 20 month period. Both static and personal monitoring were carried out at various locations around the plant production areas. Table 8.6 shows results of 'single day' air monitoring in various areas associated with use of acrylamide.

Monitoring data provided by these users of acrylamide monomer indicate that the Australian exposure standard of 0.03 mg/m^3 TWA (8h) is exceeded at times, more so during the use of crystals than the aqueous form of acrylamide. Also, some of the highest levels measured were associated with production processes specific to solid polymer production and occurred in areas where processing/cutting of the polymer gel (extruction) takes place. The company has reported that the extructor areas have now been fully enclosed and that dedicated extracted air change rooms for acrylamide decontamination have been provided. Table 8.7 shows air monitoring data at the same site following enclosure of the extructor processing area.

Figure 8.1 - Average personal acrylamide levels in air at one Australian plant Dec 98 - Aug 00 (TWA)

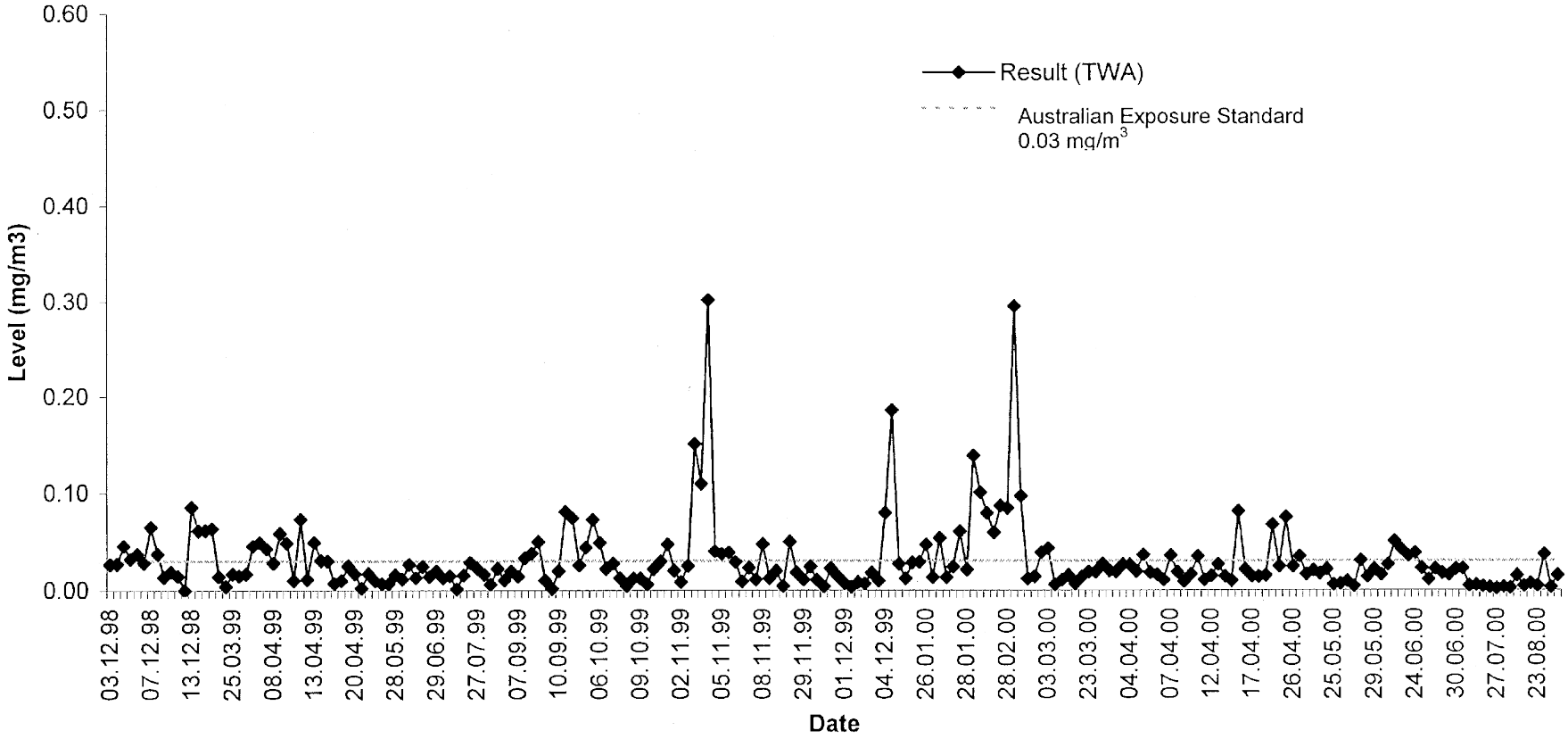


Table 8.6 - Results of static air monitoring (single day) at various `production areas of plant using solid acrylamide in the manufacture of polyacrylamide powders.

Area	Date	Flow Rate	Time	Air Vol.	Acrylamide in Air
		(mL/min)	(min)	(L)	(mg/m^3)
Debagging	4-Nov-98	100	428	42.80	0.27
Debagging	4-Nov-98	100	356	35.60	0.12
Polymerisation vessel	2-Nov-98	100	314	31.40	0.03
Change Rooms	3-Nov-98	100	523	52.30	0.03
Change Rooms	28-Oct-98	100	196	19.60	0.04
Change Rooms	26-Oct-98	100	541	54.10	0.07
Extructors	6-Nov-98	100	593	59.30	0.45
Extructors	5-Nov-98	100	556	55.60	0.62
Extructors	5-Nov-98	100	460	46.00	0.39

Table 8.7 - Acrylamide air monitoring data following enclosure of the extructor area in manufacture of polymer powders

Date	Acrylamide in air mg/m^3
27-July-99	0.16
28-July-99	0.23
02-Aug-99	0.03
Feb 1999	0.02
Oct 2000	0.03

Overseas monitoring data

Air monitoring

Table 8.8 provides a summary of air monitoring for acrylamide in the EU for manufacture of polyacrylamides.

Table 8.8 - Personal air sampling data for polyacrylamide manufacture from EU users of acrylamide, after EU report (EU, 2000)

Country	No. of samples	Arithmetic mean (mg/m^3)	Geometric mean (mg/m^3)	Range (mg/m^3)
UK	422	0.05	no data	0.01-0.77
UK	10	0.03	0.02	0.001-0.08
UK	4	0.01	0.01	0.01
Germany	no data	no data	no data	<0.03
Germany	16	0.04	0.05	<0.01-0.099

Personal air monitoring data were also reported for cleaning and maintenance tasks at one UK plant. Levels of acrylamide for dig out of reactors and holding tanks were in the range 0.14 to 1.44 mg/m^3, with mean levels in the range 0.07 to 0.24 mg/m^3.

Overseas monitoring data for polymer manufacture are consistent with Australian data in terms of mean exposure levels and highest levels monitored.

Dermal exposure

From the EU report, it is noted that operators can be dermally exposed when they come into contact with surfaces contaminated with splashes or crystal spills, with condensed vapour, or as a result of direct splashes or crystal spills on the skin. Dermal exposure was monitored for workers at one UK polymer manufacturing plant by measuring absorption onto cotton glove liners worn inside their PVC gloves. Results were reported to be a measure of actual dermal exposure to acrylamide from wearing PVC gloves and not a measure of permeation of gloves. It was not clear from the report whether the plant used solid or aqueous acrylamide. Results are presented in Table 8.9.

Table 8.9 - Dermal exposure during the manufacture of polyacrylamide at a UK plant, after EU report (EU, 2000)

Grade	Plant	No of samples	Arithmetic mean (mg/glove)	Geometric mean (mg/glove)
Bead	Monomer	33	4268	1352
	Reactors	20	483	61
	Dryers	20	172	100
Powder	Monomer	24	4297	1697

Based on an assumed surface area for hands of 820 cm^2 , the overall estimated dermal exposure for all activities at this site was between 0.0002 and 0.08 $mg/cm^2/d$. The bead grade of polyacrylamide is not produced in Australia.

8.2.3 Use in grouting applications

NICNAS received anecdotal evidence of the local use of acrylamide grouts. Whilst a number of companies involved in controlling water leaks in engineering and building applications were contacted, none reported use of acrylamide-based grouts. As no Australian data could be obtained, data from the EU report (2000) is presented on the assumption that if used, the method of use of grouts in Australia is likely to be similar to that in the EU.

The main uses of acrylamide grouts are in sewer line and manhole sealing (small scale uses) and structural water control and geotechnical applications (large scale uses).

Acrylamide grouts are prepared on site by mixing a number of constituents, including catalysts, activators, accelerators and inhibitors. The EU report indicates grouts contain either powdered acrylamide or acrylamide in an aqueous solution, consisting of a 19:1 mix of acrylamide with a cross-linking agent. A derivative of acrylamide, N-methylolacrylamide (NMA) is also used in grouting applications.

An NMA grout called Spirogel was used in 1997 in a large-scale grouting operation in a rail tunnel at Hallandsås in southeast Sweden. The grout reportedly failed to polymerise properly and leaked into the adjoining watercourse. A few weeks after grouting commenced, adverse effects symptomatic of acrylamide poisoning were observed in fish and cows downstream of the construction works. At the same time, symptoms of neurotoxicity were observed in tunnel workers. This is the only grouting operation for which monitoring data are available.

The NMA grout (Spirogel) was presented as two concentrated aqueous solutions, which were diluted with water and mixed on site. According to the manufacturer, the solutions were formulated as follows:

Solution 1

Maximum 2% acrylamide[2]

Approx. 37-38% N-methylolacrylamide (NMA)

Approx. 1% formaldehyde

Accelerator/stabiliser

Silicate hardener

Solution 2

Sodium silicate

Sodium persulphate (initiator)

[2] Later analysis showed the acrylamide content of Spirogel to be between 3.5 and 9% and not the 2% maximum claimed. The manufacturer subsequently amended its Material Safety Data Sheet to reflect this.

Once diluted and mixed, the two solutions were then injected under pressure into pre-drilled boreholes in the rock strata. Workers were potentially exposed to the neat derived monomer solutions, the mixed solution, leakage water and injected substance that sprayed back through cracks in the rock.

Inhalation exposure during the grouting operation was measured over approximately 3 h and showed personal exposure levels of between 0.05 – 0.34 mg/m^3 (total acrylamide plus NMA) and levels of between 0.05 and 0.08 mg/m^3 (acrylamide only). No data on dermal exposure were available but it was assumed to be high as workers subsequently reported having gloves and overalls so wet that they used rain suits to keep themselves dry. It is not clear whether monitored exposures occurred during or subsequent to grout injection. The grout manufacturer stated that protective gloves, goggles and thick clothing were required and when injecting, "self contained breathing protection" should be used.

The EU report also provides data obtained in two surveys on the small-scale use of acrylamide in sewer and manhole sealing in the US. A third survey was also published, however it was stated in the EU report that the exposure data was inadequate. In the first, an industrial hygiene survey for sewer line repair, grout application was carried out remotely, however grout mixing was done by workers who did not wear appropriate PPE.. Personal air monitoring for 9 h provided exposures for two workers of 0.002 and 0.009 mg/m^3. Swabs taken from inside protective gloves gave a total of 65µg of acrylamide but details of the sampling method were not given.

The second survey measured occupational inhalation and dermal exposure for manhole sealing and sewer line repair. Work practices were similar to those of the first survey except that for manhole sealing, the mixing of the components to form the polymer was done in the injection nozzle of the pumping equipment. For manhole sealing, two samples for personal air monitoring taken over approximately 160 minutes returned air levels of 0.01 and 0.12 mg/m^3 acrylamide. Five personal air samples for workers involved in sewer line sealing returned readings ranging from 0.01 to 0.08 mg/m^3 acrylamide. Dermal exposure was found to be higher for manhole sealing operations, because the grout was manually injected. As sewer line sealing was carried out remotely, the potential for dermal contact was limited to the chemical mixing operation. Wipe sampling from various pieces of equipment used in the operation gave readings ranging from zero to 7.10 mg acrylamide per 100 cm^2, the higher reading coming from the outside of the chemical mixing tank. Two samples taken after rinsing out protective gloves gave levels of 1.37 and 2.49 mg acrylamide, equivalent to a maximum exposure of 0.054 mg/kg/d. In addition, dermal exposure to trunk measured by body pads was estimated at 0.375 mg/kg/d, providing a total dermal exposure of 0.043 mg/kg/d.

An unpublished study of grouting using acrylamide solution rather than crystals in three workers over six hours on one day found acrylamide levels below the detection limit for airborne and dermal exposures. Wipe samples around the equipment used was generally less than 0.04 mg with the acrylamide tank measuring 0.054 mg acrylamide. No data is reported for the injection apparatus particularly after the grouting operation was completed. Air samples taken in the workers breathing zones were reported as less than 0.083 mg/m^3 8h TWA. Dermal pads placed at various points on the workers bare skin or protective clothing returned levels of acrylamide ranging from less than 0.100 mg to 0.390 mg. Hand-rinse samples at the end of the shift using 75ml deionised water gave acrylamide levels ranging from 1.59 to 2.48 µg/ml.(Vance, 2000).

8.2.4 Gel electrophoresis

Polyacrylamide gels are formed by co-polymerisation of acrylamide and N,N[1]-methylene-bis-acrylamide (bis-acrylamide). Acrylamide for use in gel electrophoresis is supplied either as powder or solution. Pre-cast polyacrylamide gels are also available. Recommended precautions from one suppler are to wear gloves and work in a fumehood when preparing gels. The highest exposure will occur when a user prepares gels from powdered acrylamide.

As methods of gel preparation are likely to vary between laboratories, residual monomer levels cannot be predicted and it is assumed individual laboratories do not measure gels for residual monomer. No data are available for residual monomer levels in pre-cast gels.

Bergmark (1997) measured haemoglobin adducts in laboratory workers involved in gel electrophoresis, smokers and non-smoking controls. Compared with non-smoking controls adducts were significantly increased in those exposed to acrylamide. Smokers had the highest level of adducts of the groups tested which correlated with the number of cigarettes smoked per day. The levels in laboratory workers equated to the dose of acrylamide received from 3 cigarettes per day. An unexpected result was the high level of adducts in non-smoking controls.

The EU report provides some discussion on inhalation and dermal exposure. Two measurements of inhalation exposure are reported, the highest being 0.067 mg/m^3, though not for an 8 h TWA as the procedure was performed once a day to make stock solutions. No measurements of dermal exposure are presented, although this could occur during handling of acrylamide monomer powder or aqueous solutions or in the handling of polyacrylamide gels. The way in which gel electrophoresis is performed tends to limit the extent to which gels are handled directly and gloves would generally be worn for these procedures. Significant permeation of gloves is unlikely due to the short duration of handling.

8.2.5 Uses of polyacrylamide

Paper manufacture

One manufacturer reported that polymers produced by it and used in paper manufacture have residual acrylamide levels of about 5 ppm. One paper mill reported that retention and drainage aids are dosed at approximately 0.015% to 0.08%. Polymers used as flocculants in the paper manufacturing process were reported to be used at a final dilution of about 0.08% and treatment of mill effluent used polymer at a dilution of 0.2%. Workers wear gloves, protective glasses and if using polymer powders, respirators, when dosing polymers into the process. Machine operators do not come into contact with the polymers.

The EU report estimates the maximum level of residual acrylamide in paper products to be 15 ppb, which is below the limit of detection. EASE modelling presented in the EU report provides an exposure estimate of 0.003 mg/m^3 acrylamide in air for the paper industry (assuming 0.1% monomer in polyacrylamide) and a dermal exposure of 0.0004 mg/m^2 /day from handling undiluted polymer. Typically, polyacrylamide may be diluted 1:500 for use in paper manufacture (EU, 2000).

Minerals processing

Acrylamide polymers are used as flocculants in the mining industry. As the range of polymers available is large and mineral wastewater and slurries complex, it is recommended that laboratory tests be performed prior to application to determine the best product and dilution for the purpose. The polymers are diluted for use. One manufacturer recommends powder polymers be diluted to a concentration of 0.05%, and for liquid polymers, 0.5%. These concentrations are then further diluted when added to the system being treated.

Drinking water and wastewater treatment

As in mining applications, prior laboratory testing is required for drinking water and wastewater treatment applications to determine the most appropriate product or products and their working concentration. Recommended solution strengths for polymers used in clarification are between 0.005 and 0.01%. The concentration of polymer (and hence residual monomer) in the water varies depending on the initial state of the water, for instance more polymer is used when water is dirtier following periods of heavy rain.

In use, powdered polymer is sucked from a 25 kg bag into a hopper, where it falls to a vessel for mixing with water. The resulting solution is then metered into a sedimentation tank by automated equipment and therefore further diluted. The resultant flocs either fall to the bottom of the tank as sediment or in another process, are kept floating on the surface by bubbles of air. This sediment is removed and either sent to hazardous waste disposal due to heavy metal contamination or in another case, re-used in agricultural and home gardening applications.

Monitoring and exposure

Table 8.10 summarises inhalation exposure data from various industrial users of polyacrylamide in the EU. Dermal exposure during handling of acrylamide polymers was estimated by EU using EASE to be $1 \times 10^{-4} - 1 \times 10^{-5}$ mg/cm^2/d.

Table 8.10 - Inhalation exposure data from EU users of polyacrylamide (EU 2000)

Country	Range (mg/m^3)
EASE	0.0001-0.003
UK	<0.015
Netherlands	<0.001-0.012

8.3 Public exposure

8.3.1 Consumer exposure

Some industrial processes using polyacrylamides containing residual levels of acrylamide, result in the manufacture of consumer products. These polymers may be used in the production of products such as adhesives, coatings, paper and paperboard that could be employed in food contact situations.

Polyacrylamide is also used as a thickener, or as a reagent to provide smoothness and shine in a wide range of cosmetic preparations (soap, skin care and hair grooming products). There is no information available for the use of polyacrylamide in the manufacture of cosmetics products in Australia. However, as imported cosmetic products may contain polyacrylamide, consumer exposure to the residual monomer existing in polyacrylamide is likely to occur. Based on survey information from the Cosmetic, Toiletry & Perfumery Association of the UK, polyacrylamide is currently used in rinse-off and non rinse-off cosmetic preparations at a level of up to 2%. The usual specification calls for a maximum monomer level in the polymer of below 0.01% (EU, 2001). Potential exposure is most likely via dermal contact, with inhalation exposure negligible.

The following typical use levels for polyacrylamide in cosmetics were adopted from the European Union risk assessment (EU 2000). The calculation of maximum consumer exposure is based on a level of up to 2% of polyacrylamide in the products and a maximum monomer level of 0.01% in the polymer. For non-rinse products, daily use of general purpose cream or body lotion (estimate 19.4 g), setting products (12 g) and nail products (0.25 g) for a person is associated with a daily exposure of up to 0.0635 mg/day of the monomer. For a person using rinse-off products (10% remaining on the skin), 2 g of shaving cream and 4.8 g of soap daily will cause an exposure to 0.0014 mg/day of the monomer. Hence, a total of up to 0.065 mg/day of acrylamide is expected for a person using above cosmetic products, which in combination with an dermal absorption factor of 0.3, results in approximately 0.0003 mg/kg bw/day for a man/woman with body weight of 60-70 kg.

8.3.2 Exposure via environment

Acrylamide or N-methylolacrylamide (NMA) derived monomer based grouts can be used for the construction of tunnels and for sealing water/sewer pipes to prevent leakage. Acrylamide grouts generally consist of a 19:1 mixture of acrylamide and cross-linking agent. In laboratory experiments, the amount of acrylamide diffusing into water was 0.03 to 0.17 % for prepared resin, in less than 7 days post-application. In addition, acrylamide can move through the ground to enter groundwater due to its water solubility and its inability to bind well to soil. 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. In southern Sweden in August 1997, there was large-scale use of NMA-based grout with residual acrylamide in the construction of an 8.6 km tunnel in the Vadbaken creek. At the end of September 1997, 92 mg/L of acrylamide and 342 mg/L of NMA were detected in the riverwater sample taken immediately downstream from the construction site. Concentrations of 2 mg/L acrylamide and 180 mg/L NMA were found in samples taken from fish ponds that were connected to the creek. By the end of December 1997, the concentrations for both chemicals had dropped to below 0.1 mg/L in the creek and was below the detection limit of 0.05 mg/L at the furthest point monitored downstream (EU, 2000). Hence, the concentration decreased with distance and with time.

In Australia, acrylamide grouts were used in 1962-1966 during the construction of the Geehi Dam for the Snowy Mountains Hydor-electric Scheme. Since solidified grouts (completion of the polymerisation process) contain less than 0.05% free acrylamide, the amount of acrylamide released into water from this old construction is likely to be negligible today.

Polyacrylamide is used for treatment of drinking water by flocculation. Based on the principles of colloidal suspensions to clean up water, the flocculants cause stabilised particles or coagulated suspensions to form aggregates, which can be removed by

settlement or filtration. Polyacrylamide containing a monomer level of less than 0.05% is required for this purpose. When non-ionic or anionic polyacrylamides are used in water treatment at a typical dose level of 1 mg/L, the maximum theoretical concentration of acrylamide has been estimated at 0.0005 mg/L, with practical concentrations 2 to 3 times lower. Residual levels of acrylamide from the use of cationic polyacrylamides may be higher. Assuming a maximal possible concentration of acrylamide at 0.5 µg/L in drinking water and 2 L consumption per day for a man/woman of 70 kg, it is estimated that maximum exposure would be 1.42×10^{-5} mg/kg bw/day. The *Australian Drinking Water Guidelines* (NHMRC 1996) stipulate that the concentration of acrylamide in drinking water should not exceed 0.0002 mg/L which is also the limit of detection by HPLC in combination with UV. This level is equivalent to a daily exposure limit of 5.7×10^{-6} mg/kg bw/day.

Public exposure to acrylamide may also occur via air and soil, as well as via plants and food products that have been exposed to contaminated air, water or soil either during growth or manufacture. The absorption of acrylamide by plants or fishes from contaminated water is likely to be negligible. Cooking of food and heating in food manufacture may also be a source of background dose of acrylamide in humans. Tareke et al. (2000) detected haemoglobin adducts, as seen following occupational exposure to acrylamide, in rats fed fried food. Direct release to air during industrial applications of acrylamide and polyacrylamide is controlled by engineering at manufacturing sites. The residual level of acrylamide in the atmosphere or soil will likely be washed out by rain due to its high water solubility, and gradually undergo photodegradation and biodegradation. Expected atmospheric concentrations of acrylamide are very low based on calculation and detected levels at production and processing sites in Europe. Releases of acrylamide to soil may come from applications of polyacrylamide in sewage sludge or soil treatment.

9. Kinetics and Metabolism

Most of the studies assessed in this Section and Section 10 have been summarised from the recently completed OECD SIAR (OECD 2001). However, primary sources of data were consulted where necessary. In addition, a comprehensive literature search was carried out of studies conducted from January 2000 to date, for additional material of relevance to the hazard assessment, which was not included in the OECD report or provided by importers or end-users of acrylamide and acrylamide products. References in Sections 9, 10, 11 and 12 that have not been sighted are marked with an asterisk.

9.1 Human Data

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

There is potential for acrylamide to be rapidly and extensively absorbed via oral route in humans. Deliberate oral ingestion of acrylamide resulted in severe signs of systemic toxicity despite attempts to empty the stomach within approximately 3 hours *(Donovan and Pearson, 1987).

An in vitro dermal absorption study using human skin in static diffusion cells indicated, after 24 hours, between 25 and 35% absorption of acrylamide monomer in polyacrylamide gel, containing 1.28 and 2 ppm acrylamide (Marty and Vincent, 1998).

Haemoglobin adducts N-(2-carboxyethyl)valine and N-(2-carboxy-2-hydroxyethyl)valine were identified in blood samples from a group of 41 workers occupationally exposed to acrylamide at a factory in China (Bergmark et al, 1993). Workers were exposed via inhalation at air concentrations ranging from 0.11 – 8.8 mg/m^3 (8-hour TWA). Workers were also exposed via dermal route. N-(2-carboxy-2-hydroxyethyl)valine is indicative of epoxide formation and is consistent with work carried out by *Calleman et al (1990) and Bergmark et al (1991) on haemoglobin adduct formation in rats. These studies indicate the formation of cysteine adducts from both acrylamide and the epoxide metabolite glycidamide following exposure to acrylamide. Measurement of haemoglobin adducts in acrylamide workers is currently being validated as a biomarker for exposure and hence as a possible method for biological monitoring (Section 6).

The mercapturic acid metabolite, N-acetyl-S-(propionamide)- cysteine was identified in urine of occupationally exposed acrylamide workers (Wu et al., 1993). Biomonitoring methods utilising urinary metabolites are also currently undergoing validation *(EC 2001).

9.2 Animal data

9.2.1 Absorption

Rapid absorption of acrylamide was reported following oral administration of a single dose of between 116 and 121 mg/kg [^{14}C] to male mice and pregnant females *(Marlowe et al, 1986).

Although the inhalation of radiolabelled acrylamide has been studied in F-344 rats and B6C3F1 mice by Sumner et al (2000), the extent of absorption was not reported. However, higher amounts of radiolabel were recovered from mice than rats.

Dermal absorption was investigated by Ramsey et al *(1984) in groups of rats receiving a single dermal application of 2 or 50 mg/kg aqueous [1,3-^{14}C] acrylamide. The application method was not specified i.e. whether it was applied under an occlusion and whether or not any precaution was taken to prevent oral exposure. Blood plasma analysis indicated that in the first 24 hours, approximately 25% of the applied dose was absorbed.

Sumner et al (1999) found a maximum of 2.8% of dermally (under occlusion) applied ^{13}C- acrylamide solution (137 mg/kg for 24 h) excreted as metabolites in urine of male F-344 rats. However in a subsequent study, 14 to 30% of an applied dose of 162 mg/kg acrylamide was absorbed under the same exposure conditions *(Sumner et al 2000).

Frantz et al *(1986) and Frantz et al *(1985) found that ^{14}C-radiolabelled residual acrylamide monomer from three different 1% aqueous polyacrylamide solutions was well absorbed across F-344 rat skin in vitro, with up to 93% absorption from non-ionic polymer and 41 to 46 % absorption from ionic polymers. Similarly, Diembeck and Düsing (1998) found 94 to 98% absorption of ^{14}C-acrylamide across pigskin in vitro from spiked samples of different cosmetic formulations, polyacrylamide solution (2%) and acrylamide solution (50%) after 24 hours. The horny layer accounted for 12-18%, the epidermis, 6-9% and the dermis 40-47% of the absorbed dose . Approximately 32-35% of the absorbed dose penetrated the skin. In contrast Marty and Vincent (1998) found similar relative amounts of acrylamide diffusing across human skin over 24 hours but no accumulation in the dermis or epidermis. The author also found that acrylamide diffused rapidly across human skin with equilibrium of penetration being reached after 6 hours. More acrylamide diffused across the skin when receptor fluid was replaced at 12 and 24 hours post application.

9.2.2 Distribution and macromolecular binding

Groups of male dogs and male miniature pigs received 1 mg/kg/d acrylamide administered through the diet for a period of 3 to 4 weeks followed by a single oral dose of 1mg/kg aqueous [1-^{14}C] acrylamide. The dietary administration of acrylamide continued until sacrifice *(Ikeda, 1987).

In dogs approximately 35% of the administered dose of acrylamide was found in skeletal muscle after 6 hours and smaller amounts were mainly found in the liver, blood and GI tract. The total amount accounted for after 6 hours was about 64% of the administered dose. On the second day 17% of the administered dose was found in muscle with approximately 1% in the GI tract. This study shows that acrylamide is widely distributed in all tissues except in bile and gall bladder. Less than 1% of acrylamide was found in brain and fat.

In pigs approximately 32% of the administered dose of acrylamide was found in skeletal tissue after 6 hours, 20% in GI and 5% in liver, fat and blood. The total amount accounted for after 6 hours was about 71% of the administered dose. Absorption in pigs was slower than in dogs. Fat was one of the major sites of distribution in pigs. The amount of acrylamide in bile and gall bladder were very low.

Following oral administration of acrylamide to pregnant mice, uniform distribution was observed in both dams and foetuses *(Marlowe et al, 1986). Distribution to foetus was rapid and extensive.

Between 46% and 56% of inhaled (absorbed) dose was measured in tissues of mice and rats respectively, exposed to 3ppm (15 mg/m^3) acrylamide in air for 6 hours. In rats, relative radiolabel (µmol/g tissue) 24 hours post exposure was highest in blood and skin with similar but lower levels in spleen, lung, liver, kidney and intestines, whereas in mice, the rank order was skin, testes, blood, liver, lung, spleen and brain. In rats, the radiolabel recovered immediately after exposure was similar to that recovered at 24 hours post exposure, indicating rapid distribution *(Sumner et al 2000).

Following dermal application of acrylamide to rats, 53% of absorbed dose was recovered in urine after 24 hours. Relative radiolabel (µmol/g tissue) was highest in blood with similar but lower levels in skin, spleen, liver, kidney and testes *(Sumner et al 2000).

Table 9.1 provides a summary of studies on distribution of acrylamide in various animal species. Overall, various animal studies indicate that acrylamide is rapidly and widely distributed in most tissues. In rats and mice, high levels of acrylamide were seen in the liver, kidney, testes and epididymis. In dogs and pigs, acrylamide was distributed to skeletal muscle and liver.

Macromolecular binding studies by Carlson and Weaver *(1985) and Carlson et al. *(1986) have shown acrylamide to bind to DNA, RNA and protein. Highest level of binding to DNA and RNA was seen in the liver and the highest amounts of label bound to protein were seen on skin samples. Another study by Carrington et al *(1991) showed that acrylamide or metabolites were bound to a wide range of proteins and there was a particular affinity for microtubule-associated proteins.

Calleman et al *(1990) and Bergmark et al. (1991) observed that in rats treated intraperitoneally with arcylamide, both glutathione and its metabolite glycidamide form adducts with cysteine residues in haemoglobin. The binding index for acrylamide was some three times greater than for glycidamide, indicating that glycidamide has a lower reactivity to haemoglobin cysteine than acrylamide.

9.2.3 Metabolism/biotransformation

Acrylamide is metabolised by two main pathways. The major pathway is through glutathione conjugation catalysed by glutathione-s-transferase reactions and occurs in both liver and brain. This pathway is presumed to be a detoxifying process *(Dixit et al, 1982). The second pathway is biotransformation by cytochrome P450E1 to a reactive epoxide metabolite, glycidamide. Glycidamide may also undergo glutathione conjugation and/or further metabolism (hydrolysis) to glyceramide. The proposed pathway for acrylamide biotransformation in animals is shown in Figure 9.1.

Bergmark et al (1991) determined that the amount of oxidation of acrylamide to glycidamide is inversely related to the amount of parent compound. At 5 mg/kg/bw, about 50% of acrylamide was converted to glycidamide, whereas at 100 mg/kg/bw, about 15% was converted. Barber et al *(2001) reported that 30% of plasma acrylamide was converted to glycidamide from oral dosing of 20 mg/kg/d, as opposed to 8% from ip dosing of 50 mg/kg/d in rats.

Sumner et al (1999) reported that following administration of [^{13}C] acrylamide to wild type mice approximately 30% of the administered dose was excreted in urine with 49% being metabolites from direct GSH conjugation and the remainder (50%) were metabolites formed after conversion of acrylamide to glycidamide. P450 2E1-null mice administered acrylamide also excreted a similar proportion (~30%) of acrylamide consisting only of metabolites derived from direct conjugation of GSH with acrylamide.

The authors suggest P450 2E1-null mice compensated for the deficiency by metabolising more of the acrylamide by direct conjugation with GSH. Wild type mice pretreated with 1-aminobenzotriazole (ABT), an inhibitor of cytochrome P450 enzymes, also excreted only metabolites derived from direct conjugation of GSH with acrylamide. However, the ABT treated mice did not excrete increased amounts of these metabolites over that seen in wild type mice. The authors conclude that at the dose levels used in this study P450 2E1 is possibly the only cytochrome P450 involved in the oxidative metabolism of acrylamide.

An extensive study on the metabolism of acrylamide has been done in groups of male F-344 rats and male B6C3F1 mice receiving a single oral gavage administration of 0 or 50 mg/kg aqueous [1,2,3-^{13}C] acrylamide (>99% pure) *(Sumner et al, 1992). Urine samples collected over a 24 hour period showed that 50% of the administered dose was found in rat and mouse urine as metabolites or parent compound. The identified urinary metabolites of acrylamide are shown in Table 9.2.

Table 9.1 - Summary of oral and dermal distribution studies with [^{14}C]-labelled acrylamide

Species	Organs with highest levels of radiolabel detected at specified times after dosing						
	3 h	6 h	24 h	3 - 9 d	13 d	Comments	References
Mouse (Oral)	liver, kidneys, pancreas, testes and brain[1]	liver testes		epididymis/ vas deferens		Peak levels in testes measured 1-6 h post administration	*Marlowe et al (1986)
SENCAR and BALB/c mice (Oral)		skin testes				Lowest levels were found in skin and peak concentrations in testes. No clear differences between two strains	*Carlson and Weaver (1985) and *Carlson et al (1986)
SENCAR and BALB/c mice (Oral)		stomach				No clear differences between two strains	*Carlson and Weaver (1985) and *Carlson et al (1986)
Mouse (Intraperitoneal)						Peak levels in sperm[2] 7-9 days post administration	*Sega et al (1989)
Mouse *(foetus)* (Oral)	liver, kidneys, bladder		liver, kidneys, bladder			No accumulation of radioactivity measured in peripheral nerves of adult/foetus. High levels of radioactivity seen in foetal skin.	*Marlowe et al (1986)
Rat (Oral)					erythrocytes liver, kidneys, testes, epididymis		*Ramsay et al (1984)
Dog (Oral)		skeletal muscle, liver				At 6 h, little distribution of radioactivity in brain/spinal cord	*Ikeda et al (1987)
Pig *(miniature)* Oral		skeletal muscle, liver				At 6 h, little distribution of radioactivity in brain/spinal cor.	*Ikeda et al (1987)

1. Autoradiography indicated radioactivity was highest in the cerebral cortex. Another study in mice *(Carrington et al 1991) indicated that acrylamide has a particular affinity for microtubule-associated proteins.
2. Acrylamide appears to have a particular affinity for cysteine residues in the sperm head protein, protamine *(Sega et al 1989).

Table 9.2 - Urinary metabolites in acrylamide-exposed animals *(Sumner et al, 1992).

Urinary Metabolite	Total urinary metabolites in rats and mice
N-acetyl-S- (3-amino-3-oxopropyl) cysteine	Rats – 67% Mice – 41%
N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl) cysteine	Rats – 16% Mice – 21%
N-acetyl-S-(1-carbamoyl-2-hydroxyethyl) cysteine	Rats – 9% Mice – 12%
Glycidamide	Rats – 6% Mice – 17%
2,3-dihydroxy-propionamide	Rats – 2% Mice – 5%
Small amount of parent compound	

A similar metabolic profile was found by Sumner et al *(1999) following ip administration. However, a higher percentage of glycidamide-derived metabolites was observed after dermal application, with glycidamide accounting for 17% of urinary metabolites as opposed to 6 to 7% in ip and oral studies.

N-acetylcysteine-S-propionamide methyl ester and cysteine-S-propionamide methyl ester were the two major urinary metabolites identified in rats in another study (Dixit et al 1982). This study also showed that the total amount of acrylamide excreted as thioesters was approximately 7% of the administered dose.

Mukhtar et al. *(1981) observed decreases in levels of glutathione (GSH), a decrease in activity of GSH-transferase, and aryl hydroxylase enzymes in skin and liver samples of groups of mice receiving a single dermal application of 100 mg/kg acrylamide in acetone in contact with skin for 4 h. Decreases in glutathione levels of 60% to 80% were seen in skin and liver respectively.

In studies by Tanii and Hashimoto *(1983) phenobarbital pre-treatment did not increase the rate of metabolism of acrylamide in vitro but increased the rate of reaction of acrylamide with glutathione by 40%.

9.2.4 Elimination/Excretion

In groups of rats receiving a single dermal application of either 2 or 50 mg/kg aqueous [1,3-^{14}C] acrylamide with an exposure period of up to 48 hours, the clearance of radiolabel from blood was biphasic with a half-life of about 2 hours for the first phase *(Ramsey et al 1984). The parent compound was eliminated mainly in the first phase. The second phase was due to clearance of radiolabelled metabolites with a half-life of about 10 hours. The plasma half–lives for acrylamide and glycidamide were reported to be 98 and 112 minutes respectively

following single oral acrylamide dosing, and 118 and 127 minutes respectively following repeated oral acrylamide dosing *(Barber et al, 2001).

The major route of excretion of acrylamide in both dogs and pigs is urine accounting for approximately 60% of the administered dose of 1 mg/kg/d with 7% to 27% detected in faeces. In the first 2 days, most of the radiolabel is excreted in urine with little being excreted over the 12 days. Biliary excretion is not a major route of excretion of acrylamide or metabolites in these species *(Ikeda et al, 1987).

Oral dosing (single) of rats at 1, 10, or 100 mg/kg aqueous [2,3-^{14}C] acrylamide showed that at each dose level 53 to 67% of the radiolabel administered was excreted within 24 hours and by 7 days, 65 to 82% had been eliminated. In urine sample, approximately 74% of the radiolabel was recovered and in faeces 8% *(Miller et al 1982).

Following ip injection and oral gavage doses of 50 mg/kg acrylamide, approximately 62% and 50% of the dose were excreted in urine of male F-344 rats *(Sumner et al 1992, 1999).

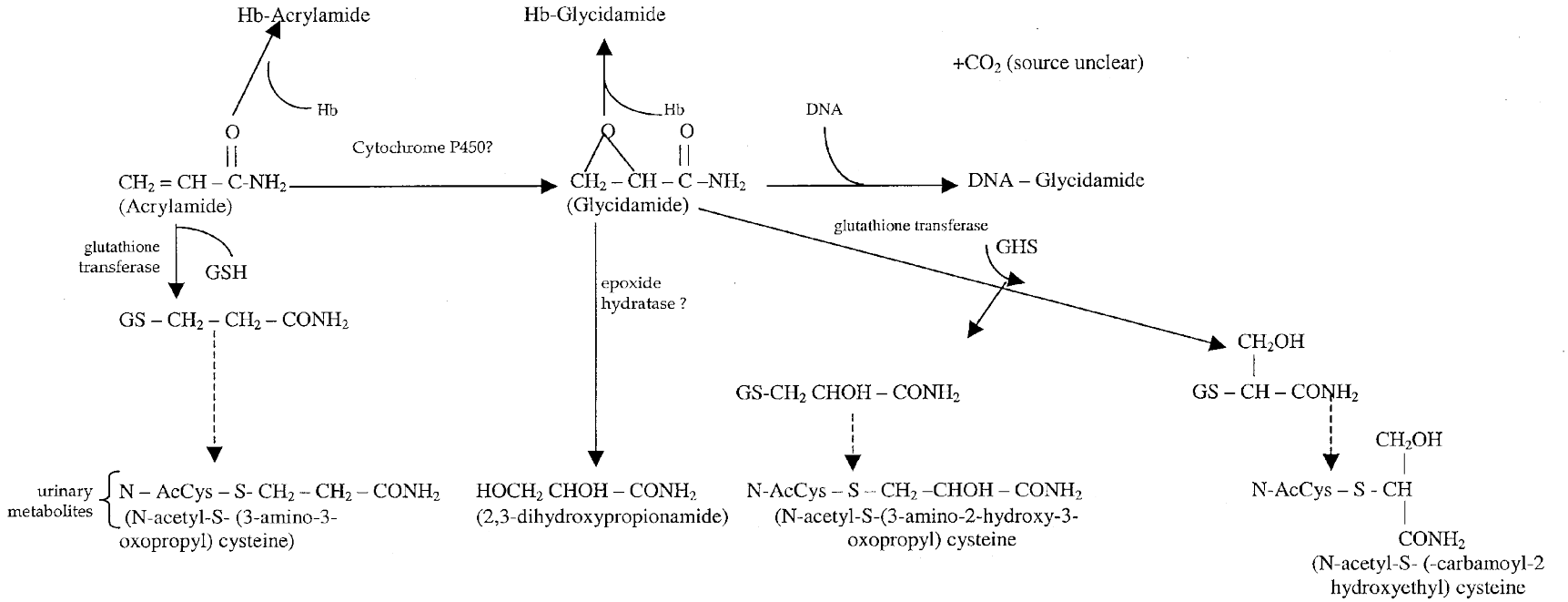
Ramsey et al. *(1984) showed that in rats receiving 0.05 or 30 mg/kg oral [1,3-^{14}C] acrylamide once per day for 13 days, that approximately 60% of the radiolabel that was administered on each day was excreted on the same day, at both the dosages. Less than 5% of the total urinary radioactivity was unchanged acrylamide.

Another study by Miller et al *(1982) showed that in groups of three male rats receiving a single oral dose of 1, 10 or 100 mg/kg aqueous [2,3-14C] acrylamide, at each dose level between 53 and 67% of the radiolabel administered was excreted in urine and faeces within 24 hours and by 7 days, 65 to 82% had been eliminated. Approximately 74% was recovered in urine samples and about 8% in faeces.

In both rats and mice, around 30% and 4% of inhaled (absorbed) dose were recovered in urine and in faeces respectively, with a small amount (<2%) as CO_2 24 hours following a 6-hour exposure to 3 ppm acrylamide. Following dermal application to rats, 36% of absorbed dose was found in urine, 0.5% in faeces with a small amount (<2%) as CO_2 (Sumner et al 2000).

Figure 9.1

Proposed metabolic pathway



Hb = Haemoglobin; AcCys = Acetyl cysteine

Adapted from IARC (1994)

10. Effects on Laboratory Animals and other Test Systems

10.1 Acute toxicity

Acrylamide has moderate acute toxicity by all routes of exposure studied. The LD50 and LC50 values in various species are shown in Table 10.1.

Table 10.1 – LD50 values for acrylamide

Route	Species	LD50/LC50	Reference
Oral	Rat	175 mg/kg bw	Tilson & Cabe, 1979
Oral	Rat	203 mg/kg bw	*Fullerton & Barnes, 1966
Oral	Rat	150 – 180 mg/kg bw	M^{c}Collister et al, 1964
Oral	Mice	107 mg/kg bw	Hashimoto et al., 1981
Oral	Guinea-pig	150 – 180 mg/kg bw	M^{c}Collister et al., 1964
Oral	Rabbit	150 – 180 mg/kg bw	M^{c}Collister et al., 1964
Dermal	Rabbit	1148 mg/kg bw	*Keeler et al., 1975

Other studies of different species and routes of administration are available, however the results are equivocal due to the methods used. An inhalation study of one hours duration exposed rats to an aqueous aerosol solution of acrylamide directly to the nose at a concentration of approximately 6 mg/L (99% of droplets less than 6μm). As one animal out of six died, an LC50 could not be determined. No toxic signs were noted during a two week observation period *(Keeler et al, 1975). McColister et al. (1964) exposed two cats to 100 mg/kg intraperitoneally. Both animals exhibited neurotoxic signs and one was sacrificed after 24 hours. The surviving animal recovered almost completely after 2 weeks. One monkey was given 100 mg/kg intraperitoneally on two consecutive days. On the third day the animal appeared to have lost its sense of balance. The authors also exposed one monkey to one daily intravenous dose of 50 mg/kg over 4 days. The animal died, but no clinical signs were described. In another part of this experiment, rabbits received dermal applications of 63, 126, 500 and 1000 mg/kg acrylamide. One animal receiving 1000 mg/kg died within two days, but there were no other deaths.

Apart from death, signs of neurotoxicity headed the clinical effects observed in rats, rabbits and mice following acute exposure. Rats exposed orally exhibited postural and motor incoordination, muscular dysfunction in the hindlimbs, hyperreflexia, tonic-clonic convulsions, tremors and dilatation of pupils. Complete recovery of hindlimb function occurred by 7 days after dosing (Tilson & Cabe, 1979). In rats given doses above 200mg/kg body weight, there was tremor and evidence of fatty liver. After 3 days, animals either died or recovered completely (Fullerton & Barnes, 1966).

A single oral administration to rabbits of 126 mg/kg resulted in tremors and pupil dilation whilst at 63 mg/kg, slight weight loss was observed. No recovery phase was described in this study (M^{c}Collister et al., 1964).

Mice in acute dosing also exhibited signs of neurotoxicity. Though an LD50 was calculated by the authors, details of the doses used are not given, nor is a recovery phase described (Hashimoto et al., 1981). Mid-range dosing (100 to 150 mg/kg bw) had a significant effect on spermatogenesis in mice *(Sakamoto et al, 1988).

Dermal application of acrylamide to rabbits also produced neurotoxicity, with tremors and incoordination of hindlimbs seen at doses of 806 and 1612 mg/kg *(Keeler et al, 1975).

10.2 Irritation and corrosivity

10.2.1 Skin

Acrylamide has shown irritant properties in some studies but not in others.

Two unpublished studies described in the OECD report found no signs of skin irritation. These studies were conducted to modern protocol standards. In the first study, 0.5 g acrylamide moistened with water was applied to the shaved, intact skin of three New Zealand White rabbits under a semi-occlusive dressing for 4 hours *(Mercier, 1997a). Skin reactions were scored at 24, 48 and 72 h post-application. No erythema or oedema was reported. The second study applied 0.5 mL of a 50% aqueous solution under the same conditions as the first experiment. Again, there were no signs of skin irritation *(Mercier, 1997b).

Keeler et al *(1975) applied an unknown volume of a 51% aqueous acrylamide solution to the shaven skin of six rabbits under a semi-occlusive dressing for 4 hours. Post-application skin reactions were scored at 24 and 72 hours. Slight reactions were reported, although individual scores were not presented. The same author describes a second experiment in which 0.5 mL of a 51% aqueous acrylamide solution was applied once per day for 10 days under a semi-occlusive dressing to shaven abdominal skin of three rabbits. Reactions were scored at 24, 48 and 72 hours. The mean score for erythema was 1.4 (maximum 2) and for oedema, the mean and maximum score was 1.0.

A 10% aqueous solution of acrylamide was applied to the shaved skin of one rabbit under an occlusive dressing. No significant responses were recorded. Application of approximately 17 mL of a 12.5% aqueous solution of acrylamide to the shaven skin of four rabbits resulted in erythema of unknown severity (M^{c}Collister et al, 1964).

10.2.2 Eye

Two unpublished studies described in the OECD report found acrylamide to be an eye irritant. These studies were conducted to modern protocols. In one experiment 82 mg of powdered acrylamide was applied to one eye of three New Zealand White rabbits *(Mercier, 1997c). Reactions were scored at 24, 48 and 72 hours and up to 21 days post-application. The mean score for iridial reactions over 24, 48 and 72 hours was 1.0; corneal opacity ranged from 2.0 to 2.3; conjunctival redness 2.0 and chemosis 1.3 to 2.0. Iridial reactions were still apparent at day 14 although there

were no abnormalities noted at day 21. The other experiment, which followed the same design, used 0.1 mL of a 50% aqueous acrylamide solution. Signs of eye irritation were observed and these reversed by day 7 *(Mercier, 1997d).

Three rabbits were exposed to 0.1 mL of a 51% aqueous acrylamide solution. Signs of eye irritation were observed *(Keeler et al., 1975).

A 10% aqueous solution applied to the eye caused slight pain and conjunctival irritation immediately on contact. There was no corneal injury and the conjunctiva was completely normal after 24 hours. Applying a 40% aqueous solution to the rabbit eye caused moderate pain, slight conjunctival irritation and significant corneal injury. The conjunctival irritation was slow in healing whilst the corneal injury healed completely within 24 hours (M^{c}Collister et al., 1964).

10.2.3 Corrosivity

There are no data investigating the corrosive effects of acrylamide.

10.3 Sensitisation

10.3.1 Skin

Two guinea-pig maximisation tests reported positive results. These studies were conducted to modern protocol standards. Groups of 20 test and 10 control animals were dermally exposed to concentrations of up to 25% aqueous acrylamide following induction of test animals with up to 50% topically and up to 3.5% acrylamide intradermally. Skin reactions were observed in most control animals but these were less severe than in test animals. A positive skin response in excess of that seen in controls was recorded in 40% of the test group *(Allan, 1995). Stockhausen *(1995) used the same test protocol and found no skin reactions in control animals but a positive response in 85% of test animals.

10.3.2 Respiratory

There are no data investigating the respiratory sensitising potential of acrylamide.

10.4 Repeated dose toxicity

Many repeated dose studies have been conducted with acrylamide in a range of species. The results of the major studies are summarised in Table 10.2. There are no inhalation studies in animals available, with oral and dermal being the routes studied. The bulk of studies are oral dosing.

10.4.1 Oral studies

Rodents

A number of studies have been carried out in rats at doses ranging from 0.05 mg/kg/d to 50 mg/kg/d of acrylamide either dissolved in drinking water or given by gavage.

In a 90-day study, electron microscopy revealed axonal and myelin degeneration (described as slight) in peripheral nerves at 1 mg/kg/d. Light microscopic lesions

seen at 5 mg/kg/d consisted of nerve degeneration, but to a lesser degree than seen at 20 mg/kg/d dosing. Electron microscopy showed nerve degeneration consistent with light microscopy at 5 mg/kg/d and 20 mg/kg/d with severity being dose related. In another study, histopathological examination revealed degeneration of the tibial nerve at 2 mg/kg/d. This consisted of swelling of individual nerve fibres, formation of vacuoles containing macrophages and eosinophilic globules and fragmentation of the myelin and axons. Other peripheral nerves were examined and changes occurred less frequently than in the tibial nerve. Overall, doses above 20 mg/kg/d produced severe lesions of the peripheral nerves, with resultant clinical signs of peripheral neuropathy. Electron microscopy revealed severe axonal degeneration and loss, myelin ovoids, lipid and cellular debris, accumulations of fine tubules in Schwann cells, invaginations of the axolemma, Schwann cell column formation. Histopathology of white matter from cervical and lumbar spine, trigeminal and dorsal root ganglia, sciatic, tibial and sural nerves showed altered diameter of axons, disruption, fragmentation and distortion of axons, and/or dilatation and fragmentation of myelin sheaths. Clinical signs seen in the various studies were consistent with nerve damage and included increases in landing foot spread, reduced locomotor activity, reduced hindlimb extensor response and reduced forelimb grip strength. Toxic effects were also seen at other sites such as skeletal muscle atrophy, decreased body weights, testicular atrophy and haematological changes at doses from 5 mg/kg/d. Duration of exposure was also relevant to toxic responses, with lower doses producing toxic effects when administered for longer time periods, or a given dose leading to more severe effects as duration of exposure increased.

Two gavage studies in mice examined doses of 36 mg/kg twice weekly and 26 mg/kg/d respectively. Clinical signs of peripheral neuropathy were seen at 36 and 26 mg/kg/d. In addition animals receiving about 36 mg/kg showed reduction in testicular weight and loss of spermatids, spermazoa and spermatocytes. Mice studies were not designed to investigate NOAELs. Another study in mice conducted for a dominant lethal assay exposed animals to dermal dosing of up to 125 mg/kg/d. No signs of toxicity were observed.

Cats

Two studies are described in the literature, with acrylamide being administered in food in both. Signs of peripheral neuropathy, exhibiting as abnormal gait and loss of use of hind limbs, were observed at 1 mg/kg/d in one study and 15 mg/kg/d in the other. This study used a single dose of 15 mg/kg/d and a control group. Histopathology and electron microscopy were undertaken in the 15 mg/kg/d study and showed decreases in numbers of myelinated fibres, abnormal membranes in the vicinity of Schwann cells and degenerating fibres of nerves supplying the left gastrocnemius muscle and of the splanchnic nerve.

Dogs

Two studies are reported with acrylamide being administered in the diet. No controls were used with animals receiving 7 mg/kg/d in one study and 6 mg/kg/d in the second. This latter study concentrated on respiratory effects and included a recovery period. Loss of use of the hind limbs occurred at both doses. In the respiratory effects study, a decreased respiratory frequency and increased tidal volume were observed as well as possible neuropathy of the vagus nerve.

Pneumonia was seen in one animal. There was restoration of the respiratory and neuropathy effects during the recovery period.

Non-human primates

Monkeys were studied in six experiments, with only one examining a range of doses. In all studies, acrylamide was given in fruit juice. Three studies concentrated on visual effects. Clinical signs of peripheral neuropathy, neuropathological effects and neurological dysfunction were observed at exposures of 10 mg/kg/d, particularly in relation to the use of limbs. Most changes were reversible after approximately 30 weeks without post-exposure to acrylamide. The visual system was markedly affected at similar dose levels. Histopathological examination revealed loss of ganglion and axonal cells in the optic tract and brain.

Only one study examined a range of doses, but the lack of detail in the reporting meant that firm conclusions could not be drawn and hence a NOAEL could not be determined.

10.4.2 Dermal studies

Rabbits

One study exposed groups of rabbits to a range of dermal dosing. Neurotoxicity was noted at 50 mg/kg/d but the lack of detail in the reporting meant that any conclusions are equivocal.

Table 10.2 - Repeated dose toxicity

Species	Exposure	NOAEL	LOAEL	Observations	References
Oral exposure					
F-344 rats, 60 males and females in each exposure group (part of a carcinogenicity study)	0, 0.01, 0.1, 0.5 and 2 mg/kg/d in drinking water for 2 years	0.5 mg/kg/d	2 mg/kg/d	Tibial nerve lesions	Johnson et al., 1986
Porton rats, male and female (numbers not stated)	0, 10, 20, 30 and 40 mg/kg/d in food for 48 weeks (estimated)	10 mg/kg/d	20 mg/kg/d	Reduced nerve conduction velocity in hind paw at doses of 20 mg/kg/d for 6 months and 40 mg/kg/d for 2 months. Recovery after 5 to 9 months without acrylamide.	Fullerton and Barnes, 1966
Wistar rats, 4 males in each exposure group	0, 7.5, 12, 19 and 30 mg/kg/d in water for 90 d (estimated)	Not determined	7.5 mg/kg/d	Reduction in body weight from 4% at lowest dose to 10% at the highest dose. Decreased rotarod performance at day 90 at the two highest doses; No other rotarod data available. Clinical signs of weakness, spreading and dragging hind limbs, urinary incontinence. Myelinated fibres of tibial and sural nerves affected at 30 mg/kg/d.	*Tanii and Hashimoto, 1983
F-344 rats, 10 males and females in each exposure group	0, 0.05, 0.2, 1, 5 and 20 mg/kg/d in drinking water for 90 d	0.2 mg/kg/d	1 mg/kg/d	Decreased body weight and water intake; decreased thymus weight in females and testes weight in males; haematological changes; peripheral nerve axon and myelin degeneration; toe curling; splayed and weak hindlimbs; incoordination.	Burek et al., 1980

Table 10.2 - Repeated dose toxicity (continued)

Species	Exposure	NOAEL	LOAEL	Observations	References
Oral exposure					
F-344 male rats, 10 in each exposure group	0, 5, 10 and 20 mg/kg/d, 3 d/week for 13 weeks by gavage	5 mg/kg/d	10 mg/kg/d	Decreased body weight gain; reduced hindlimb extension response; spontaneous locomotor activity; forelimb grip strength; peripheral neuropathy.	Tilson et al., 1979
F-344 male rats, 10 in each exposure group	0, 10 and 20 mg/kg/d, 5 d /week for 4 weeks by gavage	Not determined	10 mg/kg/d	Decreased weight gain and hindlimb extensor response; weight loss	Tilson and Cabe, 1979
SD rats, 10 males and 10 females in each exposure group	0, 10 and 30 mg/kg/d, 7 d/week for 3 weeks followed by 10 days recovery then 0, 10 and 20 mg/kg/d, 7 d by gavage	Not determined	20 mg/kg/d	Decreased food consumption, weight gain and forelimb and hindlimb grip strength at highest dose; rigidity; ptosis; impaired respiration, gait and righting reflex; inflammation of lungs; soiled fur; hunched posture; increased urination; haemorrhage in urinary bladder; altered axonal diameter and disruption of axons and myelin sheath.	Schulze and Boyson, 1991
SD rats, 10 males and 10 females in each exposure group	0, 12.5, 25 and 50 mg/kg/d, for 7 d by gavage	Not determined	12.5 mg/kg/d	Decreased activity, body weight gain, forelimb and hindlimb grip strength; splayed hindlimbs and axonal degeneration	*Newton et al., 1992 *Hughes et al., 1994

Table 10.2 - Repeat dose toxicity (continued)

Species	Exposure	NOAEL	LOAEL	Observations	References
Oral exposure					
Male mice (species not stated), 6 in each exposure group	0 and approx. 36 mg/kg twice weekly for 8 weeks by gavage	Not determined	36 mg/kg	Weakness and ataxia in hind limbs; decreased performance on Rotarod; reduced testicular weight; reduction in spermatids, spermatoza and spermatocytes; epididymides unaffected	Hashimoto et al., 1981
BALB/c female mice, 5 in each exposure group, 2 additional control groups one given saccharin and restricted food	0, and 26 mg/kg/d for 12 d in drinking water, followed by recovery then 20 mg/kg/d, 19 d	Not determined	26 mg/kg/d	Increased hind limb splay; decreased retention time on Rotarod; decreased body weight. Values returned to normal after recovery period	*Gilbert and Maurissen, 1982
Cats (species and sex not stated), 1-3 per group	0, 0.03, 0.1, 0.3, 1, 3 and 10 mg/kg/d in food 5 d per week for up to 1 year	Not determined	1 mg/kg/d	Peripheral neuropathy (abnormal gait and loss of use of hind limbs). Animals at other doses died due to intercurrent infection.	McCollister et al, 1964
Cats (species and sex not stated), 17-23 per group	0 or 15 mg/kg/d in food, 7 d/week for up to 16 weeks	Not determined	15 mg/kg/d	Hind limbs affected leading to inability to walk, weight loss, diarrhoea. Decreased conduction velocity in tibial and greater splanchnic nerves, decreased fibre density in vagus and greater splenic nerves	*Post and McLeod, 1977
Dogs (species and sex not stated), 14 in treatment group, no controls.	7 mg/kg/d in food for 10 weeks	Not applicable	7 mg/kg/d	Severe impairment of hind limb function, regurgitation	*Satchell and McLeod, 1981

Table 10.2 - Repeat dose toxicity (continued)

Species	Exposure	NOAEL	LOAEL	Observations	References
Oral exposure					
Dogs (species and sex not stated), 4 in treatment group, no controls	6 mg/kg/d in gelatin capsules for 6-7 weeks, 8 week recovery phase	Not applicable	6 mg/kg/d	The study focussed on respiratory effects. Pneumonia in one animal, decreased respiratory frequency and increased tidal volume. Possible effects on vagus nerve, loss of use of hind limbs. Effects resolved during the recovery phase.	*Hersch et al, 1989
Female monkeys (species not stated), 1 in each treatment group	0, 0.03,0.1, 0.3 (two animals), 1, 3 and 10 mg/kg/d, 5 d/week, for up to 1 year	1 mg/kg/d	3 mg/kg/d	Neuropathy, reduced knee jerk and papillary reflexes	McCollister et al, 1964
Macaque monkeys (sex not stated), 3 in treatment group, 2 controls	10 mg/kg/day in fruit juice, 5 d/week for 6-9 weeks, recovery phase for 30 weeks	Not determined	10 mg/kg/d	Loss of balance, decreased activity, hind limb weakness and forelimb tremor, elevated vibration threshold, most effects resolved during recovery.	*Maurissen et al, 1990
Macaque monkeys (sex not stated), 7 in treatment group, 2 controls	10 mg/kg/d in fruit juice, 5 d/week for up to 13 weeks, recovery phase for 20-30 weeks	Not determined	10 mg/kg/d	Study examined visual effects. Distal axonal swelling, degenerating myelin, axonal loss in fibres of optic tract	*Eskin et al, 1985
Macaque monkeys (sex not stated), 3 in treatment group, 1 control	10 mg/kg/d in fruit juice, 5 d/week for 6-10 weeks, recovery phase for 140 d	Not determined	10 mg/kg/d	Study examined visual effects. Impaired visual acuity and visual-evoked potentials reduced flicker fusion frequency. Some resolution of effects during recovery phase.	*Merigan et al, 1985

Table 10.2 - Repeat dose toxicity (continued)

Species	Exposure	NOAEL	LOAEL	Observations	References
Oral exposure					
Macaque monkeys (sex not stated), 3 in treatment group, 1 control	10 mg/kg/d in fruit juice, 5 d/week for 33-47 doses (over 6-10 weeks)	Not determined	10 mg/kg/d	Study examined visual effects. Increased cortical evoked potential, decreased visual acuity and flicker fusion frequency, affects on visuomotor coordination, most resolving during recovery phase. Hind limb weakness, gait disturbances, weight loss observed.	*Merigan et al, 1982
Macaque monkeys (sex not stated), 4 in treatment group, 2 controls	10 mg/kg/d in fruit juice, 5 d/week for 44-61 d, recovery phase for up to 146 d	Not determined	10 mg/kg/d	Loss of balance, decreased activity, hind limb weakness and forelimb tremor, elevated vibration threshold, effects on mylin sheaths and Schwann cells, most effects resolved during recovery.	*Maurissen et al, 1983
Rabbits, (sex and numbers not stated)	0, 0.5, 5 and 50 mg/kg/d, vehicle not stated, for up to 12 weeks, 50 mg/kg/d given for 5 weeks followed by a 7 week recovery phase	5 mg/kg/d	50 mg/kg/d	Clinical signs of neurotoxicity which resolved during recovery phase	*Dress et al, 1976
Male mice (species not stated), 24-30 in each treatment group	0, 25, 50, 75, 100 and 125 mg/kg/d in 70% methanol for 5 d, (part of a dominant lethal assay)	125 mg/kg/d	Not determined	No signs of toxicity were observed	*Gutierrez-Espeleta et al, 1992

Summary

In all studies, clinical signs of neurotoxicity were found to be the critical effects, with hind limbs being affected preferentially to fore limbs (where details were reported). Histopathology showed degeneration of myelin and axons, with effects on Schwann cells. In studies assessing neuropathy, effects were seen in peripheral nerves, with the tibial and sural nerves being the most affected (and studied). Centrally effects were seen on white matter from cervical and lumbar spine, trigeminal and dorsal root ganglia, sciatic, tibial and sural nerves which all showed altered diameter of axons, disruption, fragmentation and distortion of axons, and/or dilatation and fragmentation of myelin sheaths. Effects on brain regions were seen in some studies but not others. Experiments in monkeys given oral dosing, which included a recovery phase, demonstrated almost complete resolution of gross signs during the recovery period. Some nerve fibre regeneration was also noted. Dermal studies in rabbits showed resolution of gross signs during the recovery phase, but no histopathology was undertaken. The significance of results from dermal experiments is unclear.

NOAELs and LOAELs from the different species tested are presented in Table 10.3 for oral dosing, and Table 10.4 for dermal exposure (see also Table 10.2).

Table 10.3 - NOAELs and LOAELs from oral repeat dose toxicity studies

Species	NOAEL	LOAEL
Rats	0.2 mg/kg/d to 5 mg/kg/d	1 mg/kg/d to 20 mg/kg/d
Mice	Not determined	26 mg/kg/d to 36 mg/kg/d
Cats	Not determined	1 mg/kg/d to 15 mg/kg/d
Dogs	Not determined	6 mg/kg/d to 7 mg/kg/d
Monkeys	1 mg/kg/d	3 mg/kg/d to 10 mg/kg/d

Table 10.4 - NOAELs and LOAELs from dermal repeat dose toxicity studies

Species	NOAEL	LOAEL
Rabbits	5 mg/kg/d	50 mg/kg/d
Mice	125 mg/kg/d	Not determined

10.5 Genotoxicity and other related bioassays

In this section, studies relevant to the assessment of adverse effects on the genome are evaluated. Such studies include standard tests carried out to determine mutagenic and clastogenic potential and tests designed to help characterise the mechanism of acrylamide-induced carcinogenicity /reproductive effects. In vitro and in vivo genotoxicity studies for acrylamide are summarised in Tables 10.5 and 10.6 respectively.

In vitro studies

Acrylamide has been investigated in a number of in vitro assays for a number of genetic endpoints (Table 10.5).

Acrylamide was not mutagenic in standard bacterial tests with mixed results in mammalian cell assays, both with and without metabolic activation.

Alkylation of DNA has been reported in vitro, but generally significant levels of adducts are seen only after incubations of several weeks at high concentrations (Segerbäck et al., 1995).

Mainly negative results were seen for unscheduled DNA synthesis (UDS) or DNA repair in rat hepatocytes, with a positive result for UDS being reported in human mammary cell cultures. Acrylamide was weakly positive and negative in sister chromatid exchange (SCE) assays in Chinese hamster V79 and ovary cells (CHO) respectively.

Acrylamide was clastogenic in mouse and hamster cell (somatic) lines, causing an increase in chromosomal aberrations and polyploidy when tested in the presence or absence of metabolic activation systems. Equivocal results were reported for cell transformation lines in mouse and hamster cell (somatic) lines.

The main acrylamide metabolite, glycidamide, has been reported as being mutagenic in bacterial and mammalian gene mutation assays, both with and without metabolic activation. A positive result was reported for glycidamide-induced UDS in human mammary epithelial cells, with equivocal results in rat hepatocytes (Dearfield et al 1995). It has not been fully elucidated whether glycidamide is formed in metabolic activation systems employed in in vitro studies on acrylamide.

In vivo studies

Acrylamide has been investigated for a number of genetic endpoints in rats and mice (Table 10.6).

Gene mutation assays provided mixed findings. Positive results were reported in the mouse spot (somatic), heritable translocation (germ), specific locus (germ), *Drosophila Melanogaster* (somatic) tests with equivocal evidence in transgenic mouse (somatic and germ) and negative findings in a *Drosophila Melanogaster* sex-linked recessive lethal (germ) test.

Alkylation and damage to DNA, such as increases in strand breaks in spermatids, have been reported in rat and mouse studies, at doses of 25 to 50 mg/kg. DNA alkylation was determined in a number of organ tissues, with highest levels of adducts seen in liver and kidney.

Table 10.5 - Summary of in vitro genotoxicity studies

Type of test	Test system	Dose of acrylamide	Result	Comments	References
GENE MUTATION ASSAYS					
Bacterial assays					
Reversion assay	*Salmonella Typhimurium* (strains TA 98,100,102,1535, 1537, 1538)	10-100,000 µg/plate	Negative (+/- MA)	Consistent results in both liquid pre-incubation and plate incorporation assays	Dearfield et al (1995)
Gene mutation	*E.coli* (strain WP2 urvA-)	0.5-50 mg/plate	Negative (+/- MA)		*Tsuda et al (1993)
Fluctuation test	*Klebsiella pneumoniae*	1-10 mg/mL	Negative	Assay measures mutations in genes conferring streptomycin resistance	*Knaap et al (1988)
Transfection assay	*E.coli* (strain CR63)	10 µg	Positive	Assay measures inhibition of transfection. Significance of test questioned in OECD report.	*Vasavada & Padayatty (1981)
Mammalian cell assays					
Forward mutation	Mouse (L5178Y TK+/-) lymphoma cells (TK and HPRT loci)	0.5-7.5 mg/mL	Equivocal (+/- MA)	Cell survival noted as being low (<30%)	*Knaap et al (1988)
Forward mutation	Mouse (L5178Y TK+/-) lymphoma cells (TK locus)	0-850 µg/mL 10 mM	Positive (- MA)	TK mutants were small colonies. Gross aberrations (clastogenicity) were also induced.	*Moore et al (1987) *Barfknecht et al (1988)
Forward mutation	CHO cells (HPRT locus)	38-900 µg/mL	Equivocal (+/- MA)	Cell survival noted as being high (>70%)	*Godek et al (1982)
Forward mutation	CHO cells (HPRT locus)	Up to 1500 µg/mL	Negative (+/- MA)	Cell survival noted as being high (>70%)	*Godek et al (1984)
Forward mutation	Chinese Hamster V79H3 cells (HPRT locus)	0-500 µg/mL	Negative (- MA)		*Tsuda et al (1993)

Table 10.5 - Summary of in vitro genotoxicity studies (continued)

Type of test	Test system	Dose of acrylamide	Result	Comments	References
ASSAYS FOR DNA EFFECTS					
DNA alkylation	Calf thymus DNA	Test concentration not stated Incubation period = 40 d	Weakly positive	Adducts identified: Deoxyguanosine Deoxyadenosine Deoxycytidine	*Solomon et al 1985)
DNA damage	*B. subtilis* spores (rec assay)	1-50 mg/disk	Positive		*Tsuda et al (1993)
DNA damage	Neuronal cell cultures	No test data	Positive	Oxidative damage	*Benn and Thomas (in press)
UDS	Rat primary hepatocytes	0-3.55 mg/mL	Negative	Cytotoxicity seen at highest concentration	*Miller & McQueen (1986)
UDS	Rat primary hepatocytes	0-100 mg/mL	Equivocal		*Naismith & Matthews (1982)
UDS	Rat primary hepatocytes	0-71 µg/mL	Negative (- MA)	Cytotoxicity seen at highest concentration.	*Butterworth et al (1992)
UDS	Human mammary epithelial cells	0-710 µg/mL	Positive	Lowest effective concentration = 70 µg/mL (in both cell systems)	*Butterworth et al (1992)
DNA repair	Rat primary hepatocytes	0.7-710 µg/mL	Negative	Cells irradiated with UV to induce DNA damage. Cytotoxicity seen at highest concentration	*Miller & McQueen (1986)
SCE	Chinese Hamster V79H3 cells	0-213 µg/mL	Weakly positive (- MA)		*Tsuda et al (1993)
SCE	Chinese Hamster V79 cells	0-1,000 µg/mL	Weakly positive (+/- MA)	No data on cytotoxicity. Lowest effective concentration = 300 µg/mL (-MA); 1000 µg/mL (+MA)	*Knaap et al (1988)

Table 10.5 - Summary of in vitro genotoxicity studies (continued)

Type of test	Test system	Dose of acrylamide	Result	Comments	References
ASSAYS FOR DNA EFFECTS (Cont.)					
SCE	CHO cells	0-500 µg/mL	Negative	Sensitivity of study questioned in EU report	*Sorg et al (1982)
CYTOGENETICS ASSAYS					
Chromosome aberration	Chinese Hamster V79 cells	0-3,000 µg/mL	Positive (+/- MA)	No data on cytotoxicity. Lowest effective concentration = 1000 µg/mL (-MA); 100 µg/mL (+MA)	*Knaap et al (1988)
Chromosome aberration	Chinese Hamster V79H3 cells	0-355 µg/mL	Positive (- MA)	Dose-related increase in polyploid cells at 71 µg/mL. No data on cytotoxicity.	*Tsuda et al (1993)
Chromosome aberration	Mouse (L5178Y TK+/-) lymphoma cells	>750 µg/mL	Positive (+/- MA)	27% cells had chromosome and chromatid breaks	*Moore et al (1987)
Spindle effects	Chinese Hamster V79 cells	0.01-1 mg/mL	Positive (-MA)	Dose-related inhibition of mitosis & fragmented/bridged ana-telophase. Induction of polyploidy. Cytotoxicity seen at highest concentration.	*Adler et al (1993) *Sickles et al (1995)
OTHER					
Cell transformation assay	Mouse BALB/3T3, C3H/10T$^{1}/_{2}$ & NIH/3T3 cell lines	10-300 µ/mL	Equivocal (+/- MA)	Positive and negative results	*Dearfield et al (1995)
DNA amplification study	Chinese Hamster CO60 cells	0-150 µg/mL	Negative	Assay measures increased synthesis of SV40 DNA	*Vanhorick & Moens (1983)

Key:
CHO = Chinese Hamster Ovary
SCE = Sister Chromatid Exchange
UDS = Unscheduled DNA synthesis

+ MA = with exogenous metabolic activation
- MA = without exogenous metabolic activation

Table 10.6 - Summary of in vivo genotoxicity studies

Type of test	Test system	Dose of acrylamide	Result	Comments	References
GENE MUTATION ASSAYS					
Sex-linked recessive lethal (germ cell)	*Drosophila Menanogaster*	2.8 - 3.5 mg/mL (abdominal injection) 17.5 - 355 µg/mL (larvae feeding)	Negative Positive	Lowest effective concentration = 3.5 mg/mL Lowest effective concentration = 70 µg/mL	*Knaap et al (1988)
Somatic mutation and/or recombination assay	*Drosophila Menanogaster*	30- 105 µg/mL (larvae feeding)	Weakly positive	Lowest effective concentration = 70 µg/mL	Dearfield at al (1995)
Coat colour spot test (somatic cells)	Mouse (female)	0-75 mg/kg (single/repeated -gavage)	Positive	Pregnant females exposed. Doubling in number of spots of genetic relevance reported at and above 50 mg/kg single/repeated exposure.	*Neuhauser-Klaus and Schmahl (1989)
Transgenic mouse (somatic & germ cells)	*LacZ* mutation system	50 mg/kg (repeat (x5) - ip)	Equivocal	4-fold increase in mutation frequency in bone marrow. No clear increase in mutations in testicular cells.	*Hoorn et al (1993) *Myhr (1991) *Murti et al (1994)
Heritable translocation tests (germ cells)	Mouse	0-100 mg/kg (single – ip) 40-50 mg/kg/d (repeat (x5) - ip)	Positive	Dose-related increases in translocation carriers at high doses. Increase in dead implants in mated females. Lowest effective dose = 50 mg/kg (single)/40 mg/kg/d (repeat)	*Shelby et al (1987). *Adler et al (1994)
Specific locus assays (germ cells)	Mouse (male)	0-125 mg/kg (single – ip) 50 mg/kg (repeat (x5) ip)	Positive	Increased frequency of specific locus mutations in male spermatozoa and spermatid stages	*Ehling & Neuhauser-Klaus (1992) *Russell et al (1991)

Table 10.6 - Summary of in vivo genotoxicity studies (continued)

Type of test	Test system	Dose of acrylamide	Result	Comments	References
ASSAYS FOR DNA EFFECTS					
DNA alkylation	Mouse (BALB/c) Rat (Sprague-Dawley)	53 mg/kg (single - ip) 6 h before sacrifice 46 mg/kg (single - ip) 19 (rat) h before sacrifice	Positive	Formation of N-7- (2-carbamoyl-2-hydroxyethyl) guanine detected in liver, kidney and brain (mice) and liver, kidney, spleen, testes and brain (rats). N-7 adduction of guanine is suggestive of an epoxide (glycidamide).	*Sega et al (1990) *Segerbäck et al (1995).
DNA damage (germ cell) (alkaline elution method)	Mouse	0-100 mg/kg (ip)	Positive	Increase in single strand breaks in spermatids and pachytene spermatocytes. Lowest effective dose = 25 mg/kg.	*Sega & Generoso (1990)
UDS (somatic & germ cell)	Rat (F-344) (male) (Hepatocytes/ spermatocytes)	0-100 mg/kg (single/repeat (x5) - gavage)	Negative (hepatocytes) Positive (spermatocytes)	Results in hepatocytes were negative for both single and repeated exposure. Results in spermatocytes were positive following repeated exposure only (at and above 30 mg/kg).	*Butterworth et al (1992)
UDS (somatic & germ cell)	Mouse (male) (Hepatocytes/ spermatocytes)	0-125 mg/kg (single - ip) 1-24 h before sacrifice	Negative (hepatocytes) Positive (spermatocytes)	Positive results seen 6 h after thymidine injection in spermatocytes. DNA alkylation in liver was 10 fold greater than testes. Lowest effective dose = 8 mg/kg.	*Sega et al (1990)
SCE (somatic & germ cell)	Mouse (male)	50 and 125 mg/kg (single - ip)	Positive	Lowest effective dose in spleen lymphocytes and differentiating spermatocytes = 50 mg/kg.	*Russo et al (1994) *Backer et al (1989)

Table 10.6 - Summary of in vivo genotoxicity studies (continued)

Type of test	Test system	Dose of acrylamide	Result	Comments	References
CYTOGENETICS ASSAYS					
Clastogenicity (bone marrow)	Mouse (male)	0-100 mg/kg (single - ip) 1-12d before sacrifice	Positive	Increase in metaphases with chromatid breaks. Increase in frequency of aneuploidy or polyploidy vs controls.	*Cihak & Vontorkova (1988) *Kligerman et al (1991) *Shiraishi (1978)
Clastogenicity (bone marrow)	Mouse (male/female)	0-150 mg/kg (single - ip) 18-36 h before sacrifice	Positive	Increase in metaphases with chromatid breaks vs controls. Lowest effective dose = 50 mg/kg/d. Mitotic index reduced up to 27% cf controls.	*Adler et al (1988)
Clastogenicity (bone marrow)	Mouse (male)	60 mg/kg/d (repeat - diet for 1,2,3 weeks)	Weakly positive	Slight increase (>1%) in metaphases with chromatid breaks.	*Shiraishi (1978)
Clastogenicity (somatic & germ cell))	Mouse (male)	0-125 mg/kg (single - ip) 24 h before sacrifice	Negative	Slight increase (3%) in chromatid aberrations in spleen lymphocytes. No increase in chromosome/chromatid aberrations or hyperploidy in spermatogonia or primary spermatocytes.	*Backer et al (1989)
Clastogenicity (germ cell)	Mouse (male)	100-125 mg/kg (single - ip) 50 mg/kg (repeat (x5) - ip)	Equivocal	Negative in spermatogonia. Positive in spermatocytes	*Adler (1990)
Clastogenicity (germ cell)	Mouse (male)	150 mg/kg (single - ip) only males exposed	Positive	After mating, first cleavage (after spermiogenic stages) embryos (from unexposed female) assayed for aberrations.	*Valdivia et al (1989)

Table 10.6 - Summary of in vivo genotoxicity studies (continued)

Type of test	Test system	Dose of acrylamide	Result	Comments	References
CYTOGENETICS ASSAYS (Cont.)					
Clastogenicity (germ cell)	Mouse (male)	0-125 mg/kg (single – ip) 50 mg/kg (repeat (x5) - ip) only males exposed	Positive	After mating, first cleavage embryos (from unexposed female) assayed for aberrations. Dose-related increase in chromosome aberrations (fragments, dicentrics & translocations) in one-cell zygotes. Decrease (~74%) in tetraploid cells in testicular cell population.	*Pacchierotti et al (1994)
Clastogenicity (germ cell)	Mouse (male)	60 mg/kg/d (repeat - diet for 1,2,3 weeks). 0-100 mg/kg (single - ip). 1-12 d before sacrifice	Positive	Increased incidence of spermatogonia with aneuploidy, chromosome breaks (& SCE) and increase in sex-chromosome and autosomal univalents, fragments & rearrangements seen in primary spermatocytes in both exposure regimes.	*Shiraishi (1978)
Clastogenicity (germ cell)	Rat (male)	0-6 mg/kg/d (in drinking water) for 80 d.	Negative	No increase in structural aberrations. Slight increase in reciprocal translocations. Pre-implantation loss seen in mated females.	*Smith et al (1986)
Micronucleus (bone marrow, spleen & peripheral erythrocytes)	Mouse (male/female)	Up to 150 mg/kg (single/repeated - ip) 6-72 before sacrifice	Positive	Peak effects generally reported at 24 h post-exposure. Lowest effective dose ~ 50 mg/kg.	Dearfield et al (1995)
Micronucleus (bone marrow)	Mouse (male/female)	75 mg/kg (single/repeated -gavage) 30-72 h before sacrifice	Negative	Toxicity seen at all doses. Validity of results questioned in OECD report.	*Sorg et al (1982)

Table 10.6 - Summary of in vivo genotoxicity studies (continued)

Type of test	Test system	Dose of acrylamide	Result	Comments	Refs
CYTOGENETICS ASSAYS (Cont.)					
Micronucleus (germ cell)	Mouse (male)	10-100 mg/kg (single ip). 50 mg/kg (repeat (x4) - ip)	Positive	Increase in micronuclei in spermatids. Lowest effective concentration = 50 mg/kg (single exposure)	*Russo et al (1994) *Collins et al (1981)
Micronucleus (germ cell)	Rat (male)	0-100 mg/kg (single ip). 50 mg/kg (repeat (x4) - ip) 1-20 d before sacrifice	Positive	Increase in micronuclei in spermatids. Lowest effective concentration = 100 mg/kg (single exposure)	*Xiao & Tates (1994). *Lahdetie et al (1994)
OTHER					
Dominant-lethal test (germ cells)	Rat (male)	0-6 mg/kg/d (in drinking water) for 80 d Up to 100 mg/kg/d (gavage) for 5 d or 30-50 mg/kg (repeat (x5) - ip/gavage) 50-125 mg/kg (single – ip)) 25-125 mg/kg/d (dermal) for 5 d 50 mg/kg/d (repeat (x5) – ip)	Positive	Decrease in number of implantations. Increase in pre- and post-implantation loss seen in mated females. Lowest effective concentration (Rat) = 6 mg/kg/d (repeat exposure)	Dearfield et al (1995) *Smith et al (1986)
	Mice (male)	Up to 9 mg/kg/d (drinking water) for 20 wk)		Lowest effective concentration (Mouse) = 9 mg/kg/d (repeat exposure). Also, positive results seen after 5d x 25 mg/kg/d.	*Fail et al (1992)

Key:

UDS = Unscheduled DNA synthesis

SCE = Sister Chromatid Exchange

Seven days continuous treatment with acrylamide induced morphological transformation in Syrian Hamster Embryo (SHE) cells (Park et al, 2002). Acrylamide itself rather than an oxidative metabolite was thought to be involved in the transformation as inhibition of P450 metabolism by ABT did not modify acrylamide induced cell transformation in the SHE cells. However pretreatment with DL-buthionone-[S,R]-sulfoximine (BSO) to deplete GSH, enhanced acrylamide induced transformation frequency. The authors have proposed that the clastogenic activity of acrylamide and acrylamide reactivity to macromolecules causing structural and cellular functional change via GSH depletion in the cells may be involved in the SHE cell transformation.

Negative results were reported for hepatic cell UDS in rats and mice. Positive results were reported in spermatocytes in both species, following repeated exposure to doses at and above 30 mg/kg/d in rats and a single dose of 8 mg/kg in mice. Acrylamide also induced SCE in spleen lymphocytes and spermatocytes in mice at and above 50 mg/kg.

Acrylamide was clastogenic in mouse bone marrow (somatic) cells with positive results in spermatogonia and zygotes from mated females (germ cells). Increases in metaphases with chromatid breaks together with an increase in the frequency of aneuploidy and/or polyploidy were seen at and above a single dose of 100 mg/kg and repeated doses of 60 mg/kg/d. In rats, no germ cell clastogenicity was seen at doses of up to 6 mg/kg/d for 80 days.

Dose-related increases in micronucleus frequency were reported in bone marrow, spleen and peripheral erythrocytes (somatic cells) and spermatids (germ cells) of mice at and above 50 mg/kg. Positive results were also reported in rat spermatids at 100 mg/kg.

Positive results were also reported in dominant lethal tests on male mice and rats. Dominant lethality, as evidenced by increased pre- and post-implantation losses in mated females, was seen at 6 mg/kg/d (80-day dosing) in rats and 9 mg/kg/d (20 weeks) in mice.

Adler et al (2000) investigated the effects of ip administration of 125 mg/kg acrylamide on dominant lethal mutations in mice following pretreatment with 50 mg/kg ABT to determine if glycidamide was responsible for the acrylamide induced clastogenicity in mice spermatids. Males were mated 6 h after treatment. In the first experiment females were replaced every week for a total of 4 weeks and in the second experiment every 4 d for a total of 4 mating intervals. Positive results were reported in animals treated with acrylamide during the second, third and fourth mating intervals. No dominant lethal effect was seen at the second mating interval after pretreatment with ABT. At the third mating interval dominant lethality was seen in the acrylamide + ABT group that was greater than the control and ABT alone groups but less than the acrylamide alone group. At the fourth mating interval both acryamide and acrylamide +ABT group showed dominant lethality of the same order of magnitude indicating that lethality was induced despite ABT pretreatment. The authors have reported that this may be due to incomplete inhibition of P 450 or an additional mechanism may be involved in acrylamide induced clastogenicity in spermatids. Acrylamide dramatically reduced

the percentage of fast moving sperm. Addition of ABT did not inhibit this effect, in fact ABT alone also significantly reduced the percentage of fast moving sperm.

10.6 Carcinogenicity

No chronic inhalation or dermal studies have been carried out for acrylamide. Two 2-year oral studies have been carried out in F-344 rats in addition to short-term skin initiation/promotion and lung adenoma bioassays in mice.

10.6.1 Two-year studies

Study 1 (Johnson et al 1986)

This study was conducted according to modern protocol standards, with groups of 90 male and 90 female F-344 rats receiving doses of approximately 0, 0.01, 0.1, 0.5 or 2.0 mg/kg/d acrylamide in drinking water. Interim examinations and sacrifice were carried out at 6, 12 and 18 months.

Tumour incidence data at term of study (24 months) is reported in Table 10.7. Time-to-tumour data was not available, although no neoplastic or pre-neoplastic lesions were apparently observed from histopathology carried out at 12 and 18 months.

Histopathology at termination revealed an increased incidence of tumours in mammary and clitoral glands, uterus, testes, brain and spinal cord, adrenal, pituitary, thyroid and oral cavity. Of statistical significance were testicular mesotheliomas, adrenal pheochromocytomas and thyroid adenomas in males at 0.5 or 2 mg/kg/d and oral cavity papillomas and mammary and clitoral gland adenomas in females at 2 mg/kg/d. None of the tumour types showed a clear dose response relationship in this study.

A sialodacryoadenitis virus infection of experimental and control animals in this study may have affected the results.

Study 2 (Friedman et al 1995)

This study was conducted according to modern protocol standards, with groups of 75 to 204 male and 50 to 100 female F-344 rats. Male and female animals received doses of approximately 0, 0.1, 0.5 or 2.0 mg/kg/d and 0, 1.0 and 3.0 mg/kg/d acrylamide in drinking water, respectively. This study used an unbalanced design intended to detect a 5% increase in cancer incidence over a 1.3% estimated background.

No details of interim examinations and time-to-tumour data were provided, except a reported increase in palpable subcutaneous masses in both sexes from 12 months onwards. Tumour incidence data at term of study (24 months) is reported in Table 10.8. Table 10.9 provides data on incidences of tumours reported in study, together with available historical data.

Table 10.7 - Incidence of benign and malignant tumours observed in F-344 rats following exposure to acrylamide in drinking water for 24 months (Johnson et al 1986)

Study 1				Number of animals with tumours / number examined									
	Tumour description					Estimated dose (mg/kg bw/d)							
				Control		0.01		0.1		0.5		2.0	
Organ	*Tumour*	*Type*	*Origin*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*
Adrenal	Pheochromocytoma	B		3/60	-	7/59	-	7/60	-	5/60	-	10/60*	-
Brain	Astrocytoma	M	Astrocyte (glial cell)	0/60	3/60	1/60	0/60	0/60	0/60	0/60	2/60	3/60	2/60
Spinal cord	Astrocytoma	M	Astrocyte (glial cell)	1/60	1/60	0/60	0/59	0/60	0/60	0/60	0/60	3/60	3/61
Clitoral gland	Adenoma	B		-	0/2	-	1/3	-	3/4	-	2/4	-	5/5*
Mammary gland	Adenoma	B		-	10/60	-	11/60	-	9/60	-	19/58	-	23/61*
Mammary gland	Adenocarcinoma	M		-	2/60	-	1/60	-	1/60	-	2/58	-	6/61
Oral cavity	Papilloma	B		4/60	0/60	7/60	3/60	0/60	2/60	5/60	1/60	4/60	7/61*
Oral cavity	Carcinoma	M		2/60	0/60	0/60	0/60	1/60	0/60	0/60	2/60	2/60	1/61
Pituitary	Adenoma	B		-	25/59	-	30/60	-	32/60	-	27/60	-	32/60
Testes (TVM)	Mesothelioma	M		3/60	-	0/60	-	7/60	-	11/60*	-	10/60*	-
Thyroid	Adenoma	B	Follicular cell	1/60	0/58	0/58	0/59	2/59	1/59	1/59	1/58	7/59*	3/60
Thyroid	Adenocarcinoma	M	Follicular cell	-	1/58	-	0/59	-	0/59	-	0/58	-	3/60
Uterus	Adenocarcinoma	M		-	1/60	-	2/60	-	1/60	-	0/59	-	5/60

Histopathology at termination revealed an increased incidence of tumours in brain and spinal cord, mammary gland, scrotum and thyroid. Of statistical significance were scrotal mesotheliomas in males, mammary gland fibroadenomas in females and thyroid adenomas in both sexes (2 mg/kg/d in males and 3 mg/kg/d in females), at the highest doses tested in each sex. Clear dose response relationships were seen with thyroid adenomas (both sexes), thyroid adenocarcinomas, mammary gland fibroadenomas and brain meningiomas/reticulosis in females and scrotal mesotheliomas.

10.6.2 Other carcinogen bioassays

A skin initiation/promotion study was carried out in groups of 40 female Sencar mice. Animals received 6 doses of 12.5, 25 and 50 mg acrylamide per kg body weight, administered by gavage, intraperitoneal injection and dermal application, over a 2-week period. Between 2 and 20 weeks post exposure, 1µg tetradecanoyl-phorbol acetate (TPA) in acetone was applied topically to each animal, three times daily. Control animals received either acrylamide followed by no TPA or water followed by TPA. An acrylamide dose-related increase in skin tumours occurred for each dose route with TPA administration, whereas no increase in tumour incidence was seen in animals administered acrylamide without TPA administration or in those receiving TPA alone. This study indicates that acrylamide has tumour initiator properties *(Bull et al 1984a).

In a Shimkin's lung adenoma study, groups of 16 male and female A/J mice were administered doses of 1, 3, 10 and 30 mg acrylamide per kg body weight by intraperitoneal injection 3 times per week, over an 8-week period. Dose-related increases in the incidence of lung tumours were seen in both sexes. Similar results were obtained in 80 ICR mice administered oral doses of 6.25, 12.5 and 25 mg acrylamide per kg body weight 3 times per week, over an 8-week period *(Bull et al 1984b). Lung tumours are common in these species of mice and the mechanism of accelerated development of spontaneous tumours is unknown. Furthermore, it is not known whether this test has been validated with respect to carcinogen prediction (Friedman et al 1995).

10.6.3 Mode of action/mechanistic data

Notwithstanding the genotoxic potential for acrylamide (see Section 10.5), a number of epigenetic modes of action (MOA) have been proposed for acrylamide tumourigenesis (TERA 1998; Crump 1999a,b, 2000a,b).

The following is a summary of the available experimental data for acrylamide of relevance to epigenetic MOA for tumour types seen in chronic bioassays in F-344 rats.

Mammary gland tumours (females)

Mammary gland fibroadenomas were significantly increased in both Friedman et al (1995) and Johnson et al (1986) studies at an incidence ranging from 28 to 38% in high dose females. This tumour type exhibits a high spontaneous incidence in aging F-344 rats, with an incidence of up to 44% in NTP historical control rats (Table 10.9).

Table 10.8 - Incidence of benign and malignant tumours observed in F344 rats following exposure to acrylamide in drinking water for 24 months (Friedman et al 1995)

Study 2				Number of animals with tumours / number examined						
	Tumour description					Estimated dose (mg/kg bw/d)				
				Control		0.1	0.5	2.0	1.0	3.0
Organ	*Tumour*	*Type*	*Origin*	*M*	*F*	*M*	*M*	*M*	*F*	*F*
Brain	Astrocytoma	M	Astrocyte (glial)	1/204	0/100	0/98	0/50	2/75	2/100	2/100
Brain	Meningioma	B		ND	0/100	ND	ND	ND	2/100	3/100
Brain	Reticulosis	M		1/204	0/100	1/98	0/50	0/75	2/100	3/100
Spinal cord	Astrocytoma	M	Astrocyte (glial)	0/172	0/100	1/68	0/102	1/51	0/100	1/100
Clitoral gland	Adenoma	B		-	ND	-	-	-	ND	ND
Mammary gland	Fibroadenoma	B		-	9/96	-	-	-	20/94	26/95*
Mammary gland	Adenocarcinoma	M		-	2/96	-	-	-	2/94	4/95
Oral cavity	Papilloma	B		ND	ND	ND	ND	ND	ND	ND
Oral cavity	Carcinoma	M		ND	ND	ND	ND	ND	ND	ND
Pituitary	Adenoma	B		ND	ND	ND	ND	ND	ND	ND
Scrotum	Mesothelioma	M		8/204	-	9/204	8/102	13/75*	-	-
Thyroid	Adenoma	B	Follicular cell	3/204	0/100	9/203	5/101	12/75*	7/100	16/100*
Thyroid	Adenocarcinoma	M	Follicular cell	3/204	2/100	3/203	0/102	3/75	3/100	7/100
Uterus	Adenocarcinoma	M		-	ND	-	-	-	ND	ND

Table 10.9 - Percentage incidence of tumours observed in rats in studies by Friedman et al (1995) and Johnson et al (1986) together with historical control data

Organ	Tumour type	Tumour incidence (%)														
		Friedman et al study						Johnson et al study						Historical control data		
		Male		*Female*		*M + F*		*Male*		*Female*		*M + F*		*Male*	*Female*	*M + F*
		C	HD	C	HD	C	HD	C	HD	C	HD	C	HD			
Thyroid	FC ademomas	1.5	16*	0	16*	1.0	16	1.5	12*	0	5	0.7	8.5	0 – 5 (1.0) [1]	0 – 4 (0.4) [1]	
	FC adenocarc	1.5	4	2	7	1.5	5.5	NR	NR	1.5	5	-	-	0 – 7 (0.8) [1]	0 - 2 (0.4) [1]	
Brain/ Spinal cord	Malignant reticulosis	0.5	0	0	3	0.3	1.5	NR	NR	NR	NR	-	-			0.1[2]-0.2[3]
	Glial cell	0.5	2.5	0	4	0.3	3.5	1.5	10	6.5	8	4	9	0 - 7[4] 0 – 11[7]	-	
Testes/ scrotum	Mesothelioma	4	17.5*	N	N	-	-	5	16.5*	N	N	-	-	2 – 6 (3.1)[5]	N	
Mammary gland	Fibroadenoma	N	N	9.5	27.5*	-	-	N	N	16.5	37.5*	-	-	N	2 – 44 (24)[1]	
	Adenocarc	N	N	2	4	-	-	N	N	3.5	9	-	-	N	0 – 8 (2)[1] 2 – 11[8]	
Adrenal	Pheochrom	NR	NR	NR	NR	-	-	5	16.5*	NR	NR	-	-			1 – 14 (8.7)[5] 8 – 14 (11)[6]
Pituitary	Adenoma	NR	NR	NR	NR										28 – 47 (38)[5]	
Oral cavity	Papilloma	NR	NR	NR	NR	-	-	6.5	6.5	0	11.5	3.5	9			0 – 4 (1.7)[5]
Uterus	Adenocarc	NR	NR	NR	NR	-	-	N	N	1.5	8.5	-	-	0 – 2.5 (1.8)[5]		

Key to Table 10.9

* incidence reported as statistically significant in study
Unless stated the data is for F-344 rats
C = control animals
M = male
F = female
HD = high dose animals
ND = not reported
N = not relevant
FC = Follicular cell
TVM = Tunica vaginalis mesothelioma
Adenocarc = adenocarcinoma
Pheochrom = pheochromocytoma

1 NTP
2 NCI
3 *Krinke et al 1985 (SD rats)
4 NTP (CR CD rats)
5 *Dow Chemical Lab data (lab for Johnson et al 1986 study*)
6 Tarone et al (1981)
7 Zwieten et al (1988) (SD rats)
8 *Solleveld et al (1984)

The MOA for spontaneous fibroadenoma formation in ageing female F-344 rats is believed to be a consequence of failure of hormone control mechanisms of the hypothalamic-pituitary-ovary axis in maintaining blood prolactin levels resulting in hyperprolactinaemia. Hyperplolactinaemia stimulates progesterone synthesis in rats via luteotrophic effects on the ovary. Sustained elevation of prolactin/progesterone levels is associated with chronic stimulation and proliferation of tubulo-epithelial growth in stromal (fibroblast) cells of the rat mammary gland with induction of fibroadenomas (Crump 1999a).

Significant alterations in serum prolactin levels were not found in female F-344 rats administered acrylamide *(Friedman et al 1999). Acrylamide has however been shown to be a weak dopamine agonist *(Yamada et al 1995). It has been proposed that acrylamide may act on D1 receptors in rat ovary directly stimulating synthesis of progesterone, with the net effect of increasing the incidence of mammary tumours (Crump 1999a). Indirect evidence exists to support this action by acrylamide on rat ovary *(Zenick et al 1986).

Further support for a possible non-genotoxic MOA for fibroadenomas is that acrylamide does not have a significant effect on the latency of these tumours or on cell hyperplasia (reported in Friedman et al 1995), i.e. acrylamide is enhancing age-related changes in mammary gland differentiation. In addition, acrylamide did not result in significant changes in the incidence of mammary tumours in male rats.

The significance of the acrylamide-induced mammary fibroadenocarcinomas in the context of the hypothesised MOA is unclear. It is known however that increases in oestrogen levels favour formation of this tumour type in rats. It is known that ageing female rats may enter a state of constant oestrus, characterised by high levels of oestrogen secretion. This is in contrast to episodes of pseudopregnancy, which is associated with high levels of prolactin and progesterone and low levels of oestrogen.

Testicular/scrotal mesotheliomas

Tunica vaginalis mesotheliomas (TVM) were reported in both Friedman et al (1995) and Johnson et al (1986) studies at an incidence of between 16 and 18% in high dose males. Although having a relatively low spontaneous incidence in historical controls (see Table 10.9), TVMs are reported as being specific to F-344 rats in bioassay studies (Crump 1999b). No significant increases above background were found in male Sprague-Dawley, Osborne-Mendel or Wistar strains when administered a number of genotoxic carcinogens, including chemicals which produced TVMs in F-344 rats (Friedman et al 1995; Crump 1999b).

No information relating specifically to MOA with regard to acrylamide-elicited TVM was available for assessment. Available data indicate the formation of TVMs may be associated with Leydig cell tumours (LCT), which have a high spontaneous incidence in ageing F-344 rats compared to other rat strains (Friedman et al 1995; Crump 1999b). The MOA for spontaneous LCT formation in ageing F-344 rats is believed to be a consequence of failure of hormone control mechanisms of the hypothalamic-pituitary-testicular axis in maintaining blood testosterone levels. In an attempt to maintain testosterone levels, continued stimulation of Leydig cells by luteinizing hormone (LH) results in Leydig cell hyperplasia and

eventually LCT. It has been proposed that TVMs may result from a growth factor mediated autocrine response in mesothelial cells caused by changes in local androgen hormone levels in the peritesticular environment, although tumours were not increased in testes.

It has been postulated that acrylamide may act either via interference with the hypothalamic-pituitary-testicular axis or via clastogenic (genotoxic) interaction to 'initiate' transformed mesothelial cells, which through autocrine (hormone/growth factor) stimulation may progress to TVM formation *(Friedman et al 1999). Evidence in support of this hypothesis is the fact that acrylamide has been shown in a number of studies to decrease serum prolactin (inhibiting LH secretion) and testosterone levels in male F-344 rats *(Friedman et al 1999). Reduction in serum prolactin is consistent with effects of acrylamide on the dopaminergic system. In addition, indirect evidence exists to suggest that acrylamide may act on D2 receptors in rat pituitary *(Uphouse and Russell, 1981). In addition it has been shown that mesothelioma cell DNA synthesis is stimulated by transforming growth factor (TGF-β), platelet derived growth factor (PDGF) and epithelial growth factor. Mesothelioma cell lines have been shown to have increased mRNA levels of PDGF genes compared to normal mesothelial cells, suggesting these may be autocrine growth factors for mesotheliomas (Crump 1999b). Induction of transcription factors by acrylamide has not been studied in Tunica Vaginalis Mesothelium.

Further support for a non-genotoxic MOA for TVM tumours is the lack of a significant effect on the latency of these tumours (reported in Friedman et al 1995), i.e. acrylamide enhances age-related changes in the testicular environment.

Thyroid tumours

A statistically significant incidence of thyroid follicular cell adenomas occurred in high dose groups in both Friedman et al (1995) and Johnson et al (1986) studies, with a flat dose response relationship at lower doses in both sexes in the Friedman et al study. In addition, a non-statistical increase in follicular cell adenocarcinomas was seen in both sexes in the Friedman et al study and in females in the Johnson et al study at the highest dose levels. Follicular tumours are relatively uncommon in rats, with spontaneous incidence rates ranging from 0 to 5% (see Table 10.9). Although the incidence of spontaneous follicular cell tumours is low, it is a common finding in many toxicological studies from a variety of chemical agents.

Virtually all non-genotoxic thyroid carcinogens are goitrogens causing elevation of TSH via effects on either thyroid hormone production or catabolism. In addition, very few chemical mutagens have been shown to elicit thyroid tumours unless they also exert an effect on thyroid homeostasis. Modes of action identified in thyroid tumour induction include inhibition of iodine uptake, inhibition of thyroid peroxidase, direct follicular cell damage, inhibition of thyroid hormone release and enhancement of metabolism in liver by induced microsomal MFO system. There is however some evidence that increased thyroid cell proliferation can occur by MOA that do not result in TSH elevation. One such mechanism is by enhanced cAMP signal transduction.

Potential effects of acrylamide on thyroid hormone levels have been evaluated (Crump 2000a). In a 28-day study in F-344 rats and male Sprague-Dawley rats, there were no significant effects on TSH, but statistically significant non-dose related decreases in thyroid hormones T_3 and T_4 in F-344 rats were reported,

although no affects were seen after repeated exposures (*Friedman 1999; *Klaunig 2000). However, non-dose related increases were seen in BrdU incorporation and proliferating cell nuclear antigen (PCNA) expression in thyroid cells, considered to be indicative of increased DNA synthesis (i.e., proliferative response). Such findings were considered to be consistent with an effect via TSH-cAMP pathway (Crump 2000a). Induction of transcription factors by acrylamide in the thyroid is currently being studied (Friedman 2001, Personal Communication).

Brain/spinal cord tumours

Non-statistically significant increases in malignant astrocytomas (glial cell) of brain and/or spinal cord, were seen in high dose groups in both sexes in both Friedman et al (1995) and Johnson et al (1986) studies, with no clear dose response relationships. In addition, a dose-related increase (non-statistically significant) in brain meningioma and reticulosis was seen in females in the Friedman et al study. Historical data indicate a background incidence for a range of CNS glial cell tumours of up to 11%, with an incidence of up to 0.2% for malignant reticulosis in F-344 rats (Table 10.9).

Although the biochemical control of astrocyte proliferation and spontaneous tumour formation has not been fully elucidated, there is evidence in rats of contributions due to neuronal injury, hormonal perturbation and activation of transcription factors.

Both CNS and PNS are target tissues for acrylamide neurotoxic effects. Studies in mice indicated that acrylamide has a particular affinity for microtubule-associated proteins in the cerebral cortex *(Carrington et al 1991).

Crump (2000b) concluded that available evidence indicates that astrocytoma induction in the Friedman et al (1995) and Johnson et al (1986) rat bioassays is unlikely to be the result of neurotoxicity or a hormonally-mediated (hormone/growth factor) mode of action, but may be related to acrylamide activation of D2 dopamine receptors, resulting in mitogen activated protein kinase (MAPK) and tyrosine kinase cascade leading to cell proliferation and tumour formation. Experimental evidence in support of this proposed MOA is the demonstration (by measures on proliferating cell nuclear antigen (PCNA and BrdU) of acrylamide induced astrocyte proliferation in vitro and in vivo (Friedman 2001, Personal Communication; *Klaunig 2000) and the association of increased dopamine levels in brain areas where glial cell tumours were evident. In this regard, significantly increased dopamine levels were reported in caudate nucleus following acrylamide exposure, an area high in D2 receptors (*Ali 1983; *Uphouse and Russell, 1981) whereas no glial cell proliferation or astrocytomas were observed (from histopathological examination) in brain areas low in D2 receptors *(Johnson 2000). Endo et al *(1993) and Torigoe *(1997) found a significant increase in mRNA levels in the cerebral cortex of acrylamide treated rats. Studies on the effects of acrylamide on gene transcription-activating proteins, such as NFkB and AP-1 have provided conflicting results (*Endo et al 1993; *Bunting et al 1997). Further studies are currently being undertaken on acrylamide-induced RNA and protein synthesis in relation to astrocyte proliferation (Friedman 2001, Personal Communication).

An epigentic MOA, namely oxidative damage to DNA, for astrocytomas of CNS was hypothesised in some recent work on the acrylamide analogue acrylonitrile

(Whysner et al 1998a,b). However, conclusions were drawn in part due to absence of DNA adducts in rat brain. DNA adducts formed from glycidamide have been detected for acrylamide in brain tissue of rats and mice (Segerback et al 1995). Also, although DNA oxidative stress (evidenced by increased tissue levels of 8-oxodeoxyguanosine) has been reported in vitro *(Benn and Thomas, in press), it was not evident in brain tissue from acrylamide treated rats (TERA, 1998).

Other tumour types

Little MOA data were available for other tumour types seen only in the Johnson et al *(1986) study, the biological significance of which is confounded due to the viral infection reported in this study.

With regard to the increased incidence of adrenal pheochromocytomas in male rats seen in the Johnson et al (1986) chronic carcinogenicity bioassay, Lin et al (*1996; *2000) provided evidence that acrylamide directly induces neurofilament mRNA and protein synthesis in adrenal pheochromocytoma cells through involvement of gene transcription-activating proteins like AP-1. AP-1 induced gene transcription is associated with cell proliferation *(Chiu et al 1988). Although the incidence of adrenal pheochromocytomas was significantly increased in high-dose males, the fact that this tumour type was not increased in females confounds conclusions as to biological significance.

Oral cavity papillomas and pituitary adenomas were increased in females only and therefore may be epigenetic in origin. However, the incidence in high dose animals was only marginally above historical controls from the study laboratory (Table 10.9).

Uterine adenocarcinomas and adenomas of the clitoral gland are uncommon lesions in F-344 rats, with similar historical control incidences of up to 2.5% in the study laboratory. The incidence of both these tumours in the high dose groups in the Johnson et al (1986) study was 8.5%. The biological basis for these tumour types is unknown.

10.7 Reproductive toxicity

Some of the studies reported in this section contain information relevant to endpoints for fertility and developmental effects. No attempt was made to disaggregate data from different studies according to the classification endpoints i.e. studies are generally presented according to the reporting format in the EU and OECD reports.

Acrylamide is known to be distributed to male reproductive organs and to form adducts with sperm DNA and proteins. In addition, acrylamide has been shown to be a germ cell mutagen and clastogen with a propensity to cause dominant lethal mutations in mice and rats (see Section 10.6).

10.7.1 Effects on fertility

In a one-generation reproduction study *(Zenick et al 1986), groups of male and female Long-Evans rats were dosed for 10 weeks (after mating) with an estimated 0, 4, 8 and 10 mg/kg/d and 0, 5, 10 and 15 mg/kg/d acrylamide in drinking water, respectively. Females were also dosed for 2 weeks prior to mating. A crossover-

mating study was also carried out. Male rats in the high dose group (10 mg/kg/d) were sacrificed at between 5 and 6 weeks due to severe neurotoxicty. Reduction (>40%) in impregnation outcome and a statistically significant increase (24%) in post-implantation loss was seen in untreated females mated with males in the 8 mg/kg/d group, compared to those mated with control males. These parameters were not measured in other dose groups. Significant reduction in sperm count and mating ability was seen in males at 8 mg/kg/d. Sperm motility and morphology were not adequately assessed. Overt neurotoxicity (impaired hind limb function) was seen in males at 8 mg/kg/d after 8 weeks exposure, although no effects were reported on organ or body weights at terminal necropsy at 11 weeks. Maternal toxicity, as evidenced by reduced body weight gain seen at and above 10 mg/kg/d, was evident during pregnancy and throughout lactation, with overt neurotoxicity (loss of use of hind limbs) at 15 mg/kg/d. Reduced birth weights and subsequent body weight gain was seen in pups of both sexes, born to females in 10 and 15 mg/kg/d dose groups. Retardation in pup development in these dose groups was evidenced by delayed vaginal opening. The lack of a cross-fostering study prevented an assessment of whether subsequent effects on bodyweight gain in offspring were due to exposure to acrylamide in utero or due to potential lactational effects.

In a two-generation reproduction study *(Tyl 1987), groups (F0) of male and female F-344 rats were dosed with an estimated 0, 0.5, 2 and 5 mg/kg/d arylamide in drinking water for 10 weeks prior to mating. Acrylamide exposure was continued through gestation, parturition and lactation. No treatment related mortalities were seen in any dose group in F0 animals. Reduced body weight gain was seen in all exposed groups with signs of neurotoxicity seen in both sexes at 5 mg/kg/d. Reduced maternal body weight gain of up to 29% was evident during gestation and lactation at 5 mg/kg/d. Fertility index and gestation length were unaffected, although the number of implants per dam and live pups per litter were reduced by 30% and 50% respectively at 5 mg/kg/d. Reduced body weight gain (up to 9%) was seen in male (F1) pups in the 5 mg/kg/d dose group during the latter stages of lactation. No histopathological abnormalities were seen in F0 and F1 animals. F1 animals selected for the second (F2) generation study were subject to a similar dosing regime to F0 animals. Body weight gain of F1 animals was slightly reduced at and above 2 mg/kg/d acrylamide during 11 week pre-breeding period, with maternal weight gain still reduced during gestation. Signs of neurotoxicity were seen in males at 5 mg/kg/d. Fertility index and gestation length were unaffected by acrylamide exposure, although the number of implants per dam and live pups per litter was reduced at 5 mg/kg/d. Reduced body weight gain (up to 7%) was seen in F2 pups, born to females in 5 mg/kg/d dose group. Slight axonal fragmentation and swelling of sciatic and tibial nerves in F1 males, seen at 5 mg/kg/d, were the only histopathological abnormalities seen in F1 and F2 animals.

In a continuous breeding study *(NTP 1993), groups of male and female Swiss mice were dosed with an estimated 0, 0.7, 3 and 9 mg/kg/d acrylamide in drinking water for 28 weeks. A crossover-mating study was also performed after 27 weeks in F0 animals. No treatment related mortalities or effects on bodyweight or neurotoxicity were reported in any dose group in F0 animals. In addition, no effects were reported on numbers of litters, gestational length, F1 pup weight or sex-ratio. The numbers of live pups per litter was significantly reduced (15% reduction) at 9 mg/kg/d. In the crossover-mating study, there were no effects on

fertility index, although fewer pups per litter were born from unexposed females mated with males dosed with 9 mg/kg/d. However, no differences were seen in the number of live births when treated (all doses) females were mated with untreated males. No treatment-related effects were reported in sperm and spermatid counts or other measures of sperm function or oestrus cycle parameters in F0 animals. No histopathological abnormalities were seen in F0 and F1 animals. No exposure-related effects were seen in F1 post-natal survival or bodyweight gain, except after weaning (when pups were directly exposed to acrylamide in drinking water), when a slight but statistically significant reduction (8%) in bodyweight gain was seen in F1 females at 9 mg/kg/d. At terminal necropsy (about 10 weeks post-partum), no significant effects were seen in epididymal sperm or spermatid counts and sperm motility or oestrus cycle parameters in F1 animals. A significant reduction in the number of live pups per litter (45%) was also seen in F1 breeding pairs at 9 mg/kg/d, however pup weight and sex ratio of F2 offspring were unaffected.

In a continuous breeding study *(Sakamoto and Hashimoto 1986), groups of male and female ddY mice were dosed with an estimated 0, 3, 6, 9 and 12 mg/kg/d acrylamide in drinking water for 4 weeks. A crossover-mating study was also performed in F0 animals. Neurotoxicity was evident in treated males and females in high dose groups. The fertility rate, assessed on day 13 (day of delivery), was clearly affected only at 12 mg/kg/d, for males mated with untreated females. Reduced epididymal sperm count and sperm abnormalities were significant in males at 12 mg/kg/d. In addition, significant reductions in number of foetuses per dam were seen at and above 9 mg/kg/d.

Similar findings were reported in a repeat of this study (Tyl et al, 2000, where reproductive effects were also seen at 5 mg/kg/d (highest dose). However in the follow up study neurotoxicity and increased incidence of head tilt/foot splay, was reported at 0.5 and 2 mg/kg/d. Histopathology of the nerve was only carried out at the highest dose of 5 mg/kg/d. Reproductive indices relating to mating and pregnancy were unaffected at all treatment levels in F0 and F1 animals.

10.7.2 Developmental effects

Developmental (gavage) studies carried out in pregnant Sprague-Dawley rats dosed 0, 2.5, 7.5 and 15 mg/kg/d acrylamide on days 6 to 20 gestation and in pregnant Swiss mice dosed 0, 3, 15 and 45 mg/kg/d acrylamide on days 6 to 17 gestation was reported by Field et al. *(1990). In rats, apart from a slight (not statistically significant) increase in skeletal variations (extra lumbar rib) at 15 mg/kg/d, considered by author to be of limited toxicological significance, there were no apparent effects on embryo/foetal viability, growth or malformations. Apart from a reduced (12%) bodyweight gain at and above 7.5 mg/kg/d, no clear clinical signs of maternal toxicity were seen in this study. In mice, a slight but not statistically significant increase in skeletal variations (extra lumbar rib), considered by author to be of limited toxicological significance, was seen at 15 mg/kg/d. Mean foetal bodyweight was reduced amongst both sexes of offspring at 45 mg/kg/d, at which dose clinical signs of maternal neurotoxicity were evident.

A neonatal development gavage study in pregnant Sprague-Dawley rats dosed 0, 5, 10 and 20 mg/kg/d acrylamide by gavage on days 6 gestation to day 10 lactation was reported by Wise et al *(1995). Significant postnatal pup mortality was seen at 20 mg/kg/d (33% deaths in 3 days). Maternal toxicity was observed at and above 15 mg/kg/d, as evidenced by neurotoxicity and reduced bodyweight gain. Reduced

maternal bodyweight gain was also statistically significant during lactation at 10 mg/kg/d. Clear effects on numbers of live births and neonate body weight with some evidence of neurotoxicity were seen at maternally toxic doses. There were no histopathological changes in central or peripheral nervous system tissues in pups at 15 mg/kg/d.

In a study carried out by Edwards *(1976) in pregnant Porton rats administered acrylamide in diet at approximately 0, 15 and 30 mg/kg/d on 1 to 20 days gestation, there were no signs of developmental effects, apart from slight bodyweight reduction at 15 mg/kg/d and above, a dose level at which clinical signs of maternal neurotoxicity (hind limb splay) was evident.

A teratogenicity study was carried out in mice (strain not reported) by Neuhauser-Klaus and Schmahl (1989). Exposure of pregnant animals to 75 mg/kg ip acrylamide (on days 10, 11 & 12 of gestation) resulted in increased embryotoxicity and cytotoxicity. Cytotoxicity was evidenced by a high frequency of white midventral spots (WMVS). Embryotoxicity was demonstrated by a 25% decrease in litter size and high incidence (4.2% vs 1.3% in controls) of malformations (tail kink) in weaned offspring. Maternal toxicity was not mentioned in this study, despite a detailed histological examination of offspring.

10.7.3 Evidence for reproductive effects from other studies

A number of other studies of relevance to assessing reproductive effects from acrylamide have been reported. Some of these studies were carried out as part of investigations into reproductive studies reported above.

Dominant lethal studies

A statistically significant reduction in fertility index was reported in female Long-Evans rats mated with males exposed to doses at and above 15 mg/kg/d acrylamide (gavage doses of 0, 5, 15, 30, 45 and 60 mg/kg/d acrylamide for 5 days) between 1 and 4 weeks post-exposure *(Sublet et al 1989). Although systemic toxicity (neurotoxicity) was also seen at this dose level, mating behaviour of males was unaffected. In addition, there was a statistically significant reduction in the percentage of fertilised ova and increases in resorptions and post-implantation loss at and above 15 mg/kg/d. Pre-implantation loss was not affected by acrylamide. Corpora lutea counts indicated similar fertility status amongst females between groups.

Using the same species and dosing regime as Sublet et al *(1989), Tyl *(1998a) reported that mating, fertility and pregnancy indices (increased resorptions and post-implantation loss) were all affected following exposure of males at and above 15 mg/kg/d. Post-implantation loss was reported at 45 mg/kg/d. Signs of male toxicity were reported at 15 mg/kg/d, although overt neurotoxicity (impaired hind limb function) was only seen at 60 mg/kg/d. No microscopic changes were seen in the sciatic nerve from animals in any dose groups. Slightly reduced sperm count was also seen at this dose level. Tyl et al (2000) reported similar reductions in total and viable implants together with an increase in pre- and post implantation loss at 5 mg/kg/d. Neurotoxicity was seen at 5 mg/kg/d in both studies. In this study 2mg/kg/d was reported as NOAEL for prenatal (dominant) lethality.

A positive result was obtained in a dominant lethal assay from male Swiss mice exposed to 9 mg/kg/d acrylamide for 20 weeks. No treatment related effects on bodyweight or neurotoxicity were reported in any dose group in F0 animals *(NTP 1993).

In a dominant lethal assay in rats (males only dosed), there were no effects on mating index, but a reduction of viable implants and increases in resorptions was seen at 5 mg/kg/d acrylamide *(Tyl 1987).

In a dominant lethal assay in male mice exposed to between 25 and 125 mg/kg/d acrylamide, dose related increases in dead implants were seen in addition to decreases in mean number of implantations and live embryos *(Gutierrez-Espeleta et al 1992).

A significantly higher percentage of resorptions was seen in female mice following dosing of males with 0.7, 2.1 and 6 mg/kg/d acrylamide in drinking water for 20 weeks, at the high dose level *(Bishop et al, 1991).

Significantly increased pre- and post- implantation loss was seen in female rats following dosing of males with 0, 1.5, 3 and 6 mg/kg/d acrylamide in drinking water for 80 days, in high dose group *(Smith et al, 1986). An absence of overt signs of neurotoxicity (i.e. absence of hind limb slay) or pathological lesions of sciatic nerve was reported in this study.

In the above studies, dominant lethality was generally seen between days 4-12 post-administration of acrylamide. EU concluded that this was suggestive of an effect on late spermatid/early spermatozoa development, a result consistent with effects noted in testicular cell populations *(Sakamoto et al., 1988).

Translocation studies

In heritable germ cell translocation tests, Shelby et al *(1987) and Adler et al *(1994) reported increased incidence of dead implants and increases in heritable translocation carriers in mice at 40 mg/kg/d acrylamide. Between 24 and 39% male mice were either sterile or semi-sterile as a result of dosing at 40 mg/kg/d and 50 mg/kg/d respectively.

DNA effects

Sega and Generoso, *(1990) reported increased single-strand DNA breaks in male mouse spermatozoa following ip injection of 100 mg/kg acrylamide. Analysis showed that DNA breakage was occurring mainly in early and late spermatids as well as pachytene spermatocytes.

Effects on lactation

Biogenic amine levels were measured in weanlings of Wistar dams dosed 25 mg/kg/d acrylamide by gavage during days 1 to 21 lactation *(Husain et al 1987). During days 15 to 30 post-partum, statistically significant reductions were reported in noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) in whole brain samples of pups from treated dams. Limited details on pup toxicity made detailed association of alterations in brain amine levels with acrylamide exposure difficult and may be a secondary consequence of systemic toxicity in dams.

In a study repeating the conditions of the study by Husain et al *(1987), significant reduction (8%) in bodyweight gain was seen in dams at the end of lactation. In addition, overt signs of neurotoxicity were evident in dams, although histopathology revealed no lesions in sciatic nerve. On day 7 post-lactation, pup bodyweights (40% reduction) and hind limb grip strength were significantly lower in animals from treated dams compared to controls. Condition of pups in this study indicated that milk production was severely compromised or even ceased in dams. Partial recovery of pups was evident at day 70 post-lactation *(Tyl et al 1998b).

There were no studies available on acrylamide levels in animal breast milk.

11. Human Health Effects

11.1 Acute poisoning

Oral

A single abstract was available on the deliberate ingestion by a woman of approximately 18 gm acrylamide crystals (equivalent to about 375 mg/kg). No clinical signs of toxicity were seen until 5 hours post ingestion, when hallucinations and hypotension were observed. At 9 hours, seizures occurred followed after 3 days by GI bleeding, respiratory distress, hepatic effects and peripheral neuropathy, the latter still present after 2 months *(Donovan and Pearson, 1987).

No reports of acute effects were available for inhalation or dermal routes of exposure.

11.2 Irritation and corrosivity

Skin effects reported in workplace surveys and case reports were usually attributable to exposure to aqueous solutions of acrylamide. Whether these effects are due to a primary irritant effect of acrylamide is unclear.

He et al (1989) studied a group of workers exposed to aqueous solutions containing approximately 30% acrylamide. Skin effects and possible neurotoxicity were reported. Skin peeling from the hands was reported by 38/71 workers and found in 16/71 workers on clinical examination. In a control group 2/51 reported skin peeling. Erythema was seen in 16/71 workers with no reports in controls.

Exposure to acrylamide during sewer line grouting caused 3/5 workers to report skin irritation. Two reported peeling skin on the palms and the other noted an acne-like dermatitis on the face and hands *(M^{c}Hugh, 1987). A case report by Auld and Bedwell *(1967) also describes skin rash on the forearms of a worker using acrylamide in grouting operations.

Moist red raised ulcerations on the palms and soles were reported in a worker exposed to acrylamide powder. It is unclear whether the effect on the feet was due to direct contact with the chemical *(Davenport et al, 1976).

Garland and Patterson *(1967) reported six workers with neurological findings, three of whom also exhibited skin effects. One person had excessively moist hands with peeling skin within one month of starting work with acrylamide. The second person suffered peeling skin after three months exposure, but recovered completely. The third person also reported peeling skin and moist hands.

Kesson et al *(1977) described six workers with neurological findings attributed to acrylamide exposure. All six were observed to have peeling skin.

No useful data are available on the eye irritation potential of acrylamide in humans. There are no data to suggest that acrylamide is corrosive in humans.

11.3 Sensitisation

A case report describes an individual who developed itchy exudative lesions on the hands and wrists when working with acrylamide. Ten years later following a six month exposure to the chemical, the person again developed a similar reaction, despite wearing gloves *(Lambert et al, 1988).

A worker, again using latex gloves, developed eczema four months after commencing work with acrylamide. There was an improvement following a change of work. Patch testing produced equivocal results *(Dooms-Goossens et al, 1991).

There are no data available on possible respiratory sensitisation in humans.

11.4 Reproductive toxicity

There are no human studies describing effects on fertility, development or lactation.

11.5 Other studies

11.5.1 Workplace surveys

A survey was conducted of 223/242 workers thought to have been exposed to acrylamide/NMA grouts in the tunnel construction at Hallandsas in Sweden for approximately six months during 1997 *(Nordander et al, 1998). Prickling or numbness in the feet or lower legs was reported by 13% of workers and 15% reported prickling or numbness in the hands after starting work with the grout. Flaking of skin on the hands was reported by 6%, increased sweating of the hands and/or feet by 4%, and skin irritation by 21%. Irritation of the eyes occurred in 34%, the nose 25%, and the throat in 31% of workers. Other symptoms reported included cough, breathlessness, headache, nausea and dizziness. Of the 223 workers participating in the study, 50 were chosen for vibration threshold studies. No difference was found between those apparently exposed and workers apparently not exposed. In 77 workers examined for haemoglobin adducts to acrylamide the highest levels of adducts were found in those workers who apparently suffered the greatest exposure. No correlation was found between symptoms of ill health and adduct levels.

The 50 workers in the vibration threshold studies were followed up six months later. Prickling, pain and numbness in the hands feet and lower legs were still evident. Again vibration sensitivity showed nothing conclusive and adducts analysis showed an apparent increase which could not be explained *(Hagmar et al, 1998). In further follow-up examinations of 210 workers 12 and 18 months post-exposure, haemoglobin valine adduct levels were found to be directly proportional to peripheral nervous system symptoms (PNS). A NOAEL of 0.51 nmol/g globulin was estimated. Of the 210 workers, 50 reported recently developed or deteriorating PNS symptoms. Of the workers with PNS symptoms, all but two had recovered 18 months after cessation of exposure (Hagmar et al, 2001).

In the study by He et al, 1989 reported above, 71 workers producing acrylamide monomer and polymers were administered a questionnaire and medically

examined. Exposure ranged from between one and 18 months with atmospheric concentrations between 0.03 and 9 mg/m^3. It is unclear whether these levels represent 8-hour time-weighted averages. Workers also washed their hands in water contaminated with up to 410 mg/l acrylamide. A group of 51 age-matched control subjects was selected from the local area. Statistically significant increases compared with controls were seen in skin peeling from hands (54%), numbness of hands and feet (21%), lassitude (20%), sleepiness (17%), muscle weakness (15%), incoordination of hands (11%), anorexia (11%), unsteady gait (8%), coldness of hands and feet (8%), difficulty in grasping (7%), stumbling and falling (7%).

Initial effects were peeling of the skin and excessive sweating of the hands. Muscle weakness in the legs and tingling of the hands and feet appeared after 3 to 10 months of exposure. After about 12 months exposure nine workers developed lassitude, sleepiness, anorexia, loss of bodyweight, inability to hold objects, unsteady gait and loss of balance. Considerable though not complete recovery occurred after three to five months following removal from exposure to acrylamide. Sensory impairment was recorded in about 17% of workers (nil in controls) and distal skin temperature was apparently lower. Muscular atrophy in the hands occurred in 6% of workers (nil in controls) and diminished reflexes occurred in the biceps, triceps, knee and ankle. A positive Romberg test was reported in 21% of exposed workers compared with 6% in the control group. Electroneuromyographic changes were suggestive of partial denervation and axonal degeneration, but these changes were also seen in workers who had no clinical signs of neurotoxicity.

Amongst exposed workers, the H-reflex (recorded in the soleus muscle of the lower leg) and ankle tendon reflex were non-responsive in 33% and 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.

The mean nerve action potential amplitude from the sural, median and ulnar nerves was significantly decreased compared to the control group. Acrylamide exposure did not seem to affect serum β-glucuronidase, serum IgA or IgM, urinalysis or serum enzyme levels.

Another workplace survey at a polyacrylamide manufacturing plant identified 5/71 workers with clinical signs of peripheral neuropathy, with one worker also suffering cerebellar and ocular impairment. Recovery was reported to be incomplete even after five years without exposure to acrylamide. Sixty- three of the remaining workers were evaluated for neuropathological effects. At the factory there were no unexposed individuals, no engineering measures to reduce exposure and no respiratory protection. Some protective clothing was available but its efficacy was unclear.

Estimates of 8-hour TWA exposure were made but in an inaccurate way. Twenty-two workers were exposed to airborne levels of <0.3 mg/m^3, and 41 exposed to levels above this with four workers apparently subjected to levels of about 0.75 mg/m^3.

A higher prevalence of effects was reported in those exposed to >0.3 mg/m^3. Symptoms included weakness, effects on sensation, skin peeling, sweating and changes in skin pigmentation. No effects were seen on vibration threshold, tactile

or pain responses, reflexes at the ankle, knee, biceps and triceps, arm or leg coordination or Romberg test. No controls were included in this survey *(Myers and Macun, 1991).

Bachmann et al *(1992) undertook a later study of 75 workers in the same factory by way of questionnaire, physical examination and tests for vibration sensitivity. A mean 8-hour TWA of 0.16 mg/m^3 was determined (range 0.02 – 2.39 mg/m^3). Workers exposed to >0.3 mg/m^3 wore gloves. No control group was used.

There was no clinical evidence of muscle wasting, loss of spatial sense or any positive Romberg tests. Increased prevalence of symptoms such as skin peeling, sweating hands, numbness of hands and feet were observed in those exposed to >0.3 mg/m^3 compared with those exposed to lower levels of atmospheric acrylamide. No significant differences in vibration sensitivity were apparent between the two groups.

11.5.2 Case report

Davenport et al *(1976) described (Section 11.2) effects on a worker following long-term exposure to acrylamide. As well as the dermal effects, marked weight loss, fatigue, loss of appetite and unsteady gait were reported after about nine months exposure. Subsequently a number of other clinical symptoms were observed, including a tingling sensation and loss of use of the hands, impaired speech, muscle weakness in the wrists and ankles, reduced muscle tone, incoordination of upper limbs, tremor in the hands, fine nystagmus on lateral but not central gaze. There was also partial loss of pain and temperature sensation, response to touch below the forearm and below the mid-calf areas, loss of balance and coordination and reduction in tendon and plantar reflexes.

Reduced muscle action potential was recorded in the gastrocnemius and anterior tibial muscles of both legs. Sural nerve biopsy showed diffuse fibrosis, loss of nerve fibres and enlarged axons. A number of axons with randomly orientated fine bundles of filaments were seen under electron microscopy.

Clinical signs of toxicity were still evident two months after cessation of exposure. After one year there was almost complete recovery, although weakness of the ankles was still observed.

11.5.3 General public

Contamination of drinking water following grouting operations lead to five people being exposed to about 400 ppm acrylamide for approximately one month. As the level of acrylamide was only measured once, it is unclear if this level remained constant throughout the exposure period. Additionally, with no data on volumes of water consumed, the actual exposure cannot be quantified. The health effects were similar to those observed in other case reports, with recovery essentially complete after four months *(Igisu et al, 1975).

11.6 Epidemiological studies

A cohort mortality study of 357 workers employed at a site manufacturing acrylamide monomer and polymers from 1955 to 1982 was undertaken by Sobel et al *(1986). During the period 20 workers had died. Personal 8-hour TWA levels

ranged from 0.1 to 1 mg/m^3 prior to 1957, from 1957 to 1970 levels ranged from 0.1 to 0.6 mg/m^3 and were < 0.1 mg/m^3 from 1970 onwards. The extent of dermal exposure was unclear. No significant increases in the number of deaths from any cause were observed after excluding from the analysis 14 workers who had been exposed to organic dyes for a period of 5 years or more.

Collins et al *(1989) undertook a cohort mortality study investigating workers in factories in the United States and The Netherlands. The population included workers hired between 1 January 1925 and 31 January 1973, with data collected up to 31 December 1983. From the US factories 8508 men were identified. At the end of the study, 2148 had died and 513 were lost to follow-up. The cause of death was not determined in 111. In The Netherlands the population comprised 346 men. At the end of the study, 11 were dead and 20 were lost to follow-up. The cause of death could not be determined in two.

From 1977, 8-hour TWA values were available from personal monitoring and estimated for the time before 1977, based on these measured values and from a knowledge of the processes involved. Comparisons were made between those exposed to < 0.001 mg/m^3.years (approximately equivalent to one days exposure to < 0.03 mg/m^3, and those exposed to > 0.001 mg/m^3 years.

Overall the standardised mortality ratio (SMR) for all causes of death was not increased at any of the plants. Amongst the higher exposure group there was a slight but not statistically significant increase in cancer of the pancreas.

The work of Collins et al *(1989) described above was followed up by Marsh et al (1999). Only the US workers from three work sites were included in this study. Site- specific cancer risks were assessed. This follow-up contributed an additional 1115 deaths and nearly 60,000 person years from the additional 11-year period. A significantly increased risk of pancreatic cancer was found among workers with cumulative exposure to acrylamide >0.30 mg/m^3 years, however no consistent exposure/response relationship was found when exposure measures were adjusted for time since first exposure to acrylamide. This follow-up study corroborates the original cohort study, finding little evidence for a causal relationship between exposure to acrylamide and mortality from any cancer site.

12. Hazard Classification

12.1 Physicochemical hazards

Acrylamide is a non-flammable solid at STP, with a flash point of 138°C. Acrylamide tends to sublime and hence exhibits a vapour pressure at room temperature, although its vapour is denser than air. Acrylamide polymerises exothermically above its melting point (84.5°C), however it does not undergo autoignition nor is there any evidence in the literature of any explosive properties.

As with many solids, acrylamide dust may be explosive if mixed with air in critical proportions and in the presence of an ignition source. If acrylamide is handled with flammable or combustible materials, the explosion hazard may increase (Cytec 1997).

Classification status

Acrylamide does not meet the ADG Code criteria for assignment to any classes pertaining to physico-chemical hazards.

12.2 Health hazards

12.2.1 Toxicokinetics

Little information is available on the toxicokinetics of acrylamide in humans. Neurotoxic profiles in humans and experimental animals indicate similar oral, dermal and/or inhalation absorption potential between species. Oral and dermal studies in animals indicate that acrylamide is rapidly and extensively absorbed via these routes. No inhalation studies were available for assessment.

Acrylamide is widely and rapidly distributed in most tissues, with highest levels in liver, kidney and testes in both rats and mice. Binding to proteins and DNA has been shown in liver, brain and testes (spermatids). Acrylamide has a particular affinity for microtubule-associated proteins.

Metabolism of acrylamide in rats and mice suggests the major route to be conjugation with glutathione and CYP P450 2E1[3] mediated biotransformation to the epoxide, glycidamide. Glycidamide has been detected, as haemoglobin adducts, in blood samples from acrylamide-exposed humans and animals. Glycidamide has also been detected in animal urine, indicating that it is a relatively stable epoxide. The organotropic action of acrylamide cannot be explained by accumulation/DNA reactivity of glycidamide.

[3] Cytochrome P450 2E1 is the primary enzyme involved in acrylamide biotransformation to glycidamide (Sumner et al (1999).

It is not known whether the toxic effects, including mutagenicity and carcinogenicity, caused by acrylamide are due to the parent compound or a metabolite(s). Studies with inducers and inhibitors of mixed-function oxidase enzymes have provided conflicting evidence. Pretreatment of rats with SKF 525A (inhibitor) enhanced neurological effects and lethality of acrylamide (Kaplan 1973), whereas acrylamide-induced changes in CNS dopamine receptors were completely prevented (Agrawal et al 1981). Pretreatment with phenobarbital or DDT (inducers) reduced neurological and testicular effects in mice (Hashimoto and Tanii 1981), however in rats, delayed onset of neurotoxicity was accompanied by a greater degree of peripheral nerve damage (Kaplan 1973). Dominant lethal mutations (germ cells) were apparently markedly reduced by pre-treatment of animals with aminobenzotriazole, a specific inhibitor of cytochrome P450 2E1 (TERA 1998).

Phenobarbital also increased the rate of reaction of acrylamide with gluatathione by 40% in a mouse liver assay, without an increase in microsomal oxidase metabolism (Tanii and Hashimoto 1981).

The possibility of other metabolites being associated with acrylamide toxicity appears not to have been investigated, although indications are that glutathione conjugation is a detoxification pathway.

Little evidence is available to elucidate differences in metabolism 'flux' (i.e. differences in metabolite profiles) between low and high acrylamide exposures. In this regard, Bergmark et al (1991) reported a 50% conversion of acrylamide to glycidamide at 5 mg/kg, with only 15% conversion at 100 mg/kg.

It is clear that the toxicokinetics of acrylamide involve complex metabolic interactions and competing processes. The extent of metabolic conversion to critical metabolite(s) will be a function of these processes. Such processes would include: gluathione conjugation of acrylamide and glycidamide, saturation of oxidative metabolism to glycidamide and deactivation of glycidamide by epoxide hydrolase. The contribution of these metabolic processes may also vary between tissue types and between species.

12.2.2 Acute toxicity

A single case of acute oral acrylamide poisoning reported a relatively high intake (375 mg/kg). Hepatic effects and peripheral neuropathy were still present at two months following exposure.

Studies in animals show that acrylamide has an oral LD50 in rats, mice guinea pigs and rabbits ranging from 107 to 203 mg/kg bw. A single dermal study in rabbits provided an LD50 of 1148 mg/kg bw. No inhalation LC50 data were available for assessment.

The primary effect (non-lethal) reported from acute exposure to acrylamide is neurotoxicity. There is no evidence to suggest that any neurological effects seen in acute animal studies are irreversible, despite long recovery times noted in some studies.

Classification status

Acrylamide meets the NOHSC Approved Criteria (NOHSC 1999) for acute lethal effects by oral and dermal routes and is classified as harmful in contact with skin (R21) and toxic if swallowed (R25). Acrylamide is not classifiable for inhalation exposure, as no data are available.

Acrylamide does not meet the NOHSC Approved Criteria for risk of irreversible effects (other than mutagenic, carcinogenic and reproductive effects) after single exposure via oral or dermal routes.

12.2.3 Irritation

Dermatological effects have been reported in humans exposed to acrylamide and acrylamide solutions. Whether effects, particularly skin peeling, were due to primary irritant effects is unclear. However, other effects such as rash and ulcerations on hands and feet have been reported in workers using acrylamide. Eye irritation is apparently the most frequently reported adverse effect in workers using acrylamide grouts.

Tests in rabbits on acrylamide applied dermally under occlusive or semi-occlusive conditions indicate a lack of skin irritation potential.

Tests in rabbits found acrylamide to be an eye irritant when applied in powdered form or in aqueous solutions (>40% acrylamide). All signs of irritation were normal seven days post-application.

Classification status

Evidence from human exposures indicate that acrylamide meets the NOHSC Approved Criteria for skin irritant effects and is classified as irritating to skin (R38).

Acrylamide meets the NOHSC Approved Criteria for eye irritant effects and is classified as Irritating to eyes (R36).

12.2.4 Sensitisation

Apart from a few case reports of workers developing allergic-type skin reactions, including dermatitis and eczema, following re-exposure to acrylamide, little data is available for skin sensitisation in humans. Patch testing has also provided equivocal results. No data on potential respiratory sensitisation was available for humans.

Two maximisation tests carried out in guinea pigs reported positive results in up to 85% of test animals. No studies were available on the sensitisation potential of acrylamide in animals by inhalation.

Classification status

Acrylamide meets the NOHSC Approved Criteria for skin sensitisation and is classified as May cause skin sensitisation by skin contact (R43).

12.2.5 Repeated dose toxicity

Effects from repeated exposure to acrylamide are well documented in workers and there is one report of adverse effects in people having consumed drinking water contaminated with acrylamide. Effects in humans are similar to those seen in animals repeatedly exposed to acrylamide. Critical effects are PNS and CNS neuropathies. Peripheral nerve degeneration is the critical pathological lesion in all species tested.

Although peripheral nerve damage appears to be reversible, longer exposures may also lead to loss of ganglion and axonal cells in the optic tract and brain. Such effects are less well investigated in terms of reversibility.

Critical exposure levels/doses are not well characterised in humans, as it is likely that both inhalation and dermal absorption may have contributed to effects seen in workers.

The LOAEL for peripheral neuropathy in rats and cats was determined at 1 mg/kg/d in subchronic oral studies. Chronic studies in rats indicated a higher LOAEL of 2 mg/kg/d. Sub-chronic oral LOAELs in monkeys and dogs were 3 mg/kg/d and 6 mg/kg/d respectively.

Only rabbits and mice have been tested for repeated dose toxicity from dermal application of acrylamide. No signs of toxicity were detected in a short-term mouse study. The LOAEL in rabbits was 50 mg/kg/d in a sub-chronic study.

No repeated dose animal inhalation studies were available for assessment.

Classification status

The NOHSC Approved Criteria (1999) for severe effects after repeated exposure require the demonstration of a clear functional disturbance or morphological change(s) of toxicological significance, which although potentially reversible, includes major functional changes in the central and peripheral nervous system.

The available evidence indicates that acrylamide meets the NOHSC Approved Criteria for classification as harmful: danger of serious damage to health by prolonged exposure in contact with skin (R48/21) and Toxic: danger of serious damage to health by prolonged exposure if swallowed (R48/25).

Because it is considered likely that inhalation of acrylamide contributed to reported cases of human poisonings, it is considered prudent to also classify acrylamide as Harmful: danger of serious damage to health by prolonged exposure through inhalation (R48/20).

12.2.6 Genotoxicity

Overall, the weight of evidence from in vitro and in vivo studies indicates that acrylamide is genotoxic in both somatic and germ cells.

According to the NOHSC Approved Criteria (NOHSC 1999), a substance known to be mutagenic in humans should be classified as Category 1. No human mutation epidemiology studies are available i.e. there is no evidence to establish a causal relationship between human exposure to acrylamide and *heritable* genetic damage.

For classification as Category 2 (substances which should be regarded as mutagenic to humans), positive results are needed from assays demonstrating a mutagenic effect or other interactions relevant to mutagenicity in *germ* cells of mammals in vivo. In this regard, acrylamide has been shown to:

- induce heritable mutations in a mouse-specific locus and heritable translocation tests;
- cause effects on progeny/defects in developing embryo, as evidenced by positive results in dominant lethal tests in rats and mice following single or repeated exposure;
- increase the frequency of chromosomal aberrations including aneuploidy and micronuclei (detected by cytogenetic analysis) in spermatogonia;
- induce unscheduled DNA synthesis (UDS) in rat and mouse spermatocytes following single or repeated exposure;
- bind (covalent) to DNA in rat testes; and
- increase the frequency of single strand breaks in spermatids and spermatocytes (pachytene) in mice.

Classification status

Based on the above evidence, acrylamide meets the NOHSC Approved Criteria for classification as a Category 2 mutagen - May cause heritable genetic damage (R46).

12.2.7 Carcinogenicity

In rats administered acrylamide in drinking water, there was clear evidence of an increase in tumour incidence in several organs in both sexes, although bioassays in mice indicate that acrylamide may not act as a complete carcinogen.

The IPCS Conceptual Framework for Cancer Risk Assessment was utilised to evaluate the available data in the context of postulated modes and mechanisms of action (MOA). IPCS define MOA as a description of key events and processes (cellular and anatomical) resulting in tumour formation. This is contrasted with mechanism of action, which implies a more detailed knowledge of causal relationship between such events and tumour formation. According to the NOHSC Approved Criteria (NOHSC 1999), mechanism of action is required for classification purposes.

Weight-of-evidence from appropriate bioassays indicates that acrylamide is genotoxic, causing gene mutations in somatic and germ cells in vivo. Acrylamide is also an aneuploidigen and/or clastogen in vivo.

In both Friedman et al (1995) and Johnson et al (1986) studies, increased tumour incidence was seen primarily in tissues, such as adrenal, thyroid, mammary gland, ovary, uterus, testes, considered to be under hormonal control. Thus a possible relationship with disturbed endocrine function has been proposed as a mode of action (MOA) for acrylamide carcinogenicity in rats.

It is well known that non-genotoxic carcinogens can induce tumours in these organs. Mechanisms of action associated with endocrine-mediated tumourigenesis

in hormonally sensitive organs have been well investigated, particularly in rats and humans.

Species differences in endocrine function are of primary importance in determining the relevance of tumours seen in animals to humans. In this regard, it is considered that mammary fibroadenomas and testicular TVMs seen in F-344 rats are unlikely to be relevant to humans due to their high spontaneous incidence in this species/strain of rat and/or known differences in endocrine biochemistry and tumour potential at these sites between species.

For other tumour types, in particular, thyroid adenomas, CNS glial cell tumours and oral cavity papillomas, all of which were statistically increased above controls, a number of modes of action (MOA) have been hypothesised for tumour induction, with supporting evidence in some cases. However, the available data are insufficient to support a consensus view on a clear biological mechanism(s) of action for acrylamide-induced tumours and it may be that different mechanisms are acting concurrently or in different organs/tissues.

According to the NOHSC approved criteria (NOHSC 1999a), a substance known to be carcinogenic in humans should be classified as Category 1. No evidence of increased tumour incidence was found in three cohort studies (epidemiological) for acrylamide workers.

For classification as a Category 2 carcinogen i.e. substances which should be regarded as if they are carcinogenic to humans, clear positive evidence of carcinogenicity is needed in two animal species or clear positive evidence in one species, together with supporting evidence from genotoxicity, metabolic or biochemical studies, induction of benign tumours and structural relationship with other known carcinogens.

Substances may be classified as a Category 3 carcinogen i.e. substances which cause concern for humans owing to possible carcinogenic effects, but in respect of which the available information is not adequate for making a satisfactory assessment, if there is some evidence from animal studies, but insufficient to classify in Category 2.

The criteria set out in S4.86 of the Approved Criteria provide a 'distinction' between classification as a Category 2 or 3 carcinogen. In this regard, acrylamide:

- elicited carcinogenic effects at doses not exceeding the maximal tolerated dose;
- induced tumours in organs not associated with a high spontaneous incidence of tumours;
- did not induce tumours only at site of application;
- was genotoxic in short-term tests in vivo and in vitro;
- did not exhibit a clear (practical) threshold (in any organ) or clear evidence of a secondary (epigenic) mechanism of action (despite effects reported on endocrine hormones and growth factor regulation) for all tumorigenic effects seen; and
- the mode of action postulated for acrylamide-induced mammary tumours and TVMs may be biologically plausible, however no mechanisms of

action have been characterised for any of the tumour types seen in acrylamide-exposed rats, i.e. there is no evidence to suggest that tumours are species specific.

Classification status

Based on the above evidence, acrylamide meets the NOHSC Approved Criteria (NOHSC 1999a) for classification as a Category 2 carcinogen (Risk Phrase R45 – May cause cancer).

12.2.8 Reproductive effects

Effects on fertility

According to the NOHSC Approved Criteria (NOHSC 1999a), a substance known to impair fertility in humans should be classified as Category 1. There are no data available for human reproductive effects from acrylamide exposure.

For classification as Category 2 (substances which should be regarded as if they impair fertility in humans), clear evidence is required from animal studies of impaired fertility in the absence of toxic effects or evidence of impaired fertility occurring at around the same dose levels as other toxic effects, but which is not a secondary non-specific consequence of the other toxic effects. In this regard, acrylamide exposure has been associated with:

- significant reduction in male sperm count at 8 mg/kg/d in rats (Zenick et al 1986) and 12 mg/kg/d in mice (Sakamoto and Hashimoto 1986);

  Paternal toxicity: Neurotoxicity seen at both doses. No effects on organ/body weights

- significant reduction in fertility and pregnancy indices in untreated female rats and mice (Tyl 1998; Sublet et al 1989; Sakamoto and Hashimoto 1986) mated with treated males at 15 mg/kg/d (rat) and 12 mg/kg/d (mouse);

  Paternal toxicity: Neurotoxicity seen at these doses. Mating ability/behaviour was not affected in males in these studies

- significant reduction in fertilised ova in unexposed female rats mated with males at 15 mg/kg/d;

  Paternal toxicity: Neurotoxicity seen at 15 mg/kg/d. Mating ability/behaviour was not affected in males at this dose

- significant increase in pre- and post-implantation loss in unexposed female rats mated with males treated at 6 mg/kg/d (Smith et al 1986);

  Paternal toxicity: No neurotoxicity/pathological lesions seen at this dose

- significant post-implantation loss also seen in untreated females by Zenick et al 1986, Tyl 1998 and Sublet et al 1989 at doses associated with male toxicity;

- significant reduction (15 (F0) to 45% (F1) in numbers of live pups per litter from female mice (F0 and F1 breeding pairs) exposed to 9 mg/kg/d.

No difference from controls in pups per litter from exposed female mice (F0 animals) mated with unexposed males (NTP 1993);

Paternal/Maternal toxicity: No neurotoxicity or reduction in body weights at this dose

Dominant lethal effects may be related to reduction in live births.

- significant reduction in numbers of foetuses per dam in female mice exposed to 9 mg/kg/d (Sakamoto and Hashimoto 1986);

  Maternal/Paternal toxicity: neurotoxicity (both sexes) and sperm abnormalities seen at 12 mg/kg/d.

In summary, there is sufficient evidence to indicate that fertility effects seen in either male or female animals are not secondary to either generalised or specific toxicity, such as neurotoxicity or hormonal effects. In addition, many of these fertility effects have been demonstrated at relatively low exposure levels.

Evidence indicates that such effects may be primarily seen in males. Although the mode of action is not known, acrylamide has been shown to reduce sperm counts in some studies. In addition, effects on male germ cells have also been reported in a number of in vivo genotoxicity assays (see Section 10.5) and it has been demonstrated that acrylamide has a particular affinity for binding to 'protamine' a protein present in sperm-heads.

Despite the clear effects demonstrated in animals, the relevance of the animal data to humans is questionable with regard to the dosing regime employed i.e. the route of administration and magnitude of the LOAEL for effect. The LOAEL associated with changes to fertility was 5 mg/kg/d (NOAEL = 2 mg/kg/d), which is some 50-fold greater than the highest estimated intake (not taking into account PPE) from polymer manufacture (see Table 14.2).

Classification status

Based on the above evidence and in accordance with S4.108 of the NOHSC Approved Criteria, acrylamide meets the criteria for classification as a Category 3 reprotoxicant – May impair fertility (R60).

Developmental effects

According to the NOHSC Approved Criteria (NOHSC 1999a), a substance known to cause developmental toxicity to human progeny should be classified as Category 1. There are no data available for human reproductive effects from acrylamide exposure.

For classification as Category 2 (substances which should be regarded as if they cause developmental toxicity in humans), clear evidence is required from animal studies where effects have been observed in the absence of marked maternal toxicity or at around the same dose levels as other toxic effects, but which are not a secondary non-specific consequence of the other toxic effects. In this regard, acrylamide exposure has been associated with:

- retardation in pup development (delayed vaginal opening) in rats at maternal dose of 10 mg/kg/d (Zenick et al 1986);

  Maternal toxicity: 10% reduction in maternal weight gain during lactation only. Minimal neurotoxicity

- significant reduction (15 (F0) to 45% (F1) in numbers of live pups per litter from female mice (F0 and F1 breeding pairs) exposed to 9 mg/kg/d. No difference from controls in pups per litter from exposed female mice (F0 animals) mated with unexposed males (NTP 1993);

  Paternal/Maternal toxicity: no neurotoxicity or reduction in body weights at this dose

  Dominant lethal effects may be related to reduction in live births

- increased embryotoxicity and incidence of malformations (tail kink) in mice (Neuhauser-Klaus & Schmahl, 1989).

  Maternal toxicity: not reported

It is not clear from the above studies whether the effects seen are lactational or in utero effects, or secondary to maternal toxicity and/or reduced food intake or in the case of decreased numbers of live births, whether dominant lethality is the primary effect. The relevance of embryotoxicity and the malformations reported in the Neuhauser-Klaus & Schmahl study is confounded by lack of details on maternal toxicity in addition to the lack of relevance of the route of administration (intraperitoneal) to humans.

Classification status

Based on the available evidence, acrylamide does not meet the NOHSC Approved Criteria for classification as a developmental toxicant.

Effects on lactation

For the purposes of classification, substances which are determined to cause toxic effects on reproduction and which also cause concern due to their effects on offspring resulting from exposure via maternal milk should be classified with risk phrase R64 – May cause harm to breastfed babies.

In this regard, although acrylamide exposure during lactation has been associated with marked toxicity (weight loss and neurotoxicity) in pups, no conclusions could be drawn from available studies as to whether effects were caused by exposure to acrylamide in utero or via transmission through maternal milk (Zenick et al 1986; Tyl 1987), or in the case of effects seen in pups only exposed during lactation, whether the effects seen were secondary to maternal toxicity and/or compromised (quality/quantity) milk production (Husain et al 1987; Tyl 1988).

The lack of cross-fostering data from available studies precludes any conclusions regarding the contribution of acrylamide in breast milk to effects seen in pups.

Classification status

Acrylamide does not meet the NOHSC Approved Criteria for classification for lactational effects.

13. Effects on Organisms in the Environment

The information presented in the next section summarises the ecotoxicological studies that were discussed in the EU risk assessment report (EU 2000).

13.1 Aquatic plants

In a study using the fresh water alga *Selenastrum capricornutum*, a 72-hour EC50 of 67.7 mg/L (growth inhibition) and a NOEC of 32 mg/L (growth inhibition) are reported *(SEPC, 1997). The test was performed using OECD 201 Guidelines and EEC Directive 92/69 Method C.3. It should be noted that a 50% acrylamide solution was used. Therefore the EC50 and NOEC values should be divided by two to give the toxic effect due to acrylamide. This gives a 72-hour EC50 of 33.8 mg/L (growth inhibition) and a NOEC of 16 mg/L (growth inhibition). The EC50 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 IC50 of 72 mg/L for *Selenastrum capricornutum* with acrylamide in an algal growth inhibition test, however no study details were reported.

13.2 Aquatic invertebrates

In Table 13.4 summarises the available toxicity data for aquatic invertebrates.

In acute tests, the water flea *Daphnia magna* is the most sensitive species with a 48-hour LC50 of 98 mg/L. Tests with the salt water shrimp *Mysidopsis bahia* showed a very similar sensitivity (48-hour LC50 of 109 mg/L). Long-term toxicity data are only available for salt-water species, with a 28-day NOEC of 2.04 mg/L being reported for *Mysidopsis bahia*.

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. At the end of the study, the authors concluded that acrylamide appeared to have a selective adverse effect on invertebrates, but more research was needed to adequately define the effect of acrylamide on rivers.

During 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 noted that spates and high mica-dam discharges (the site was downstream from a china clay site) would lead to lower concentrations whilst low flows and discharges would produce higher concentrations. 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.

Table 13.4 - Summary of aquatic invertebrates toxicity studies

Species	Method	Effect concentration (mg/L)	Reference	Validity
Daphnia magna	Standard method for acute toxicity test with fish, macroinvertebrates and amphibians	48 h LC50 = 98 (m) [Mortality] 48 h EC50 = 98 (m) [Immobilisation] 48 h NOEC = 60 (m) [Immobilisation and mortality]	ABC Labs (1983b)	Valid
Paratanytarsus parthenogenetica	Standard method for acute toxicity test with fish, macroinvertebrates and amphibians	48 h LC50 = 4 10 (m) [Mortality] 48 h EC50 = 230 (m) [Immobilisation] 48 h NOEC=60 (m) [Immobilisation and mortality]	ABC Labs (1983c)	Use with care
Mysidopsis bahia (Salt water species)	Acute toxicity test	96 h LC50 = 78 (m) 96 h NOEC = 5.2 (m)	*EG&G Bionomics (1986)	Use with care
	Prolonged toxicity test	96 h NOEC = 2.04 (m) [Mortality in F1 generation] 28-day NOEC = 2.04 (m) [Mortality] 28-day NOEC > 4.4 (m) [Reproduction]	*Springborn Bionomics (1985)	Use with care

Notes: m = Measured concentration

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). 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. From a comparison with the data collected, it could be seen that a natural reduction to similar levels might have been expected in any case.

13.3 Fish

Table 13.5 summarises the available toxicity data for fish.

Acute toxicity

The bluegill sunfish (*Lepomis macrochirus*) appears to be the most sensitive freshwater species with a 96-hour LC50 of 100 mg/L. The toxicity to other fish species appears to be in a similar range (96-hour LC50s between 100 and 180 mg/L).

Long-term toxicity

There are only a limited number of reported long-term toxicity studies on fish. Unfortunately, they are not well documented.

Hermans and Leeuwaugh *(1982) reported a 14-day LC50 of 34.8 mg/L for the Guppy (*Poecilia reticulata*). No details of the test method were given.

Edwards *(1975) reported a 7-day LC100 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*) 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). At 50 mg/L fish had difficulty in orientating themselves when swimming, and based upon this effect an EC100 of 50 mg/L was quoted.

13.4 Toxicity to micro-organisms

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 due, it was thought, to acrylamide having a toxic effect on the micro-organisms used within the test. Based upon this result, it is suggested that 2 mg/L is taken as a NOEC for micro-organisms.

Table 13.5 - Summary of fish toxicity studies

Species	Flow/ Static	Method	Effect concentration (mg/l)	Reference	Validity
Lepomis macrochirus	Flow	Standard method for acute toxicity test with fish, macroinvertebrates and amphibians	96 h LC50 = 100 (m) 96 h NOEC = 35 (n) 96 h EC50 = 85 (n)	ABC Labs (1982a)	Valid
Pimephales promelas	Flow	Standard method for acute toxicity test with fish, macroinvertebrates and amphibians	96 h LC50 = 120 (m) 96 h NOEC = 41 (n) 96 h EC50 = 86 (n)	ABC Labs (1983a)	Valid
	Static	Acute toxicity test (No details)	96 h LC50 = 124 96 h NOEC = 56	*Batchelder (1975)	Not valid
Oncorhynchus mykiss	Flow	Standard method for acute toxicity test with fish, macroinvertebrates and amphibians	96 h LC50 = 110 (m) 96 h NOEC = 37 (n) 96 h EC50 = 88 (n)	ABC Labs (1982b)	Valid
	Static	OECD Guideline 203 Fish, Acute Toxicity Test	96 h LC50 = 180 (n)	*United States Testing Company (1990)	Valid
	Static	Acute toxicity test (No details)	96 h LC50= 162 (m)	*Petersen et al (1985)	Use with care
Salmo trutta	Static	Acute toxicity test (No details)	48 h LC50 = 400 (n)	*Woodiwiss and Fretwell (1974)	Use with care
Carassius auratus	Static	APHA Guideline	24 h LC50 = 460 (m) 96 h LC50 = 160 (m)	*Bridié et al (1979) and *Bridié et al (1973)	Use with care
		Acute toxicity test (No details)	72 h LC50 = 140	*Paulet and Vidal (1975)	Not valid
Heteropneustes fossilis	Static	APHA Guideline	48 h LC50 = 87 48 h NOEC = 15	*Shanker and Seth (1986)	Use with care
Rasbora heteromorpha (Salt water species)	Flow	MAFF Guideline Standard constant flow procedure	96 h LC50 = 130 (n)	*Tooby et al (1975)	Use with care

Notes: EC50 and NOEC values based upon behaviour and mortality.
n-Nominal concentration
m-Measured concentration

Starostina et al *(1983) studied the effect of treating bacterial cells with acrylamide. The 16-hour EC100 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 the cell, thus inhibiting the synthesis of nucleic acids and disturbing the cell wall synthesis. The authors concluded that acrylamide is one of the major toxic factors affecting microbial cells during their immobilisation in polyacrylamide.

Spraggs et al *(1982) reported an EC50 of 13,500 mg/L for *Photobacterium phosphoreum* with acrylamide in a photoluminiscence test.

13.5 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.

13.6 Seawater studies

Chet and Mitchell *(1976) studied the control of marine fouling by chemical repellents. Motile marine bacteria identified as *Pseudomonads* were isolated from sea water 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, which slowed the growth of algae. The colonisation by marine mussels was significantly retarded when the plates were stored in the dark.

13.7 Toxicity to terrestrial plants

In his study on the impact of toxic substances on pollen germination and tube growth using the pollen of *Impatiens sultanii*, Bilderback *(1981) found that when acrylamide was added to the basal medium at concentrations ranging from 10 to 2000 ppm, there was no significant effect upon germination, tube formation, or tube growth.

Using the seeds from a number of higher plants (7 species approximately), Kuboi and Fujii *(1984) found that at 100 mg/L acrylamide retarded root elongation by 61% compared to the control and the EC50 was calculated as 220 mg/L. No significant effect on seed germination was observed.

In a study of the potential for the uptake and accumulation of ^{14}C labelled acrylamide into plant tissue using lettuce plants (*Lactuca saliva* L), it was found that 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 *(Hazeleton Labs, 1987).

Overall, acrylamide shows a slight toxic effect on plant growth at concentrations of 10 mg/kg soil. No effects on seed germination were observed.

13.8 Atmospheric effects

Because of its reactivity with hydroxyl radical in the atmosphere, concentrations of acrylamide in ambient air are expected to be low and short lived. It is not expected that these levels will cause effects on organisms exposed through this route.

13.9 Summary of effects, PNECs and hazard classification

Using Mensink (1995), it can be seen that acrylamide is slightly toxic to aquatic plants (lowest EC50=33.8 mg/L) and organisms (lowest *Daphnia* EC50=98 mg/L and lowest fish EC50=85 mg/L). From the data available, it appears that acrylamide does have some toxic effect on micro-organisms and terrestrial plants but only to a slight degree.

The testing of acrylamide as a marine antifouling agent indicates that it does exhibit a repelling action to colonisation by marine bacteria and mussels, but its toxicity to these organisms cannot be concluded from the studies.

In the atmosphere, acrylamide is highly reactive with hydroxyl radicals and therefore the concentrations will be low and very short lived.

13.9.1 Predicted no-effect concentrations (PNECs)

With limited available data, a predicted no-effect concentration (PNEC) is calculated using the lowest valid NOEC (or LC50) and dividing it by the appropriate assessment factor. Determination of the assessment factor depends on the number and type of valid toxicity studies. The following PNECs are calculated using valid toxicity studies as indicated in the SIAR, which have been agreed on within the OECD Chemicals Program by member countries including Australia.

Aquatic PNEC

Valid acute LC50 values are reported for fish (lowest 96-hour LC50=100 mg/L, *Lepomis macrochirus*), aquatic invertebrates (the lowest 48-hour EC50=98 mg/L, *Daphnia magna*), and freshwater algae (the lowest 72-hour EC50=33.85 mg/L, *Selenastrum capricornutum*). Since there is valid acute toxicity data for three trophic levels and only a valid long-term (72-hour) NOEC for freshwater algae, an assessment factor of 1000 is used (in accordance with EU guidance). Based on the

72-h EC50 for freshwater algae (the most sensitive species in short term tests), the aquatic PNEC is 33.85 μg/L.

PNEC for micro-organisms in wastewater treatment plants

Adverse effect on microbial activity in wastewater treatment plants can occur, therefore a micro-organisms' PNEC can be calculated. The assessment factor depends upon the microbial effect data available, which for acrylamide is limited. For *E. coli* a 16-hour EC100 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 micro-organisms 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 micro-organisms in wastewater treatment plants.

PNEC for terrestrial organisms

In calculating the PNEC for terrestrial organisms only data on plants, earthworms and bacteria are usually considered, however for acrylamide only data on plant germination and growth is available. Only a short-term toxicity test using higher plants grown in a soil medium is available. This gave an EC50 of 220 mg/l. An assessment factor of 1000 is used to give a PNEC of 220 μg/l. This PNEC should only be used as an indication since the plants were not grown in 'real' soil.

13.9.2 Hazard classification

There is currently no environmental hazard classification system in Australia. In accordance with the OECD Harmonised Integrated Hazard Classification System for Chemical Substances and Mixtures, acrylamide would be classified in the Acute III Class (OECD 2001). This classification is based on the 96 h LC50 (fish) >10 - ≤100 mg/L and/or 48 h EC50 (for crustacea) >10 - ≤100 mg/L and/or 72 or 96 h ErC50 (for algae or other aquatic plants) >10 - ≤100 mg/L.

14. Risk Characterisation

In this section, critical data on environmental effects and health hazards are analysed with regard to estimated/measured exposure levels of relevance to environmental/human exposures. The resultant risk characterisations provide a basis for risk management strategies.

14.1 Environmental risk

The level of risk in general is calculated by PEC/PNEC ratio. If this ratio is less than 1, then there is negligible risk.

14.1.1 Aquatic risk

PNEC for aquatic organisms	33.85 μg/L
Estimated PEC	3 μg/L
PEC/PNEC ratio	ratio 0.088
Risk level	<1, negligible.

14.1.2 Risk to wastewater treatment plant micro-organisms

PNEC for micro-organisms	200 μg/L
Estimated PEC	15 μg/L
PEC/PNEC ratio	0.075
Risk level	<1, negligible.

14.1.3 Risk to terrestrial organisms

This cannot be calculated with the information available. However as indicated by the Mackay distribution model, less than 0.1% of the environmentally available acrylamide is expected to partition to soil.

14.2 Occupational health risks

14.2.1 Occupational exposures

All acrylamide used in Australia is imported either as powder or aqueous solution and is used almost exclusively in production of liquid and solid grade polyacrylamide. In Australia, occupational exposure to acrylamide is reported to be limited to workers engaged in the production of polyacrylamide and laboratory workers using acrylamide in the preparation of polyacrylamide gels for electrophoresis.

During manufacture of polymers, exposure to acrylamide may occur during tasks such as bagging/debagging, sparging of liquid monomer tanks, pouring of

monomer into reactor, polymer gel drying, cutting, mincing, packaging, maintenance and spills.

During use of acrylamide, exposure via inhalation may occur to vapours from aqueous solutions of acrylamide or from sublimation of the solid. Dermal exposure may also occur from direct contact of solid or solutions with the skin or where workers come into contact with contaminated surfaces. It is considered unlikely that generation of aerosol droplets will occur during its reported uses in Australia.

Occupational exposure to residual acrylamide may also occur from polyacrylamide use in water/sewage treatment, paper and minerals processing, textile dyeing, surface coatings/paints, cosmetics and soil treatment during farming. Levels of residual monomer are usually kept below 0.1%, although levels of up to 2% have been reported for polyacrylamide used in some surface coatings/paints.

Exposure monitoring data were available for levels of acrylamide in air for different occupational exposure scenarios, however only data for polyacrylamide manufacture were provided for acrylamide uses in Australia.

14.2.2 Critical health effects

Acute effects

Acrylamide has been shown to cause skin and eye irritation in both humans and animals. Animal evidence also indicates a skin sensitisation potential. In rats, acrylamide is toxic and harmful by oral and dermal routes of administration respectively, and neurotoxic effects were reported in one human case report of accidental ingestion. Doses of acrylamide causing acute systemic effects, including neuropathy, in animal studies were around two orders of magnitude greater than those eliciting effects in repeated dose studies.

Effects from repeated exposures

The key toxicological endpoints for repeated exposure to acrylamide are neurotoxicity, genotoxicity, carcinogenicity and reproductive effects, although only neurotoxicity has been reported/observed in humans. No firm conclusions could be drawn from available human cohort mortality studies on potential carcinogenicity of acrylamide to humans.

Limited quantitative data are available relating adverse effects to exposure in humans. Although air-monitoring data was available for some workplaces, the contribution of dermal exposure to total exposure has not been quantified.

Most toxicological studies carried out in animals are oral studies, with no data on inhalation toxicity. NOAELs have been identified from animal studies for neurotoxicity and reproductive effects.

From the available data, there are no clear species differences in sensitivity to neurotoxic effects of acrylamide, however lower (critical) NOAELs were determined for rats. A critical NOAEL of 0.2 mg/kg/d was determined for F-344 rats in a 90-day drinking water study, with a LOAEL of 1 mg/kg/d, where slight changes in nerve tissue were seen by electron microscopy (Burek et al 1980). A chronic study NOAEL of 0.5 mg/kg/d was determined in the same species/strain, based on lack of histopathological effects in tibial nerve (Johnson et al 1986). It

was reported that neurological investigation in this study was not as extensive as that carried out in the Burek et al study.

For reproductive effects, seen in both rats and mice, a critical NOAEL of 2 mg/kg/d was determined in F-344 rats in two-generation reproduction (drinking water studies), with a LOAEL of 5 mg/kg/d (Tyl 1987, Tyl 2000).

Acrylamide produced an increase in incidence of a number of benign and malignant tumours, in a number of organs in F-344 rats (Johnson et al 1986; Friedman et al 1995) and was genotoxic in a number of in vivo studies. Despite the fact that statistical significance was only apparent at high doses (lowest LOAEL = 0.5 mg/kg/d for scrotal mesothelioma), it was not possible to identify a clear NOAEL from the available carcinogenicity data.

14.2.3 Risk estimate(s)

Risks from physicochemical hazards

Acrylamide is a non-flammable and non-explosive solid at STP, with a flash point of 138°C. Acrylamide tends to sublime above 25°C, forming a vapour that is denser than air hence an asphyxiation hazard may exist. Acrylamide polymerises exothermically above its melting point (84.5°C). Although it does not undergo autoignition or explosion, run-away reaction temperature is possible during industrial polymer production and temperatures need to be carefully controlled.

Acrylamide is relatively stable under normal storage conditions. Although contact with air causes some polymerisation to occur, this is very slow at temperatures below its melting point. Acrylamide has no oxidising properties. Acrylamide powder, like other dusts, may be explosive if ignited when present at a critical concentration in air.

Risks from physicochemical hazards during storage and use of acrylamide and acrylamide solutions are considered to be low.

Acute health risks

For acute occupational effects, a formal risk characterisation is of limited value due to irritation and sensitisation potential of acrylamide, where dose response data is unavailable. In addition, it was not considered appropriate to attempt to differentiate between acute and chronic risks associated with exposure to a potential genotoxic carcinogen.

In addition, the toxicodynamics of neurotoxicity are insufficiently understood i.e. it is unknown whether intermittent exposures to higher doses of acrylamide are equivalent in terms of risk to low-level continuous exposure. It is therefore considered that risks of systemic effects (e.g. neurotoxicity) from acute exposure to acrylamide are likely to be reflected by those calculated for repeated exposures e.g., if margins of exposure (MOE) are high for repeated exposure, then risks of acute effects would also be considered to be low and vice versa. Such an assumption is vindicated by the fact that, for occupational exposure, no delineation is made between acute and repeated exposures/risks in terms of personal protective equipment used.

Health risks from repeated exposures

Because of the uncertainties in MOA for carcinogenicity and the lack of a clear threshold for effect or NOAEL for acrylamide-elicited tumourigenicity and genotoxicity, it was considered that, for the purposes of risk characterisation, a MOE approach would be appropriate only for estimating non-cancer risks from repeated exposure. This approach is consistent with that utilised by the EU (EU 2000). For carcinogenic risks, the methodologies used and risk estimates determined for acrylamide by other national/international bodies are reviewed (see below).

Non-cancer risks

The critical effect from repeated exposure to acrylamide is neurotoxicity. The critical NOAEL was determined as 0.2 mg/kg/d (see Section 14.2.2). It was considered that risk estimates carried out for neurotoxicity would 'control' for other non-cancer effects, including reproductive effects, as this was the lowest NOAEL.

Table 14.1 provides MOEs calculated from Australian occupational monitoring data, with MOE estimates for EU data reported in Table 14.2. Where exposure data (for air levels or dermal deposition) were unavailable, these were calculated using the UK EASE model. Internal doses (body burden) were calculated using the appropriate formulae detailed in Appendix 1. Neither amelioration by PPE nor inter-species differences e.g. dose adjustments for differences in metabolic rate/surface area etc, were factored into the MOE calculations.

Cancer risks

The following is a summary of available risk estimates for carcinogenicity carried out by other national/international bodies.

US North Carolina State Scientific Advisory Board on Toxic Air Pollutants (SAB) determined that mammary and scrotal tumours (TVMs) were the two most critical tumour endpoints in Johnson et al (1986) and Friedman et al (1995) studies. Incidence data for each of these tumour types was combined from these studies and analysed using the linearised multistage model for carcinogenesis. Multiple cancer risk estimates were generated for each tumour type based on 95% upper confidence limits (UCL) or the maximum likelihood for effect (MLE). The estimated effective inhalation exposures associated with a risk of 1×10^{-5} for mammary tumours were 0.17 $\mu g/m^3$ and 0.25 $\mu g/m^3$ for UCL and MLE, respectively. For scrotal mesotheliomas, these values were 0.16 $\mu g/m^3$ and 0.23 $\mu g/m^3$ for UCL and MLE, respectively. Estimated uptake associated with these exposures (excluding potential dermal exposure) ranged from 0.023 to 0.036 μg/kg/d (US SAB 2001).

An analysis of cancer risk from available epidemiological studies by Granath et al (2001) determined a risk of 1.6×10^{-3} for an estimated cumulative exposure for workers in USA to 0.25 $mg/m^3.y$ (corresponding to an uptake of 0.35 μg/kg/d), utilising the EPA risk model and an estimated 5×10^{-3} based on a multiplicative model. As uptake via dermal absorption was not factored into these calculations, the actual risk increments may be considerably higher.

Table 14.1 - Margins of exposure (MOE) for non-neoplastic effects for occupational exposures to acrylamide from Australian monitoring data

Occupational scenario	Estimated exposure (mg/kg/d)			MOE (based on NOAEL)
	Inhalation	*Dermal*	*Total*	
Polyacrylamide manufacture (plant) (using aqueous acrylamide)	0.001 - 0.0002	very low	0.001 – 0.0002	<200 - 1000
Polyacrylamide manufacture (plant) (using acrylamide crystals)	0.004 – 0.085[1]	very low	0.004 – 0.085	<2.5 - 50
Polyacrylamide manufacture (manual debagging) (using acrylamide crystals)	0.023[2]	very low	0.023	<8.5
Polyacrylamide manufacture (automated debagging) (using acrylamide crystals)	0.001[2]	very low	0.001	<200

Key:
Dermal exposure was estimated using UK EASE (Version II). No quantitative estimate was derived and hence MOEs are expressed as 'less than' estimates.
[1] combined data from static and personal monitoring.
[2] personal monitoring data

NOAEL = 0.2 mg/kg/d

Table 14.2 - Margins of exposure (MOE) for non-neoplastic effects for occupational exposures to acrylamide from overseas data (EU 2000)

Occupational scenario	Estimated exposure (mg/kg/d)			MOE (based on NOAEL)
	Inhalation	*Dermal*[1]	*Total*	
Polyacrylamide manufacture (plant)	0.007[7] (personal monitoring)	1.94 [6]	0.1	2
Polyacrylamide manufacture (plant maintenance and cleaning)	0.01 - 035[8] (personal monitoring)	Not reported/ estimated	>0.035	<5.5 - 20
Polyacrylamide manufacture (packaging)	0.002	0.004[2,3]	0.006	33
Polyacrylamide use (paper manufacture)	0.0004[3]	0.004[2,3]	0.0044	45
Preparation of polyacrylamide gels	0.01	0.004[2,3]	0.014	14
Grout application (small scale use)	0.017[10] (personal monitoring)	0.43[4]	0.45	0.5
Grout application (large scale use)	0.007 -0.01[9] (personal monitoring)	0.43[5]	0.44	<0.5

Key:

[1] An absorption factor of 0.75 was utilised by EU in estimation of dermal doses for EU occupational exposures

[2] estimated by EU using UK EASE

[3] handling undiluted polymer

[4] measured by body pad/glove wipe sampling

[5] estimate for small-scale use as no dermal data available for large-scale use

[6] estimated from a mean value of 0.01 mg/cm^2/d (highest value was 0.08 mg/cm^2/d)

[7] estimated from a mean value of 0.05 mg/m^3 TWA (highest value was 0.77 mg/m^3)

[8] estimated from mean values for reactor/holding tank "dig-out" of 0.07 -0.24 mg/m^3 (highest value was 1.44 mg/m^3)

[9] estimated for levels of acrylamide only (NMA was also present in product used)

[10] highest personal sampling measurement in 3 surveys (0.12 mg/m^3)

NOAEL = 0.2 mg/kg/d LOAEL = 1.0 mg/kg/d (Neurotoxicity)

Crump (1999b) evaluated the carcinogenic potency of acrylamide using the T25 method (used by EU). T25 values are not estimates of risk as such, but are reported here for completeness. The T25 value represents the chronic dose, equivalent to an expected 25% increase in the incidence of a specified tumour above background. Tunica vaginalis (scrotal) mesothelioma was selected for analysis. Three methods of calculating T25 values utilised in this study were multistage dose response linear extrapolation, benchmark dose multistage model and Weibull dose response model. T25 estimates for these three methods were 1.6, 3.4 and 5.2 mg/kg/d respectively.

Similarly, in a draft report by Toxicology Excellence for Risk Assessment (TERA 1998), US EPA Benchmark Dose software[4] was used to generate ED_{10}[5] and LED_{10} (the lower 95% confidence limit of dose associated with the ED_{10}) from combined tumour data from Johnson et al (1986) and Friedman et al (1995) studies. The models used for calculating these values were multihit, multistage and Weibull distributions. LED_{10} doses calculated for thyroid and mammary tumours in humans were 0.265 and 0.20 mg/kg/d respectively.

Although TVMs and mammary tumours were not considered relevant for humans (see Section 12.2.7), they were utilised in the above cancer risk estimates to provide conservative estimates.

14.2.4 Uncertainties in occupational risk estimate(s)

Uncertainties arise in any risk characterisation process due to factors, such as quality of critical animal/human studies, lack of exposure information and assumptions made. Large uncertainties are involved in the characterisation of human cancer risk from acrylamide, as the MOA in animal studies has not been fully characterised. Not only is acrylamide not a proven carcinogen, but also it is not known whether a threshold for effect exists. Uncertainties also arise from lack of sufficient data to validate the mathematical models used to estimate risks at different levels of exposure.

In regard to MOEs for neurotoxicity, the following were identified as sources of uncertainty in their calculation:

- lack of personal air monitoring data (for certain scenarios);
- lack of dermal exposure data and inconsistent data on dermal absorption potential;
- lack of knowledge on relative contribution of dermal and inhalation exposure to body burden in humans i.e., confounding dose response estimation assumption of 75% dermal and 100% absorption from skin and lung respectively;

[4] Version 2 (test version only)

[5] effective dose at 10% response

- lack of an inhalation NOAEL for animals i.e. default to an oral NOAEL determined for MOE estimates; and
- lack of knowledge on the cumulative neurotoxicity of acrylamide (i.e. the contribution to toxicity of short intermittent high exposure vs continuous exposure over longer periods to lower levels).

14.2.5 Areas of concern

The risk characterisation indicates that the highest risks of neurotoxicity are associated with acrylamide exposure during grouting applications. The exposure data used to calculate MOEs for grouting indicated that dermal exposure accounted for at least 90% of total intake. Although no monitoring data were available for grout use in Australia, it is assumed that exposures monitored overseas are likely to be representative of any Australian grout-use scenarios. It is also acknowledged that high dermal exposures in some overseas grouting scenarios may have arisen from incomplete polymerisation of acrylamide due to certain conditions of application. Of interest is the knowledge that the estimated/monitored levels of acrylamide exposure for grout workers in the EU have been associated with worker neurotoxicity, thus providing some validation of the NOAEL selected for the risk characterisation. However, it should be noted that for scenarios and grout products associated with neurotoxicity in the EU i) N-methylolacrylamide (NMA) may have contributed to neurotoxicity and ii) unpolymerised NMA is known to hydrolyse to acrylamide` under certain conditions, which may have lead to higher levels of acrylamide than would normally be encountered using other acrylamide-based grouts (see Section 8).

MOEs for workers involved in polyacrylamide production in Australia range from <2.5 to 1000. The lower levels of MOE in this range are considered likely to be overestimates due to the fact that they were determined from static air sampling and relate to exposures from a process (extruction) in solid polymer production, which is not relevant to liquid polymer production, and has since been partially enclosed. Although very low dermal exposure was determined from EASE modelling, limited overseas monitoring data indicate that dermal exposure may contribute up to 90% of total body burden (i.e. a similar contribution to overall exposure as grouting applications). Although few details were available on working scenarios or length of exposure for the dermal monitoring, it should be noted that the results were for actual dermal exposure resulting from wearing gloves, thus causing greater concern for this route of exposure.

It is clear from the MOE estimates for Australian manufacturing scenarios that risks associated with handling acrylamide solid (crystals) are significantly higher (between one and two orders of magnitude) than for handling acrylamide solutions. Similarly, risks during production of solid polymers appear to be higher than for liquid polymers. With regard to the use of acrylamide crystals, an area of concern is the manual debagging process, where risks are significantly reduced for the corresponding automated process. Risks during manual debagging processes were controlled by wearing full body PPE and SCBE at one plant visited by NICNAS.

Low inhalation MOEs (range 2.5 to 9) were determined for certain processes during the transfer and cutting/mincing (extruction) of solid polymer products. However, not only were exposures measured by static sampling, but workers are reported to be exposed for only 1.5 hours during such processing on a daily basis.

In addition, the Australian manufacturer of solid polyacrylamide has advised that this process is now largely enclosed. Limited post-enclosure monitoring data indicate a 10-fold reduction in exposure levels to around the current Australian exposure standard for this process.

An MOE of 33, calculated from EU data for polyacrylamide packaging (assumed to be for solid polymer), provides a moderate level of risk, which would again be expected to be significantly reduced for liquid polymers (due to dilution) and wearing of PPE.

Occupational exposure at polymer plants is likely to be highest during maintenance and cleaning activities. An MOE of between 5 and 20 was obtained from EU inhalation exposure data for removal (dig out) of solidified polymer from reaction and holding vessels. As respiratory equipment is worn for such tasks, actual intake from inhalation is likely to be considerably lower than the value used in this risk estimation.

Information provided by applicants indicates that PPE (including respiratory protective equipment) is worn for debagging, packaging and maintenance and clean up of spills. Although PPE is not worn during the production process itself, it appears that entry to plant is infrequent as the process is automated and controlled by computer operation at a location away from the reactor. Even though risks from inhalation exposure are low for certain processes in polymer manufacture, it is considered that all processes are of concern, with the possible exception of polymer packaging because of the uncertainties relating to dermal exposure, particularly where crystalline acrylamide is used.

Although no Australian monitoring data were available for use of acrylamide in the preparation of polyacrylamide gels, processes and exposures would be expected to be similar to those reported overseas. In this regard, an MOE of 14, estimated by EU, is considered representative of a worst-case scenario for laboratory workers, where gels are prepared from raw materials, including acrylamide powder. Although this risk is considered reasonably high, it would be significantly reduced by PPE and by using pre-cast gels or acrylamide solutions (EC 2001). In addition, exposure of laboratory workers from this activity would be expected to be intermittent in most cases.

Occupational exposure from use of polyacrylamide was not investigated in detail in this report. The main uses reported in Australia include water treatment, paper and minerals processing. In most cases the polymer is diluted to between 0.05 and 0.5% for these applications. A single exposure estimate was available from EU data for paper manufacturing where exposure was calculated for a worst-case exposure scenario for undiluted polymer. An MOE of 45 suggests there is a low risk for concern (of non-neoplastic effects) for workers using polyacrylamide products in these applications.

The above risk evaluations are based on risks of non-neoplastic effects, and in particular, neurotoxicity. With regard to carcinogenicity, there are reasons for concern in all workers repeatedly exposed to acrylamide, as no threshold for effect has been established for either carcinogenicity or genotoxicity. Based on the available risk estimates for inhalation exposure from LMS UCL method (see Section 14.2.2), cancer risks from exposure to acrylamide to workers involved in polyacrylamide production (uptake range 1 – 85 μg/kg/d) range from 6.3×10^{-5} to 5.3×10^{-3}, which corresponds to a worst-case estimate of approximately 5 extra

cases of cancer per 1000 workers exposed. It should be pointed out that such estimates were for tumour types (seen in animal studies) considered not to be relevant for humans and are therefore of limited use in quantitative risk estimation.

14.3 Public health risks

14.3.1 Consumer products

Public exposure to acrylamide is likely to occur from use of cosmetic/toiletries products containing polyacrylamide, with dermal contact the main route of exposure. No information on acrylamide-containing copolymers in cosmetics was available for the quantitative risk assessment. The following typical use levels for polyacrylamide in cosmetics were adopted from the E U risk assessment (EU 2000). The calculation of maximum consumer exposure is based on a level of up to 2% of polyacrylamide in the products and a maximum monomer level of 0.01% in the polymer. For non-rinse products, daily use of general purpose cream or body lotion (estimate 19.4 g), setting products (12 g) and nail products (0.25 g) for a person is associated with a daily exposure of up to 0.0635 mg/day of the monomer. For a person using rinse-off products (10% remaining on the skin), 2 g of shaving cream and 4.8 g of soap daily will cause an exposure to 0.0014 mg/day of the monomer. Hence, a total of up to 0.065 mg/day of acrylamide is expected for a person using above cosmetic products, which in combination with an dermal absorption factor of 0.3, results in approximately 0.0003 mg/kg bw/day for a man/woman with body weight of 60-70 kg. This level of exposure would represent a safety margin of approximate 15000 for neurotoxicity based on the dermal NOAEL of 5 mg/kg bw/day. It is also 600-fold lower than the lowest oral NOAEL of 0.2 mg/kg bw/day for neurotoxicity, and 6000-fold lower than the oral NOAEL of 2 mg/kg bw/day for reproductive toxicity. These margins offer reassurance that the risk to reproduction and of neurotoxicity arising from consumer exposure to acrylamide is low. For a genotoxic carcinogen, it is not possible to reliably identify a threshold level of exposure, below which there is no increased risk. However, in animal studies, no tumours were found at an oral dose of 0.1mg/kg bw/day, and the estimated dose of 0.0003 mg/kg bw/day from cosmetic products represents a 300-fold margin of safety. Based on concerns for its carcinogenicity, the European Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers has recently (1999) recommended a tolerable level of < 0.1 and < 0.5 ppm acrylamide for non-rinse and rinse-off cosmetic products respectively. The change will result in a reduction of exposure from 0.000055 to 0.000275 mg/kg bw/day which is 4 to 18-fold lower. A life-long exposure to this low residual level of acrylamide is not considered to pose a significant risk of cancer and mutagenicity to consumers.

Australian food standards do not include a maximum level for acrylamide present in food as a result of contact with articles and materials containing the chemical (ANZFA, 2000). According to US FDA, $\leq$ 5% polyacrylamide existing in some other specified polymers as articles or components of articles may be safely used in contact with food (US Code of Federal Regulations 21 CFR 2001). Polyacrylamide used in the imprinting of soft-shell gelatin capsules, or in wash water for fruits and vegetables should contain acrylamide at $< 0.2\%$ (US Code of Federal Regulations 21 CFR 2001, 172.255; MISC.REG.GMP 173.315). Modified polyacrylamide resin used as flocculent in the clarification of beet or cane sugar

juice is limited to less than 5 ppm (0.0005%) by weight of juice (US Code of Federal Regulations 21 CFR 2001, MISC.REG 173.10).

14.3.2 Indirect exposure via the environment

Another potential source of public exposure to acrylamide is through drinking water. For drinking water treatment by flocculation, a monomer level of < 0.05% in polyacrylamide dosed at 1 ppm (1 mg/L) is required. It results in a maximal possible concentration of acrylamide at 0.5 µg/L in drinking water. Assuming 2 L consumption per day for a man/woman of 70 kg, it is estimated that maximum exposure would be 1.42×10^{-5} mg/kg bw/day. Based on its toxicity and human health considerations, the *Australian Drinking Water Guidelines* (NHMRC 1996) stipulate that the concentration of acrylamide in drinking water should not exceed 0.0002 mg/L, which is also the limit of detection by HPLC in combination with UV. This level is equivalent to a daily exposure limit of 5.7×10^{-6} mg/kg bw/day. The impact of this low level of exposure to public health, including the risk of cancer, is negligible. In fact, acrylamide has not been detected in Australian drinking waters (NHMRC 1996).

Residue acrylamide in plants and food products (including seafood) may result from contaminated air, soil and water either during growth or manufacture. The residual level of acrylamide in the atmosphere or soil will likely be washed out by rain due to its high water solubility and gradually undergo photodegradation and biodegradation. Expected atmospheric concentrations of acrylamide are very low based on calculations and detected levels at production and processing sites in Europe. Contamination of food products during manufacture is only likely to occur as a result of accidental contamination of water supplies. The absorption of acrylamide by plants or fishes from contaminated water is likely to be negligible.

15. Risk Management

15.1 Assessment of current control measures

According to the NOHSC *National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994), exposure to hazardous substances should be prevented or, where this is not practicable, adequately controlled so as to minimise risks to health and safety. The NOHSC *National Code of Practice for the Control of Workplace Hazardous Substances* (NOHSC, 1994a) provides further guidelines in the form of a hierarchy of control strategies, namely:

- elimination;
- substitution;
- isolation;
- engineering controls;
- safe work practices; and
- personal protective equipment (PPE).

These measures are not mutually exclusive and effective control usually requires a combination of these strategies.

15.1.1 Elimination and substitution

Elimination is the removal of a chemical from a process and should be the first option considered in minimising risks to health. Information provided during assessment indicates that about 5000 tonnes of acrylamide have been imported annually since 1998.

The main use of acrylamide is to manufacture polymers and no information was received indicating a move away from the use of acrylamide polymers, nor did any manufacturer of acrylamide polymers who provided information indicate that alternatives were being investigated. Therefore elimination and substitution do not appear to be being locally pursued.

The evaluation of alternative substances for acrylamide and polyacrylamide use is beyond the scope of this report, as a full hazard assessment is required prior to considerations regarding substitution. Some acrylamide related monomers used in polymer production are also known to cause neurotoxic effects (e.g. NMA) and should therefore be considered as candidates for substitution, rather than possible alternatives to acrylamide.

The following information on potential alternatives was made available during this assessment. It should not be considered as a recommendation or evaluated source of data.

Polyacrylamide gel electrophoresis

There have been no generally acceptable substitutes identified for polyacrylamide in gel electrophoresis. Agarose gels can be used as an alternative in certain applications. However, these cannot be used for the full range of research applications in which polyacrylamide gel electrophoresis is used. In addition, a very large body of historical background information has been developed using polyacrylamide gels, which would become redundant should polyacrylamide be substituted with alternative agents (EC 2001). Pre-cast polyacrylamide gels are available, which reduce exposure to acrylamide for laboratory workers.

Acrylamide grouts

A number of alternatives to acrylamide are reportedly used in grouting operations overseas. These include acrylic, acrylate, polyurethane, epoxy, silicate, formaldehyde and methyl acrylate. The choice of compound depends mainly on the conditions for use (i.e. physical/chemical conditions that will affect the application and stability of the grout). Polyurethane and acrylate are the main alternatives for sewer-line sealing with the former compound also used in manhole sealing. Silicates and polyurethanes are reported to be the main alternatives for structural water control and geo-technical grouting. Information on health and environmental effects of alternative grouts is limited and is further confounded by variations in formulation. For example, polyurethane grouts have a wide range of compositions including a range of hazardous ingredients such as toluene di-isocyanate (TDI), dibutyl phthalate and methylene-bis-ortho-chloroaniline (MOCA) (EC 2001).

Flocculants

Acrylamide polymers are only a part of a water and wastewater treatment regimen while other flocculants not based on acrylamide are also used, not as substitutes for polyacrylamide, but because of the particular properties of the non-acrylamide-based compounds. Polyacrylamides however form the majority of flocculants used for treatment of non-drinking water. In addition to flocculants, coagulants are also used and these are not acrylamide based. Treatment of water by flocculation with polyacrylamide covers both drinking water and sewage, as well as waste water from industrial processes such as mining and paper making.

15.1.2 Isolation

Isolation as a control measure aims to separate employees, as far as practicable, from the chemical hazard. This can be achieved by distance, use of barriers or enclosure. In this regard, the following controls were identified for polymer manufacture:

- polymer manufacture is undertaken in closed vessels;
- cutting/mincing (extruction) of solid gel is carried out in an enclosed area and away from other work activities; and
- operators monitor polymer reaction from a remote control room with a dedicated conditioned external air supply.

15.1.3 Engineering controls

Engineering controls are plant or processes which minimise the generation and release of hazardous substances. They include enclosure or partial enclosure, local exhaust ventilation and automation of processes.

A number of engineering controls are in place to prevent exposure to acrylamide. The controls vary and include:

Polymer manufacture

- automatic debagging line during manufacture of polymer using crystalline acrylamide;
- maintenance of debagging line under negative pressure;
- local exhaust ventilation located at mixing vessel charging point during manual debagging;
- local exhaust ventilation provided on monomer make-up units, polymerisation vessels and gel mincing machinery;
- mechanical pump for transfer of acrylamide solution from IBC to reaction vessel;
- enclosure of gel processing/cutting (extruction) for solid polymer production;
- scrubber through which exhaust ventilation is directed prior to discharge to the atmosphere;
- use of oxygen to stop the polymerisation reaction at any time;
- continuous monitoring of temperature and pH of monomer solutions with alarms to alert operators of problems;
- provision of quenching water to arrest temperature rises in monomer storage tanks;
- pilot batches made in sealed pressure reactor in a specially designed room with extraction ventilation; and
- operators to monitor polymer reaction from a remote control room with a dedicated conditioned external air supply.

Research laboratory formulation of polymers for gel electrophoresis

- Stock solutions made from crystalline acrylamide prepared under a fume hood.

Water treatment

- At water treatment facilities, polymer metered into the treatment stream.

15.1.4 Safe work practices

Safe work practices have an important role in reducing dermal and inhalation exposure to acrylamide whether in its crystalline form or in an aqueous solution. Work practices vary and include:

Polymer manufacture

- imports of chemical are not in general stored by the importer, but despatched from the wharves direct to the customers' premises;
- industrial users of acrylamide store the chemical in designated dangerous goods stores. When required for manufacturing, the chemical is moved to the required area by forklift;
- 50% monomer solutions are stored between 13.9 ^{0}C and 32.2 ^{0}C with transfer lines maintained within a similar temperature range;
- empty solution containers are stored in a protected location;
- storage of acids, bases, oxidising, reducing and chelating agents in an area separate from acrylamide;
- continuous air sparging of bulk stored acrylamide monomer solutions;
- checking of levels of copper inhibitor in monomer solution storage tanks;
- storage and shipping containers of monomer solution are not completely filled to maintain adequate levels of dissolved oxygen;
- workers follow written procedures for charging acrylamide to reaction vessels and receive training in the safe handling of the chemical;
- operators are required to shower and change clothing after dispensing monomer to reaction vessels;
- after use, operators clean air hoods with an alcohol wipe;
- equipment used to manufacture polymers or handle monomers is thoroughly washed and cleaned before handover to maintenance crews;
- during charging of reaction vessels the area is signposted to restrict entry;
- immediately resealing broken bags of crystals to prevent sublimation;
- delivery containers of solution are neutralised with sodium metabisulphite after emptying and prior to washing;
- prior to maintenance, reaction vessels, transfer lines and pumps are decontaminated with warm water and air purging;
- any bags of crystals that are dusty on delivery are returned to the supplier; and
- processes using acrylamide crystals are calculated to consume full bags so that no partly used open bags remain.

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- polymerise unused solutions prior to disposal.

15.1.5 Personal protective equipment

Personal protective equipment (PPE) is used to minimise exposure to or contact with chemicals. PPE should be used in conjunction with other controls and not as a replacement. Where other control measures are not practicable or adequate to control exposure, then PPE should be used. Exposure to acrylamide is mainly by inhalation and skin contact and the PPE selected aims to give workers protection against exposure by these routes.

Acrylamide exhibits no warning properties at concentrations at or below the permissible exposure level. Airborne exposure is controlled by use of an enclosed air hood or a cartridge respirator. Air hoods are used when charging mixing vessels with crystalline acrylamide monomer in manual debagging. Cartridge respirators are used when handling polymers. Respirator cartridge service life tests showed that organic vapour cartridge provide protection from airborne levels of acrylamide up to 9 mg/m^3. No breakthrough at this concentration was detected after 8 hours at 85% relative humidity. Cartridges are changed at the beginning of each shift.

Dermal exposure is controlled by the use of protective gloves, overalls and/or full chemical resistant suits where necessary. It is important to select materials that are resistant to all chemicals used in the process. Tyvek ^{TM} disposable overalls are used when charging mixing vessels with crystalline acrylamide monomer in manual debagging. They are used once only then discarded.

Recommendations on types of glove to be used with particular chemicals are provided by many glove manufacturers and in a number of books and databases. Recommendations are usually based on tests of degradation, which present as changes in physical properties of the glove following contact with the chemical, such as swelling, hardening and permeation. A major importer of acrylamide monomers provides product stewardship manuals for crystals (Cytec, 1997) and aqueous solutions (Cytec, 1999). The manuals recommend impervious rubber or plastic gloves such as neoprene, polyethylene or PVC and rubber or neoprene shoes or boots. In addition impervious disposable overalls and a hardhat or disposable hat are also recommended. Polymer manufacturers recommend PVC, neoprene or rubber gloves. Forsberg and Mansdorf (1977) in their guide for selection of protective clothing do not recommend natural rubber or neoprene for handling aqueous acrylamide solutions. Suitable materials given are butyl rubber, nitrile rubber, polyvinyl chloride, Viton ^{TM}, 4H ^{TM} (PE/EVAL) and Tychem 10 000 ^{TM}. No recommendations were provided for the crystalline form.

Data provided by local polymer manufacturers indicate that the level of PPE used depends on the activity being undertaken. For dispensing acrylamide crystals on an automated debagging line, operators wear overalls and gloves. If the bags of crystals are opened manually, the operators wear a chemical resistant suit, PVC gloves, Wellington boots and use a respirator hood. Acrylamide can penetrate leather boots. When using monomer solutions, one manufacturer requires head covering, long-sleeved shirts and impervious neoprene gloves and footwear be worn. Workers handling the product after polymerisation at polymer manufacturer

sites wear gloves, safety glasses, boots and overalls. Respirators are not used at this stage.

For laboratory production of polymers, staff wear a lab coat and gloves.

At some municipal water treatment plants, workers wear gloves, overalls and a dust mask with a filter canister, but no specific PPE at other sites during use of polymers containing acrylamide for water treatment.

15.2 Hazard communication

15.2.1 MSDS

Under NOHSC *National Model Regulations for the Control of Workplace Substances* (NOHSC 1994) and the corresponding State and Territory legislation, suppliers are required to provide MSDS to their customers for all hazardous substances. Employers must ensure that a MSDS, prepared in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC 1994b), is readily accessible to employees with potential exposure to acrylamide used in the workplace. A sample MSDS (for solid acrylamide only) prepared in accordance with this Code is provided in Appendix 1. This sample MSDS is for guidance only. Under the NOHSC MSDS Code, manufacturers and importers have the responsibility of compiling their own MSDS and to ensure information is up-to-date and accurate.

A number of MSDS for acrylamide and acrylamide-containing products were provided for assessment. MSDSs provided for assessment fall into four main categories:

1) acrylamide monomer (solid and solution);

2) acrylamide co-monomer mixtures (solid and solution);

3) acrylamide monomer analogues containing residual acrylamide (solid and solution; and

4) polyacrylamide and acrylamide co-polymer mixtures containing residual acrylamide (solid, emulsion, solution and resin).

For the purpose of this assessment, it was not considered appropriate to attempt to differentiate between 1), 2) and 3) as only information/data relevant to acrylamide per se is of relevance to the scope of this report. It was however considered appropriate to differentiate between monomers and polymers due to the considerable differences in hazards and hence importance of appropriate handling and control instruction.

The content and format of MSDS were assessed according to NOHSC guidelines (NOHSC 1994). This assessment focused on the adequacy of the information provided in relation to the 'core' elements; product identification, health hazard information; precautions for use and safe handling information.

MSDS for products containing other hazardous substances in addition to acrylamide should address the hazards of all ingredients/residues, taking into account combined/additive effects of chemicals where relevant. Such an assessment is outside the scope of this report. The approach used was to evaluate

each MSDS for the required information/data pertaining to the level of acrylamide in the product. However, product identification (including ADG Code classification), precautions for use and safe handling information were evaluated in the context of the overall hazard for the product where reported in the MSDS. The quality/adequacy of information presented in MSDS for acrylamide and polyacrylamide products is summarised in Appendix 3, Tables 1 and 2 respectively.

Assessment of MSDS for acrylamide and co-monomer mixtures

A total of 15 MSDS were provided for assessment. Appendix 3 provides a summary of this assessment against 'core' elements as described above. The following is a discussion of the key findings of this assessment.

Product identification

The most common deficiency was incorrect reporting of the Hazchem Code for acrylamide. In many cases, this was the result of reporting the product under a generic UN Number for Toxic organic liquid, N.O.S, as opposed to using the UN number for acrylamide. The two most common alternative Hazchem codes reported were 2PE and 2X. In addition, some MSDS reported that the ADG Code Class was either not applicable or not available for acrylamide solutions. It would only be appropriate to utilise alternative Class labelling where toxicity testing data existed for the specific concentration of acrylamide solution or co-monomer product under consideration and where results were outside of the criteria for classification in Class 6.1.

Some MSDS failed to identify acrylamide in the list of ingredients. None of the MSDS for acrylamide provided details on impurities (e.g. acrylonitrile).

Health hazard information

Overall, health effects were well covered and reflected the latest classification endpoints reported in the NOHSC *List of Hazardous Substances* (NOHSC 1999a). However, some errors in the risk phrases were apparent in some MSDS. The most common error was the incorrect reporting of risk phrases for acrylamide solutions, due to misinterpretation of the concentration cut-off levels prescribed in the List. Appropriate risk phrases for co-polymer mixes were not assessed. Some MSDS for co-monomer mixtures failed to adequately address potential hazards of other ingredients/impurities (e.g. formaldehyde).

None of the MSDS reflected changes in the classification resulting from the recent EU revisions, however there is no requirement to do so until taken up in the NOHSC List.

Precautions for use

Overall, the information on personal protective equipment was considered satisfactory. Some MSDS did not report the existence of the NOHSC atmospheric exposure standard for acrylamide.

Safe handling information

Notwithstanding the issue of ADG Code classification and Hazchem code (see *Product identification* above), adequate information was provided on storage and transport. Handling of spills and disposal was also adequately addressed, however there was a general lack of consistency between MSDS in procedures recommended. Fire/explosion hazards and procedures were also generally adequately addressed, however, the potential for dust explosion was less well dealt with for some solid acrylamide products.

Assessment of MSDS for polyacrylamide and co-polymer products

A total of 11 MSDS were provided for assessment. Appendix 3 provides a summary of this assessment against 'core' elements as described above. The following is a discussion of the key findings of this assessment.

Product identification

The majority of MSDS assessed did not contain information on levels of residual acrylamide monomer. Although this is not strictly a requirement where levels are below 0.1%, it was considered that this information should be provided, as regulations are in place with respect to permissible acrylamide monomer content for specified uses of polyacrylamide.

Health hazard information

Health hazard information and hazard statements were deemed inadequate (I) for products that did not contain information on levels of residual acrylamide monomer, unless they specifically addressed the potential for carcinogenicity/mutagenicity of residual acrylamide monomer. It is interesting to note that trace levels of other carcinogenic chemicals (e.g. styrene and formaldehyde) were often identified in MSDS without reference to acrylamide. Other MSDS, although addressing the quantity of residual acrylamide in the polymer, provided an incorrect hazard statement.

Precautions for use

The majority of MSDS either reported an incorrect exposure standard for acrylamide or did not report the standard.

Overall, the information on personal protective equipment was considered satisfactory, although in many cases it was not clear when particular PPE (e.g. respirator) should be worn.

Safe handling information

Handling of spills and disposal were also adequately addressed, however there was a general lack of consistency between MSDS in procedures recommended.

15.2.2 Labels

Under the NOHSC *National Model Regulations* (NOHSC 1994) and NOHSC *National Code of Practice for the Control of Workplace Hazardous Substances* (NOHSC 1994a) and the corresponding State and Territory legislation, suppliers are required to provide labels in accordance with the NOHSC *Code of Practice for*

the Labelling of Hazardous Substances (NOHSC 1994) for all hazardous substances.

Labels submitted for assessment were assessed for requirements under this Code. The assessment took the form of a qualitative appraisal, which included the following categories of information:

- substance identification;
- hazard category/signal word;
- ADG Code classification/packaging group;
- risk information (or phrase)[1];
- safety information (or phrase)[2];
- information on spills/leaks or fires; and
- reference to MSDS.

[1]Risk phrases resulting from this priority existing chemical assessment are provided in Section 12. The labels provided were assessed against those appearing in the *List of Designated Hazardous Substances* (NOHSC 1999a).

[2]Safety phrases recommended for acrylamide in the NOHSC *List of Designated Hazardous Substances* (NOHSC 1999a) are:

S45 – *In case of accident or if you feel unwell, seek medical advice immediately (show label whenever possible)*

S53 – *Avoid exposure – obtain special instructions for use*

The NOHSC *List of Designated Hazardous Substances* (NOHSC 1999a) also prescribes the 'Note D'. Note D requires the manufacturer or importer/supplier to include the words 'non-stabilised' on the label to warn of susceptibility to spontaneous polymerisation where appropriate e.g. acrylamide 50% solution (non-stabilised).

A total of 10 labels were provided for acrylamide and/or acrylamide co-monomer products. All labels contained the concentration or range of acrylamide in product, correct risk phrases or hazard information and reference to the suppliers MSDS. Only one label contained the correct Hazard category/signal word, ADG Code classification/packaging group or first aid information. None of the labels complied with the safety phrases prescribed in the List, however safety precautions included were considered adequate. It was noted that where acrylamide contained an inhibitor, this was stated on the label, but where it didn't, the words 'non-stabilised' were not included as stipulated under NOHSC regulations. Most labels contained adequate information on spills.

A total of 11 labels were provided for polyacrylamide and/or co-polymer products. All labels contained safety precautions and first aid instructions and most contained spillage instructions. Only one label contained the concentration or range of acrylamide in product and only three labels contained information on ingredients, risk phrases or hazard information. Two labels contained ADG Code information, but this may not be applicable to other products (i.e. not classified in ADG Code).

15.2.3 Education and training

Guidelines for the induction and training of workers exposed to hazardous substances are provided in the National Commission's *National Model Regulations for the Control of Workplace Hazardous Substances (NOHSC 1994)* (the Model Regulations). Under these regulations, employers are obliged to provide training and education to workers handling hazardous substances.

The Model Regulations stipulate that training and induction should be appropriate for the workers concerned. It is important that each workplace implement a program that is suitably designed to accommodate the needs of different workers.

It is important that training be given to the workers at induction and repeated at regular intervals to reinforce the information. Review of training and education needs for workers on a regular basis is useful.

For acrylamide, induction programs address:

- acute and potential chronic health effects of acrylamide;
- sublimation of acrylamide;
- potential for exothermic polymerisation;
- lung and skin absorption potential of acrylamide;
- explanation of MSDS and labelling for acrylamide and acrylamide polymers;
- correct selection, use and maintenance of personal protective equipment;
- emergency procedures;
- basic plant operation;
- procedures to be followed for cleaning tanks and lines prior to maintenance;
- chemistry of monomers; and
- quality assurance.

Information obtained for assessment indicates that very few polymer manufacturers have written instructions or formal training for workers. Those that did provided comprehensive training, which was ongoing. Ongoing training reinforces what was taught at initial induction training and also covers areas of:

- quality assurance;
- general site training;
- PPE;
- hazard awareness;
- product applications;
- emergency plans;
- computer skills; and

- first aid.

External training is also utilised in addition to in-house instruction.

The Cytec product stewardship manuals provided with supply of acrylamide monomers (see above) also give recommendations on handling, methods of analysis, medical surveillance and emergency procedures. Some polymer manufacturers also provide written training manuals.

15.3 Occupational monitoring and regulatory controls

15.3.1 Monitoring

Both personal and static monitoring of acrylamide in air is carried out at a number of plants manufacturing polyacrylamide in Australia. Current personal and static air monitoring data for facilities handling or using acrylamide are reported in Section 8 of this report.

In order to correctly assess the risks posed to workers from acrylamide, airborne concentrations should be determined by sampling in the breathing zone of the worker.

Dermal monitoring has been carried out overseas using body pads and glove wipe techniques. The usefulness of such monitoring as a routine control measure for exposure scenarios where little dermal exposure has been identified is limited.

Although not yet used routinely, a considerable amount of work has been carried out on developing biomonitoring techniques for assessing exposure to acrylamide, in particular the analysis of acrylamide-haemoglobin adducts in blood and metabolites in urine. Validation of methodology for urine and blood analyses is currently being undertaken by HSE in UK in conjunction with Ciba. For further details see Section 15.3.3.

15.3.2 Exposure standards

According to the NOHSC *Exposure Standards for Atmospheric Contaminants in the Occupational Environment* (NOHSC, 1995), exposure to Category 2 carcinogens such as acrylamide should be minimised to the lowest practicable levels and a program of routine air monitoring should be implemented to ensure the effectiveness of relevant control measures.

The current national occupational exposure standard for acrylamide in Australia is 0.03 mg/m^3 expressed as an 8 hour TWA airborne concentration with a 'skin notation'. The standard was adopted from documentation developed by the American Conference of Governmental Industrial Hygienists (ACGIH, 1986, 1991). The basis for the standard was the consistent production of tumours in animals and the suspicion of cancer in humans raised by epidemiological studies. The skin notation implies that special measures are required to prevent absorption through the skin as the national standard only considers absorption via inhalation.

Historically a 8 h TWA exposure limit of 0.3 mg/m^3 was originally derived by the American Conference of Governmental Industrial Hygienists (ACGIH) for acrylamide as early as 1966, based on its central nervous system toxicity in animals. Subsequent to this determination, the TWA for acrylamide was re-

evaluated in the 1980's based on new data that indicated it had carcinogenic activity in rats and could be absorbed through the skin. The 8 hour TWA exposure limit of 0.03 mg/m^3 was derived from the available carcinogenicity data.

In Australia, there is no short-term exposure limit (STEL) for acrylamide. However, according to the NOHSC Exposure Standards (NOHSC, 1995a), a process is not considered to be under reasonable control if short-term exposures exceed three times the TWA exposure standard for more than 30 minutes per 8 hour working day, or if a single short-term value exceeds five times the TWA exposure standard.

Rohm and Haas Australia recommend an exposure standard for acrylamide of 0.03 mg/m^3 8 hour TWA with a STEL of 0.09 mg/m^3.

Current occupational exposure standards for acrylamide in Australia and other countries are summarised in Table 15.1.

Table 15.1 - National occupational exposure standards for acrylamide

	Exposure limit		
Country	**8 h TWA**	**STEL**	**Year Adopted**
Australia[SK]	0.03 mg/m^3	-	1993
Belgium[SK]	0.3 mg/m^3	-	1993
Canada[SK]	0.03 mg/m^3	0.6 mg/m^3	
Denmark[SK]	0.03 mg/m^3	-	1999
Finland	0.3 mg/m^3	0.9 mg/m^3	1993
France	0.3 mg/m^3	-	1993
Hungary[SK]	-	0.3 mg/m^3	1993
Ireland[SK]	0.3 mg/m^3	-	1997
Israel	0.03 mg/m^3	-	
Japan[SK]	0.3 mg/m^3	-	1999
Mexico	0.3 mg/m^3	0.6 mg/m^3	
Netherlands[SK]	0.3 mg/m^3	-	1993
Norway	0.3 mg/m^3	-	1999
Philippines	0.3 mg/m^3	-	1993
Poland	0.1 mg/m^3	-	1998
Russia	-	0.2 mg/m^3	1993
Sweden	0.03 mg/m^3	0.1 mg/m^3	1999
Switzerland[SK]	0.03 mg/m^3	-	1999
United Kingdom[SK]	0.3 mg/m^3	0.6 mg/m^3	2000
USA (NIOSH)[SK]	0.03 mg/m^3	-	1997
USA (OSHA)[SK]	0.3 mg/m^3	-	1989

NIOSH = National Institute of Occupational Safety and Health (recommended limits)
OSHA = Occupational Safety and Health Administration (statutory limits)
STEL = short-term (15-min) exposure limit
TWA = time-weighted average
[SK] = with skin notation

No biological exposure standard (BEI) has been set for acrylamide in Australia or overseas.

15.3.3 Health surveillance

Acrylamide is not listed in schedule 3 of the NOHSC Model Regulations (NOHSC 1994) as a substance requiring health surveillance. However, in accordance with NOHSC regulations, employers have a responsibility to provide health surveillance to those workers where exposure to a substance may lead to an identifiable disease or adverse health effects. Adverse effects have been reported in workers exposed to acrylamide.

Some polymer manufacturers provided details of active medical surveillance whilst other did not. Where detailed, surveillance consisted of pre-placement and/or annual medical examinations for potentially exposed workers. Some facilities handling acrylamide provide weekly medical examinations consisting of checks of hands/feet for signs of skin peeling, excessive sweating, muscle tremor and impaired touch or vibration sense, all of which are early indicators of neurological effects.

Other methods of health surveillance for acrylamide neurotoxicity reported in the literature include electrophysiological measurements of nerve conduction velocity and vibration sensation in toes/fingers. The most sensitive conduction velocity test is reported to be reduction in nerve action potential amplitude in distal sensory nerves. For these tests, pre-exposure baseline studies are required for individual workers for comparison purposes.

Techniques have also been developed for biological monitoring of acrylamide metabolites in blood and urine (see Section 6). Given the persistence of haemoglobin adducts of acrylamide in red blood cells, considerable effort has gone into developing monitoring techniques and validating this biomarker for predicting acrylamide-induced peripheral neuropathy. In a recent study, a clear-cut dose response relationship was found between haemoglobin (Hb) adduct levels and symptoms of peripheral neuropathy in a population of over 200 workers using acrylamide grouts for a period of up to 2 months. In this study, a normal background range of haemoglobin adducts was determined in the range 0.02 - 0.07 nmol/g globin. Around 40% of workers with haemoglobin adduct levels exceeding 1 nmol/g globin experienced tingling and numbness of hands/feet, with a NOAEL determined at 0.51 nmol/g globin. Workers with Hb-adduct levels exceeding 0.3 nmol/g globin attended follow-up examination at 6, 12 and 18 months after exposure cessation, which included 23 workers with strong evidence of PNS impairment. In almost all cases, PNS symptoms were found to be reversible (Hagmar et al 2001).

Validation of biomonitoring methods for urinary metabolites (mercapturic acids) and haemoglobin adducts is currently being undertaken in trials in UK in a collaborative program between Ciba and UK HSE (Ciba submission 2000).

15.3.4 Transportation regulations

Acrylamide is imported in 25 kg paper bags, 20L steel drums and 1000 L IBCs.

Under the ADG Code, acrylamide (UN Number 2074) is classified in Class 6.1, Packing Group III (FORS, 1998). Class 6.1 comprises toxic substances. Acrylamide is assigned Packing Group III because of its low level of acute toxicity.

The ADG Code sets out various requirements relating to the transport of acrylamide by road or rail.

Drums and bags must be labelled with the proper shipping name of the dangerous goods, the UN Number, the class label and the name and address in Australia of the manufacturer, consignor or their agent (Clause 7.2.3).

IBCs must be placarded with class label 6.1 ('toxic') and an Emergency Information Panel containing additional information such as the Proper Shipping Name of the chemical ('acrylamide'), its UN Number, Hazchem Code and the name and telephone number of the consignor of the goods (Division 7.5). The Hazchem Code for bulk loads of acrylamide is 2WE. The Code reflects the initial emergency response recommended in case of fire, leakage or spillage. The number '2' indicates that water fog should be used for firefighting. In the absence of fog, a fine spray may be used. The letter 'W' means that there is a risk of violent reaction or explosion, that emergency personnel should wear full protective clothing (breathing apparatus, protective gloves, appropriate boots and a chemical splash suit) and that any spillage should be contained so as to prevent the chemical from entering drains or watercourses. The letter 'E' denotes need for consideration of evacuation of people from the neighbourhood of an incident.

IBCs must be designed according to the requirements of the ADG Code and approved for use (Clause 4.6.1).

The ADG Code prescribes that an IBC must be inspected prior to filling and details requirements for filling, loading and methods of restraining an IBC on a vehicle (Clause 4.6.3).

Design and performance-testing requirements for bags and drums are given in Chapter 3 of the ADG Code.

The ADG Code also contains detailed provisions for the inner packaging and marking of packages containing small quantities (less than 500 ml if liquid or 500 gm if solid) of the dangerous goods, such as bottles of reagent grade acrylamide distributed by road or rail.

Exemptions to the labelling requirements of IBCs transported in freight containers (Clause 7.5.6) and pallet loads of bags or drums (Clause 7.3.5), are also provided in the AGD Code.

15.4 Public health regulatory controls

Acrylamide occurs as a minor impurity in polyacrylamide. It may be present in drinking water through the use of polyacrylamides as flocculant aids in water treatment and through the use of grouting agents containing acrylamide. Overseas studies have reported concentrations of up to a few micrograms per litre in drinking water, but none has been detected in Australian drinking water.

According to the *Australian Drinking Water Guidelines* (NHMRC 1996), the concentration of acrylamide in drinking water should not exceed 0.0002 mg/L, based on health considerations.

15.5 Environmental regulatory controls

Users of acrylamide must abide by States and Territory Regulations.

15.5.1 Disposal and waste treatment

Two companies provided detailed acrylamide disposal information.

Company 1

Residues from acrylamide containers are either dried out or chemically fixed before being taken to approved landfill. This accounts for approximately 120 L of acrylamide annually.

When bulk storage tanks require cleaning, the acrylamide in the tank is assessed to determine if it can be used in products or resold. If neither of these is possible, it is disposed of by incineration or dried and disposed of to approved landfill. If the acrylamide waste is to be landfilled, it will be chemically treated to produce a stable polymer.

At the site, all processing and handling areas are bunded with drains going to the effluent treatment plant. The company has indicated that approximately 60000 kL/year of effluent is released from the plant. The concentration of acrylamide in the effluent is less than 1 mg/L, which equates to less than 60 kg of acrylamide per year.

Approximately 0.2 kg of acrylamide will be released to air via tank filling and spills.

Company 2

Imported solid acrylamide comes in paper bags with plastic liners. The empty bags, with minimal residue acrylamide, are placed in a double plastic bag and when this is full it is sealed and disposed of to landfill.

Polyacrylamide (liquid) that does not meet manufacturing specifications is generally reworked to meet the specifications. If this is not possible, then it is disposed of. This entails the solidification of the material on-site then disposal to landfill or transfer to an approved off-site trade waste treatment plant. Before the waste polyacrylamide leaves the site, it is tested to ensure that the level of residue acrylamide is less than 0.1%. Any waste acrylamide monomer is reacted with catalyst to produce polyacrylamide then disposed of to an off-site waste treatment plant.

At one site, equipment washwater is diluted to between 20 and 1000 ppm and then disposed of the sewer under a trade waste agreement with council. At another site, liquid wastes containing acrylamide are polymerised and diluted (<0.2% acrylamide) before being sprayed over a designated, monitored on-site grassed area to allow natural biodegradation.

At both sites, any spills of acrylamide are treated with sand or other inert filler, collected and disposed of to landfill.

Air emissions are minimised by the use of water scrubbers, with the subsequent scrubber water generally being recycled into the acrylamide circuit.

15.6 Emergency procedures

Recommendations for dealing with spills involving dry crystals and aqueous solutions are provided in MSDSs and are the similar for both forms and state:

- isolate the spill or leak area immediately;
- do not touch damaged containers unless wearing appropriate PPE;
- wear approved full facepiece, positive pressure, self-contained breathing apparatus, two piece PVC suit with hood or PVC coveralls with hood, impervious rubber or plastic gloves (e.g. neoprene, polyethylene, PVC) and rubber or neoprene boots;
- spills of acrylamide to be promptly removed;
- for crystalline acrylamide, use vacuum cleaner equipped with charcoal exhaust scrubber or hose from mechanical exhaust ventilation system, or if unavailable, sweep up spills and place in a covered waste disposal container;
- cover spills of solution with an inert absorbent material. Sweep up and place in a waste disposal container. Do not permit liquid spill to dry;
- after vacuuming or sweeping up spills, flush spill area with plenty of water to a chemical sewer;
- in the event that acrylamide crystals begin to polymerise, isolate the area and use water fog or spray to control vapours, if possible without wetting product. Take defensive action only. Let the reaction run its course. Once the reaction is complete, normal precautions for acrylamide should be followed; and
- if a spill partly polymerises, the resulting acrylamide polymers formed are very slippery when wet. The spill area should be re-cleaned if slipperiness remains.

Recommendations for fighting fires involving dry crystals and aqueous solutions are provided in MSDSs and are the same for both forms and state:

- for small fires, use water spray, carbon dioxide or dry chemical to extinguish fires;
- for large fires , use water spray or fog or dry chemical;
- move containers from fire area if it can be done without risk;
- dike fire control water for later disposal; do not scatter the material;
- fight fire from maximum distance or use unmanned hose holders or monitor nozzles;
- cool containers with flooding quantities of water until well after fire is out;

- if a truck, rail car, shipping container or other large quantity of acrylamide is involved in a fire, isolate for 800 metres in all directions and consider initial evacuation for 800 metres in all directions; and
- in the event that the fire or heat causes acrylamide to begin to polymerise, isolate the area, use water fog or spray to control vapours if possible without wetting product. Take defensive actions only. Let the reaction run its course. Once the reaction is complete, normal precautions for acrylamide should be followed.

Site-specific procedures provided by polymer manufacturers are:

- sweep up powder spills;
- react liquid spills with catalyst to allow polymerisation and flush residue to site effluent treatment plant;
- for liquids, barricade area and contain the spill with inert material. Ventilate if in a confined area. Transfer contaminated materials to designated waste containers and label for disposal; and
- after donning correct PPE, seal torn bags of crystals with masking tape.

In the event of an uncontrolled polymerisation reaction occurring, the following are undertaken:

- introduce air to the reaction vessel;
- evacuate if temperature reaches 65^0 C; and
- monitor reaction temperature remotely.

16. Discussion and Conclusions

16.1 Importation and use

In Australia, acrylamide monomer is used mainly in the manufacture of polymers for a variety of applications, including water and waste (sewage) treatment, mining and mineral processing, paper and textile processing and as ingredients in surface coatings (paints and resins) and adhesives. Acrylamide monomer is also used to prepare electrophoresis gels for laboratory applications, such as amino acid separation and DNA analysis. Acrylamide is not manufactured in Australia and is imported in either solid or aqueous forms, either as the pure monomer or as a co-monomer mixture (i.e. with other monomers such as styrene, N-methylolacrylamide and other acrylamide analogues).

The concentration of acrylamide in polyacrylamides varies according to intended use. For the majority of applications, levels are kept to below 0.1%. Highest levels are reported for polymers used in surface coatings and adhesives with up to 2% monomer. Acrylamide monomer in polyacrylamide manufactured for use in drinking water treatment is regulated at <0.05%.

Occupational and environmental exposure may occur from any of the above uses and also during transportation and disposal. Exposure to the general public may occur from drinking water and from residual monomer in imported cosmetic products and possibly from food packaged in polyacrylamide containing materials. Levels of acrylamide blood adducts are also higher in smokers.

16.2 Health hazards

Limited information on toxicokinetics of acrylamide in humans is available. Animal studies indicate that acrylamide is readily absorbed by oral, dermal and inhalation routes. Absorbed acrylamide is distributed rapidly and throughout the body. Acrylamide is metabolised via conjugation with glutathione and via oxidative metabolism to the epoxide metabolite, glycidamide. Glutathione conjugation of glycidamide also occurs. Between 50 and 70% of administered dose is eliminated in 24hours, with the majority via urinary route and a small amount in faeces. Studies show that acrylamide and glycidamide bind to DNA, RNA and a wide range of proteins. In addition, both acrylamide and glycidamide form adducts with blood haemoglobin.

The critical adverse effect in humans and in animals from acute and repeated exposure is neurototoxicity, with effects being seen in CNS and PNS. Chronic oral studies in rats indicate that acrylamide is clearly carcinogenic, although no firm conclusions regarding carcinogenicity in humans could be drawn from available epidemiological studies. Similarly, no conclusions could be drawn regarding the mode of action for tumourigenicity in animals and relevance to humans, as evidence indicates both genotoxic and epigenetic effects.

Acrylamide has also been shown to have effects on fertility in rats and mice and to be a skin irritant and sensitiser.

Currently, acrylamide is listed on the NOHSC *List of Designated Hazardous Substances* (NOHSC, 1999a) as both a carcinogen and mutagen, Category 2. In addition, it is classified as *toxic by skin contact and if swallowed* and *toxic: danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed*. Based on the assessment of health effects in this report, additional classifications are to be recommended to NOHSC. These are *irritating to eyes and skin; may cause sensitisation by skin contact* and *may impair fertility.* In addition, it is recommended that the classifications *toxic by skin contact* and *toxic: danger of serious damage to health by prolonged exposure through inhalation and in contact with skin* be amended to *harmful by skin contact* and *harmful: danger of serious damage to health by prolonged exposure through inhalation and in contact with skin*, respectively.

This classification is consistent with that proposed by EU in their recent risk assessment of acrylamide (EU 2000), except that EU has recommended use of *Harmful by inhalation* for acute effects and use of *toxic* as opposed to *harmful* for the classification*: danger of serious damage to health by prolonged exposure through inhalation and in contact with skin*. In this regard, NICNAS considered that it was only appropriate to classify acrylamide as harmful by inhalation for repeated exposures, due to the lack of acute (LD50) and repeat-dose animal inhalation data and limited human exposure data. With regard to dermal effects from prolonged exposure, the available toxicological LOAEL data indicate classification as harmful and not toxic.

16.3 Occupational health and safety

Occupational exposure to acrylamide may arise from polymer manufacture, preparation of electrophoresis gels, and from residual monomer during use of polymers. Occupational risks were characterised based on potential for neurotoxic effects. Exposures of most concern were associated with polyacrylamide production and grout application. Risks were significantly higher where solid (crystals) acrylamide was used and where manual handling was evident in the process. During manufacture of solid polymers, high risks were identified during transfer of polymer gel and during cutting (extruction) of polymer. Installation of automated debagging equipment and isolation and enclosure of the extruction process have been shown to significantly reduce exposure risks during polymer manufacture.

Although limited data were available for dermal exposure, available data indicate that skin absorption may be significant even when protective clothing (e.g. gloves) is used.

No information was provided on use/exposure from acrylamide-containing grouts. Overseas monitoring data indicate risks are high for workers involved in grouting operations, largely due to potential for skin absorption. Because of health problems experienced in EU and US in grout workers, considerable effort is currently going into development of risk reduction practices. In 1991, the US EPA proposed a ban on acrylamide and NMA grouts (US EPA, 1991), which was subsequently withdrawn, and similar consideration has been given to prohibition by EU (EC 2001).

A risk reduction strategy currently being considered by the EU covers use of acrylamide grouts, polymer manufacture and electrophoresis gel preparation. A

number of alternative chemicals were identified by the EU for grout and electrophoresis applications, but little data was available on their suitability for specific uses. The assessment of alternative substances for acrylamide is beyond the scope of this report and selection by industry should involve a full hazard evaluation. EU reports that a number of companies in Europe have switched to alternative grouts over the past few years. Some acrylamide co-monomers used in polymer production, and acrylamide related monomers, are also known to cause neurotoxic effects (e.g. NMA) and some are known to form acrylamide on degradation. It should not be assumed therefore that using these monomers confers a greater degree of safety than acrylamide per se.

Overseas monitoring data indicate that use of acrylamide in preparation of electrophoresis gels may not provide adequate margins of exposure for some methods of preparation. The EU risk reduction strategy recommends use of pre-weighed packs of acrylamide in either solid or aqueous form or pre-cast gels to avoid exposures during weighing operations.

Risks from occupational use of polyacrylamide products were considered low due to the low levels of residual polymer and the fact that in most cases, polymer products are diluted for use.

In summary, until risks from skin absorption are better characterised, all current uses of acrylamide should be considered as high-risk operations/practices.

The NOHSC occupational atmospheric exposure standard of 0.03 mg/m^3 is considered to provide a sufficient margin of exposure with regard to acrylamide inhalation. It is considered that a review of the exposure standard by the National Occupational Health and Safety Commission is not warranted at this stage. The standard, adopted from ACGIH, is among the lowest set internationally. As contribution to overall exposure from dermal exposure is not well characterised for any occupational scenario and in view of the fact that acrylamide is classified as a Category 2 carcinogen, it is considered that exposures should be kept as low as practicably possible. From the monitoring data provided, it appears that OHS control measures are available to control exposures to the current exposure standard, although certain processes in polymer manufacture indicate excursion of around an order of magnitude higher.

Because of concerns over dermal absorption and the adequacy of atmospheric monitoring as an indicator of overall worker exposure, ongoing efforts overseas are focussing on developing a biological exposure index (BEI). Methods based on urinary glutathione metabolites and haemoglobin adducts are currently undergoing validation.

Provision of adequate hazard information is considered central to occupational risk reduction. The MSDS and labels for Australian acrylamide monomer and polymer products were generally considered to be adequate. However, these will need to be updated to reflect additional hazards identified in this assessment, which are not currently included in the NOHSC *List of Designated Hazardous Substances* (NOHSC 1999a).

It is considered that information on the amount of residual monomer in polyacrylamide products, which was lacking in many cases, should be included on labels and MSDS, even if below the current concentration-cut off level.

16.4 Environment

The main use of acrylamide in Australia is in the production of polymers. Fugacity modelling (Section 8.1.2 Distribution) indicates that more than 99% of any acrylamide released to the environment will partition to water. Due to its high water solubility and moderate volatility, it is unlikely to be removed from water but will undergo dilution and biodegradation. Whilst acrylamide may be taken up in fish, it is also eliminated in its unchanged form. The likelihood of accumulation is low.

In this assessment, grouting use has not been considered due to the lack of Australian use and exposure information available. However, the OECD Program determined that the chemical was a candidate for further work as follows:

"National or regional exposure information gathering and risk assessment may need to be considered for grouting applications based on an existing regional risk assessment for Europe".

The environmental assessment undertaken in this report is based on the release of acrylamide monomer. Concerns with respect to the use of polyacrylamide flocculants, as outlined in Section 7.2, are considered to be outside the scope of this assessment.

There is currently no environmental hazard classification system in Australia. In accordance with the OECD Harmonised Integrated Hazard Classification System for Chemical Substances and Mixtures, acrylamide would be classified in the Acute III Class (OECD 2001).

The examination of environmental risk (Section 14.1) indicates that the risk level for aquatic organisms and wastewater treatment plant micro-organisms is less than 1 and therefore negligible. Therefore, the expected low levels of acrylamide released are not expected to result in adverse effects on aquatic organisms.

The current waste disposal methods used in Australia are adequate. Generally this entails the polymerisation of the acrylamide to polyacrylamide with less than 0.1% residual acrylamide.

While acrylamide is on the list of NPI chemicals to be reported, information on the amounts released will not be available until January 2003.

16.5 Public health

The public health assessment concluded that a potential exists for contamination of drinking water by acrylamide-containing grouts, but risks could not be characterised due to lack of exposure data. The use of polyacrylamide containing <0.05% monomer and dosed at 1 mg/L as prescribed for drinking water treatment is not considered to present a significant hazard to public health. Based on available overseas data, the use of polyacrylamides at 2% or less in consumer products does not present a significant hazard to public health.

Polyacrylamide is used extensively in cosmetics and is present in some food packaging materials. Overseas regulations limit the amount of acrylamide monomer in polymers used for cosmetics and food packaging, however acrylamide is not currently listed in the Australian Food Standards Code or the SUSDP. No

data on levels of acrylamide in Australian cosmetics and food products or food packaging materials were available for assessment.

16.6 Data gaps

This report identifies a number of gaps in the available information for acrylamide. These include:

- dermal exposure monitoring data (i.e. to provide estimates of total intake);
- Australian exposure data (particularly personal exposure monitoring) for certain occupational scenarios;
- validated biological monitoring technique(s);
- data on levels of acrylamide in cosmetic and food products in Australia;
- knowledge of mechanism of action for carcinogenicity (i.e. whether certain tumour types seen in rats are relevant to humans);
- a formal (with cross-fostering) lactation study; and
- data on human metabolism/toxicokinetics.

17. Recommendations

This section provides the recommendations arising from the priority existing chemical assessment of acrylamide. Recommendations are directed at regulatory bodies and users (employers and employees) of acrylamide and polyacrylamide products. Implicit in these recommendations is that best practice is implemented to minimise occupational and public exposure and to minimise environmental impact.

Recommendation 1: Use of acrylamide-containing grouts

Based on data provided in the EU risk assessment report, concerns are expressed with regard to occupational, public and environmental health from the use of acrylamide (including NMA) containing grouts. However this assessment was unable to identify any remaining grout use in Australia and therefore recommendations to control this use are not necessary at this time. Should acrylamide-based grouts be introduced/used in Australia in future, section 64 of the Industrial Chemicals (Notification and Assessment) Act requires notification to NICNAS (refer to Section 18 of this report).

Recommendation 2: Hazard classification

In accordance with the NOHSC Approved Criteria (NOHSC 1999), acrylamide is classified as:

- R21 Harmful in contact with skin
- R25 Toxic if swallowed
- R48/25 Toxic: danger of serious damage to health by prolonged exposure if swallowed
- R48/20/21 Harmful: danger of serious damage to health by prolonged exposure through inhalation and in contact with skin
- R36/38 Irritating to eyes and skin
- R43 May cause sensitisation by skin contact
- R45 (Cat 2) May cause cancer
- R46 (Cat 2) May cause heritable genetic damage
- R62 (Cat 3) Possible risk of impaired fertility

It is recommended that this classification be adopted by NOHSC according to the usual process for updating the *List of Designated Hazardous Substances* (NOHSC 1999a).

In line with this revised classification, it is recommended that the concentration cut-off data in the *List of Designated Hazardous Substances* (NOHSC 1999a) also be amended as follows:

Conc $\geq$25%: T; R25; R21; R36/38; R43; R45; R46; R48/25, R48/20/21; R62

≥20% Conc <25%; T; R22; R36/38; R43; R45; R46; R48/25, R48/20/21; R62

≥10% Conc <20%: T; R22; R43; R45; R46; R48/25; R48/20/21; R62

≥5% Conc <10%: T; R22; R43; R45; R46; R48/22; R62

≥3% Conc <5%: T; R22; R43; R45; R46; R48/22

≥1% Conc <3%: T; R43; R45; R46; R48/22

≥0.1% Conc <1%: T; R45; R46

Consequent to the above classification, it was considered that the safety phrases prescribed in the *List of Designated Hazardous Substances* (NOHSC 1999a) adequately reflect the overall hazards. These are:

- S45 In case of accident or if you feel unwell, seek medical advice immediately (show label whenever possible)
- S53 Avoid exposure – obtain special instructions for use.

Recommendation 3: Hazard communication

Material Safety Data Sheets

This assessment found that compliance with MSDS requirements was generally satisfactory. It is recommended that particular attention be paid to the following:

- risk phrases and hazard information should be updated to reflect the hazard classification in Recommendation 1 (not necessary for polymer products containing <0.1% acrylamide);
- MSDS for polymer products contain information on concentration of residual acrylamide monomer;
- the NOHSC atmospheric exposure standard be included in MSDS for acrylamide;
- handling of spills and disposal be consistent with information provided in Recommendation 9.

A sample MSDS for acrylamide is provided at Appendix 1.

Labels

This assessment found that compliance with labelling requirements was generally satisfactory. It is recommended that particular attention be paid to the following:

- inclusion of Hazard Category (Toxic) or Signal word (Hazardous) for acrylamide products and polymers containing >0.1% acrylamide;
- inclusion and updating of risk phrases to reflect the hazard classification in Recommendation 1 (not necessary for polymer products containing <0.1% acrylamide);

  inclusion of NOHSC recommended safety phrases (S45 and S53) for acrylamide products;

- inclusion of First Aid information (in addition to S45);
- use of the word "Non-stabilised" in acrylamide products which do not contain a polymerisation inhibitor;
- handling of spills and disposal consistent with information provided in Recommendation 9.

For labels of acrylamide products used for the preparation of polyacrylamide gels for laboratory application (e.g. electrophoresis and chromatography), it was considered that inadequate instruction for safe use appeared in MSDS and labels for such applications. It is recommended that additional instruction be included as follows:

- particular care should be taken when handling this chemical, both in powder and liquid form;
- when using acrylamide, wear gloves and safety glasses at all times;
- weigh and decant dry powder in a FUMEHOOD and wear a facemask;
- never mouth pipette acrylamide solutions;
- promptly wash spilt acrylamide off skin with soap and copious amounts of water. Remove contaminated gloves and clothing immediately.

It is recommended that State/Territory occupational health and safety authorities monitor compliance with these recommendations to improve industry performance.

Recommendation 4: Workplace control measures

Where and when safer alternatives have been identified for intended uses, it is recommended that use of acrylamide/polyacrylamide products be phased out. Some acrylamide co-monomers, and acrylamide related monomers used in polymer production are also known to cause neurotoxic effects (e.g. NMA) and should similarly be considered as candidates for substitution, rather than as possible alternatives for acrylamide. Because higher airborne exposures are associated with use of acrylamide solid, it is recommended that where possible, monomer solutions or gels are used in place of solid/crystalline products.

Attention should be paid to areas where risk of exposure to acrylamide has been identified as high (i.e. debagging and maintenance/cleaning) during workplace assessment and monitoring. Engineering controls should be implemented where possible to reduce exposure. In particular:

- weighing and decanting of acrylamide solid (powder) for electrophoresis gel preparation should take place using a fumehood/cupboard;
- automated bag removal (debagging) equipment for handling solid acrylamide;
- isolation/enclosure of equipment used for processing of solid polyacrylamide e.g. gel cutting (extrusion) equipment;
- local exhaust ventilation at locations where airborne acrylamide has been detected e.g. debagging area, mixing vessel charging point, monomer make-up units, polymerisation vessels and gel processing machinery.

Engineering controls should be supplemented by safe work practices and the use of PPE.

The current NOHSC standard for acrylamide in air is 0.03 mg/m^3. A 'skin notation' indicates that special measures are required to prevent skin absorption (NOHSC 1995).

Although this standard is considered to provide a sufficient margin of exposure (approximately 50) with regard to neurological and other non-neoplastic effects from inhalation exposure, it should be noted that because the extent of skin exposure has not been well characterised for certain occupational scenarios, overall MOEs might be significantly lower. In addition, it is, at present, not possible to reliably estimate the risk of carcinogenic effects from exposure to acrylamide.

Therefore, it is recommended that exposure to acrylamide be minimised to the lowest practicable levels.

Recommendation 5: Air monitoring

Routine air monitoring is recommended for acrylamide to ensure the effectiveness of relevant control measures. In order to correctly assess the risks posed to workers from acrylamide, airborne concentrations should be determined by personal monitoring i.e. by measuring the concentration in the breathing zone of the worker.

For estimation of TWA exposures, it is recommended that such monitoring should employ at least the minimum sampling times as recommended by NOHSC (NOHSC 1995). The frequency of routine air monitoring will depend on the results obtained and can be reduced once it has been established that levels do not exceed the NOHSC exposure standard and/or once other complimentary monitoring techniques for assessing worker exposure (e.g. biological monitoring) are available in the workplace.

Because levels of exposure and the current standard are relatively low, sampling and analytical methods of appropriate sensitivity should be employed.

It has been suggested that air sampling using silica gel tubes to collect acrylamide does not pick up dust particles, only vapour, and that in addition to silica gel tubes a separate filter should also be used. Noting the OSHA method described in the report utilises a separate filter it is recommended that organisations using air sampling through silica gel tubes only, validate the method to ensure that it will measure both dust and vapour concentrations of acrylamide.

Recommendation 6: Health surveillance

Although exposure to acrylamide may be controlled by engineering controls, safe work practices and the use of PPE, risks for certain workplace scenarios, such as debagging and maintenance/cleaning are high. In addition, because of the significant contribution to risks from dermal exposure, reliance on air monitoring in the assessment of worker exposure is a matter of concern.

Current health surveillance techniques employed for acrylamide workers in Australia are aimed at early identification of symptoms of neurotoxicity. Techniques employed are variable with respect to sensitivity in detecting adverse effects.

It is therefore recommended that NOHSC give consideration to the establishment of formal health surveillance guidelines for acrylamide. In addition to an assessment of available surveillance techniques, such as nerve conduction velocity and vibration sensation measurement, it is recommended that NOHSC consider the setting of a biological exposure standard (BEI) aimed at prevention of acrylamide-induced neurotoxicity. Current techniques for measurement of haemoglobin adducts in blood or metabolites (mercapturic acids) in urine should be evaluated with view to setting a health-based BEI (see Section 15.3.3). Validation of methods for biological monitoring is currently being undertaken overseas. Therefore NOHSC should monitor the progress of this work before considering the establishment of a BEI.

Any industry-based biological monitoring strategy should be targeted at those industries where there is a heavy reliance on PPE and where the potential for dermal contact is high (i.e. high risk activities).

Recommendation 7: Public health protection

Based on the potential for dermal absorption and the potential for carcinogenicity of the acrylamide monomer, it is recommended that this priority existing chemical report be forwarded to the National Drugs and Poisons Schedule Committee (NDPSC) for their consideration.

Overseas regulations exist limiting the amount of acrylamide monomer in polymers used for food packaging. No data on levels of acrylamide in Australian food products or food packaging materials were available for assessment. It is recommended that this report also be forwarded to the Australia New Zealand Food Authority (ANZFA) for their consideration.

18. Secondary Notification

Under section 64 of the Industrial Chemicals (Notification and Assessment) Act, the secondary notification of a chemical that has been assessed under the Act may be required where an applicant or other introducer (importer) of a chemical becomes aware of any circumstances which may warrant a reassessment of its hazards and risks. In the case of acrylamide, specific circumstances include

- the function or use of acrylamide has increased, or is likely to change, significantly;
- import/use of acrylamide-based grouts;
- the amount of acrylamide introduced into Australia has increased, or is likely to increase significantly;
- manufacture of acrylamide has begun in Australia; or
- additional information has become available to the applicant/notifier as to the adverse health and/or environmental effects of acrylamide.

The Director (Chemicals Notification and Assessment) must be notified within 28 days of the manufacturer/importer becoming aware of any of the above or other circumstances prescribed under section 64(2) of the Act.

Appendix 1

Sample Material Safety Data Sheet for Acrylamide

Date of issue		Page 1	of Total 7

A crylamide is classified as hazardous according to the National Occupational Health and Safety Commission's *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(1999)].

Company Details

Company name

Address

State Postcode

Telephone number Emergency telephone number

Identification

Product Name

Acrylamide (dry crystals)

Other names

Acrylic acid amide; 2-propenamide; Propenoic acid amide; Vinyl amide; Ethylene carboxamide

Manufacturer's product code

UN Number

UN 2074

Dangerous goods class and subsidiary risk

6.1

Hazchem code

2WE

Poisons Schedule Number

None allocated

Use

Reactive monomer for production of co-monomers, polymers & co-polymers.

Physical description and properties

Appearance

White crystalline odourless solid

Boiling Point

Acrylamide polymerises above its melting point (84.5 °C) and does not boil at standard atmospheric pressure

Freezing Point

Not applicable

Vapour pressure

0.9 Pa at 25 °C

Specific Gravity

1.122 at 30 °C

Flashpoint

138 °C

Flammability Limits

Not applicable

Solubility in water

2.16 g/mL at 30 °C

Other properties

Odour: Odourless

Odour threshold: Not applicable

Density (d^{20}): 1.0329 kg/L

Vapour density: 2.46 (relative to air = 1)

Evaporation Rate: Material sublimes

Partition Co-efficient log $P_{o/w}$: Measured values range from - 1.24 to - 0.67

Explosive Limits: Not applicable, but material generates dust which may be explosive if mixed with air in critical proportions and in the presence of an ignition source. All equipment should be grounded to avoid static discharge.

Reactivity: At temperatures above its melting point, acrylamide polymerises in a rapid, highly exothermic reaction. Exposure to uv light also results in ready polymerisation.

Ingredients/impurities

Chemical entity	CAS Number	Proportion
Acrylamide	79-06-1	
Impurities		

Health hazard information

HEALTH EFFECTS

Acute

Inhalation: No data.

Skin: Irritating to skin. Redness and peeling of the skin most common complaint in workers. Harmful in contact with skin.

Eye: Acrylamide is an eye irritant in rabbits.

Swallowed: Toxic in animals by oral route. Effects reported from ingestion in humans include hallucinations, seizures, breathing difficulties, liver and nervous system effects. Nervous system effects may persist for several weeks/months following exposure.

Chronic

Inhalation: No human or animal data. However, acrylamide should be considered as dangerous to health by repeated or prolonged exposure by inhalation.

Skin: Acrylamide is dangerous to health by repeated or prolonged exposure by the dermal route. May also cause skin sensitisation. The main effects in humans and animals are on the nervous system*.

Swallowed: Acrylamide is dangerous to health by repeated or prolonged exposure by the oral route. The main effects in human and animals are on the nervous system*.

Acrylamide has been shown to be carcinogenic and to cause heritable genetic damage in animal studies.

Acrylamide has been shown to cause effects on fertility (reproduction) in animal studies.

*Acrylamide is a neurotoxicant and can affect both the central and peripheral nervous systems. Repeated exposure can cause numbness and tingling of the limbs, peeling of the skin on the fingertips, generalised fatigue, sweating on the hands and feet, muscular weakness, decreased reflexes. Such effects may persist for several weeks/months after cessation of exposure but are usually reversible.

Contraindications:

Smoking is associated with increased levels of acrylamide blood adducts in non-exposed individuals. Smoking may therefore exacerbate acrylamide toxicity.

Health hazard information (cont.)

FIRST AID

Inhalation: Remove from exposure. Keep warm and at rest until fully recovered. If breathing laboured and patient cyanotic, give oxygen. Apply artificial respiration if not breathing.

Skin: Remove and dispose of contaminated clothing. Wash immediately with copious quantities of water.

Eye: Irrigate immediately with copious quantities of water for at least 15 minutes.

Swallowed: Do not induce vomiting.

Contact a Poisons Information Centre (Phone 13 1126) or a doctor immediately.

First aid facilities: Safety shower and eye washbasin desirable.

ADVICE TO DOCTOR

Treat symptomatically. No specific antidote. As ingestion may cause central and peripheral nervous system depression, do not induce vomiting because of the danger of aspiration. Acute or chronic exposure may lead to weak or absent reflexes.

Precautions for use

EXPOSURE STANDARD

Australian (NOHSC) Exposure Standard: 0.06 ppm (0.03 mg/m^3) 8 hour TWA with skin notation.

The 'skin notation'(Sk)indicates that absorption through the skin may be a significant source of exposure.

ENGINEERING CONTROLS

Utilise an enclosed process. If enclosure is not feasible, airborne concentrations should be controlled to below the NOHSC exposure standard. Use only with adequate ventilation. Local exhaust ventilation may be necessary for some operations.

Laboratories using acrylamide should be equipped with both exhaust (mechanical) ventilation and fumehoods/cupboards.

Precautions for use (cont.)

PERSONAL PROTECTION

Avoid exposure and obtain special instructions for use of acrylamide.

Protective overalls (preferably disposable), gloves and boots (not leather) should be worn in accordance with manufacturers' recommendations. When charging mixing vessels with acrylamide crystals/powder, an enclosed air hood with a separate air supply should be used unless the operator is isolated from exposure to the substance.

Ensure good personal hygiene. All non-disposable protective clothing should be washed at the end of each work shift.

Fire fighting: wear self-contained breathing apparatus & complete protective clothing.

Safe handling information

STORAGE and TRANSPORT

Store in a cool dry place away from heat sources, ignition sources and direct sunlight. Polymerisation liberating heat can occur if the temperature of the crystals exceeds 50 ^{0}C.

Acrylamide should not be stored with or near acids, bases, vinyl polymerisation initiators, copper, aluminium, brass, bronze, oxidising and reducing agents.

Shipping name: Acrylamide

Packaging group: III

Transport label required: Toxic

Initial Emergency Response Guide: 36P

Safe handling information (cont.)

SPILLS and DISPOSAL

Crystals sublime at room temperature. Spills should be immediately swept up with personnel wearing PPE comprising PVC gloves, overalls, safety boots, glasses and an organic vapour/particulate half-face or full-face cartridge respirator. Waste should be placed in a covered waste disposal container.

Should dry crystals begin to polymerise, isolate the area and use water fog or spray to control vapours. Avoid wetting the product if possible. The reaction should be allowed to run its course and the solidified polymer disposed of to landfill.

Unused acrylamide should be polymerised prior to disposal.

FIRE/EXPLOSION HAZARD

Acrylamide crystals can generate dusts, which are flammable in air and can explode. Minimum ignition energy is 7 millijoule (850 g/m^3 dust concentration in air, moisture 0.1-0.8%, particle 0.1mm). Acrylamide may polymerise violently on melting.

Fire fighting:

- Use dry chemical, CO_2 or water fog/fine spray
- Wear SCBA and full protective clothing

Other information

Toxicological Information

Acute (oral) LD_{50}	150 - 203 mg/kg bw (rat)
Acute (dermal) LD_{50}	1148 mg/kg bw (rabbit)

Repeat dose:

Neurotoxicity	NOAEL (oral) 0.2 mg/kg bw (rat)
	LOAEL (oral) 1.0 mg/kg bw (rat)
Carcinogenicity	significant increase in tumours in various organs at 2.0 mg/kg bw (rat - oral). No NOAEL determined.
Reproductive effects	NOAEL (oral) 2.0 mg/kg bw (rat)
	LOAEL (oral) 5.0 mg/kg bw (rat)
Genotoxicity	mutagenic in in vitro and in vivo studies.

Other information (cont.)

Environmental data

Acrylamide is mainly distributed in the environment to water (>99.9%), with <0.1% in air, water and soil.

Acrylamide is moderately mobile in most soil types and is unlikely to volatilise from water at ambient temperatures.

Acrylamide is readily biodegradable, biotic & abiotic degradation occurs in water and soil.

Acrylamide has a low potential for bioaccumulation (BCF (fish) range 0.26 – 2.53)

Aquatic toxicity

Daphnia magna (48h LC_{50}) = 98 mg/L

Mysidopsis bahia (48h LC_{50}) = 78 mg/L

Bluegill sunfish (96h LC_{50}) = 100 mg/L. NOEC (96 h) = 35 mg/L.

Rainbow trout (96h LC_{50}) = 110 mg/L. NOEC (96 h) = 37 mg/L.

Classification

R21	Harmful in contact with skin
R25	Toxic if swallowed
R48/25	Toxic: danger of serious damage to health by prolonged exposure if swallowed
R48/20/21	Harmful: danger of serious damage to health by prolonged exposure through inhalation and in contact with skin
R36/38	Irritating to eyes and skin
R43	May cause sensitisation by skin contact
R45 (Cat 2)	May cause cancer
R46 (Cat 2)	May cause heritable genetic damage
R62 (Cat 3)	Possible risk of impaired fertility
S45	In case of accident or if you feel unwell, seek medical advice immediately (show label whenever possible)
S53	Avoid exposure – obtain special instructions for use

Further information

National Industrial Chemicals Notification and Assessment Scheme (NICNAS). Assessment report on Acrylamide: Priority Existing Chemical Assessment Report No. 23

The full report can be downloaded from http://www/nicnas.gov.au/publications/car/pec/pecindex.htm

Contact Point

Contact name	Telephone number
Position title	
Address	
State Postcode Country	

Appendix 2

Details of calculations of exposures (body burden) used in the calculation of margins of exposure (MOE) in Table 14.1 and 14.2 (Section 14).

Inhaled dose (D_{inh}) was calculated using the following formula:

$$\mathbf{D_{inh} = \frac{C \times R \times B}{BW}\ mg/kg/d}$$

where:

C = concentration of acrylamide in air (mg/m^3)

R = worker respiration volume per 8 h shift (10 m^3/day)

B = bioavailability of acrylamide across the lung (1 = 100%)

BW = average body weight of worker (70 kg)

The dermal uptake was calculated using the following formula:

$$\mathbf{D_{sk} = \frac{C \times A \times B}{BW}\ mg/kg/d}$$

where:

C = deposition of acrylamide per cm^2 skin surface area per 8 h shift ($mg/cm^2/d$)

A = skin surface area exposed (hands only = 820 cm^2)

B = bioavailability of acrylamide across skin (0.75 = 75%)[6]

BW = average body weight of worker (70 kg)

Table 14.1

The following calculations are estimated intake from *inhalation* of acrylamide using Australian exposure monitoring data. Estimated dermal exposures using EASE provided 'very low' estimates and hence the inhalation intake estimates were also considered as 'total' intake estimates.

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6 As per EU calculations (EU 2000).

1) Polymer manufacture (general plant - using aqueous acrylamide)

$\frac{(0.0015 – 0.008) \times 10 \times 1}{70}$ = **0.001 - 0.0002 mg/kg/d**

2) Polymer manufacture (*general plant - using acrylamide solid*)

$\frac{(0.03 – 0.62) \times 10 \times 1}{70}$ = **0.004 - 0.085 mg/kg/d**

3) Polymer manufacture (*manual debagging process - using acrylamide solid*)

$\frac{0.16 \times 10 \times 1}{70}$ = **0.023 mg/kg/d**

4) Polymer manufacture (*automated debagging process - using acrylamide solid*)

$\frac{0.01 \times 10 \times 1}{70}$ = **0.001 mg/kg/d**

Table 14.2

The following calculations are for estimated intake from inhalation and dermal absorption using EU exposure monitoring data and/or EASE estimates:

1) Polymer manufacture (*plant - general*)

Inhalation

$\frac{0.05 \times 10 \times 1}{70}$ = **0.007 mg/kg/d**

Dermal

$\frac{0.01 \times 820 \times 0.75}{70}$ = **0.09 mg/kg/d**

2) Polymer manufacture (*plant maintenance*)

Inhalation

$\frac{0.07 – 0.24 \times 10 \times 1}{70}$ = **0.01 – 0.035 mg/kg/d**

Dermal

Not estimated

3) Polymer manufacture (*packaging*)

Inhalation

$$\frac{0.015 \times 10 \times 1}{70} = \textbf{0.002 mg/kg/d}$$

Dermal

$$\frac{0.0004 \times 820 \times 0.75}{70} = \textbf{0.004 mg/kg/d}$$

4) Polymer use (*paper manufacture*)

Inhalation

$$\frac{0.003 \times 10 \times 1}{70} = \textbf{0.0004 mg/kg/d}$$

Dermal

Estimate as per 3) above

5) Acrylamide use (preparation of polymer gels - laboratory use)

Inhalation

$$\frac{0.067 \times 10 \times 1}{70} = \textbf{0.01 mg/kg/d}$$

Dermal

Estimate as per 3) above

6) Acrylamide use (*grout application – small scale*)

Inhalation

$$\frac{0.12 \times 10 \times 1}{70} = \textbf{0.017 mg/kg/d}$$

Dermal

Estimated in EU report as follows:

body

$\frac{5 \text{ mg/h} \times 7 \text{ h/d} \times 0.75}{70 \text{ kg}} = 0.375 \text{ mg/kg/d}$

hands

$\frac{0.006 \times 820 \times 0.75}{70} = 0.053 \text{ mg/kg/d}$

hands and body = 0.375 + 0.053 = **0.43 mg/kg/d**

7) Acrylamide use (grout application – large scale)

Inhalation

$\frac{0.05 – 0.08 \times 10 \times 1}{70}$ = **0.007 – 0.01 mg/kg/d**

Dermal

Estimate as per 6) above

Appendix 3

Table 1A - Assessment of MSDSs for acrylamide and co-monomer products

Data/ information	Details	MSDS number														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
NOHSC hazard statement		X	□	□	□	□	□	□	□	□	□	□	□	□	□	**X**
Product name		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
UN number		□	□	□	**NA**	□	□	□	**NA**	□	□	□	□	**I**	□	□
ADG Code Class		□	□	□	**I**	□	□	□	**I**	□	□	□	□	**I**	□	□
Hazchem Code		**X**	□	□	**I**	**I**	**I**	**I**	**I**	**X**	**I**	□	**I**	**I**	□	**X**
Formulation	P	□	□	□	□	□	□	□	□	□	□	□	**X**	**X**	□	□
	R	□	□	**X**	□	□	□	□	□	□	□	□	**X**	**X**	**X**	□
Health effects[1]		**I**	□	**I**	□	□	**I**	**I**	**I**	**I**	**I**	□	□	□	□	**I**
First aid		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Exposure standard[2]		**X**	□	□	□	□	□	□	□	□	□	□	**X**	**X**	□	**I**
Advice on PPE		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
Safe handling	S/T	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
	S/D	**I**	□	□	□	□	□	□	□	□	**I**	□	□	□	**I**	□
	F/E	□	□	□	□	□	□	□	□	□	□	□	**I**	**I**	□	□
Company details		□	□	**X**	□	□	□	□	□	□	□	□	□	□	**I**	**I**

Key:

√ = Adequate

I = addressed but inadequate or incomplete

X = No data or not present

NA = not allocated (as stated in MSDS)

1 = according to NOHSC List of Designated Hazardous Substances (1999a)

2 = according to NOHSC Occupational Exposure Standard (1995)

P = reporting presence of acrylamide

R = data on concentration/range acrylamide

S/T = storage/transport

S/D = Spills/disposal

F/E = Fire/explosion

Table 2A - Assessment of MSDSs for polyacrylamide and co-polymer products

Data/information	Details	MSDS number										
		1	2	3	4	5	6	7	8	9	10	11
NOHSC hazard statement		X	I	□	I	□	□	□	□	I	I	□
Product name		□	□	□	□	□	□	□	□	□	□	□
UN number		NA	NA	NA	NA	NA	NA	□	□	NA	NA	□
ADG Code Class		NA	NA	NA	NA	NA	NA	□	□	NA	NA	□
Hazchem Code		NA	NA	NA	NA	NA	NA	□	□	NA	NA	□
Formulation	P	X	□	X	X	X	□	X	X	X	X	X
	R	X	X	X	X	X	□	X	X	X	X	X
Health effects[1]		I	I	I	I	I	I	I	I	I	I	I
First aid		□	□	□	□	□	□	□	□	□	□	□
Exposure standard[2]		□	X	X	X	X	I	X	X	X	X	X
Advice on PPE		□	I	□	□	□	□	□	□	I	I	
Safe handling	S/T	I	□	□	□	□	□	□	□	□	□	□
	S/D	I	□	I	□	I	□	□	□	□	□	□
	F/E	□	□	□	□	□	I	□	□	□	I	□
Company details		□	□	□	□	□	□	□	□	I	I	□

Key:

√ = Adequate

I = addressed but inadequate or incomplete

X = No data or not present

NA = not allocated (as stated in MSDS)

1 = according to NOHSC List of Designated Hazardous Substances (1999a)

2 = according to NOHSC Occupational Exposure Standard (1995)

P = reporting presence of acrylamide

R = data on concentration/range acrylamide

S/T = storage/transport

S/D = Spills/disposal

F/E = Fire/explosion

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