Substance name: Acrylamide
EC number: 201-173-7
CAS number: 79-06-1

MEMBER STATE COMMITTEE SUPPORT DOCUMENT FOR IDENTIFICATION OF ACRYLAMIDE AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS CMR PROPERTIES

Adopted on 27 November 2009

CONTENTS

TABLES

Substance Name: Acrylamide

EC Number: 201-173-7

CAS number: 79-06-1

The substance is identified as a carcinogenic and mutagenic substance according to Article 57 (a) and (b) of Regulation (EC) No 1907/2006 (REACH).

Summary of how the substance meets the CMR (Cat 1 or 2), PBT or vPvB criteria, or is considered to be a substance of an equivalent level of concern

Acrylamide is listed as carcinogen category 2, R 45 and mutagen category 2, R46 in Annex VI, part 3, Table 3.2 (the list of harmonised classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC) of Regulation (EC) No 1272/2008[1]. This corresponds to a classification as carcinogen (1B) and mutagen (1B) in Annex VI, part 3, Table 3.1 of Regulation (EC) No 1272/2008 (list of harmonised classification and labelling of hazardous substances) - see section 3 of this document for full details on classification and labelling.

Registration number(s) of the substance or of substances containing the substance:

-

[1] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

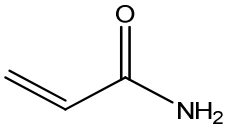
JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name:	Acrylamide
EC Number:	201-173-7
CAS Number:	79-06-1
IUPAC Name:	Prop-2-enamide

1.2 Composition of the substance

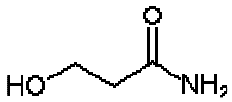
Chemical Name:	Acrylamide
EC Number:	201-173-7
CAS Number:	79-06-1
IUPAC Name:	Prop-2-enamide
Molecular Formula:	C_3H_5NO
Structural Formula:	
Molecular Weight:	71.09
Typical concentration (% w/w):	> 98 (in solid form)
Concentration range (% w/w):	

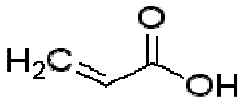
The major impurities reported as present (% w/w on basis of acrylamide solid) are as follows:

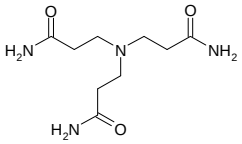
3-hydroxypropionitrile	< 0.5
3-hydroxypropionamide	< 0.5
acrylic acid	< 0.3
tris-nitrilopropionamide	< 0.3
acrylonitrile	< 0.1
water	< 1

Chemical Name:	3-hydroxypropionitrile
EC Number:	203-704-8
CAS Number:	109-78-4
IUPAC Name:	3-hydroxypropanenitrile

Molecular Formula:	C3H5NO
Structural Formula:	
Molecular Weight:	71.08
Typical concentration (% w/w):	
Concentration range (% w/w):	<0.5

Chemical Name:	3-hydroxyproprionamide
EC Number:	
CAS Number:	2651-43-6
IUPAC Name:	3-hydroxypropanamide
Molecular Formula:	C3H7NO2
Structural Formula:	
Molecular Weight:	89.09
Typical concentration (% w/w):	
Concentration range (% w/w):	<0.5

Chemical Name:	Acrylic acid
EC Number:	201-177-9
CAS Number:	79-10-7
IUPAC Name:	prop-2-enoic acid
Molecular Formula:	C3H4O2
Structural Formula:	
Molecular Weight:	72.06
Typical concentration (% w/w):	
Concentration range (% w/w):	<0.3

Chemical Name:	Nitrilotrispropionamide[2]
EC Number:	220-196-3
CAS Number:	2664-61-1
IUPAC Name:	3-[bis(3-amino-3-oxopropyl)amino]propanamide
Molecular Formula:	C9H18N4O3
Structural Formula:	
Molecular Weight:	230.26
Typical concentration (% w/w):	
Concentration range (% w/w):	<0.3

Chemical Name:	Acrylonitrile
EC Number:	203-466-5
CAS Number:	107-13-1
IUPAC Name:	prop-2-enenitrile
Molecular Formula:	C_3H_3N
Structural Formula:	
Molecular Weight:	53
Typical concentration (% w/w):	
Concentration range (% w/w):	<0.1

Chemical Name:	Water
EC Number:	231-791-2
CAS Number:	7732-18-5
IUPAC Name:	Oxidane
Molecular Formula:	H_2O
Structural Formula:	

[2] This name has been indicated in the IPCS report (1985). In the EU RAR, the name of Tris-nitrilopropionamide was used without detailed information on its identity. In this report, nitrilotrispropionamide is used instead of the name of Tris-nitrilopropionamide.

Molecular Weight:	18
Typical concentration (% w/w):	
Concentration range (% w/w):	<1

Acrylic acid and nitrilotrispropionamide are by-products of the production process or the polymerisation reaction. Acrylonitrile is present as unconverted material from the production process. Copper is added typically in amounts less than 100 ppm as an inhibitor to polymerisation.

1.3 Physico-chemical properties

Table 1: Summary of physico- chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	Reference
VII, 7.1	Physical state at 20°C and 101.3 kPa	4.1	White crystalline solid	EU RAR, 2002
VII, 7.2	Melting/freezing point	4.2	84.5°C	EU RAR, 2002
VII, 7.3	Boiling point	4.3	125°C at 25 mm Hg or 3.3 kPa	EU RAR, 2002
VII, 7.4	Relative density	4.4	1.127 g/cm^3 at 30°C	EU RAR, 2002
VII, 7.5	Vapour pressure	4.6	0.9 Pa at 25°C	EU RAR, 2002
VII, 7.7	Water solubility	4.8	2,155 g/l at 30°C	EU RAR, 2002
VII, 7.8	Partition coefficient n-octanol/water (log value)	4.7	-1.0	EU RAR, 2002

2 CLASSIFICATION AND LABELLING

2.1 Classification in Annex VI of Regulation (EC) No 1278/2008

Acrylamide has index number 616-003-00-0 in Annex VI, part 3, Tables 3.1 and 3.2 of Regulation (EC) No 1272/2008.

Its classification according to part 3 of Annex VI, Table 3.2 (the list of harmonised classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC) of Regulation (EC) No 1272/2008 is:

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

Xi; R36/38	Irritating to eyes and skin
R43	May cause sensitisation by skin contact

Notes: D[3], E[4]

Specific concentration limits: none

Its harmonised classification according to Regulation (EC) No 1272/2008 (Annex VI, Table 3.1) is:

Carc. 1B, H350	May cause cancer
Muta. 1B, H340	May cause genetic defects
Repr. 2, H361f	Suspected of damaging fertility
Acute Tox. 3, H301	Toxic if swallowed
STOT RE 1, H372	Causes damage to organs through prolonged or repeated exposure
Acute Tox. 4, H332	Harmful if inhaled
Acute Tox. 4, H312	Harmful in contact with skin
Eye Irrit. 2, H319	Causes serious eye irritation
Skin Irrit. 2, H315	Causes skin irritation
Skin Sens. 1, H317	May cause an allergic skin reaction

Note: D[3]

Specific concentration limits: none

3 ENVIRONMENTAL FATE PROPERTIES

Not relevant for this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Not relevant for this dossier.

4.2 Acute toxicity

Not relevant for this dossier.

[3] Certain substances which are susceptible to spontaneous polymerisation or decomposition are generally placed on the market in a stabilised form. It is in this form that they are listed in Part 3. However, such substances are sometimes placed on the market in a non-stabilised form. In this case, the supplier must state on the label the name of the substance followed by the words 'non-stabilised'.

[4] Substances with specific effects on human health (see Chapter 4 of Annex VI to Directive 67/548/EEC) that are classified as carcinogenic, mutagenic and/or toxic for reproduction in categories 1 or 2 are ascribed Note E if they are also classified as very toxic (T+), toxic (T) or harmful (Xn). For these substances, the risk phrases R20, R21, R22, R23, R24, R25, R26, R27, R28, R39, R68 (harmful), R48 and R65 and all combinations of these risk phrases shall be preceded by the word 'Also'.

4.3 Irritation

Not relevant for this dossier.

4.4 Corrosivity

Not relevant for this dossier.

4.5 Sensitisation

Not relevant for this dossier.

4.6 Repeated dose toxicity

Not relevant for this dossier.

4.7 Mutagenicity

Acrylamide is listed in Annex VI, part 3, Table 3.2 (the list of harmonised classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC) of Regulation (EC) No 1272/2008 as mutagen category 2. This corresponds to a classification as mutagen (1B) in Annex VI, part 3, Table 3.1 of Regulation (EC) No 1272/2008 (list of harmonised classification and labelling of hazardous substances).

The following information on mutagenicity was taken from the Risk Assessment Report, published by the ECB in 2002. The references from the 2002 EU RAR [cited between brackets] are not given in the SVHC Support Document but can be found in the RAR.

4.7.1 In vitro data

Studies in bacteria

A number of well-conducted published and unpublished liquid preincubation and plate incorporation bacterial mutagenicity tests exist in which 100-50,000 μg/plate acrylamide was assayed; results were consistently negative using *Salmonella typhimurium* tester strains TA 98, TA 100, TA 102, TA 1535, TA 1537, and TA 1538, and *E.coli WP2 uvrA-* in the presence and absence of metabolic activation [Bull et al., 1984a; Godek et al., 1982a; Hashimoto and Tanii, 1985; Jung et al., 1992; Knaap et al., 1988; Lijinsky and Andrews, 1980; Muller et al., 1993; Tsuda et al., 1993; Zeiger et al., 1987].

In a fluctuation test (to determine mutations in genes conferring resistance to streptomycin) in *Klebsiella pneumoniae* the mutation frequency was not significantly altered by 100-10,000 μg/ml acrylamide [Knaap et al., 1988].

In a bacterial transfection assay using *E.coli* CR63 cells, a linear increase in percentage inhibition of transfection (apparently indicative of mutagenic potential in this assay system) was noted using up to 10 μg acrylamide [Vasavada and Padayatty, 1981]. The significance of this finding is uncertain, particularly in view of the negative results in standard bacterial assay systems.

Studies in mammalian cells

Cytogenetics assays

A well-conducted *in vitro* cytogenetics assay was available using V79 Chinese hamster cells exposed to 0-3,000 µg/ml acrylamide (>98% purity) with and without metabolic activation [Knaap et al., 1988]. The exposure period was for 3 hours with fixation following a further 15 hours. A dose-related significant increase in the number of metaphases with chromosome aberrations was observed with and without metabolic activation. There was no clear information available regarding cytotoxicity.
Another *in vitro* cytogenetics assay used V79H3 Chinese hamster cells exposed to 0-5 mM (0-355 µg/ml) acrylamide (>99% purity) without metabolic activation [Tsuda et al., 1993]. The exposure period was 24 hours and fixation followed a further 20 or 40 hours later. Again, there was a dose-related, statistically significant increase in the number of metaphases with chromosome aberrations. There was also a dose-related, statistically significant increase in polyploid cells at 20 and 40 hours (29% and 24% respectively at 4mM, 284 µg/ml). The incidence of chromosome aberrations and polyploid cells was less than 2% in negative controls, and the frequencies amongst acrylamide-exposed cells exceeded those of the positive control, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). There was no clear information available regarding cytotoxicity.
Acrylamide is clearly a direct-acting clastogen in mammalian cells *in vitro*.

Gene mutation assays
A number of published and unpublished *in vitro* gene mutation assays using mouse lymphoma L5178Y and Chinese hamster ovary cells (TK and HPRT deficiency) were available [Godek et al., 1982b; 1984; Knaap et al., 1988; Moore et al., 1987; Tsuda et al., 1993].

In an assay using Chinese hamster V79H3 cells (HPRT locus), cells were exposed without metabolic activation to 0-7 mM (0-500 µg/ml) acrylamide for 24 hours [Tsuda et al., 1993]. The number of mutants per 10_6 survivors was 9, 8, 6, 5, 1, 2 at 0, 71, 213, 355, 416, 500 µg/ml, respectively. At 213 µg/ml survival was 59%, at 355 µg/ml or more, survival was 21% or less.
Acrylamide was not mutagenic in this assay.

The following two assays reported either an equivocal or negative result but cell survival was noted to be high (about 70%). The current practice for *in vitro* mammalian cell gene mutation assays is to produce assay conditions in which survival is reduced to 20-50% and hence there are some doubts regarding the stringency of the test conditions employed.

Chinese hamster ovary cells (HPRT locus) were exposed to 37.5-900 µg/ml acrylamide (unknown purity) with and without S9 for 5 hours [Godek et al., 1982b]. A slight dose-related increase in mutant frequency (less than 50% above the solvent control value of about 10 mutants/10^6 survivors) with S9 was observed at 300 µg/ml and above and at all exposure levels without S9.
In a follow-up study, a negative result was obtained using up to 1,500 µg/ml acrylamide with and without S9 [Godek et al., 1984]. The frequency of mutants amongst acrylamide-exposed cells was not significantly greater than the negative controls. Survival was approximately 70%, cytotoxicity was observed at 1,500 µg/ml with S9, and mutant frequency was 285-330 mutants/10^6 survivors in the positive controls EMS and DMN.

In a mouse lymphoma L5178Y TK +/- assay, cells were exposed to 0-7,500 µg/ml Acrylamide with and without metabolic activation for 2, 4, or 20 hours [Knaap et al., 1988]. At each of the time-points, with and without metabolic activation (+ or - S9), there was a slight dose-related increase in mutant frequency: 19-36 mutants/10_6 survivors in controls; with 2 hours exposure 39/10_6 at 2,500 µg/ml -S9 and 119/10_6 at 7,500 µg/ml +S9, with 4 hours exposure 160/10^6 at 2,000 µg/ml -S9 and 189/10^6 at 2,500 µg/ml +S9; with 20 hours exposure 89/10^6 at 300 µg/ml - S9 and 57-95/10^6 at 300 µg/ml in the presence of metabolic activation (from primary rat liver or Syrian hamster ovary cells). These increases were associated with low survival (usually less than 30% survival). A positive result was obtained in this assay.

Similar increases associated with low survival were noted by Moore et al. [1987] in an assay using

0-850 μg/ml acrylamide (>99% pure) for 4 hours without metabolic activation studying large and small colonies. An increased mutant frequency (70-400 mutants/10_6 survivors, approximately $20/10^6$ in controls) was observed at 500 μg/ml acrylamide and above, and percentage survival was 40% at 500 μg/ml, 10% at 850 μg/ml. There was a dose-related increase in the frequency of small colonies and at 750 μg/ml and above mutants were mainly small colonies. The increase in small colony formation is suggestive of clastogenicity, and a separate assay for chromosome aberrations confirmed the increase (27% cells had chromosome and chromatid breaks or rearrangements). The overall pattern from mammalian cell gene mutation assays is that acrylamide is a directacting mutagen, probably causing clastogenic effects rather than gene mutations.

DNA synthesis and repair

In an *in vitro* assay to measure unscheduled DNA synthesis (UDS) 0-100 mg/ml acrylamide was incubated with freshly isolated rat hepatocytes for 18-20 hours [Naismith and Matthews, 1982]. Viability of the harvested cells was 88%. The positive control was 2-acetamidofluorene (2-AAF). Net nuclear grain counts were statistically significantly increased above negative controls (26-45 at 1-33 mg/ml, compared to about 1 in the negative controls) and in the first assay were close to the count achieved by 2-AAF. There was no clear dose-response for UDS.
These results were confirmed by a repeat experiment.

An *in vitro* UDS assay was available in which 0-50 mM (0-3.55 mg/ml) acrylamide was incubated with rat hepatocytes for 18 hours [Miller and McQueen, 1986]. Cytotoxicity (cell death) was observed at the highest concentration. A negative result was obtained in this and in a repeat experiment in that there was not an increase of 5 net nuclear grain counts over negative control values and there was no clear dose-response in the counts observed. The positive control 2-aminofluorene gave a clear increase in UDS.

In another *in vitro* rat hepatocyte UDS assay, 0-10 mM (0-710 μg/ml) acrylamide was incubated with the cells for 17-19 hours without metabolic activation [Butterworth et al., 1992]. Amongst acrylamide-exposed cells there was no significant increase in the number of net nuclear grains when compared with the negative control. Toxicity (assessed by the morphological appearance of cultures) was observed at the highest concentration level. The sensitivity of the assay was confirmed using DMN, which gave a clear increase in net nuclear grains. Acrylamide did not induce UDS in this assay.

Two abstracts were available which briefly described rat hepatocyte UDS assays using acrylamide concentrations of up to 30 mM (about 2 mg/ml) [Barftnecht et al., 1987; Barftnecht et al., 1988]. Acrylamide reportedly gave positive results in the presence and absence of metabolic activation at the higher exposure levels used in these experiments. In the absence of any other experimental details it is difficult to draw any firm conclusions about the validity and reliability of these results.
Rat hepatocytes previously irradiated with ultraviolet (UV) light to induce DNA damage were incubated with 10^{-3}-10 mM (0.7-710 μg/ml) acrylamide and tritiated thymidine [Miller and McQueen, 1986]. The incorporation of tritiated thymidine was then assessed autoradiographically. A dose-dependent increase in net nuclear grain counts was observed following UV irradiation, and 10 mM (710 μg/ml) acrylamide only slightly increased the amount of DNA repair (from about 60 net nuclear grains to about 75). Lower levels of acrylamide than this did not affect DNA repair, and higher levels were reported to be cytotoxic. In addition, density gradient centrifugation of hepatocyte DNA on a caesium chloride gradient following incubation with BrdU and 10 mM (710 μg/ml) acrylamide showed that acrylamide did not induce DNA repair under the conditions of that system.
Overall, there are inconsistent findings in the available *in vitro* UDS studies and it is difficult to draw a definite conclusion.

Sister chromatid exchange

In an assay for sister chromatid exchange (SCE), V79H3 Chinese hamster cells were exposed to 0-3 mM (0-213 μg/ml) acrylamide (>99% purity) without metabolic activation for 24 hours followed by an additional 28 hours for bromodeoxyuridine (BrdU) incorporation; 50 cells per exposure level were counted [Tsuda et al., 1993]. A slight, but dose-related and statistically significant increase in SCE was seen (50% increase compared to an 800% increase by the positive control, mitomycin C). Similar findings were observed by Knaap et al., 1988 testing V79 cells in the range 0-3,000 μg/ml with and without metabolic activation with a 3-hour exposure period followed by 21-25 hours BrdU incorporation.

Chinese hamster ovary cells were exposed to 0-500 μg/ml acrylamide (purity not stated) with and without metabolic activation [Sorg et al., 1982b]. The cells were treated for 5 hours followed by a 27-hour BrdU incorporation time and 30 cells per exposure level were counted for SCE. The results did not show statistically significant or dose-related increases in SCE compared to the solvent control. The positive controls ethyl methanesulphonate (EMS) and dimethylnitrosamine (DMN) doubled the number of SCE seen per cell in comparison with control. Comparing the results of positive controls in the previous experiment [Tsuda et al., 1993] it is possible that, under the conditions used, this assay may not have been sensitive enough to detect clear dose-responses in an increase in SCE. Therefore, the validity of the negative result obtained in this report is unclear.

Cell transformation

Cell transformation is not a reliable indicator of genotoxicity. Nevertheless, the studies have been summarised in this section for convenience. A number of published and unpublished mammalian cell transformation assays are available using BALB/3T3, C3H/10T½, and NIH/3T3 cell lines with and without metabolic activation [Banerjee and Segal, 1986; Microbiological Associates, 1984a, 1982a, 1982b; Tsuda et al., 1993]. Positive results were obtained in all assays except those reported in Microbiological Associates [1982b]. Overall, acrylamide increased cell transformation frequency in the *in vitro* cell lines tested, generally in the presence or absence of metabolic activation.

Other in vitro studies

A dose-related increase in the percentage of cells with spindle disturbances (increases in c-mitoses and fragmented or bridged ana-telophase figures) was observed by Adler et al. [1993] using Chinese hamster V79 cells exposed to up to 1 mg/ml acrylamide for 6 hours without metabolic activation.

Amongst controls, the incidence of c-mitoses was 1% compared to 91% at 0.5 mg/ml. The incidence of fragmented or bridged ana-telophase figures was 0.2% in controls compared with 1.1% at 0.1 mg/ml with no such figures being observed at higher concentrations due to the blocking of metaphase. For example, at 0.5 mg/ml more than 90% of metaphase figures were damaged, with a large number of figures scattered in the cytoplasm. In addition, the polyploidy index (the number of polyploid cells as a percentage of total mitoses observed) increased in a dose-related manner (from 1.4% in controls to 4.4% at 0.5 mg/ml and 9.8% at 1 mg/ml. At concentrations up to 0.5 mg/ml there were no clear signs of cytotoxicity, and at 1 mg/ml pyknosis was observed indicating the onset of a cytotoxic effect. The results of this study indicate that acrylamide is a potent spindle poison.

Human fibrosarcoma cells were exposed to 0-10 mM (0-710 μg/ml) acrylamide for 4 hours [Sickles et al., 1995]. Cells were processed by Giemsa staining of chromosomes and then immuno-fluorescence staining to visualise microtubules. Colchicine was used as a positive control.

In negative controls 2% of cells were observed to be in mitosis. Colchicine dispersed the chromosomes throughout the cytoplasm and increased the percentage of cells in mitosis to 10% as expected from a substance that arrests cells in mitosis by disassembly of microtubules. Acrylamide also caused a concentration-dependent increase in the number of cells in mitosis, but unlike with colchicine the chromosomes in cells exposed to acrylamide appeared to be condensed at the metaphase plate at all exposure levels used (>1mM, 71 μg/ml). This effect was more pronounced at

higher concentrations. Hence with acrylamide, the formation of the mitotic spindle and chromosome alignment on the spindle was not apparently adversely affected, although subsequent chromsome segregation and migration were affected.

In an SV40-DNA amplification study CO60 Chinese hamster cells were exposed to 0-150 µg/ml acrylamide [Vanhorick and Moens, 1983]. Cells were incubated with acrylamide for 24 hours before hybridization with ^{32}P-labelled SV40-DNA. Increased synthesis of SV40 DNA (DNA amplification) was seen at acrylamide concentrations of >50 µg/ml, concentrations that were associated with cell survival of 57% or less. The authors considered this result to indicate that acrylamide had little or no ability to induce SV40-DNA amplification (i.e. little or no potential to directly damage DNA in this system). The true significance of the findings in this invalidated system is unclear.

Summary of *in vitro* studies

Acrylamide is not mutagenic in standard bacterial assays when tested in the presence or absence of metabolic activation systems. However, acrylamide was clearly clastogenic (direct-acting) in mammalian cells *in vitro*, producing chromosome aberrations and polyploidy in two different cell systems investigated. Supporting evidence for *in vitro* clastogenicity was also evident in mammalian cell gene mutation assays.

4.7.2 In vivo data

Studies in Drosophila

Published and unpublished reports were available using different assay systems (investigating somatic and germ cells), with a mixture of positive and negative results [Batiste-Alentorn et al., 1991; Foureman et al., 1994; Knaap et al., 1988; Microbiological Associates, 1984b; Tripathy et al., 1991]. The overall significance of these results for human health is unclear and much greater weight can be put on the mammalian cell system results, both *in vitro* and *in vivo*.

Somatic cells

Cytogenetics

Groups of 5 male mice received a single intraperitoneal (ip.) injection of 0 or 100 mg/kg aqueous acrylamide and 50 bone marrow cells per mouse were analysed for chromosome aberrations at 6, 18, 24, and 48 hours post-administration [Cihak and Vontorkova, 1988]. No positive control was used. A clear, statistically significant increase in the number of metaphases with chromosome and chromatid breaks (3-11% excluding gaps compared to 1% in controls) was noted at the three later time-points with a maximum value at 24 hours.

Groups of 5 male and 5 female mice received single ip. injections of 0 or 100 mg/kg acrylamide in saline [Adler et al., 1988]. Samples of bone marrow were taken at 12, 18, 24, 30, and 36 hours for analysis of chromosome aberrations. Statistically significant increases in the number of metaphases with chromosome and chromatid breaks were observed (2.6-4.4% excluding gaps compared to 0.7% in controls). The maximal response was at 18 hours in this assay. An additional dose-response assay was performed with mice receiving 0, 50, 100, and 150 mg/kg acrylamide with a sampling time of 18 hours. A positive control group received cisplatin.

Statistically significant increases in the frequency of aberrant cells were seen at all exposure levels (2.1%-4.1% excluding gaps compared to 0.3% in the negative control and 3.6% for the positive controls). Mitotic index was reduced by up to 27% compared with negative controls.

In another study, groups of 5 male mice received 500 ppm acrylamide (approximately 60 mg/kg/day assuming 25g bodyweight and food consumption of 3g/day) by dietary administration

for 1, 2 or 3 weeks, or a single ip. injection of 0 or 100 mg/kg [Shiraishi, 1978]. At least 100 bone marrow cells were scored for chromosome breaks per animal at 1/2, 1, 11 and 12 days post-administration in the case of ip. treated mice, and immediately after sacrifice at weeks 1, 2, and 3 for animals receiving dietary acrylamide (animals were pretreated with colchicine). There was no mention of the use of positive controls in this study. Following single-exposure there was an increase in metaphases with chromosome breaks (2.7% in negative controls, 3.5-7% in treated animals).

There was an increase in the frequency of cells with aneuploidy or polyploidy (3.7% in negative controls, 5-10.5% in treated animals). For animals treated by the dietary route there were similar slight increases. In addition, there was also a slight, but not statistically significant increase in SCE/cell (2.9 in controls and 3.5-3.7 in treated animals). It was not clear from the results that were presented whether or not results at each time point were compared with control groups or if any statistical comparisons had been performed. The author concluded these changes to be negative responses. However, there are some doubts about the validity of this assertion due to the limitations in the presentation of information. The values presented for chromosome breaks would, by current standards, indicate a positive result.

In a further study using cells from an unconventional source, groups of 4 male mice received single ip. injections of 0, 50, 100, or 125 mg/kg acrylamide [Backer et al., 1989]. Positive controls received cyclophosphamide. Mitoses were analysed from 100 spleen lymphocytes at each exposure level 24 hours post-administration only. There was no clear increase in the frequency of metaphases with chromosome aberrations but there was a non-statistically significant increase in chromatid aberrations: 5% at 125 mg/kg compared to 2% in negative controls at 125 mg/kg. There was also a significant dose-related increase in the frequency of SCE/cell. In view of the non-validated nature of this assay system no firm conclusions can be drawn from the results.

Overall, the results of these studies indicate that acrylamide produces chromosome aberrations in somatic cells *in vivo*.

Micronucleus assays

In addition to the chromosome aberration analysis performed on bone marrow cells taken from acrylamide-exposed mice a micronucleus assay was conducted [Adler et al., 1988, see Cytogenetics section above for details). There was no significant change in the ratio of polychromatic to normo-chromatic erythrocytes (P/N ratio). However, clear, statistically significant increases in micro-nucleus frequency were observed following single intraperitoneal administration of 100 mg/kg acrylamide at 18, 24, and 30 hours with maximum values at 24 hours (0.66% compared to 0.13% in negative controls). Subsequently, groups of 5 male and 5 female mice received 0, 50, 75, and 125 mg/kg acrylamide with samples taken at 24 hours. Clear, statistically significant, and dose-related increases in micronucleus frequency were seen at all exposure levels. A frequency of 1% was observed with the positive control, cisplatin, and at 125 mg/kg acrylamide the frequency was 0.9%.

A number of other *in vivo* micronucleus assays in male and female mice were available all giving positive results using cells taken from bone marrow, spleen or peripheral blood with up to 150 mg/kg aqueous acrylamide administered by the intraperitoneal route singly or repeatedly [Backer et al., 1989, Cao et al., 1993, Cihak and Vontorkova, 1988, Cihak and Vontorkova, 1990, Knaap et al., 1988, Russo et al., 1994]. Sampling times ranged from 6-72 hours with the peak effects generally observed at 24 hours.

A negative result was obtained in an unpublished *in vivo* bone marrow mouse micronucleus assay using males and females receiving 75 mg/kg aqueous acrylamide by oral gavage singly or repeatedly [Sorg et al., 1982a]. Clinical signs of toxicity were observed following single and repeated (2x) administration of 75 mg/kg. Sampling times were 30, 48, and 72 hours. The positive control used was triethylenemelamine, given intraperitoneally. Overall, there are some doubts about the validity of the negative result obtained as no sampling times less than 30 hours were used.

Liver UDS assay

In an *in vivo* liver UDS test groups of rats received single or repeated (5x) ip. injections of 0, 30, or 100 mg/kg acrylamide [Butterworth et al., 1992]. In the case of single exposure, animals were sacrificed 2 or 12 hours post-administration and for repeated-exposure animals sacrifice was 4 hours after the last injection. Hepatocytes were isolated and incubated with tritiated thymidine for 4 hours. There were no increases in net nuclear grain counts. The positive control, DMN, produced a clear response. Thus, ACR did not cause UDS in liver cells *in vivo*.

Mammalian spot test

As part of a study investigating potential mutagenic and developmental effects, groups of 31-93 pregnant female mice received single or 3 daily ip. injections of 0, 50 or 75 mg/kg aqueous ACR on day 12 or days 10, 11, and 12 [Neuhauser-Klaus and Schmahl, 1989]. Approximately 220-300 offspring per dose level were available for examination. A positive result (doubling in the number of spots of genetic relevance compared to negative controls) was reported following single exposure to either 50 or 75 mg/kg and to repeated exposure to 50 mg/kg/day. Repeated exposure to 75 mg/kg resulted in increased embryotoxicity and cytotoxicity.

Studies in transgenic mice

As part of a validation for a new test method, groups of mice received 5 daily ip. injections of 0 or 50 mg/kg acrylamide - 3, 7, and 10 days later the *LacZ* mutation system was used to determine mutant frequency (MF) in bone marrow only (Hoorn et al., 1993 and Myhr 1991). An increase in MF was noted (62-89 . 10^6 compared to 15-26 . 10^6 in controls), although with no clear pattern with respect to sampling time. Procarbazine and ethyl nitrososurea gave more substantial increases. Although demonstrating positive results, the full significance of the results in this as yet unvalidated assay is unclear but the result does provide support for the view that acrylamide is an *in vivo* genotoxicant.

Summary of *in vivo* mammalian somatic cell assays

Acrylamide is clearly mutagenic *in vivo*, producing positive results particularly in the bone marrow micronucleus assay. The pattern of results indicates clastogenicity or interference with mitosis rather than gene mutation activity.

Germ cells

Cytogenetics

Groups of 5-16 male mice received single ip. injections of 0 or 75 mg/kg acrylamide and were mated with untreated females 7 days later, or received 125 mg/kg with mating 7 or 28 days later, or 5 daily injections of 50 mg/kg with mating 7 days post-administration [Pacchierotti et al., 1994]. Chromosome aberrations from one-cell zygotes were scored at 5 hours with at least 100 metaphases scored per group except for animals receiving repeated exposure to Acrylamide where chromosome effects were very common and fewer metaphases were analysed. In addition, flow cytometry was performed for cells taken from testicular preparations at 3 and 35 days after treatment with up to 150 mg/kg acrylamide (elongated spermatids, round spermatids, diploid cells, S-phase cells, tetraploid cells, and elongated/elongating diploid spermatids were counted).

From one-cell zygotes a statistically significant, dose-related increase in the frequency of aberrations was noted following mating at 7 days and to a lesser degree at 28 days after mating with chromosome fragments, dicentrics and translocations being prominent. For repeated exposure 85% of zygotes were reported to contain chromosome aberrations.

From testicular cell populations there was a marked decrease (~74% of control values) in tetraploid cells 3 days after treatment amongst animals receiving a single exposure. The total cell number was not apparently affected. At 35 days there was a statistically significant, dose-related decrease in the percentage of elongated spermatids (70% of control values at 150 mg/kg) and a statistically significant increase in elongated/elongating diploid spermatids suggesting impaired segregation during mitosis. A decrease in diploid spermatids was noted 3 days post-exposure following single exposure but not after repeated exposure; the authors suggest that this effect was due to spermatocytes being affected during meiosis by the initial injections.

Following on from the assay reported in the section on somatic cell effects, groups of 5 male mice received 500 ppm acrylamide (approximately 60 mg/kg/day assuming 25g body weight and food consumption of 3g/day) by dietary administration for 1, 2 or 3 weeks or a single ip. injection of 0 or 100 mg/kg [Shiraishi, 1978]. At least 100 metaphases from spermatogonia were scored for chromosome aberrations per animal at 12 and 24 hours, and 11 and 12 days postadministration in the case of ip. treated mice and immediately after sacrifice for animals receiving dietary acrylamide. In addition, 50-100 spermatocytes in the diakinesis-metaphase *I* stage were examined from each animal.

An increased incidence of spermatogonia with aneuploidy, chromosome breaks, and sister chromatid exchanges was seen using both exposure regimes. Similarly, amongst primary spermatocytes there was a marked increase in sex-chromosome and autosomal univalents, fragments and rearrangements observed in both exposure regimes.

Also following work in somatic cells, groups of 4 male mice received single ip. injections of 0, 50, 100, or 125 mg/kg acrylamide [Backer et al., 1989]. Chromosome and chromatid aberrations were scored in spermatogonia and spermatocytes 24 hours post-administration only. There were no significant changes in the number of chromosome/chromatid aberrations or hyperploidy compared to negative controls. This study is limited by the use of only one sampling time.

As part of a dominant lethal assay summarised below [Smith et al., 1986] cytogenetic examination was performed on rat spermatocytes taken from 10-11 males exposed to 0, 1.5, 3, or 6 mg/kg/day acrylamide in drinking water for 80 days and after a 12-week recovery period. No increase in structural aberrations was observed on completion of 80 days although a slight increase in reciprocal translocations was noted amongst treated animals (0, 1, 1, and 2 in each of the groups respectively). The significant increase in pre-implantation loss would suggest that an adequate exposure level was used. However, no further details were available regarding the conduct of this investigation hence it is difficult to draw any firm conclusions regarding the potential to form chromosome aberrations in rat spermatocytes from this report.

Germ cell micronucleus assays

Micronucleus formation in spermatids was studied using groups of 5 male rats receiving single ip. injections of 0, 50, or 100 mg/kg acrylamide [Lahdetie et al., 1994]. Animals were sacrificed on days 1, 3, 18 and 19 post-administration and 2000 spermatids/animal were analysed. The positive control used was mitomycin C. following this, groups of 5 male rats received 4 daily ip. injections of 50 mg/kg acrylamide and were sacrificed on days 1, 3, 18 and 19 postadministration. Analysis of spermatids at these sacrifice times correspond to cells that would have been exposed to acrylamide as spermatocytes in diplotene-diakinesis (day 1), late pachytene (day 3), and preleptotene stages of meiosis (day 18) and as intermediate and type B spermatogonia (days 18 and 19). A statistically significant increase in the number of micronuclei was observed from cells sampled on day 18 following exposure to 4.50 mg/kg (2.0/1000 early spermatids compared to 0.55/1000 in negative controls).

Groups of 4-5 male rats received 0, 50, or 100 mg/kg acrylamide by single ip. exposure or 4 daily injections of 50 mg/kg [Xiao and Tates, 1994]. Spermatocytes were isolated and analysed for

micronucleus formation on days 1, 3, 15, 18, 19, and 20 after acrylamide administration. Statistically significant increases in micronucleus formation were observed 18-20 days after single ip. exposure to 0, 50 or 100 mg/kg acrylamide and the effect was more marked following 4 daily ip. injections of 50 mg/kg. For single exposures, the frequency of micronucleus formation amongst acrylamide-treated animals was 3.3-4.2/1000 early spermatids compared to 1.5/1000 in the negative controls 18-20 days after acrylamide-administration. Slight, but not statistically significant, increases were also observed 1-3 days after administration (up to 2/1000 at 100 mg/kg). The most significant increases (day 18-20) would have corresponded to spermatids initially exposed in the preleptotene stage - a result consistent with a number of other studies. Amongst repeated-exposure animals, the frequency of micronucleus formation reached 6.4/1000 on day 19.
A similar increase in micronucleus formation was reported by Russo et al. [1994] using Golgiphase and Cap-phase spermatids (post-meiotic developmental stages) from acrylamide-exposed mice. In addition, differentiating spermatogonia were assessed for SCE – a statistically significant, exposure-related increase in SCE was noted.

DNA synthesis and repair
Groups of 4-6 male mice received single ip. injections of 0, 8, 16, 31, 63, or 125 mg/kg acrylamide [Sega, 1990]. Tritiated thymidine was injected into the testes 0-48 hours after acrylamide administration and sperm from the caudal epididymides were recovered 16 days post-administration for UDS analysis. In addition, groups of 4-6 male mice received a single ip. injection of 0 or 125 mg/kg acrylamide with tritiated thymidine injected into the testes 6 hours later and sperm from caudal epididymides (spermatozoal to early spermatocytes at the time of treatment) was recovered at 2-3 day intervals for up to 30 days post-administration for UDS analysis. Also, groups of 4 male mice received ip. injections of 46 mg/kg [^{14}C]-acrylamide. DNA was extracted from liver and testes samples 1-24 hours post-administration and analysed for radioactivity.

In the first experiment, there was a clear increase in UDS, the maximum response of one order of magnitude greater than that of controls occurring 6 hours after tritiated thymidine injection. This peak response related to sperm, which would have been in the early spermatid stage at the time of acrylamide exposure. For the second experiment, no significant amounts of tritiated thymidine were incorporated during the first 10 days following exposure to 125 mg/kg acrylamide but from days 12-27 a positive UDS response was seen. In the third experiment DNA alkylation was observed, which reached maximum levels 4-6 hours post-administration in the testes and 1-2 hours post-administration in the liver, with levels being substantially (10-fold) lower in testes than liver.

In an *in vivo* spermatocyte UDS test groups of F344 rats received single or repeated (5x) ip. injections of 0, 30, or 100 mg/kg acrylamide (Butterworth et al., 1992). In the case of single exposure, animals were sacrificed 2 or 12 hours post-administration and for repeatedexposure animals sacrifice was 4 hours after the last injection. Following repeated administration of 30 mg/kg there was a statistically significant increase in the number of net nuclear grain counts (5.4 and 5.6 compared to 0 in controls). The positive controls MMS and cyclophosphamide gave counts of 4.9 and 6.5 respectively. For single administration, the increase was not significant (1.5 and 1.6). Overall, the results indicate that acrylamide caused an increase in UDS in rat spermatocytes following repeated exposure.

Dominant lethal assays
• Studies in mice
Groups of 24-30 male mice received 0, 25, 50, 75, 100, or 125 mg/kg/day acrylamide in 70% aqueous methanol by the dermal route for 5 consecutive days [Gutierrez-Espeleta et al., 1992]. Males were then mated with untreated females from day 7-10 after the last exposure. There was a slight, but treatment-related decrease in the mean number of implantations per pregnant female (21 in controls, 16 at 125 mg/kg/day). The percentage of dead implants (3%, 11%, 20%, 46%, 61%, and

76% respectively at 25, 50, 75, 100, and 125 mg/kg/day) and number of pregnant females with one or more dead implant was statistically significantly increased amongst all treated animals when compared with controls (20/78 in controls, 29/36, 40/43, 51/57, 40/42, and 27/27 respectively). The number of live embryos per pregnant female was approximately 10 in controls and decreased in a treatment-related manner to approximately 2 at 125 mg/kg/day. There was a corresponding treatment-related increase in dominant lethality (up to 91% at 125 mg/kg/day).
Overall, these results indicate that repeated dermal exposure of male mice to 25 mg/kg/day or more acrylamide for 5 days resulted in dominant lethal effects in the progeny, and the reduced number of pregnant females is suggestive of reduced male fertility.

Male mice received a single ip. injection of 125 mg/kg or 5 daily injections of 50 mg/kg acrylamide (>99% pure) prior to mating with females [Shelby et al., 1986]. In addition there was an assay performed with mating over a limited period (days 6-10 after acrylamide-treatment) such that the available sperm would derive from cells exposed as late spermatids or epididymal sperm. Dominant lethality, observed as an increased frequency of dead implants particularly between days 4-12 post-administration, was noted following single and repeated exposure. The early increase was suggestive of an effect on late spermatids and early spermatozoa, a result consistent with DNA alkylation studies reported by Sega and Generoso [1990a], effects noted in testicular cell populations by Sakamoto et al. [1988], and the distribution studies by Sega et al. [1989], and Marlowe et al. [1986].

In a study reported as a brief abstract, groups of male mice received approximately 0, 0.7, 2.1, or 6 mg/kg/day acrylamide in drinking water for 140 days (20 weeks) [Bishop et al., 1991]. Males were then mated with untreated females which, 16 days later, were examined for implantations, live/dead foetuses, and resorptions. A significantly higher percentage of resorptions was found amongst animals receiving 6 mg/kg/day (13% compared to 7% in controls). This report should be treated with a little caution due to insufficient reporting detail, but the results provide further support that acrylamide induces dominant lethal mutations in the germ cells of male mice.

Other positive results for dominant lethality in mice were obtained as part of studies to investigate heritable translocations [Shelby et al., 1987; Adler et al., 1994; Ehling and Neuhauser- Klaus, 1992]. In addition positive results for dominant lethality were also obtained in a combined dominant lethal/two-generation reproduction study [NTP, 1993; Chapin et al., 1995] and in another crossover breeding study [Sakamoto and Hashimoto, 1986].

- Studies in rats

Groups of 10-11 male rats were exposed to 0, 1.5, 3, or 6 mg/kg/day acrylamide in drinking water for a total of 80 days [Smith et al., 1986]. After 72 days males were mated with untreated females. Significantly increased pre-implantation loss was noted amongst females mated with high dose males. Post-implantation loss was increased at 3 and 6 mg/kg/day. There were no clinical or histopathological signs of neurotoxicity which may have affected male fertility.

Other positive results for dominant lethality were reported in studies in which rats received up to 100 mg/kg/day for 5 days by oral gavage as part of investigations into potential reproductive effects [Sublet et al., 1989; Tyl, 1998a] and also as part of a combined two-generation/dominant lethal assay in which rats received up to 5 mg/kg/day acrylamide in drinking water for 10 weeks [Tyl, 1987].

Heritable translocation assays

Groups of 120 male mice received 5 daily ip. injections of 0, 40 or 50 mg/kg acrylamide (99% pure) and were mated with untreated females 7-10 days after the last injection [Shelby et al., 1987]. Male progeny were weaned and females discarded. The males were then mated with additional untreated females. If reduced fertility was observed, the males were again mated with additional females, which were sacrificed on day 14 for examination of the uterine contents. In addition, spermatocytes

from males with reduced fertility underwent cytogenetic analysis. The repeated administration of 50 mg/kg/day acrylamide resulted in a "high level" (percentage not stated) of dominant lethal mutations, and 40 mg/kg/day produced approximately 70% dominant lethality. For the heritable translocation studies, 49/125 (39%) males at 50 mg/kg/day and 39/162 (24%) at 40 mg/kg/day were either sterile or semisterile. This compared with a historical control incidence of 17/8095 (0.2%). All 10 males selected for cytogenetic analysis were confirmed as translocation carriers. In addition, 31-85% of females mated with semisterile males carried dead implants, compared with 0-9% in females mated with apparently non-sterile males.

A single ip. injection of 0, 50 or 100 mg/kg or 5 daily injections of 50 mg/kg acrylamide in male mice [Adler et al., 1994]. There was a similar selection of sterile/semisterile animals to the previous study. Dominant lethal effects were observed at the highest exposure level and there was an exposure-related increase in heritable translocations; 3/8700 (0.04%) in controls, 2/362 (0.6%) at 50 mg/kg, 10/367 (2.7%) at 100 mg/kg, 23/105 (22%) at 5 . 50 mg/kg.
The results of these two studies demonstrate that acrylamide caused heritable translocations in mice.

Specific-locus assays
Groups of male mice received ip. injections of 0, 100, or 125 mg/kg aqueous acrylamide [Ehling and Neuhauser-Klaus, 1992] and were serially mated with untreated females (homozygous for a number of key physical features). A high frequency of specific-locus mutations was noted for males mated with females 5-8 days and 9-12 days after injection (6-14 mutations per locus per 10^5 gametes for males receiving 100 and 125 mg/kg Vs 1.3 per 10^5 gametes in controls). This would indicate that specific-locus mutations occurred in male spermatozoa and spermatids.

In another assay for specific-locus mutations, male mice received 5 repeated ip. doses of 50 mg/kg [Russell et al., 1991]. Increased frequencies of specific-locus mutations were observed for males mated with females 8-14 and 15-21 after injection suggestive of specific-locus mutations amongst the late spermatid and spermatozoal stages.

Studies in transgenic mice
As part of study to validate a new test method, groups of male mice received 5 daily ip. injections of 0 or 50 mg/kg acrylamide with assays performed on testicular cell preparations examining the *LacZ* mutation system 21-23 days later [Murti et al., 1994]. There was no clear increase in mutations noted in this system. Microscopic examination showed an increase in the number of "unusually large cells" which were speculated, but not proven by the authors, to be due to interkinetic delay during meiosis caused by acrylamide. Overall, no firm conclusions can be drawn on the nature and significance of effects seen.

Other germ cell studies
Groups of male mice received a single ip. injection of 0 or 100 mg/kg acrylamide (99.9% pure) following testicular injection of tritiated thymidine, and mature spermatozoa were removed daily for 21 days post-administration [Sega and Generoso, 1990]. DNA was removed by alkaline elution and a significant increase in single-stranded breaks was observed in treated animals, the greatest elution rate being observed in the second week post-exposure. Further analysis showed that DNA breakage following acrylamide exposure was occurring mainly in early and mid-late spermatids as well as pachytene spermatocytes.
Other information regarding DNA binding, distribution to male reproductive organs, and effects on testicular cell populations was available in the studies by Sega et al. [1989] and Marlowe et al. [1986], Sakamoto et al. [1988].

Summary of *in vivo* mammalian germ cell assays

Acrylamide is clearly positive in a number of different germ cell assays (chromosome aberrations, micronucleus assays, UDS, dominant lethal assays, heritable translocation, and specific locus assays) indicating that it is a germ cell mutagen.

4.7.3 Human data

No data available.

4.7.4 Other relevant information

No data available.

4.7.5 Summary and discussion of mutagenicity

All in all, it was concluded in the EU RAR (2002) that a substantial body of information is available covering many genotoxicity end points. Although acrylamide is not mutagenic in bacteria, its mutagenic potential is clearly shown in mammalian systems *in vitro.* It is a direct-acting mutagen and there is also a large body of evidence clearly demonstrating that acrylamide is genotoxic *in vivo* to both somatic cells and germ cells. In the case of germ cells, acrylamide has been demonstrated to induce heritable mutations.
The published evidence available since 1995 (date of last literature search for the 2002 EU RAR), which recently has been reviewed by the UK Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (COM, 2009), extends the effects of acrylamide to include identifiable glycidamide DNA adducts and gene mutations, detectable in cultured mammalian cells and somatic cells *in vivo*. These effects appear to be due to metabolism of acrylamide to glycidamide. The UK Committee confirmed that acrylamide is an *in vivo* mutagen, and concluded that there are multiple potential mechanisms (such as extensive protein binding/enzyme inhibition, oxidative stress and DNA adduct formation) which may contribute to the genotoxicity of acrylamide. The default approach is to assume no threshold for the genotoxic effects of acrylamide (COM, 2009).

4.8 Carcinogenicity

Acrylamide is listed in Annex VI, part 3, Table 3.2 (the list of harmonised classification and labelling of hazardous substances from Annex I to Directive 67/548/EEC) of Regulation (EC) No 1272/2008 as carcinogen category 2. This corresponds to a classification as carcinogen (1B) in Annex VI, part 3, Table 3.1 of Regulation (EC) No 1272/2008 (list of harmonised classification and labelling of hazardous substances).

4.8.1 Carcinogenicity: oral

In the EU RAR (2002) the following two studies have been described. NB: The references from the 2002 EU RAR [cited between brackets] are not given in the SVHC Support Document but can be found in the RAR.

In a combined chronic toxicity/carcinogenicity study conducted according to modern protocol standards, groups of 90 male and 90 female F344 rats received 0, 0.01, 0.1, 0.5, or 2.0 mg/kg/day aqueous acrylamide (>96% pure) in drinking water for up to 2 years [Johnson et al., 1984; 1986]. Routine observations included clinical signs of toxicity, bodyweight, food and water consumption,

haematology, urinalysis, blood biochemistry, macroscopic and microscopic pathology. Groups of 10 animals were selected at 6, 12, and 18 months for interim sacrifice.
A statistically significant increase in mortality was noted from 21 months onwards amongst males and females receiving 2 mg/kg/day. A slight decrease in bodyweight (up to 4%) was noted amongst males at 2 mg/kg/day and there were no significant effects on food and water consumption and no clinical signs of toxicity. There were no significant adverse effects on haematology, blood biochemistry, or urinalysis examinations, organ weights, or macroscopic pathology at 6, 12 and 18 months. However, at 24 months, there was an increase in the number of subcutaneous and mammary gland masses amongst females at 2 mg/kg/day. Histopathologically, there were no abnormalities at 6 months. At examinations performed from 12 months onwards there was an increase in the incidence and severity of tibial nerve degeneration amongst males at 2 mg/kg/day and from 18 months onwards in females at 2 mg/kg/day (focal swelling of nerve fibres with fragmentation of myelin and axon, and the formation of vacuoles containing small round eosinophilic globules and macrophages). There were no clear changes amongst animals at lower exposure levels or amongst other peripheral nerve samples (saphenous branch of the femoral nerve and brachial plexus).

In males, there was a statistically significantly increased incidence of benign follicular cell adenomas of the thyroid at the highest dose level (1/60, 0/58, 2/59, 1/59, 7/59). In females there was a non-significant increase in the incidence of benign follicular cell adenomas of the thyroid (0/58, 0/59, 1/59, 1/58, 3/60) and malignant adenocarcinomas (1/58, 0/59, 0/59, 0/58, 3/60). In females there was a statistically significant increase in the incidence of malignant adenocarcinomas in the uterus (1/60, 2/60, 1/60, 0/59, 5/60, or 1.7%, 3.3%, 1.7%, 0, 8.3%). The historical control range was stated to be 0-2.3%. In males there was a statistically significant increase in the incidence of malignant testicular mesothelioma at 0.5 and 2 mg/kg/day (3/60, 0/60, 7/60, 11/60, 10/60 or 5%, 0, 12%, 18%, 17%). The historical control incidence was 3.1% with a range of 2-6%.

In males there was a non-significant increase in the incidence of malignant astrocytomas in the spinal cord (1/60, 0/60, 0/60, 0/60, 3/60). There were also non-significant increases in malignant astrocytotomas in the brain of females (0/60, 1/60, 0/60, 0/60, 3/60), glial proliferation in the brain suggestive of an early tumour (0/60, 0/60, 0/60, 1/60, 3/60), and malignant astrocytomas in the spinal cord (1/60, 0/59, 0/60, 0/60, 3/61). In addition, malignant astrocytomas were also observed in the brain (3/60, 0/60, 0/60, 2/60, 2/60), and glial proliferation (suggestive of an early tumour) in 0/60, 0/60, 0/60, 1/60, 1/60. The effects in astrocytomas for brain and spinal cord in males and females do not show any clear dose-response but there are some concerns as these tumours are occurring in potential target tissues and, according to the authors, the concurrent control values may have been abnormally high so trends would not have been clear. Also, the group sizes used in this study may not have been sufficiently large enough to detect clear increases. Overall, because of these limitations, the toxicological significance of the presence of these astrocytomas in this study is unclear.

For females, there was a statistically significant increase in the incidence of benign papillomas in the oral cavity at 2 mg/kg/day (0/60, 3/60, 2/60, 1/60, 7/61) and a non-significant increase in focal hyperplasia (1/60, 2/60, 1/60, 0/60, 4/61). The incidence of malignant carcinomas did not show any clear dose-response (0/60, 0/60, 0/60, 2/60, 1/61). For males, the incidence of tumour formation in the oral cavity did not show any clear exposure relationship (carcinomas 2/60, 0/60, 1/60, 0/60, 2/60, and papillomas 4/60, 7/60, 0/60, 5/60, 4/60) although there was a statistically significant increase in focal hyperplasia of the hard palate (0/60, 1/60, 1/60, 1/60, 4/60, 5/60). Again, although effects are not clear, there are some concerns as there is a possibility that hyperplasia and subsequent, but unclear, tumour formation may have arisen as a result of local effects due to the route of exposure employed.

In females there were increases in benign and malignant tumours of mammary glands (10/60, 11/60, 9/60, 19/58, 23/61 and 2/60, 1/60, 1/60, 2/58, 6/61 respectively or 17%, 18%, 15%, 33%, 38% and 3%, 2%, 2%, 3%, 10%), benign pituitary gland adenomas (25/59, 30/60, 32/60, 27/60, 32/60 or 42%, 50%, 53%, 45%, 53%), and benign tumours of the clitoral gland (0/2, 1/3, 3/4, 2/4, 5/5). In males there were increased incidences of benign tumours in the adrenal glands (pheochromocytoma) (3/60, 7/59, 7/60, 5/60, 10/60 or 5%, 12%, 12%, 8%, 17%). The increased incidences of mammary tumours, benign pituitary adenomas and adrenal pheochromocytomas are of doubtful toxicological significance due to the poor dose-response and high historical control incidence (18% for benign mammary tumours, 2% for malignant mammary tumours - NTP data only, 28-47% for pituitary adenomas, 1-14% for pheochromocytomas). For clitoral adenomas the total number of tissues examined was too small to draw any firm conclusions.

A second, carcinogenicity study was conducted according to modern protocol standards using larger group sizes to clarify the tumour profile. Groups of 75-204 male F344 rats received 0, 0.1, 0.5, and 2 mg/kg/day acrylamide (99.9% pure) in drinking water for up to 2 years, and groups of 50-100 females received 0, 1, and 3 mg/kg/day [American Cyanamid Co., 1989; Friedman et al., 1995]. Two control groups were used for each sex. Routine observations included clinical signs of toxicity, food and water consumption, bodyweight, macroscopic- and microscopic pathology.

Increased mortality was noted amongst males receiving 2 mg/kg/day from 17 months onwards and in females at 3 mg/kg/day in the final month (75% vs 44-53% in males, 49% vs 28-40% in females at termination). There was an increased incidence of palpable subcutaneous masses in males and females during the last 6 months. There were no clinical signs of toxicity and no adverse effects on food and water consumption. Bodyweight was statistically significantly reduced by up to 9% amongst all treated males and by up to 7% in females (although not attaining statistical significance at 1 mg/kg/day) compared to the pooled control values at termination.

An increase (about 20-30%) in testicular weight was noted in males at 0.5 and 2 mg/kg/day. However this was considered to be of uncertain importance due to the high background incidence of interstitial cell tumours in this strain of rat - testes with large tumours which would have distorted testicular weight values were not excluded from organ weight analysis.

Histopathologically, an increased incidence of minimal to mild degeneration of the sciatic nerve (vacuolation of nerve fibres) was observed amongst females at 3 mg/kg/day and in males at 2 mg/kg/day.

There were increases in thyroid follicular adenomas (attaining statistical significance at 2 mg/kg/day) and a non-significant increase in carcinomas amongst males (3/204, 9/203, 5/101, 12/75 and 3/204, 3/204, 0/102, 3/75 respectively). Similarly, in females, there were increases in thyroid follicular adenomas and carcinomas (0/100, 7/100, 16/100 and 2/100, 3/100, 7/100 respectively). In males, there was a statistically significant increase in malignant scrotal mesothelioma at 2 mg/kg/day (8/204, 9/204, 8/102, 13/75).

In the brain the following increased incidences of benign and malignant tumours were noted: astrocytoma (1/204, 0/98, 0/50, 2/75 or 0.5%, 0, 0, 3% at 0, 0.1, 0.5, and 2 mg/kg/day respectively amongst the males and 0/100, 2/100, and 2/100 or 0, 2%, 2% at 0, 1, and 3 mg/kg/day respectively in females), meningioma (0/100, 2/100, and 3/100 in females, no significant increase in males), malignant reticulosis (0/100, 2/100, and 3/100 in females, no significant increase in males). These tumours may not be related to acrylamide exposure; combined historical control data from NTP studies exist with a range of glial cell tumours in the brain of up to 4%. Individual occurrences of astrocytomas in the spinal cord were observed in males and females but at very low incidence (0/172, 1/68, 0/102, 1/51 in males and 1/100 at 3 mg/kg/day, none in other groups for females). These findings do not show clear dose responses, and do not attain statistical significance. However, some concerns do remain, as there is a suggestion, although not convincing, of some

changes at the highest dose levels and because the brain and spinal cord represent possible target tissues for acrylamide.

In females, there were increased incidences of mammary gland fibroadenomas (9/96, 20/94, 26/95 respectively) and adenocarcinomas (2/96, 2/94, 4/95). As with the previous study, these tumours are of doubtful toxicological importance as mammary tumours occur at a high spontaneous incidence in rats. There were no significant increases in the incidence of neoplastic findings in the uterus, clitoral gland, pituitary gland and oral cavity. Unfortunately, sections did not appear to be taken from the oral cavity of all available animals making it difficult to draw firm conclusions regarding potential tumour formation at this site.

Overall, for acrylamide-exposed rats there are clear increases in tumours in several organs. Some of the tumour types observed in these two rat studies show a possible relationship with disturbed endocrine function (for example thyroid, testicular mesothelioma, adrenals) and raise the possibility of a hormonal mechanism. However, acrylamide is clearly genotoxic and it is possible that such tumours could have arisen following direct damage to the hormone-producing organ. There is also a suggestion of tumours in the brain and spinal cord and, although the picture is not clear, these are possibly acrylamide-induced.

4.8.2 Carcinogenicity: inhalation

No carcinogenicity studies were available using the inhalation route of exposure.

4.8.3 Carcinogenicity: dermal

No carcinogenicity studies were available using the dermal route of exposure.

4.8.4 Carcinogenicity: human data

Two industrial epidemiological studies have been described in the EU RAR (2002). NB: The references from the 2002 EU RAR [cited between brackets] are not given in the SVHC Support Document but can be found in the RAR.

A cohort mortality study was available investigating populations of workers exposed to acrylamide at 3 factories in the United States and one in the Netherlands [Collins et al., 1989]. The cohort was defined as all workers hired between January 1, 1925 and January 31, 1973 with data collected up to December 31, 1983. From the US factories 8,508 men were identified, with complete follow-up for 94% - of this group 5,847 were still alive at the end of the study, 2,148 were dead (with the cause of death not determined in 111), and 513 were lost to the follow-up. In the Netherlands 346 men were identified, 315 were alive at the end of the study, 11 were dead (with the cause of death not determined in 2), and 20 lost to follow-up.

Occupational exposure information was available from 1977 (8-hour TWA values from personal monitoring). Estimates were made of exposure before this time on the basis of these values and from the knowledge of processes involved. The extent of dermal exposure was unclear. Comparisons were made against an internal population where cumulative airborne exposure was less than 0.001 mg/m3-years (approximately equivalent to one day exposure to 0.3 mg/m3). There were 2,293 in the “acrylamide-exposed” group (those exposed to >0.001 mg/m3-years) and 8,094 people in the group of “unexposed” workers (those exposed to <0.001 mg/m3-years). Amongst the 2,293 “acrylamide-exposed” workers, there was no clear breakdown of the numbers in each of the sub-groups used (0.001-0.03, 0.03-0.3, >0.3 mg/m3-years). For the “acrylamide-exposed” group, approximately half of the person years of exposure were at 0.3 mg/m3-years or more [Collins et al.,

1990]. In this group the median exposure was around 5 mg/m3-years (equivalent to about 15 years exposure to 0.3 mg/m3).

Overall, taking into account the entire cohort membership, the standardised mortality ratio (SMR) for all causes of death was not increased at any of the four plants. There were no statistically significant increases in the SMR for cause-specific mortality amongst the workers exposed to >0.001 mg/m3-years ("acrylamide-exposed"). When this group was further subdivided there were no statistically significant increases in deaths resulting from any cause in any of the three exposure categories (overall 33 deaths observed against 44 expected at 0.001-0.03 mg/m3, 97 observed vs. 97 expected at 0.03-0.3 mg/m3, and 169 observed vs. 158 expected at >0.3 mg/m3). Amongst "acrylamide-exposed" workers, there was a slight, but not statistically significant increase, in cancer of the pancreas (8 observed, SMR=2.03, 95% confidence intervals, CI = 87-400). This was further broken down to show the following observed/expected (O/E) ratios: 19/21 in the "non-exposed" group, 1/0.7 in the 0.001-0.03 mg/m3-year group, 2/1.8 in the 0.03-0.3 mg/m3-year group, and 5/3.8 in the >0.3 mg/m3-year group. No clear exposure-response emerges from this data.

It was stated that this study would have been able to detect a 25% increase in total cancer, 50% increase in respiratory cancers, and a 3-fold increase in cancer of the brain and central nervous system with a power of 80%.

Overall, this study did not reveal any significant increase in mortality from any given cause, including site-specific cancer, amongst the workers potentially exposed to acrylamide at these plants.

A second, but much smaller, cohort mortality study was available [Sobel et al., 1986] amongst 371 workers potentially exposed to acrylamide from 1955 in the manufacture of Acrylamide monomer and polyacrylamide. The cohort included all those employed up until and including December 31, 1982. Of the total population at this site, 357 had no exposure to organic dyes, and from these, a total of 20 had died. Personal 8-hour TWA airborne exposure levels were available - before 1957 these ranged from 0.1-1 mg/m3, from 1957-1970 from 0.1-0.6 mg/m3 and from 1970 were <0.1 mg/m3. Again, the extent of dermal exposure was unclear. Exposure also potentially involved acrylonitrile and organic dyes - data were presented separately for those exposed to organic dyes for more than 5 years.

For the total cohort (including those exposed to organic dyes) 29 deaths were observed against 38 expected. However, there was an increase in the number of deaths from all cancers (O/E=11/7.9) related to increases in death due to cancer of the digestive tract (O/E = 4/1.9, SMR=2.02, CI=57-539) and also cancer of the respiratory system (O/E = 4/2.9, SMR=1.38, CI=38=353). When workers who had been exposed to organic dyes for more than 5 years were excluded (14 workers were excluded, leaving a group size of 357 workers) no statistically significant increases in the number of observed deaths due to any cause were observed. The largest value was death due to cancer of the digestive tract in which there were 2 mortalities against 1.6 expected, SMR = 1.24, CI=15-452.

The authors stated that a 2-fold increase in total cancer could have been observed with 80% power. The power of this study in respect of detecting cancer at any specific site was severely limited by small numbers.

In an 11-year follow-up (1984-1994) of the original cohort from the 1989 Collins et al. study (Marsh et al., 1999), 1115 additional deaths and nearly 60000 person-years were added to the original study. For the 1925-1994 study period, excess and deficit overall mortality risks were found for cancer sites of interest: brain and other areas of the central nervous system, thyroid gland, testis and other male genital organs, and cancer of the respiratory system. However, none was significant or associated with exposure to acrylamide. A significant (2.26-fold) increased risk for pancreatic cancer mortality among U.S. workers with cumulative exposure to acrylamide > 0.30 mg/m^3.years

was seen (a total of nine deaths). However, no consistent dose-response relationship to acrylamide exposure was found.
The last update of this cohort was published by Marsh et al. (2007) and covered the period 1925-2002. The updated analysis of the previous exploratory findings for pancreatic cancer in the U.S. cohort revealed much less evidence of a possible exposure-response relationship with acrylamide. The authors concluded that acrylamide exposure at the levels present in the study sites was not associated with elevated cancer mortality risks.

4.8.5 Other relevant information

In the EU RAR (2002) another two studies have been described. Not being true carcinogenicity bioassays, they provide some, albeit limited, information. NB: The references from the 2002 EU RAR [cited between brackets] are not given in the SVHC Support Document but can be found in the RAR.

In a skin initiation/promotion assay, groups of 16-40 female SENCAR and ICR mice received 0 or up to 50 mg/kg aqueous acrylamide (>99% pure) or acrylamide in ethanol (for dermal studies) 3 days/week for 2 weeks by oral gavage, ip injection or topically [Bull et al., 1984a; 1984b]. Tetradecanoyl-phorbol acetate (TPA) was then administered dermally 3 days/week for 20 weeks to most groups (there were some non-TPA controls) and animals were sacrificed on completion of 52 weeks. An acrylamide dose-related increase in tumour formation was noted for all routes of acrylamide administration when TPA was administered subsequently but there was no increase in tumour incidence in mice treated with acrylamide but not subsequently with TPA. These results suggest that acrylamide was “initiating” tumour formation.

In addition, in a lung adenoma bioassay [Bull et al., 1984a], groups of 16-40 male and female A/J mice received up to 25 mg/kg aqueous acrylamide by oral gavage or up to 60 mg/kg by the ip route 3 days/week for 8 weeks. These animals were sacrificed after 8-9 months. In the adenoma bioassay, there was also an exposure-related increase in the formation of lung tumours. The enhancement of benign lung tumour incidence by acrylamide in a mouse strain showing a high background incidence of such tumours is of doubtful significance in relation to human health.

4.8.6 Summary and discussion of carcinogenicity

It was concluded in the EU RAR (2002) that acrylamide is carcinogenic in animals producing increased incidences in a number of benign and malignant tumours identified in a variety of organs (for example thyroid, adrenals, testicular mesothelioma). The tumour types observed show a possible relationship with disturbed endocrine function and raise the possibility of a hormonal mechanism. There is also a suggestion of tumours in brain and spinal cord and, although the picture is not clear, these are possibly acrylamide-induced. Given the genotoxicity profile of acrylamide, genotoxic activity cannot be discounted from contributing to tumour formation. There are no mechanistic arguments to indicate that these findings would be restricted to animals and could not occur in humans.

Two human cohort mortality studies did not show any clear increase in cause-specific mortality as a result of acrylamide exposure although there were clear inadequacies in one of the two studies available. No firm conclusions can be drawn from these studies.

4.9 Toxicity for reproduction

Not relevant for this dossier.

4.10 Other effects

Not relevant for this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not relevant for this dossier.

6 PBT, VPVB AND EQUIVALENT LEVEL OF CONCERN ASSESSMENT

Not relevant for this dossier.

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