European Commission

Recommendation from the Scientific Committee on Occupational Exposure Limits for Acrylamide

SCOEL/SUM/139
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Employment, Social Affairs and Inclusion

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8 hour TWA:	not assigned (see "*Recommendation*")
STEL (15 mins):	not assigned (see "*Recommendation*")
Additional classification:	'skin' notation
BLV:	not assigned (see "*Recommendation*")
BGV:	See Annex (December 2012)
SCOEL carcinogen group:	B (genotoxic carcinogen, for which the existence of a threshold cannot be sufficiently supported at present.)

Substance identification:

Synonyms: 2-propenamide, acryl acid amide, ethylene caroxamide, propenoic acid amide, vinyl amide

EINECS No.: 201-173-7

EU-Classification:

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

CAS No.: 79-06-1

Molecular formula: C_3H_5NO

Structural formula: $CH_2=CH-CONH_2$

MWt: 71.09 $gmol^{-1}$

Conversion factor: At 25°C 1 ppm = 2.907 mgm^{-3};
1 mgm^{-3} = 0.344 ppm

This document is largely based on the EU-RAR from 2002 with the inclusion of some more recently published studies and of the evaluations of DFG (2007, 2008, 2009).

1. Occurrence/use and occupational exposure

Acrylamide is produced in the European Union as a 30 to 50% aqueous solution via the catalytic hydration of acrylonitrile using either (a) a low-temperature enymatic process, or (b) a copper catalyst at 100 to 150 °C. The production capacity within the EU is estimated at between 150,000 to 200,000 tonnes per annum.

The major use of acrylamide (more than 99%) is in the production of polyacrylamides. These are high molecular weight polymers that are produced to have non-ionic, cationic or anionic properties depending on specific uses. The residual acrylamide content of these polymers is controlled to below 0.1% to avoid classification as a Class 2 Carcinogen under the Dangerous Preparations Directive (88/379/EEC). Both solid and aqueous grades of acrylamide are used in polymer manufacture to produce various solid and liquid grade polymers. The main uses of polyacrylamides are in wastewater treatment, paper and pulp processing and mineral processing. They are also used in a wide range of other applications including crude-oil production processes, cosmetics, soil and sand stabilisation, dispersants and bindings in coatings, textile processing, as thickeners and in adhesives.

Acrylamide monomer is also used in the preparation of polyacrylamide electrophoresis gels in hospital, university and research labs.

Acrylamide is also used in the formulation of acrylamide grouting agents.

The occurrence of acrylamide in food has been the subject of recent reviews (e.g. Dybing et al. 2005, UBA 2008).

2. Health significance

2.1 Toxicokinetics

2.1.1 Human data

The association of acrylamide exposure by inhalation, skin contact or ingestion with neurotoxic effects in humans indicates that acrylamide is absorbed and that it or its metabolites are distributed to skeletal muscle and/or nerves associated with affected muscles (EU-RAR, 2002).

Non-smoking human volunteers ingested 0.94 mg acrylamide and urine was collected for the following 72 hours (Fuhr et al 2006). About 60% of the dose was recovered as metabolites in the urine indicating that acrylamide is well absorbed by the oral route. Unchanged acrylamide, its mercapturate metabolite *N*-acetyl-*S*-(2-carbamoylethyl) cysteine, and the mercapturate metabolite of glycidamide, *N*-acetyl-*S*-(2-hydroxy-2-carbamoylethyl) cysteine, accounted for 4.4%, 50% and 5.9% of the dose respectively. The authors concluded that the metabolism of acrylamide to glycidamide in humans is 2-fold lower than in rats and 4-fold lower than in mice.

In another recent human volunteer study a comparison was made of haemoglobin adduct levels following oral and dermal exposures to acrylamide (Fennell et al 2005, 2006). After dermal exposure the formation of haemoglobin adducts was only about 6.6% of that compared to oral ingestion of an equivalent dose. In this study, approximately 86% of the urinary metabolites detected following oral administration were derived through glutathione conjugation. Although the results of this study suggest a relatively low degree of dermal absorption of acrylamide in humans, reports

of neurotoxicity in workers exposed to acrylamide and polyacrylamide highlight the importance of dermal absorption under practical workplace conditions.

2.1.2 Animal data

There are few inhalation data. In a radiolabel study, Sumner *et al* (2003) exposed rats and mice nose-only to a mixture of [1,2,3 ^{13}C] and [2,3 ^{14}C] 2.9 ppm (8.4 mg.m^{-3}) acrylamide vapour for 6 hours. In rats and mice respectively, 56% and 46% of the [^{14}C] was recovered in the tissues and 31% and 27% was eliminated within 24 hours in the urine. These results indicate that an overwhelming portion of inhaled vapour of acrylamide is absorbed across the respiratory tract. In rats, glutathione conjugates accounted for 68% of the urinary metabolites, compared with only 27% in mice. The remaining urinary metabolites were glycidamide derivatives.

Acrylamide is rapidly absorbed following oral administration and is distributed to all organs and tissues including the developing foetus. There is evidence that acrylamide has an exceptionally high affinity for the male reproductive tract. Whole body radiography in mice showed a high uptake of the radioactive label in testes, then epididymis then glans penis in mice during 1-9 days post-dosing (Marlowe et al., 1986). Measurements of high levels of DNA adducts in the testes of rats confirms the distribution of acrylamide to the testes (Doerge et al 2005). Evidence of binding of acrylamide or its metabolites to RNA, DNA and protein has been found in a range of tissues (EU RAR 2002). There is some evidence of accumulation of acrylamide in erythrocytes following repeated administration.

In relation to dermal absorption, a comparison of the results for dominant lethal and heritable translocation tests in mice following dermal and intraperitoneal administration suggested that about 39% of the dermal dose was absorbed (Adler *et al*, 2004). In a radiolabel study with [C^{14}] acrylamide, between 14-30% of a dermal dose was absorbed in rats over 24 hours (Sumner et al 2003). In a study by Frantz et al (1994), as cited by Shipp et al (2006), it was reported that 30% of a dermal dose of aqueous acrylamide was absorbed in rats based on recovery in tissues and excreta. A further 31% of the dermal dose was recovered in skin segments; the authors considered that this would have been available for systemic distribution, had the experiment continued beyond 24 hours.

There are two main routes for metabolism of acrylamide; direct conjugation with glutathione and CYP2E1 mediated oxidation to glycidamide, a reactive epoxide. Glycidamide can also undergo subsequent conjugation with glutathione. Both acrylamide and glycidamide bind covalently to the N-terminal valine residue in haemoglobin. The haemoglobin adduct caused by Michael addition to acrylonitrile itself is *N*-2-carbamoyl-ethyl-valine [*N*-(2-carbonamide-ethyl)-valine]. The corresponding adduct of glycidamide is *N*-(*R,S*)-2-hydroxy-2-carbamoyl-ethyl-valine. There is a collation between the quantities of both adducts (DFG 2008). The long half-life of haemoglobin means that the level of adducts (given) reflects cumulative exposure over the lifetime of the red blood cell. Thus, similar levels of adducts could arise from the same total exposure over an extended time period as over a short period. Acrylamide is rapidly excreted in the urine primarily in the form of mercapturate derivatives of acrylamide and glycidamide. Most studies have reported that about 50% of the administered dose is eliminated in the urine in the first 24 hours following administration (Boettcher et al. 2006a,b).

2.1.3 Biological monitoring

For the analysis for urinary metabolites, Wu et al (1993) described an HPLC Assay developed for the mercapturic acid metabolite of acrylamide, mercapturic acid, N-

Acetyl-s-(propionamide)-cysteine (APC) in urine. Boettcher and Angerer (2005) and Li et al (2005) have described methods of analysis for mercapturic acid metabolites of acrylamide in urine using LC-MS/MS.

For the determination of low levels of the haemoglobin adducts a GC-MS method has been optimised (Schettgen et al. 2004), which was based on the method of Paulsson et al. (2003) and included a modified Edman degradation of the alkylated N-terminal valine. Calibration is performed with the dipeptide *N*-2-carbamoyl-ethyl-valine-leucine-anilide. Although this method is time-consuming and expensive, it is currently performed in specialized laboratories on a routine basis, with reliable results.

Several studies have used the formation of haemoglobin adducts as a marker of exposure. Jones et al (2006) took blood samples from 60 workers exposed to acrylamide monomer and polymer and measured acrylamide haemoglobin adducts, comparing the results with acrylamide levels in personal air samples and glove linings. Measurements were taken twice over a three-month interval and indicated that airborne exposures were relatively stable over this time. The results showed a good correlation between acrylamide-haemoglobin adducts, and personal inhalation exposures. Dermal exposures showed a wide range and some were below the limit of detection, although a significant correlation with dermal exposures and adduct levels was obtained. Inhalation exposures in these workers were low, and consistently below the UK occupational exposure limit of 0.3 $mg.m^{-3}$ (8-hr TWA). The 90^{th} percentile of adducts was 0.514 nmol/g globin, and there were no symptoms of peripheral neuropathy in any workers (although no details of any health investigations were reported). Long-term exposure to 0.3 $mg.m^{-3}$ was calculated to lead to a mean acrylamide haemoglobin adduct level of 1.550 nmol/g globin (95% CI 1.150 -1.950 nmol/g globin). There was no unexposed control group in this study, but 13 workers had very low inhalation exposures consistently below 0.01 $mg.m^{-3}$. Among these workers there were 8 non-smokers and 5 smokers with mean acrylamide haemoglobin adduct levels of 0.032 and 0.051 nmol/g globin respectively. These data correspond to those of Schettgen et al. (2004), reporting mean levels (+/- SD) of 19 +/- 7 pml/g globin in 13 non-smokers and 80 +/- 48 pmol/g globin in 18 smokers for the acrylamide adduct. The repesctive values for the glycidamide adduct were 17 +/- 4 and 53 +/- 30 pmol/g globin. Hence, the haemoglobin adducts discriminate effectively between occupational exposures and environmental exposures arising from smoking and the diet.

Compared to the measurement of urinary metabolites, the haemoglobin adduct monitoring has the advantage that it provides a measure of cumulative exposure over several weeks. At the present time, there are insufficient data from field studies to establish biological limits for excretion of the mercapturic acids derived from acrylamide. A recent assessment has been presented by DFG (2008).

2.2 Acute toxicity

2.2.1 Human data

There are no data about acute effects following inhalation exposure to high concentrations of acrylamide (EU-RAR, 2002). There is a single case report of acrylamide poisoning following deliberate ingestion. Despite attempts to empty the stomach, the patient experienced hallucinations, hypotension and subsequently seizures within 9 hours of ingestion followed by gastrointestinal bleeding, respiratory distress and symptoms of peripheral neurotoxicity and liver toxicity during the following three days. Symptoms of neurotoxicity persisted for at least two months following the incident (EU RAR 2002).

2.2.2 Animal data

Acrylamide is toxic by the oral route of administration with LD50 values of around 200 mg/kg in rats and 107 mg/kg in mice. The principal effects prior to death are neurotoxicity. A dermal LD50 of 1148 mg/kg has been obtained in rabbits. There is only one acute inhalation toxicity study available in rats, in which no clear evidence of toxicity was observed following a 1-hour exposure to 6000 $mg.m^{-3}$ of aerosolised aqueous droplets of acrylamide (EU RAR 2002).

2.3 Irritation and corrosivity

2.3.1 Human data

There is evidence from case-reports and workplace surveys of effects such as skin rashes, skin peeling and acne-like dermatitis associated with dermal exposure to acrylamide. Skin peeling has been observed mainly on the palms of the hands, but also on the feet, and has been reported in workers exposed to monomeric acrylamide powder, to polymeric acrylamide, to aqueous solutions of acrylamide and also to acrylamide used in sewer grouting work. There was a high prevalence of self-reported symptoms such as eye irritation and respiratory tract irritation in workers exposed to aqueous solutions of acrylamide used in tunnel construction (Hagmar et al 2001).

2.3.2 Animal data

Skin

A number of studies have been carried out in rabbits as summarised in the EU RAR (2002). The results showed either slight or no skin reactions following single or repeated dermal exposures to either aqueous solutions of acrylamide (10-51%) or with powdered acrylamide moistened with water.

Eyes

Results of studies in animals have been summarised by EU-RAR (2002). Tests for ocular irritation and reversibility in rabbits gave different results between crystalline and dissolved acrylamide. In case of acrylamide crystals, effects on conjunctiva, iris and cornea were moderate to severe and did not show total reversibility until day 21. With solutions of acrylamide, the effects were not more than moderate, and there was total reversibility by day 7.

Respiratory tract

There are no studies in animals that have investigated the potential irritant effects of acrylamide on the respiratory tract.

2.4 Sensitisation

2.4.1 Human data

There are no reports of respiratory sensitisation in humans despite the widespread use of acrylamide and acrylamide products (EU-RAR, 2002).

There have been only two published case reports of skin sensitisation in workers exposed to acrylamide despite widespread workplace exposure to products containing acrylamide (EU-RAR, 2002). These case reports refer to exposure to polyacrylamide gels. In a human volunteer study into the toxicokinetics of acrylamide, one of 24 subjects developed a delayed hypersensitivity response following 3 daily dermal

applications of a 50% aqueous solution of acrylamide indicative of skin sensitisation; the skin response took 3 weeks to resolve (Fennell et al 2005).

2.4.2 Animal data

Tests in guinea pigs conducted to meet appropriate guidelines have demonstrated that acrylamide is a skin sensitiser in animals (EU-RAR, 2002).

2.5 Repeated dose toxicity

2.5.1 Human data

There are a number of case reports of individuals with occupational exposure to acrylamide who developed clinical signs of neurotoxicity including unsteady gait and impaired speech. These signs were accompanied by tingling sensations and loss of use of hands, reduced muscle tone, incoordination of upper limbs and tremor of the hands. Following cessation of exposure there appears to be recovery over a period of months although some muscle weakness was still noted in one individual after one year. Among the individual case-reports available, no clear information is available regarding the levels and route(s) of exposure to acrylamide (EU RAR).

Extensive clinical examinations were carried out in 71 workers at a Chinese factory producing acrylamide monomer and polymers (He et al 1989). The workers had been exposed to acrylamide for between 1-18 months. One year prior to the examinations, air concentrations of acrylamide were reported to be 5-9 $mg.m^{-3}$ during peak production (no further details available). The workers were also reported to wash their hands in water contaminated with up to 410 mg/l acrylamide. Examinations revealed clear signs of peripheral neuropathy as well as effects on balance and nystagmus, and decreases in action potential amplitude in sural, median and ulnar nerves. Other findings included muscle wasting, numbness/tingling of hands and feet, and loss of tendon reflexes. There were also reports of increased sweating, skin peeling and skin erythema. The relative contributions to these findings from dermal and inhalation exposure cannot be determined from this study.

Two publications were based on the examination of Norwegian workers in tunnel construction projects terminated more than 2 years prior to examination, therefore demonstrating persistent nervous system effects caused by acrylamide exposure. The first study (Goffeng et al. 2008a) showed reduced sural sensory nerve conduction in the legs, a reduced amplitude at ERG 30 Hz flicker stimulation, and a prolonged VEP N75 component. ERG 30 Hz primarily reflects retinal cone and adjoining bipolar cell activity located centrally in the visual field. The VEP N75 component reflects the time period from a stimulus eliciting an impulse until the impulse reaches the visual cortex, thus reflecting the optic nerve function. In the second study (Goffeng et al. 2008b) a reduced visual light sensitivity centrally in the visual field, with a possible component of reduced colour vision, was shown. Though statistically significant, the effects were regarded as being sub-clinical. The effects were seen in accordance with animal studies, in that primarily thin fibres of the optical nerve are affected.

Following reports of clinical signs of neurotoxicity in 5 workers, investigations were carried out in 71-75 workers from a polyacrylamide-manufacturing factory in South Africa (Myers and Macun 1991, Bachmann et al 1992). Personal exposure levels ranged from 0.02-2.39 $mg.m^{-3}$ with an overall mean of 0.16 $mg.m^{-3}$ (presumably 8-hr TWA). There was no unexposed control group, but findings were compared in those exposed to <0.3 and >0.3 $mg.m^{-3}$. There was no evidence of muscle wasting, loss of position sense or any positive Romberg tests in either group. No differences in vibration sensitivity were noted across the two groups. Slightly impaired tactile responses were noted in workers from both exposure groups. There was a higher

prevalence of reports of skin peeling and sweating, and numbness of hands and feet in those exposed to >0.3 versus <0.3 $mg.m^{-3}$. It is not possible to assess the relative contributions of dermal and inhalation exposures to the observed effects among these workers.

Hagmar *et al* (2001) obtained blood samples for measurement of haemoglobin adducts and carried out clinical investigations in 210 tunnel workers exposed for about 2 months to a grouting agent containing acrylamide and N-methylolacrylamide. Fifty workers reporting recently developed neurological symptoms with or without objective findings at clinical examination were referred for further neurophysiological tests. Those workers with peripheral nervous system symptoms and acrylamide haemoglobin levels $\geq$ 0.3 nmol/g globin were followed up with clinical and neurological examinations up to 18 months post-exposure.

Among 18 non-smoking non-acrylamide exposed controls, the reported range of acrylamide-haemoglobin adducts was 0.02-0.07 nmol/g globin. Forty-seven tunnel workers had adduct levels within this range. The remaining 163 workers had adduct levels up to a maximum of 17 nmol/g globin. Repeat sampling from five workers showed a decline in adduct levels over a five month period that was consistent with the 120-day life span of human red blood cells The authors concluded that there was a clear-cut dose-response relationship between the presence of peripheral nervous system symptoms and the levels of acrylamide-Hb adducts. A no-observed adverse effect level of 0.51 nmol/g globin (upper-bound confidence limit) was estimated for numbness/tingling in the feet or legs. Thirty-nine percent of those with Hb-adducts exceeding 1 nmol/g globin experienced tingling or numbness in the hands or feet. All but two workers had recovered by 18 months post-exposure.

Of the 50 workers referred for neurological examination, 29 had adduct levels >0.3 nmol/g globin. Two of these 29 showed evidence of polyneuropathy, 8 had skight impairment of nerve conduction or amplitude, 9 had increased sensory perception thresholds, and 9 were neurophysiologically "normal".

Ten of the workers showed dermatitis of the hands. Only one worker showed a positive skin sensitisation response to N-methylolacrylamide There was a high prevalence of self-reported symptoms such as eye irritation and respiratory tract irritation in the most highly exposed workers

The blood levels reported by Hagmar *et al* (2001) were about 10% of those reported in Chinese workers (0.3 to 34 nmol/g) exposed to acrylamide levels of between 0.11 and 8.8 mgm^{-3} (0.039-3.03) although the Chinese workers also had extensive dermal contact with acrylamide (Bergmark *et al*, 1993). It must be noted that exposure in this Chinese study was much more prolonged than in the study by Hagmar et al. (2001). Bergmark *et al* noted that the highest adduct levels found in their study were similar to those found in rats following injection of 3 mg/kg.

Kjuus et al. (2004) evaluated the effects on the peripheral nervous system of 24 tunnel workers exposed to acrylamide and N-methylolacrylamide, 4 and 16 months after the cessation of grouting operations. Fifty tunnel workers not involved in grouting operations served as referents. The exposed workers reported a higher prevalence of symptoms during grouting work than they did in an examination 16 months later. A statistically significant reduction in the mean sensory nerve conduction velocity of the ulnar nerve was observed 4 months post exposure when compared with the values of the reference group (52.3 versus 58.9 m/s, $p = 0.001$), and the mean ulnar distal delay was prolonged (3.1 versus 2.5 ms, $p = 0.001$). Both measures were significantly improved when measured one year later. Exposure-related improvements were observed from 4 to 16 months post exposure for both the median and ulnar nerves. A significant reversible reduction in the mean sensory amplitude of the median

nerve was also observed, while the mean sensory amplitude of the sural nerve was significantly reduced after 16 months. The changes were slight and mostly sub-clinical.

In summary, workplace studies indicate that exposure to acrylamide is associated with symptoms of peripheral neuropathy. The importance of inhaled acrylamide versus acrylamide absorbed through the skin is unclear as in all of the workplace studies workers have had extensive skin contact with acrylamide.

2.5.2 Animal data

Inhalation

There are no data for exposure by inhalation.

Oral

Repeated oral exposures in rats, mice, cats, dogs and non-human primates reveal that the nervous system is the principal target for the effects of acrylamide. There is also evidence for adverse effects on the male reproductive organs. Doses of 5-6 mg/kg/day and above produce overt signs of neurotoxicity including loss of use of hind limbs, ataxia and tremor. Morphological changes are observed at doses of 5 mg/kg/day and above and include loss of axons, axonal swelling, degenerating myelin, degenerative changes in peripheral and optic nerves, swollen astrocytes, and degenerative changes in the lateral geniculate nucleus. Studies in non-human primates also reveal reduced visual acuity at 10 mg/kg/day.

Among the studies in experimental animals, the key study that provides the most useful dose-response information and included the most detailed neuropathological investigations was a 90-day drinking water study in rats (Burek et al 1980). In this study rats were administered acrylamide doses of 0, 0.05, 0.2, 1, 5 and 20 mg/kg/day. At 20 mg/kg/day there were losses in body weight gain and severe clinical signs of neurotoxicity including loss of use of hind limbs. Among males at this dose there was evidence of testicular atrophy and mineralisation of the seminiferous tubules; at 5 mg/kg/day and below there were no clinical signs of toxicity and no neurobehavioural abnormalities. However, peripheral nerve lesions were observed in most animals at 5 mg/kg/day. Haematological investigations on day 76 and at the end of the study revealed reductions in packed cell volume, red blood cells and haemoglobin values in rats of both sexes at 20 mg/kg/day and in females at 5 mg/kg/day. At 1 mg/kg/day, there were no neuropathological changes visible by light microscopy but electron microscopic evaluations revealed axolemmal invaginations, which were completely reversible after 25 days. This minimal effect may be regarded as an LOAEL. Doses below 1mg/kg/day were without any effects, compared to the controls. Overall, a well-established experimental NOAEL of 0.2 mg/kg/day can be derived from this study.

In a combined chronic toxicity/carcinogenicity study in F344 rats in which acrylamide was administered via the drinking water, at the top dose of 2 mg/kg/day there was tibial nerve degeneration in males observed at 12 months onwards and in females at 18 months onwards (Johnson et al 1986).

Information on neurological effects is also available from a 2-generation/dominant lethal study in rats (Tyl et al 2000). In this study, rats were exposed to acrylamide via the drinking water for 10 weeks pre-breeding at doses of 0, 0.5, 2 or 5 mg/kg/day. In the F0 males, increased incidences of head tilt and foot splay were reported at all doses, but in the F1 males the only clinical sign was head tilt at 5 mg/kg/day.

Several studies have investigated the mechanisms underlying acrylamide neurotoxicity since the EU-RAR including LoPachin *et al* (2003), Lehning *et al* (2002, 2003) and Ko *et al* (2002). The mode of action appears to be complex, including interference with neurotransmitters and interference with axonal transport. A recent discussion of these effects has been presented by DFG (2007, 2009).

Limited experimental information is available concerning the effects of repeated dermal exposure. In a study reported as an abstract only, rabbits were administered dermal doses of 0. 0.5, 5 or 50 mg/kg/day for up to 12 weeks (Drees et al 1979). Clinical signs of neurotoxicity (no details given) were reported at 50 mg/kg/day; these signs resolved after 7 weeks recovery. There were no signs of toxicity reported in other dose groups although the extent of investigations was unclear. In a dermal dosing study in mice designed to investigate dominant lethal effects, no signs of neurotoxicity were reported with doses up to 125 mg/kg/day for 5 days (Gutierrez-Espeleta et al 1992).

2.6 Genotoxicity

2.6.1 In vitro

Acrylamide gives negative results in standard bacterial mutation tests. Studies in mammalian cells consistently indicate positive results for chromosomal aberrations and polyploidy (EU RAR 2002; see also COM 2009). There are a number of gene mutation studies in mammalian cells; these mainly yielded negative or equivocal results. Positive results have been reported in some studies (e.g Barfknecht et al 1988, Besaratinia and Pfeiffer 2003). The latter study used cells from a a transgenic mouse mode and the authors concluded that acrylamide was a weak mutagen, causing mutations at cytotoxic concentrations.

Zeiger et al. (2009) evaluated the low-dose micronucleus response in mouse bone marrow and the shape of the dose-response curve. Mice were treated orally with acrylamide for 28 days using logarithmically spaced doses from 0.125 to 24 mg/kg per day, and micronuclei were assessed in peripheral blood reticulocytes and erythrocytes. Liver glycidamide DNA adducts and acrylamide and glycidamide N-terminal valine hemoglobin adducts were also determined. Acrylamide produced a weak micronucleus response, with statistical significance at 6.0 mg/kg per day in reticulocytes and at 4.0 mg/kg per day or in normochromatic erythrocytes. The adducts increased at a much different rate than the micronuclei. When the values for micronuclei in reticulocytes were compared to administered dose, the response was consistent with a linear dose-response model. However, when hemoglobin- or DNA-adducts were used as the dose metric, the response was significantly nonlinear, and models that assumed a threshold dose of 1 or 2 mg/kg per day provided a better fit. These data were taken to suggest a threshold for acrylamide genotoxicity in the micronucleus test.
In addition, Friedman et al. (2008) provided data suggesting that inhibition of the microtubule motor protein kinesin is responsible for the acrylamide-induced clastogenicity and aneuploidy. Two kinesin motors, KIFC5A and KRP2, which are responsible for spindle assembly and disassembly of kinetochore MT, respectively, were inhibited by acrylamide. The inhibitory concentration for a response was below the levels shown to adversely affect the cytogenetic parameters. The kinesin proteins as site of action of acrylamide were again taken as an argument for a threshold effect of the chromosomal genotoxicity of acrylamide.

2.6.2 In vivo – Human data

Kjuus et al. (2005) examined chromosome aberrations in whole-blood cultures from 25 tunnel workers exposed to acrylamide-containing grout in injection work and 25 control persons. The chromosome examinations showed no statistically significant differences for cells with chromosome aberrations or for chromatid breaks,

chromosome breaks, and chromosome gaps. The exposed workers had a significantly higher number of chromatid gaps (mean 10.6, SD 5.6) than the unexposed workers (mean 6.4, SD 4.4, p=0.004), but there was no exposure-response relationship.

2.6.3 In vivo – Animal data

Studies in rodents with acrylamide yield positive results for chromosomal aberrations, micronucleus formation and aneuploidy in bone marrow assays.The EU-RAR (2002) concluded that acrylamide is a direct-acting mutagen but probably causes clastogenic effects rather than gene mutations.

Acrylamide is clearly a germ cell mutagen in males but there are no studies of germ cell mutagenicity in females. In relation to germ cell mutagenicity, positive results have been obtained for a range of endpoints including chromosomal aberrations, micronuclei, sister chromatid exchange, unscheduled DNA synthesis, dominant lethal mutations and heritable translocations (EU RAR 2002). The germ cell effects are quite striking. Marchetti et al., (1997) administered acrylamide at 50 mg/kg/day for 5 consecutive days via intraperitoneal injection to male mice, and this treatment led to chromosomal aberrations in 76% of first metaphase zygotes from matings 6.5 days post-dosing, corresponding to exposure of early spermatocytes. Consistent with these results, Paccheriotti (1994) also found a high percentage of chromosomal aberrations (85%) in one-cell zygotes from male mice administered acrylamide 7 days prior to mating. Tyl et al (2000) observed dominant lethal effects in rats exposed to acrylamide in the drinking water at doses of 5 mg/kg/day.

Overall, there is an overwhelmingly large body of evidence for the germ cell mutagenicity of acrylamide, and this has been reviewed by Favor and Shelby (2005). From the available evidence it is clear that different spermatogenic stages have differential sensitivity to the genotoxic effects of acrylamide. Positive results have been reported for effects on spermatogonia and spermatocytes but not consistently. In contrat, there is very clear and consistent evidence for genotoxic effects in late spermatids and early spermatozoa, which in mice correspond to treatment periods of around 12.5 and 6 days pre-mating respectively. One possible reason for the enhanced sensitivity of these cell types is that spermatids and spermatozoa are post-meiotic cells that are deficient in DNA repair capability. It has also been suggested that alkylation of sulfhydryl groups of protamines (which replace nuclear histones in these stages of sperm development) may explain the enhanced effect of acrylamide on these spermatogenic stages. On one hand, it may be possible that the germ cell mutagenicity of acrylamide could be related to direct DNA alkylation. On the other hand, reproductive endpoints appear more sensitive than genotoxic endpoints; this has been taken as an argument for the significance of protein interaction (Allen et al. 2005).

There is some evidence to suggest that the germ cell mutagenicity of acrylamide may be mediated by its glycidamide metabolite rather than by acrylamide directly. For example, Ghanayem et al (2005) showed that acrylamide caused dominant lethal mutations in wild type mice, but negative results were obtained in CYP2E1-null mice.

2.7 Carcinogenicity

2.7.1 Human data

In the EU RAR (2002) it was considered that the carcinogenicity of acrylamide had not been adequately investigated in epidemiological studies and there were felt to be clear inadequacies in one of the two occupational cohort studies available. Since then, the two cohort studies in acrylamide manufacturing workers have been updated and there has also been a number of epidemiological studies of the carcinogenic potential of acrylamide via the diet.

In relation to the occupational cohort studies, Swaen et al (2007) updated the study originally reported by Sobel et al (1986), extending the years of follow-up until 2001. The updated analysis was based on 141 deaths as compared to only 24 deaths in the original study. The results showed no increases in cause-specific cancer deaths other than for a non-statistically significant increase in pancreatic cancer (SMR = 222.2 95% CI: 72.1 – 518.5). There was an increase in deaths from diabetes (SMR = 288.7 95% CI: 138.4 - 531.0) but this was considered to be unrelated to acrylamide because there had been a deficit of deaths from diabetes in the other larger-scale cohort study (Marsh et al., 2007). Overall, no firm conclusions can be drawn from this study due to the limited number of deaths available for analysis.

Marsh et al (2007) updated the cohort study of 1 Dutch and 3 US plants that was first reported by Collins et al., (1989), and subsequently updated to 1994 by Marsh et al., (1999). The Marsh et al (2007) update covers an additional 8-year period from 1995-2002 providing an additional 275 deaths for analysis compared to the previous update. About 50% of the US cohort and 25% of the Dutch cohort were short-term workers with less than 1 year of employment, and all US workers had terminated employment before 1995. The largest of the US plants comprising about 85% of the total cohort had ceased production of acrylamide in 1985.

A worker was counted as "exposed" to acrylamide if his cumulative exposure value was greater than 0.001 mg/m^3-years, the equivalent of one day of average exposure to 0.3 mg.m^{-3}. Using this criterion, about 22% of the total cohort was counted as being exposed to acrylamide. In view of the high numbers of short-term workers and the low exposures, the durations and cumulative exposures to acrylamide were generally low. The average intensity of exposure among the US plants ranged from 0.007 to 0.115 mg.m^{-3}.

In relation to the mortality data, for the US cohort, SMRs were calculated based on local and on national US rates and for both methods there were no increases in cause-specific mortality other than for respiratory system cancer (SMR = 1.17, CI: 1.06-1.27). In the Dutch plant, there were no increases in cause-specific mortality. Specific causes of death were analyzed in relation to exposed/non-exposed, and showed no statistically significant excesses of cancer mortality in the exposed group for either the US or Dutch cohort. For the US cohort, there was also an exposure-response analysis of SMRs in relation to duration of exposure and cumulative exposure, and this showed no trend of increasing risk of any type of cancer with any acrylamide exposure variable. Previous suggestions of an increased risk of pancreatic cancer were not confirmed. Overall, in this study there was no evidence for any increases in cancer that could be attributed to acrylamide. However, it should be noted that exposures were low.

Dietary studies: Reviews of dietary studies on acrylamide are available from Rice (2005) and Wilson (2006). The available studies show no link between increased cancer risk and exposure to acrylamide. A possible limitation of dietary studies is that acrylamide is present in a wide variety of foods and, it may be difficult (on the basis of food questionnaires) to reliably discriminate among subjects with low, medium and high intakes. In the US it has been reported that the 90th percentile of intake is 0.9 micrograms per kilogram body weight per day, and the average intake is 0.48 micrograms per kilogram (Wilson et al 2006). Acrylamide is found in a wide range of foods and generally, potato products, bread and coffee are the dominant sources. Smoking is also an important source of exposure; blood levels of acrylamide are approximately 4-times higher in smokers than in non-smokers.

Mucci et al (2003a) reanalysed an existing Swedish population-based case-control study consisting of 591 incident cases of bowel cancer, 263 cases of bladder cancer

and 133 cases of kidney cancer, searching for possible associations with dietary acrylamide exposure. Exposure estimates were based on food questionnaires and reported levels of acrylamide in frequently consumed food products. No associations with dietary acrylamide and excess risk cancers at these sites were observed. It is noted that none of the cancers seen in the rat studies were investigated, and that food questionnaires may lack precision in relation to exposure estimation. Smokers and non-smokers were analysed separately with no increased risk found for either group in relation to acrylamide. Mucci et al (2003b) reanalysed the data to take account of coffee and crispbread consumption, and again found that neither colorectal, bladder nor kidney cancer were associated with acrylamide exposure. Rather, colorectal and bladder cancer showed an inverse relationship with estimated exposure to acrylamide.

Mucci et al (2004) (cited in the review by Wilson 2006) reanalysed the data from a larger case-control study of renal cell cancer using data from the Swedish component of an international collaborative population-based study. The authors identified 379 incident cases and 353 controls matched for age and sex. Using similar methods to those above, again no associations were found between estimated dietary intake of acrylamide and renal cell cancer.

Pelucci et al (2003) found no association between fried/baked potato consumption and cancer at a number of sites (including oral cavity, bowel, breast and ovary) in a series of case-control studies in Italy and Switzerland. This study may be limited by the fact that fried/baked potatoes are not the only dietary source of acrylamide, and also by the fact that the degree of browning of the food may lead to differences in acrylamide content from 20-1000 micrograms/kg and this could not be taken into account from the data available.

As discussed by Wilson et al (2006), case-control studies are potentially subject to recall and selection bias, particularly in dietary studies and for this reason prospective studies may offer advantages. Wilson et al., summarise two prospective studies both by Mucci et al. One was a study of breast cancer, and comprised a cohort of 43,404 Swedish women followed from 1991-2002, yielding 490,000 person-years of follow-up with 667 incident cases. The other study examined the risk of colorectal cancer using data from 61,467 women in the Swedish Mammography cohort (Mucci et al 2006). These studies revealed no associations between estimates of dietary exposure to acrylamide and cancer risk.

Overall, there are limitations in the general population dietary studies; in particular not all possible current and past sources of acrylamide exposure may have been taken into account and there is the potential for exposure misclassification. Furthermore only a limited range of cancer sites have been investigated. However, the dietary studies taken together with the occupational cohort studies do not provide any evidence for an increased risk of cancer from exposure to acrylamide in humans.

2.7.2 Animal data

No inhalation data are available to assess the cancer risks for acrylamide. See Table 1 for a summary of the tumour findings from the two drinking water cancer bioassay studies in rats (Johnson et al 1986, Friedman et al 1995).

Johnson *et al* (1986) exposed rats to 0, 0.01, 0.1 or 2.0 mg/kg/day aqueous acrylamide in drinking water for up to 2 years. In males there was a significantly increased incidence of benign follicular cell adenomas of the thyroid at the highest dose level. In females there was a non-significant increase in benign follicular cell adenomas and malignant adenocarcinomas of the thyroid. There was a statistically significant increase of malignant adenocarcinomas at doses of 0.1 mg/kg/day or

greater. In males there was a statistically significant increase in malignant testicular mesotheliomas at 0.5 and 2 mg/kg/day. In females there was a statistically significant increase in uterine adenocarcinoma. Non-significant increases in the incidence of malignant astrocytomas in the spinal cord of males and brain of females were also reported. There was also an increased incidence of benign papillomas in the oral cavity of females exposed to 2 mg/kg/day and evidence of focal hyperplasia of the hard palate in males. The tunica vaginalis mesothelioma tissues from this study were re-read later (Damjanov 1998, Shipp 2006). It was discussed that the tumours were benign in nature, likely a result of an acceleration of a background tumour and induced by a hormonal and not a genotoxic mode of action (Shipp 2006).

In a subsequent study, groups of 75-204 male F344 rats received 0, 0.1, 0.5, and 2 mg/kg/day acrylamide in drinking water for up to 2 years, and groups of 50-100 females received 0, 1, and 3 mg/kg/day (Friedman *et al* 1995). Statistically significant increases in thyroid follicular adenomas and non-significant increases in carcinomas were found at 2 mg/kg/day. In males there was a statistically significant increase in malignant scrotal mesothelioma at 2 mg/kg/day. Increased incidences of benign and malignant tumours were found in the brain, but no dose-response relationships were observed and the relationships were not statistically significant. 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). There were no statistically significant increases in the incidence of neoplastic findings in the uterus, pituitary gland and oral cavity. However, histological 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.

There are two additional studies summarised in the EU RAR that inform on carcinogenicity; a skin tumour initiation/promotion assay and a lung adenoma assay. These studies were both conducted in mice and were reported in the EU RAR (Bull et al 1984). In the skin tumour assay, acrylamide led to an increased rate of skin tumours following dermal, oral and intraperitoneal doses but only when the skin tumour promoting agent tetradecanoyl-phorbol acetate was also administered. Acrylamide alone did not lead to skin tumours. Acrylamide also led to an increased rate of lung adenomas following oral and intraperitoneal dosing but as the study involved a strain of mouse with a high background incidence of lung adenomas, no useful conclusions concerning human health can be drawn from this finding.

Overall, the only useful data on carcinogenicity in experimental animals derive from two drinking water studies in rats, in which the results show increases in tumours in variety of organs. Some of the tumour types show a possible relationship with disturbed endocrine function (thyroid, mammary glands, testicular mesothelioma, adrenals) and raise the possibility of a hormonal mechanism. However, acrylamide is clearly genotoxic and a role for genotoxicity in the development of the tumours cannot be excluded. It is also noted that there are no published studies that provide definitive evidence for a hormonal mechanism that could account for this tumour profile, although a number of hypotheses have been elaborated (Shipp et al 2006). There is also a suggestion of an increased risk of glial cell tumours in the brain and spinal cord and, although the picture is not clear, these are possibly acrylamide-induced. It has been suggested (see Shipp et al 2006) that the astrocytomas may develop as a result of acrylamide-induced chronic cytotoxicity in neuronal cells leading to the release of growth factors which stimulate glial cell proliferation, rather than via a genotoxic mechanism.

An in-depth evaluation of the proposed modes of action of acrylamide carcinogenesis has been presented by DFG (2007, 2009) and the U.K. Committee on Mutagenicity (COM 2009).

Table 1. Summary of tumour findings from two cancer bioassays in F344 rats in which acrylamide was administered via the drinking water (Johnson et al 1986, and Friedman et al 1995).

Dose mg/kg/day		0	0.01	0.1	1	2	3
ADRENAL PHAEOCHROMOCYTOMA							
Johnson	males	3/60	7/59	7/60	5/60	10/60	
GLIAL CELL TUMOURS IN BRAIN AND SPINAL CORD							
Astrocytomas in brain							
Johnson	females	0/60	1/60	0/60	0/60	3/60	
Friedman	males	1/204	N/a	0/98	0/50	2/75	
Friedman	females	0/100	N/a	N/a	2/100	N/a	2/100
Astrocytomas in spinal cord							
Johnson	males	1/60	0/60	0/60	0/60	3/60	
Friedman	females	1/60	0/59	0/60	0/60	3/61	
Friedman	males	0/172		1/68	0/102	1/54	
Friedman	females						1/100
Meningioma							
Friedman	females	0/100			2/100		3/100
Malignant reticulosis							
Friedman	females	1/100			2/100		3/100
Glial cell proliferation in brain suggestive of early tumour							
Johnson	males	0/60	0/60	0/60	1/60	1/60	
Johnson	females	0/60	0/60	0/60	1/60	3/60	
ORAL CAVITY PAPILLOMAS							
Johnson	females	0/60	3/60	2/60	1/60	7/61	

MAMMARY GLAND TUMOURS (females)							
Johnson	Adenoma	10/60	11/60	9/60	19/58	23/61	
	Carcinoma	2/60	1/60	1/60	2/58	6/61	
Friedman	Adenoma	9/96			20/94		26/95
	Carcinoma	2/96			2/94		4/95
PITUITARY GLAND ADENOMA							
Johnson	females	25/59	30/60	32/60	27/60	32/60	
TESTICULAR MESOTHELIOMA							
Johnson	males	3/60	0/60	7/60	11/60	10/60	
Friedman	males	8/204	N/a	9/204	8/102	13/75	
THYROID FOLLICULAR ADENOMA							
Johnson	males	1/60	0/60	2/59	1/59	7/59	
Johnson	females	0/58	0/59	1/50	1/58	3/60	
Friedman	males	3/204	N/A	9/203	5/101	12/75	
Friedman	females	0/100			7/100		16/100
THYROID ADENOCARCINOMA							
Johnson	females	1/58	0/59	0/59	0/58	3/60	
Friedman	males	3/204		3/204	0/102	3/75	
Friedman	females	2/100			3/100		7/100
UTERINE ADENOCARCINOMA							
Johnson	females	1/60	2/60	1/60	0/59	5/60	

2.8 Reproductive toxicity

2.8.1 Human data

There are no studies of the effects of acrylamide on reproductive parameters in humans although there is limited information to suggest that placental transfer of acrylamide to the foetus occurs in humans (Schettgen *et al,* 2004) and also data that suggest acrylamide is secreted in human milk (Sorgel *et al,* 2002).

2.8.2 Animal data

Fertility

The EU-RAR (2002) reviewed a number of studies in rats and mice that demonstrated adverse effects on male fertility including reduced sperm counts at doses of 5 mg/kg/day or more (principally Tyl et al 2000, Sublet et al 1989, Chapin et al 1995). Impaired mating performance arising from loss of use of hind limbs has also been reported at similar levels of exposure. In multi-generational studies, increases in post-implantation losses and reductions in the numbers of live pups at each mating stage are consistent with dominant lethal mutations in male germ cells. Acrylamide appears to have no effects on female fertility as demonstrated in cross-over studies (Sakamoto and Hashimoto 1986, Zenick et al 1986).

The study that provides the most useful dose-response data for effects on fertility is that of Tyl et al (2000). These authors conducted a combined 2-generation/dominant lethal study in which male and female rats were exposed to 0, 0.5, 2 or 5 mg/kg/day acrylamide via drinking water. In both the F0 and F1 generations there were reductions in the numbers of implantations per dam and the number of live pups per litter at 5 mg/kg/day. Survival of Fo and F1 pups was also reduced at 5 mg/kg/day. In the dominant lethal study, the number of viable implantations was reduced at 5 mg/kg/day and there was an increase in resorptions at this dose level (14% compared to 6% in controls), indicating a positive result. Overall, acrylamide has an adverse effect on fertility at 5 mg/kg/day consistent with a dominant lethal effect; a NOAEL for effects on fertility can be identified at 2 mg/kg/day.

Developmental toxicity

The developmental toxicity of acrylamide has been investigated in rats and mice as detailed in the EU RAR (2002). Results show minor signs of developmental toxicity (increased incidence of skeletal variations and reduced body weight gain) at doses that were associated with maternal toxicity (about 15 mg/kg/day and above for rats and 45 mg/kg/day for mice). There was no evidence of selective developmental toxicity. There are studies that have investigated whether or not acrylamide induces toxicity in rat pups during lactation but as the doses used caused significant maternal toxicity no conclusions can be drawn regarding specific effects mediated via lactation.

3. Recommendation

As evidenced in animal experiments, acrylamide possesses a number of hazardous properties including neurotoxicity, impairment of male fertility, somatic and germ cell mutagenicity, and carcinogenicity. In relation to neurotoxicity, acrylamide causes impairment of axonal transport, leading to axonal swelling, loss of axons, degenerating myelin and changes to glial cells in the brain, spinal cord and peripheral nervous system. In workers, exposures to acrylamide have led to clinical signs of

neurotoxicity such as tremor, in-coordination and reductions in nerve conduction velocity, and symptoms such as tingling and numbness in the hands and feet.

There are reports of skin irritation with an unusual presentation, i.e peeling of the skin on the hands and feet.

Rodent carcinogenicity studies in which acrylamide was administered in the drinking water showed increased tumour incidences in a number of organs/tissues including the testicular mesothelium, adrenals, mammary glands, and thyroid. This tumour profile suggests that the mechanism of carcinogenesis may involve hormonal disturbance but there is no definitive evidence for endocrine disturbance that could account for the diverse range of tumours seen. The fact that acrylamide does not affect fertility in females does not add support to a proposed hormonal mechanism for tumour development. It is also noted that there is evidence suggestive of an increase in glial cell tumours in the brain and spinal cord. In view of the genotoxic properties of acrylamide, a role for genotoxicity cannot be excluded.

In relation to the evidence for carcinogenicity in humans, there are two cohort studies in workers, and a number of studies of dietary exposure in the general population. None of these studies reveal any link between acrylamide and cancer, but there are possible limitations in these studies (low cumulative exposures in the occupational studies and the possibility of exposure misclassification in the dietary studies). Overall, in view of the animal evidence for carcinogenicity, as well as the evidence for somatic and germ cell mutagenicity, there has to be a concern for carcinogenic potential in humans and concern for the possibility of heritable mutations. A clear threshold for such effects cannot be identified. The very clearcut and strong neurotoxicty of acrylamide has led to an avoidance of higher exposures in industrial practice also in the past. Therefore, it is plausible why no direct evidence for acrylamide in exposed humans has been obtained.

The uncertainties surrounding the risk of cancer and genotoxicity (in particular heritable mutations) in workers exposed to acrylamide suggest that a health-based OEL cannot be derived. Although attempts have been published to explain the modes of action of the various tumour types observed in experimental animals (Shipp et al. 2006), the multiplicity of target sites, combined with the directly genotoxic nature of the metabolic intermediate, glycidamide, add significant uncertainties to these explanations (see also the detailed discussion by DFG 2007, 2009). *Considering this situation, acrylamide is categorized in the SCOEL carcinogen group B (Bolt and Huici-Montagud 2008), as a genotoxic carcinogen, for which the existence of a threshold cannot be sufficiently supported at present. In consequence, a health-based OEL and BLV cannot be recommended.*

A reasonable quantitative cancer risk assessment for humans is not feasible for acrylamide, because of two reasons: (1) Human cancer studies do not provide reliable figures as a basis of a risk quantitation. (2) The cancers observed in rats (testicular mesotheliomas, mammary tumours, glial cell tumours, thyroid tumours, adrenal phaeochromocytomas) are significantly influenced by species-specific factors, which make meaningful quantitative extrapolations to humans almost impossible.

However, it is important that any regulation that may be established for acrylamide should also be protective against the development of neurotoxicity, given that there is a wealth of evidence for acrylamide-induced neurotoxicity in workers.

Experimentally, a minimal effect level (LOAEL) for neurotoxicity of 1 mg/kg per day and a NOAEL at the next lower dose of 0.2 mg/kg/day was established in a 90-day drinking water study in rats (Burek et al 1980). Johnson et al. (1984, 1986) repeated

this study as part of a chronic study with a dose of 0.5 mg/kg per day and found no effect. Both studies included electron microscopic examinations. Hence, an established NOAEL for neurotoxicity in the rats is 0.5 mg/kg. This would correspond, for the rat (8 h respiratory volume of 0.38 m^3/kg) to an inhalation concentration of 1.32 $mg.m^{-3}$, i.e. 0.45 ppm (8-hr TWA).

In occupational studies it is difficult to establish a dose-response for neurotoxicity due to the inability to distinguish the relative contributions of dermal and inhalation exposure. However, a dose-response between neurological symptoms and acrylamide-haemoglobin adducts was reported in a study in tunnel workers (Hagmar et al 2001). The workers in this study were mainly exposed to acrylamide via dermal contact. By using biological monitoring of the haemoglobin adduct of acrylamide (*N*-2-carbamoyl-ethyl-valine), a NOAEL for neurotoxicity of 0.5 nmol adduct/g globin was reported with respect to neurotoxicity in occupationally exposed persons. This is seconded by another notion that no symptoms of peripheral neuropathy were reported in workers with this same adduct level in a biological monitoring study (Jones et al 2006). The adduct level based on these studies of 0.5 nmol/g globin would correspond to an airborne exposure of about 0.1 $mg.m^{-3}$ or 0.035 ppm (8-hour TWA).

Dermal absorption is important in relation to workers under practical working conditions, and a 'skin' notation is therefore warranted. Indeed, in view of the low volatility of acrylamide there is the possibility that the skin is the dominant exposure route.

An annex on possibilities for biological monitoring of occupational exposures to acrylamide will be produced by SCOEL as a matter of urgency.

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Annex to SCOEL/SUM/139, December 2012: Recommendation from the Scientific Committee on Occupational Exposure Limits for a Biological Guidance Value for Acrylamide

BGV:	Acrylamide haemoglobin adducts (AAVal Hb adducts): 80 pmol/g globin (for non-smokers)

1. Substance identification

Name:	Acrylamide
Synonyms:	2-Propenamide, acryl acid amide, ethylene carboxamide, propenoic acid amide, vinyl amide
Molecular formula:	C_3H_5NO
Structural formula:	CH_2=CH-$CONH_2$
EC No.:	201-173-7
CAS No.:	79-06-1
Molecular weight:	71.09 g/mol
Boiling point:	no value (polymerises above melting point)
Melting point:	84.5 °C
Vapour pressure (20 °C):	0.9 Pa 25 °C
Conversion factors: (25 °C, 101.3kPa)	1 ppm = 2.907 mg/m^3 1 mg/m^3 = 0.344 ppm

EU classification:

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

2. Background

This document is an annex to the SCOEL recommendation for acrylamide (SCOEL/SUM/139). It gives a recommendation for a biological guidance value (BGV) for acrylamide. Biological guidance values can be established when toxicological data cannot support a health-based BLV. It represents the upper concentration of the substance or a metabolite of the substance in any appropriate biological medium corresponding to a certain percentile (generally 90th or 95th percentile) in a defined (non-occupationally exposed) reference population. A value exceeding the BGV might help to identify the need for an expert consideration of the working conditions.

The use and toxicity of acrylamide is summarised in SCOEL/SUM/139. In view of the animal evidence for carcinogenicity, as well as the evidence for somatic and germ cell mutagenicity, there is a concern for a carcinogenic potential in humans and for possible heritable mutations. A clear threshold for such effects could not be identified. The uncertainties surrounding the risk of cancer and genotoxicity (in particular heritable mutations) in workers exposed to acrylamide suggested that a health-based

OEL cannot be derived. Acrylamide was categorised in the SCOEL carcinogen group B, as a genotoxic carcinogen, for which the existence of a threshold cannot be sufficiently supported at present. In consequence, a health-based OEL and a BLV were not recommended, but an annex on biological monitoring was seen as a matter of urgency.

Therefore, this document summarises the possibilities for biomonitoring of occupational exposures to acrylamide and gives recommendation on a biological guidance value for acrylamide. Biomonitoring can be used as a complimentary method to air monitoring to assess workers' exposure to acrylamide.

3. Metabolism and excretion of acrylamide

The toxicokinetics of acrylamide is discussed in SCOEL/SUM/139. Once absorbed, acrylamide is either directly conjugated with glutathione or transformed by CYP2E1 to its epoxide intermediate glycidamide [1]. According to the current knowledge, glycidamide is the DNA-reactive moiety responsible for the genotoxicity (and carcinogenicity) of acrylamide [2, 3]. Both acrylamide and glycidamide form adducts with sulphhydryl groups on haemoglobin (Hb) and other proteins [4]. Reaction of acrylamide and glycidamide via Michael addition with the N-terminal valine residue in Hb results in the formation of N-(2-carbamoylethyl)valine (AAVal) and N-(2-carbamoylhydroxyethyl)valine (GAVal), respectively [5].

Glutathione conjugation of acrylamide and glycidamide results in the formation of mercapturic acid metabolites, i.e. N-acetyl-S-(2-carbamoylethyl)cysteine (AAMA) and N-acetyl-S-(2-hydroxy-2-carbamoylethyl)cysteine (GAMA). AAMA is the main metabolite recovered in the urine in humans [6-8]. Fuhr et al [8] evaluated the toxicokinetics of acrylamide in six young healthy volunteers after the consumption of a meal containing 0.94 mg of acrylamide. During the next 72 hours 60 % of the dose was recovered in the urine. AAMA accounted for 50 % of the dose recovered in the urine, whereas GAMA accounted for 6 %, and unchanged acrylamide for 4 %. Excretion of unconjugated glycidamide in urine is low; in the Fuhr et al study [8] no free glycidamide was detected in the urine, whereas in another study low amounts (< 3 % of the total urinary metabolites) of glycidamide were detected [7].

About 50 % of the administered dose is eliminated in the urine during the first 24 hours following ingestion [6]. Fuhr et al [8] reported urinary elimination half-lives of 2.4, 17.4, and 25.1 hours for acrylamide, AAMA and GAMA, respectively, in humans following a single oral dose of acrylamide. There is some interindividual variation in the excretion of AAMA and GAMA in urine. Individual coefficients of variation ranging from 20 % to 30 % for urinary excretion of AAMA and GAMA following controlled doses in humans has been reported [8].

4. Acrylamide biomarkers

Several potential biomarkers are available for assessing internal exposure to acrylamide. These have been reviewed by Dybing *et al* [9] and Hays *et al* [10].

4.1 Urinary metabolites

It is possible to measure acrylamide and glycidamide in serum, but their short half-lives (< 2 hours in rodents, [3, 11]) makes interpretation difficult. Therefore, these are not considered optimal biomarkers for internal exposure assessment. Both acrylamide and glycidamide have been detected in urine from humans and rodents given to acrylamide orally but the levels are low, accounting for only < 5 % of the

dose recovered in urine. The main urinary metabolite in humans is AAMA, accounting for approximately 50 % of the dose recovered in the urine. AAMA is also eliminated more slowly than the parent compound, providing a more stable marker of exposure [8]. Boettcher and Angerer [12] developed a specific liquid chromatography-tandem mass spectrometry (LC–MS/MS) based method for the determination of both AAMA and GAMA in human urine and evaluated urinary AAMA and GAMA levels of 29 non-occupationally exposed individuals [13]. The median levels in smokers (n = 13) were found to be about four times higher than in non-smokers (n = 16): 127 µg/l vs. 29 µg/l for AAMA and 19 µg/l vs. 5 µg/l for GAMA. The levels of AAMA in the whole population (n = 29) ranged from 3 to 338 µg/l, the levels of GAMA from < LOD to 45 µg/l [13].

Huang *et al* [16] reported a repeated-measurement study investigating the correlation between occupational exposure to airborne acrylamide and the time-dependent behavior of urinary AAMA, GAMA2, and GAMA3 (two different isomers of glycidamide mercapturic acid metabolites). The study involved 8 acrylamide exposed workers and 36 controls. Pre- and post-shift urine samples were collected from the exposed group in parallel with personal sampling for 8 consecutive days and analysed using LC-electrospray ionisation-MS/MS. According to personal exposure monitoring, acrylamide 8-hour TWA concentrations for the workers ranged from 4.63 to 76.64 µg/m^3 with a mean of 18.10 µg/m^3. The metabolite AAMA was detected in 100 % and 88.9 % of urine samples collected from the workers and controls, respectively. GAMA2 and GAMA3 were detected in 56.5 and 76.5 % of workers and not in controls. Post-shift mean levels of AAMA, GAMA2, and GAMA3 of exposed workers were 2 972.5 (range 98.9–66 975.1) µg/g creatinine, 32.1 (5.1–337.9) µg/g creatinine, and 93.9 (12.0–2 109.2) µg/g creatinine, respectively. Mean AAMA levels of exposed workers were significantly higher than those of controls [mean of 114.6 (range ND–661.7) µg/g creatinine]. According to analysis of data using Spearman correlation and a linear mixed-effects model, airborne acrylamide exposure was significantly influencing post-shift urinary AAMA levels. A linear mixed-effects model incorporating airborne exposure and smoking status predicted post-shift urinary AAMA levels of 3 729.8 µg/g creatinine for smokers and 2 983.2 µg/g creatinine for non-smokers exposed to 30 µg/m^3 acrylamide in air [16].

There is an alternative method for the biomonitoring of urinary acrylamide metabolites. Callemann *et al* [14] and Bull *et al* [15] used a method based on the acid hydrolysis of urinary AAMA to *S*-carboxyethyl-cysteine (CEC) and consecutive analysis by high performance liquid chromatography (HPLC).

Callemann *et al* [14] demonstrated a correlation between urinary mercapturic acid metabolite levels collected for 24 hours starting at the beginning of the worker's shift and signs and symptoms of acrylamide neurotoxicity in 41 heavily exposed Chinese workers in acrylamide and polyacrylamide production [14].

Bull *et al* [15], on the other hand, made an industrial hygiene survey in manufacture, handling and polymerisation of acrylamide at Ciba in Bradford, which can be considered to represent a typical workplace where acrylamide exposure is well-controlled. The total of 260 airborne samples showed exposures ranging from < 0.004 mg/m^3 (detection limit) to 0.282 mg/m^3. Over half (130) of the measurements were < 0.014 mg/m^3, and the mean airborne concentration was 0.028 mg/m^3. Urine samples were collected at the start and end of each shift, the total numbers of samples being 275 pre-shift samples and 247 post-shift samples. The exposed workforce exhibited slightly higher mean results than controls (1.64 mmol/mol vs. 1.40 mmol/mol creatinine). Ninety-nine out of 227 post-shift samples from the exposed workers remained below the detection limit (1 mmol/mol creatinine). A linear mixed effect model predicted that smoking increases urinary CEC levels by 0.8

mmol/mol, whilst an airborne concentration of 1 mg/m^3 increases post-shift urine results by 8 mmol/mol. When only personal airborne exposure data and smoking habits were incorporated into the model, it predicted post-shift urinary levels of 4.26 mmol/mol creatinine for smokers and 3.46 mmol/mol creatinine for non-smokers exposed to acrylamide at the air levels of 0.3 mg/m^3. The 90th percentile of CEC in urine samples collected post-shift was 3.4 mmol/mol creatinine for the entire population studied. For the exposed smokers, the value was 3.9 mmol/mol creatinine. Based on these results the authors proposed 4 mmol CEC/mol creatinine, in urine samples collected at the end of shift, as a pragmatic biomonitoring guidance value associated with good occupational hygiene practice [15].

4.2 Haemoglobin adducts

The most popular method to quantify internal acrylamide exposure is, however, the measurement of acrylamide Hb adducts. Also glycidamide Hb adducts have sometimes been measured in conjunction with acrylamide adducts. Mainly the *N*-terminal valine adducts of acrylamide and glycidamide, AAVal and GAVal, respectively, have been used for routine biomonitoring in humans.

The analytical method is based on Edman degradation to cleave the *N*-terminal valine Hb adduct of acrylamide/glycidamide [17]. Briefly, red blood cells are separated and globin precipitated followed by Edman degradation. Adducts from acrylamide and glycidamide are measured as derivatives of AAVal and GAVal, respectively [17]. GC/MS–MS, in negative ion chemical ionisation (NICI) mode or electron impact (EI) mode and LC/MS–MS has been used to quantify the adduct levels.

This method has shown sufficient sensitivity and reproducibility in several studies. The main advantage of this method over other biomonitoring methods is that it provides a measure of cumulative exposure over several weeks (the life-time of erythrocytes is 120 days). The carcinogenicity of acrylamide has been linked with glycidamide and with the area under the glycidamide serum curve (AUC) [4, 10]. Although neurotoxicity is less well defined in terms of the mechanism of action, it is likely associated with the AUC or peak serum for acrylamide, glycidamide or both [4, 10]. The disadvantage of this method when compared to the analysis of urinary metabolites is the invasive nature of sampling.

Since AAVal adduct analysis has been a most commonly used method for acrylamide biomonitoring, there is a large database available on the variation of acrylamide adduct levels in normal populations. In some studies, GAVal adducts and the ratio of acrylamide/glycidamide adducts have also been evaluated. The studies on the variation of AAVal and GAVal adduct levels in normal, non-occupationally exposed populations are presented in Table 1.

Table 1. AAVal/GAVal adduct levels in occupationally non-exposed populations.

Reference	No. of non-smokers (ns) /smokers (s)	AAVal adduct level, pmol/g globin			GAVal adduct level, pmol/g globin		
		Mean/ *median	Range	95th percentile	Mean/ *median	Range	95th percentile
Bergmark 1997 [18] (Sweden)	8 ns 10 s	31 116	24—49 27—148				
Hagmar *et al* 2001 [19] (Sweden)	18 ns		20—70				
Schettgen *et al* 2003 [20] (Germany)	25 ns 47 s	21* 85*		46 159			
Paulsson *et al* 2003 [21] (Sweden)	5 ns	27±6 (SD)			26 ±6 (SD)		
Schettgen *et al* 2004 [22] (Germany)	13 ns 16 s	19 80	7—31 25—199		17 53	9—23 22—119	
Kjuus 2004 [23] (Norway)	6 ns 2 s	33 154	20—47				
Bader *et al* 2005 [24]** (Germany)	296 ns 99 s	15* 55*	11—44 11—442	30 140			
Hagmar *et al* 2005 [25] (Sweden)	70 ns 72 s	31* 152*	20—100 30—430				
Urban *et al* 2006 [26] (Germany)	60 ns 60 s	28 82	18—51 19—210				
Vesper *et al* 2006 [27] (USA)	96 (ns+s)	129*	27—453	306	97	27—240	221
Bjellaas *et al* 2007 [28] (Norway)	44 ns 6 s	38 154	18—66 99—211		20 77	7—46 29—99	
Vesper *et al* 2007 [29] (USA)	73 ns *** 88 s	51* 194*	7—610 60—584	155 403	34 107	4—319 17—364	117 215
Chevolleau *et al* 2007 [30] (France)	52 ns 16 s	27 53	9—70 16—163		22 34	12—47 15—62	
Olesen *et al* 2008 [31] (Denmark)	235 ns 139 s *(women only)*	35* 122*		88 277	21* 60*		53 126
Vesper *et al* 2008 [32] (Europe)	255 ns 255 s	43* 121*		88 285	40* 93		83 198
	By country:						
	France 15 ns	40*	27—92		36*	21—60	
	Italy 30 ns	45*	21—81		42*	14—93	
	Spain 30 ns	42*	23—70		41*	13–75	
	UK 30 ns	65*	34—177		58*	27—151	
	Netherlands 30 ns	49*	25—171		50*	24—77	
	Greece 30 ns	41*	15—150		40*	10—112	
	Germany 30 ns	40*	15—92		34*	11—97	
	Sweden 30 ns	35*	20—97		34*	15—69	
	Denmark 30 ns	37*	17—98		27*	8—100	

Table 1. AAVal/GAVal adduct levels in occupationally non-exposed populations.

Reference	No. of non-smokers (ns) /smokers (s)	AAVal adduct level, pmol/g globin			GAVal adduct level, pmol/g globin		
		Mean/ *median	Range	95th percentile	Mean/ *median	Range	95th percentile
Hartmann *et al* 2008 [33] (Germany)	91 ns	30*	15—71	51	34*	14—66	52
Duale *et al* 2009 [34] (Norway)	43 ns 6 s	37* 166	18—97 99—211		18 83	7—46 29—99	
Kütting *et al* 2009 [35] (Germany)	Adults (>18 y) 749 ns 149 s	27 83	3—68 8—331	45 198			
Wilson *et al* 2009 [36] (Sweden)	296 ns *(women only)*	44	14—148		49	23—157	
Vikström *et al* 2010 [37] (Sweden)	61 ns 19 s	51 152	16—179 65—275		40 95	14—122 25—200	
Vesper *et al* 2010 [38] (USA)	All (age 3- >60): AAVal: 5 686 ns	50 (95 % CI 49—52)					
	GAVal: 5 809 ns				51 (95 % CI 49—53)		
	AAVal: 1 316 s ***	113 (95 % CI 103—123)					
	GAVal:1 357 s ***				94 (95 % CI 87—101)		
Outzen 2011 [39] (Denmark)	537 ns *(women only)*	35*		89	21		49

* Median.
** Calculated from µg/l blood.
*** Smoking status based on plasma cotinine levels.

As shown in Table 1, smokers have usually 3–4-fold higher levels of acrylamide and glycidamide Hb adducts than non-smokers. Diet is another main factor affecting the adduct levels observed in different individuals or population groups [35, 38-40]. Vesper *et al* [32] investigated acrylamide exposure among adults 41–60 years of age from nine European countries and showed significant differences in acrylamide adduct levels between different populations with Dutch and British showing the highest median levels. Also high inter-individual variation was seen. High variation between different individuals or population groups was also seen in studies among the US population [29, 38]. In some studies, women have shown lower AAVal adduct levels than men, but this difference was not consistent across the studies. Children have higher AAVal adduct levels, probably due to the larger intake of food per body mass [38, 41]. Also body mass index (BMI) has been suggested to affect the adduct level; higher BMI being associated with lower adduct levels [32]. Current data do not show any clear effect of genetic polymorphisms of glutathione transferases.

Some variation in GAVal/AAVal level ratios between individuals has also been noted [22, 32]. Usually the ratio has varied between 0.5 and 1. One factor affecting ratio is alcohol, which inhibits acrylamide metabolism to glycidamide (CYP2E1) and thereby decreases the GAVal/AAVal adduct ratio [37].

Occupational exposures to acrylamide have resulted in Hb-adduct levels up to 3 000–30 000 pmol/g globin (Table 2) [17, 19, 42-44].

Bergmark *et al* [17] were the first to describe increased acrylamide adduct levels in workers exposed to high levels of acrylamide. Control workers had acrylamide adduct

Table 2. Hb adduct levels in occupationally exposed individuals.

Reference	Number of controls/workers	AAVal/GAVal adduct level pmol/g globin
Bergmark *et al* 1993 [17]	41 workers involved in AA and poly-AA production	AA adduct levels in the exposed workers were 300–34 000 pmol/g Hb. GA adducts in 5 workers analysed were 1 600-32 000 pmol/g Hb.
Bergmark *et al* 1997 [18]	22 SDS-PAGE laboratory workers	Higher levels of AA adducts were seen among non-smoking PAGE workers when compared to the non-smoking controls. Mean adduct levels in controls were 31 and 116 pmol/g Hb, smokers (n = 10) and non-smokers (n = 8) and in PAGE workers 54 pmol/g Hb (non-smokers, n = 15, range 24-116) and 71 pmol/g Hb (smokers, n = 7).
Hagmar *et al* 2001 [19]	121 tunnel workers 18 controls	47 workers had adduct levels within the background range (20–70 pmol/g Hb) whereas 74 tunnel workers had AAVal adduct levels > 300 pmol/g globin, the two highest values being 17 700 and 4 300 pmol/g globin. Dose-response was observed with adduct levels and PNS symptoms. A NOAEL of 500 pmol/g Hb for PNS effects was proposed. 18 controls had adduct levels ranging from 20–70 pmol/g globin.
Licea Pérez *et al* 1999 [44]	11 workers in AA production plant (7 ns/4 s)	AAVal adducts varied between 74–1 854 pmol/g globin, GAVal adducts varied between 5–177 pmol/g globin.
Jones *et al* 2006 [42]	60 workers from the production of AA monomer and polymer	At mean air levels of 0.03 mg/m^3 mean AA adduct levels were 32 and 51 pmol/g globin in non-smokers and smokers, respectively. The 90th percentile was 0.514 nmol/g globin. Long-term exposure to 0.3 mg/m^3 was calculated to lead to a mean adduct level of 1 550 pmol/g globin (95 % CI 1 150–1 950 pmol/g globin).
Paulsson *et al* 2006 [43]	17 workers in contaminated soil transport, 3 from glass work, 1 highly exposed sealing worker	*Transport*: Mean levels were similar to those observed in non-occupationally exposed individuals: 29 and 110 pmol/g globin in 11 non-smokers and 6 smokers, resp. *Glass work*: 94-310 nmol/g globin. *Tunnel worker*: 9 months after high exposure 23 000 pmol/g globin.

AA: acrylamide, GA: glycidamide, PNS: peripheral nervous system, SDS-PAGE: sodium dodecyl sulphate polyacrylamide gel electrophoresis, ns: non-smokers, s: smokers.

levels below 10 pmol/g globin, whereas workers exposed to acrylamide in the production of acrylamide or polyacrylamide had levels ranging from 300 to 34 000 pmol/g globin. Adduct levels (as well as urinary mercapturic acid metabolite levels) in these heavily exposed workers were correlated with symptoms of acrylamide neurotoxicity [14]. No threshold for these effects could, however, be identified.

Hagmar *et al* 2001 [19] studied tunnel workers exposed for about 2 months to a chemical grouting agent containing acrylamide and *N*-methylolacrylamide. Out of 121 workers, 74 had AAVal adduct levels > 300 pmol/g globin, the two highest values being 17 700 and 4 300 pmol/g globin. Others had levels similar to normal background levels. Levels of > 500 pmol/g globin were associated to peripheral nervous system symptoms in these workers [19].

Similarly, increased levels of acrylamide adducts were observed by Licea Pérez *et al* 1999 [44] in 11 workers in acrylamide production and by Paulsson *et al* 2006 [43] in 3 workers in glass industry (exposure arising from acrylamide containing window sealing agent). Slightly higher mean levels of acrylamide adducts were seen among non-smoking SDS PAGE workers when compared to the non-smoking controls in the study by Bergmark *et al* [18].

Jones *et al* [42] performed an occupational hygiene survey involving 60 workers in the production of acrylamide monomer and polymer in workplaces where exposure was well-controlled. This is the only study providing information on the correlation of acrylamide air levels and Hb adduct levels. The airborne samples (N = 285) at these workplaces were all below the UK maximum exposure limit (MEL) of 0.3 mg/m^3

(maximum 0.28 mg/m^3) and the mean exposure was about 0.03 mg/m^3. Of the workers, 13 showed airborne acrylamide exposures consistently below 0.01 mg/m^3, which resulted in no detectable increase in AAVal Hb adduct levels from the normal background levels [mean acrylamide adduct levels were 32 and 51 pmol/g globin in non-smokers (n = 8) and smokers (n = 5), respectively]. A good correlation between mean airborne acrylamide levels and mean acrylamide Hb adduct levels was seen (r = 0.61 least squares linear regression). The 90th percentile of Hb adducts in this study was 514 pmol/g globin. Long-term exposure to 0.3 mg/m^3 was calculated to lead to a mean adduct level of 1 550 pmol/g globin (95 % CI 1 150–1 950 pmol/g globin) [42].

5. Recommendation and derivation of a BGV

Biomonitoring can be used as a complimentary method to air monitoring to assess workers' exposure to acrylamide especially in those situations in which there are no comprehensive workplace air monitoring system in place or in which exposure via skin contact (including skin-mount contact) is suspected. Acrylamide-derived Hb-adduct measurement is currently the most commonly used method for acrylamide biomonitoring, although adduct analysis involves invasive sampling and is more complicated than measuring metabolites in urine. In addition, it provides information on cumulative acrylamide exposure over several weeks, while urinary metabolite analysis can give us information only on very recent exposure.

There is a large database available on AAVal adduct levels in normal populations. The study by Vesper *et al* [32] suggests a 95th percentile for AAVal adducts in normal European non-smoking populations of 88 pmol/g globin. In other studies, 95th percentiles for AAVal adducts varied between 30 and 89 pmol/g globin. *Based on the available data, a biological guidance value of 80 pmol/g globin is suggested for non-smoking adults.* Smokers have shown a 3–4-fold higher adduct level than non-smokers. In the Vesper *et al* [32] study, the 95th percentile for smokers was 285 pmol/g globin.

However, it should be noted that there is a large inter-population variation in acrylamide adduct levels. The German Federal Environment Agency [41] has given a value of 1.2 µg AAVal/l blood (i.e. 45 pmol/g globin) to describe the background exposure for non-smoking adults based on the available data from German populations. Also high inter-individual variation has been described, the diet being the main factor affecting the adduct levels.

Acrylamide air levels higher than ~10–15 µg/m^3 have been shown to result in AAVal Hb adduct levels higher than background levels in normal non-smoking populations.

The present Annex was adopted by SCOEL on 13 December 2012.

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