ANNEX 1

Background document

in support of the Committee for Risk Assessment (RAC) evaluation of limit values for acrylonitrile in the workplace

Prepared by the European Chemicals Agency (ECHA)

ECHA/RAC/ O-0000001412-86-188/F

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Preamble

The Commission, in view of the preparation of the third and fourth proposals for amendment of Directive 2004/37/EC on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (CMD), and in line with the 2017 Commission Communication '*Safer and Healthier Work for All*' - *Modernisation of the EU Occupational Safety and Health Legislation and Policy*'[1], asked the advice of RAC to assess the scientific relevance of occupational exposure limits for some carcinogenic chemical substances.

Therefore, the Commission made a request (8 March 2017[2]) in accordance with Article 77 (3)(c) of the REACH Regulation, to evaluate, in accordance Directive 2004/37/EC, the following chemical compounds: 4,4'-methylenebis[2-chloroaniline] (MOCA), arsenic acid and its inorganic salts, nickel and its compounds, acrylonitrile and benzene.

In support of the Commission's request, ECHA prepared a proposal concerning occupational limit values for acryonitrile at the workplace. This proposal was made publically available at: 'https://echa.europa.eu/echas-executive-director-requests-to-the-committees-previous-consultations' on **13 October 2017** and interested parties were invited to submit comments by **10 November 2017**.

The Committee for Risk Assessment (RAC) developed its opinion on the basis of the proposal submitted by ECHA. During the preparation of the opinion on occupational limit values for acrylonitrile, the ECHA proposal was further developed as the Background Document. In addition, stakeholders were able to provide comments on the RAC opinion during the evaluation process.

Following adoption of an opinion on 9 March 2018, recommending an Occupational Exposure Limit for acrylonitrile by RAC, this Background Document was amended to align it appropriately with the view of RAC. It supports the opinion of RAC and gives the detailed grounds for the opinion[3].

1 http://ec.europa.eu/social/main.jsp?langId=en&catId=148&newsId=2709&furtherNews=yes

2 https://echa.europa.eu/documents/10162/13641/ec_note_to_echa_oels_en.pdf/f72342ef-7361-0d7c-70a1-e77243bdc5c1

3 https://echa.europa.eu/documents/10162/13579/interim_wponevaluation_oel_agreed_rac_42_en.pdf/021bc290-e26c-532f-eb3f-52527700e375

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Literature search

A comprehensive search was carried out of available registration dossiers, recent scientific literature (post 2002[4]) and documents from authoritative bodies, that are related, but not limited, to acrylonitrile carcinogenicity. Identified documents were also assessed for information relating to the mode of action to support derivation of dose-response relationships and quantitative risk estimates for acrylonitrile. Tailored search strings relating to (i) the substance acrylonitrile; (ii) exposure; (iii) hazard; (iv) bioavailability and (v) non-carcinogenic endpoints were defined by an information scientist and used to interrogate a number of databases, namely: Web of Science™; PubMed; TOXLINE®; OECD eChemPorta. Data mining of 'grey literature' was also performed (e.g. ATSDR; US National Toxicology Program Reports (NTP)) to identify proprietary data, where available, and provide a broad overview for the identification of other potentially relevant academic studies, via the cited references.

For each element identified in the searches, screening was carried out on the publication title and abstract to identify potentially relevant studies to be included for full text screening, reliability scoring, and data extraction.

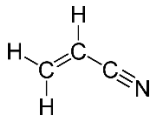
Additional information that was submitted in the public consultation on the ECHA proposal is also included in this Background Document.

1. Chemical Agent Identification and Physico-Chemical Properties

Acrylonitrile is a mono constituent substance of organic origin, having the appearance of a clear colourless liquid and a characteristic, slightly pungent odour.

The substance acrylonitrile has the following characteristics and physical–chemical properties:

Table 1: Substance identification

Endpoint	Value
IUPAC Name	Prop-2-enenitrile
Synonyms	Vinyl cyanide, cyanoethylene, acrylonitrile
Chemical structure	
Chemical formula	C_3H_3N
CAS No.	107-13-1
EINECS No.	203-466-5
Molecular Weight	53.06 g/mol
Appearance	clear colourless to pale yellow liquid
Melting point	-83.5
Boiling point	77.3 °C

[4] Time period chosen to overlap with SCOEL, 2003.

Endpoint	Value
Density	0.806 g/cm^3 at 20 °C
Vapour pressure	115 hPa at 20 °C
Partition coefficient (log Pow)	0.25 at 25 °C
Water solubility	73 g/L at 20 °C
Viscosity	0.34 mPa s at 25 °C.
Conversion factor	1 mg/m^3 = 0.45 ppm; 1 ppm = 2.2 mg/m^3

Source: Chemical Safety Report

2. EU Harmonised Classification and Labelling - CLP (EC)1272/2008

The classification of acrylonitrile based on EC Regulation 1272/2008 on classification, labelling and packaging of substances and mixtures is presented in Table 2. No concentration limits are specified for acrylonitrile.

Table 2: EU classification: CLP (EC) 1272/2008, Annex VI listing of acrylonitrile

Index No	Annex VI of CLP hazard class and category	Hazard statement code	Note
608-003-00-4	Flam. Liq. 2 Carc. 1B Acute Tox 3* Acute Tox 3* Acute Tox 3* STOT SE 3 Skin Irrit. 2 Eye Dam. 1 Skin Sens. 1 Aquatic Chronic 2	H225 H350 H331 H311 H301 H335 H315 H318 H317 H411	D

* indicates the minimum classification for a category in Annex VI; D – 'Certain substances which are susceptible to spontaneous polymerisation or decompensation 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'.
Source: https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/77896 [accessed July 2017]

3. Chemical Agent and Scope of Legislation - Regulated uses of acrylonitrile in the EU

The use of acrylonitrile in the workplace is not covered by an indicative or a binding occupational exposure limit (IOEL, BOEL).

3.1 Directive 98/24/EC and Directive 2004/37/EC

Acrylonitrile is a hazardous chemical agent in accordance with Article 2(b) of Directive 98/24/EC, and falls within the scope of this legislation.

Acrylonitrile is also a carcinogen or mutagen for humans in accordance with Article 2(a) and (b) of Directive 2004/37/EC, and falls within the scope of this legislation.

3.2 REACH Registrations

There is one joint submission under the REACH[5] registration for acrylonitrile, as listed[6] below in Table 3. The registration dossier lists 95 active registrants.

Table 3: REACH Registrations

Substance	Tonnage	Type	Status	Worker DNELs
Acrylonitrile	1 000 000 – 10 000 000	FULL	Active	Inhalation systemic long term – 2.7 mg/m³ Inhalation systemic acute/short term – 10 mg/m³ Inhalation local effects long term – 1.8 mg/m³ Inhalation local effects acute/short term – 10mg/m³ Dermal systemic long term – 1.4 mg/m³

The registration dossier advises not to use the substance in any process other than as an intermediate or monomer. Direct use of the substance by the general public or use resulting in wide and dispersive release are also not advised (see Section 3.4).

3.3 Authorised uses under Annex XIV of REACH

Acrylonitrile is not listed under Annex XIV and is therefore not subject to Authorisation.

3.4 Restricted uses under Annex XVII of REACH

Acrylonitrile is included under Entry 28[7] of Annex XVII to REACH, meaning that the substance shall not be supplied to the general public when the individual concentration in the substance or mixture is equal to or greater than generic or specific concentration limits. In addition, the packaging of such substances and mixtures shall clearly state “restricted to professional users”.

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[5] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (OJ L 396 of 30 December 2006, p. 1; corrected by OJ L 136, 29.5.2007, p. 3)

[6] ECHA https://echa.europa.eu/information-on-chemicals/registered-substances accessed 4th July 2017

[7] https://echa.europa.eu/documents/10162/caa50aef-640d-43b6-8eb0-6c9c542afa70

3.5 Plant Protection Products Regulation (EC)1107/2009

Acrylonitrile is not listed as an active substance in Annex I to Directive 91/414/EEC.

3.6 Biocidal Products Regulation (EU)528/2012

No application for approval has been submitted under Directive 98/8/EC or Regulation (EU) No 528/2012 for acrylonitrile.

4. Existing Occupational Exposure Limits

In various EU Member States, as well as outside the EU, occupational exposure limits (OELs) are established. These OELs are presented in Table 4. The list should not be considered as exhaustive. The primary sources of OELs for EU Member States were the following documents:

a) European Risk Observatory Report: Exploratory Survey of Occupational Exposure Limits for Carcinogens, Mutagens and Reprotoxic Substances at EU Member States Level (EU-OSHA[8])

b) SER OEL Database[9]

c) RMOA conclusion document by Germany (BAUA 2014)[10].

Table 4: Existing Occupational Exposure Limits (OELs) for acrylonitrile

Country/ Organisation	Level mg/m³	Time-relation	Comments
Austria[a,b,c]	4.5 (2 ppm) 18 (8 ppm)	TWA value (8hr) STEL (15 min)	C2 Skin notation
Belgium[a,b,c]	4.4 (2 ppm)	TWA value (8hr)	C2 Skin notation
Czech Republic[a]	2 6	TWA value (8hr) STEL (15 min)	C2 Skin notation
Denmark[a,b,c]	4 (2 ppm)	TWA value (8hr)	C2
Estonia[a]	4.5 (2 ppm) 13 (6 ppm)		
Finland[a,b,c]	4.4 (2 ppm) 8.8 (4 ppm)	TWA value (8hr) STEL (15min)	C Skin notation
France[b,c]	4.5 (2 ppm) 32.5 (15 ppm)	TWA value (8hr) STEL (15 min)	
Germany[d]	2.6 (1.2 ppm) 0.26 (0.12 ppm)	Tolerable conc Acceptable conc	C Skin notation
Greece[c]	4.5 (2 ppm)	TWA value (8hr)	
Iceland[c]	4.5 (2 ppm)	TWA value (8hr)	
Ireland[c]	4.5 (2 ppm)	TWA value (8hr)	
Latvia[a]	0.5	TWA value (8hr)	C2

[8] European agency for Safety and Health and Work (EU-OSHA). European Risk Observatory Report: Exploratory Survey of Occupational Exposure Limits for Carcinogens, Mutagens and Reprotoxic Substances at EU Member States Level.

[9] https://www.ser.nl/en/sitecore/content/internet/nl/grenswaarden/acrylnitril.aspx

[10] BAUA (2014). Risk Management Options Analysis Conclusion document for Acrylonitrile.

Country / Organisation	Level mg / m³	Time-relation	Comments
Lithuania[a]	4.5 (2 ppm) 13 (6 ppm)	TWA value (8hr) STEL (15min)	C
Norway[b,c]	4 (2 ppm)	Threshold limit value	
Poland[a]	2 10	TWA value (8hr) STEL (15min)	C2 Skin notation
Portugal[a,c]	4.4 (2 ppm)	TWA value (8 hr)	
Romania[11]	5 (2.3 ppm) 10 (4.6 ppm)	TWA value (8 hr) STEL (15 min)	C1B Skin notation
Slovakia[a]	7 (3 ppm)	TWA value (8 hr)	C Skin notation
Slovenia[a]	7 28	TWA value (8 hr) STEL (15 min)	C2 Skin notation
Spain[a,b,c]	4.4 (2 ppm)	TWA value (8 hr)	Skin sens
Sweden[a,b,c]	4.5 (2 ppm) 13 (6 ppm)	TWA value (8hr) STEL (15 min)	C Skin notation
Switzerland[b,c]	4.5 (2 ppm)	TWA value (8 hr)	
UK[a,b,c]	4.4 (2 ppm)	TWA value (8 hr)	C2 Skin notation
OSHA	4.4 (2 ppm) 22 (10 ppm)	TWA value (8 hr) STEL (15 min)	C2 Skin notation
NIOSH	2.2 (1 ppm) 22 (10 ppm)	TWA value (8 hr) STEL (15 min)	C2 Skin notation
ACGIH	4.3 (2 ppm)	TWA value (8 hr)	C Skin notation
Japan	4.3 (2 ppm)	TWA value (8 hr)	C2A Skin notation
China	2.0 (0.92 ppm)	MAC (maximum allowable concentration)	C
Australia	4.3 (2 ppm)	TWA value (8 hr)	C2

Sources: [a] EU-OSHA; [b] SER OEL Database; [c] BAUA, 2014
Notes: C- considered carcinogenic; C2 – category 2 carcinogen

Slovakia recommend a biomonitoring limit value (BLV) for acrylonitrile exposure of 420 µg/L N-2-Cyanoethylvaline (CEV) in blood (erythrocyte)[12]. Germany[13] recommend an 'acceptable concentration' of 650 pmol CEV/g globin (equivalent to a statistical risk level of 4:10 000 for developing cancer and a 'tolerable concentration' of 6 500 pmol CEV/g globin (risk level of 4:1 000).

[11] Romanian Labour Inspection 2017 – Government Decision no. 1218/2006 completed and modified in 2015

[12] European agency for Safety and Health and Work (EU-OSHA). European Risk Observatory Report: Exploratory Survey of Occupational Exposure Limits for Carcinogens, Mutagens and Reprotoxic Substances at EU Member States Level.

[13] GMBl-Bek.-TRGS 910 Seite 1 von 4. 8th June 2017

5. Occurrence, Use and Occupational Exposure

5.1 Occurrence

Acrylonitrile is used almost exclusively as a monomer in the production of polymeric materials (EC, 2004). It is an important industrial raw material used for the synthesis of polymers and resins (e.g. polyacrylonitrile, butadiene–styrene–acrylonitrile mixtures and nitrile rubber) and for basic chemicals (e.g. hexamethylenediamine and acrylamide). The substance is a clear, colourless liquid at normal temperature and pressure and has a pungent odour.

Small amounts of acrylonitrile are released during the combustion of plant matter such as biomass, timber and tobacco and several studies have quantified emissions of acrylonitrile from tropical fires and the burning of biomass (Yokelson *et al.*, 2007 and Warneke *et al.*, 2011). The major source of release to the environment is from the organic chemicals industry, with a smaller contribution from municipal sewage treatment plants (WHO, 2002). Research on the reactivity of acrylonitrile with hydroxyl radicals and chlorine atom predicts a short atmospheric lifetime (~12 h) that would indicate degradation close to the emission source (Teruel *et al.,* 2007). Concentrations measured in ambient outdoor air are detailed in Section 5.4.2. Environmental tobacco smoke is a potentially important source of acrylonitrile in indoor air.

5.2 Production and Use Information

Acrylonitrile is manufactured in substantial amounts in the EU at a volume of 750 000 tons per year.

Acrylonitrile is produced in a closed system by catalytic “ammoxidation” of ammonia and propylene. The predominant process used is the Sohio process, which achieves >85% conversion rates from stoichiometric quantities of ammonia and propylene in the presence of air at 400-500 °C at 20-200 kPa. Fractional distillation of the crude product, following scrubbing to remove ammonia, yields 99.9% pure acrylonitrile (SCOEL, 2003).

The primary use of acrylonitrile is as the raw material for the manufacture of acrylic and modacrylic textile fibres (315 000 tpa). Other major uses include the production of plastics; acrylonitrile and styrene are also used together in the production of styrene–acrylonitrile (SAN) and acrylonitrile–butadiene–styrene (ABS) resins (Scélo *et al.*, 2004); this use accounts for ~24% of the acrylonitrile manufactured in the EU (179 300 tpa). Acrylonitrile is also used as a feedstock in the production of nitrile rubbers (53 000 tpa). Use of acrylonitrile as an intermediate for the manufacture of bulk chemicals (e.g. adiponitrile and acrylamide), materials and resins accounts for 136 000 tpa.

The manufacture and uses of acrylonitrile given in the Chemical Safety Report are detailed below:

- Manufacture of acrylonitrile
- Production of acrylic and modacrylic textile fibres
- Production of acrylonitrile-butadiene-styrene (ABS) and styrene-acrylonitrile (SAN) plastics
- Monomer for production of nitrile rubbers
- Intermediate for the production of bulk chemicals, resins and adiponitrile/acrylamide synthesis
- Lab reagent

Historically, acrylonitrile has been used, in a mixture with carbon tetrachloride as a fumigant for flour milling and bakery food processing equipment and for stored tobacco. However, this use of acrylonitrile was discontinued due to concerns over its potential health effects (HPA, 2007).

5.3 Occupational exposure

Occupational exposure to acrylonitrile may occur during its production and use in the manufacture of products such as textiles and plastics. However, as manufacture occurs within a closed system (SCOEL, 2003) it is considered that the greatest potential for exposure occurs during its use as a chemical intermediate, where it may not be as easily contained.

It is widely considered that workplace exposure to acrylonitrile has greatly decreased since the early 1980s due to the implementation of effective industrial hygiene actions and the systematic use of personal protection in work situations involving possible exposure (EC 2004, Swaen *et al*., 1998). Currently, occupational exposure to acrylonitrile is minimised through rigid process isolation together with engineering controls to reduce emissions, waste streams and leaks from the closed system. Workers routinely use eye/skin protection and respiratory protection is deployed where isolation cannot be maintained (EC, 2004).

The EU RAR (EC, 2004) details monitoring data for six European manufacturers of acrylonitrile. Mean personal exposure concentrations ranged from <0.12-0.49 ppm (0.26-1.06 mg/m^3) with the overall range being between <0.1-2.21 ppm (<0.22-4.86 mg/m^3).

The EU RAR (EC 2004) reports that the manufacturing process for acrylic fibres comprises four mains steps that are listed below together with typical exposure levels:

i) receiving the monomer into bulk storage,
ii) polymerisation (0.4 ppm, i.e. 0.88 mg/m^3),
iii) spinning (0.5 ppm, i.e. 1.1 mg/m^3), and
iv) finishing including drying and baling (0.1 ppm, i.e. 0.22 mg/m^3).

Generally, slightly higher exposure to acrylonitrile was recorded for the use when compared to its manufacture. This is consistent with manufacturing of acrylonitrile being initially in a closed system, while manufacture of e.g. ABS polymers is carried out in a partially closed system, with local exhaust ventilation and higher potential for emission (EC, 2004).

The EU RAR (EC 2004) reports mean exposure levels from five European ABS processing plants during the 1990s as 0.046-0.3 ppm (0.1-0.66 mg/m^3) with a 95th percentile of 0.64 ppm (1.4 mg/m^3) from over 700 measurements. The Swaen *et al.* (1998) epidemiological study of workers in the Netherlands reports pre-1980 average 'exposure ranges' as 1.1 mg/m^3 for a combined acrylonitrile and ABS plant and 0–2.2 mg/m^3 for two other ABS plants.

NICNAS (2000) report measurements at an Australian SAN processing plant during normal operations; personal exposure to acrylonitrile was <0.01 ppm (<0.022 mg/m^3) in 16/28 samples, <0.1 ppm (<0.22 mg/m^3) in 21/28 samples and <0.2 ppm (<0.44 mg/m^3) in 100% of the samples. During maintenance operations much higher, short-term concentrations were measured from grab samples; these ranged from <0.5 to >120 ppm (<1 to >264 mg/m^3).

NICNAS (2000) report personal exposure measurements during tanker unloading of acrylonitrile at four different sites in Australia in the 1990s. All measurements were less than the LoD[14] except for two readings of 0.07 and 0.08 ppm (0.154 and 0.176 mg/m^3).

Jet room operators and maintenance workers were reported as having typical exposures of 0.4 ppm and 0.2 ppm, respectively (i.e. 0.88 mg/m^3 and 0.44 mg/m^3).

It is stated in the Chemical Safety Report that a number of improvements in the workplace have been introduced since the measurements reported in the EU RAR were taken and that these have significantly reduced the extent of inhalation and dermal exposure. The improvements include: (i) delivery of acrylonitrile by pipeline (reducing exposure during unloading); (ii) redirection of vents away from occupied areas of the workplace; (iii) continuous air monitoring in areas of greater risk; (iv) engineering controls to pumps seals to reduce potential leakage; and (v) improvements in ventilation including extraction at critical locations.

An HSE (2010) study involved a number of plastics and processes that detected very low levels of acrylonitrile under certain conditions for the processing of acrylonitrile-butadiene-styrene (ABS). The site manufactures various items such as car roof boxes, caravan panels etc, primarily from ABS and high-density-polyethylene (HDPE) using vacuum forming techniques. Analytes of particular interest such as butadiene, acrylonitrile and styrene that would clearly demonstrate polymer degradation were absent from the air samples.

Rongzhu *et al.* (2005) measured concentrations of acrylonitrile during its production and use in acrylic fibre production to assess possible neurobehavioural effects in Chinese workers. Acrylonitrile concentrations were measured at one site by the company's health and safety department employing 'periodic short-term area sampling´ (no details provided) between 1997 and 1999 (no personal samples were taken). 390 samples were taken in the area of the plant producing acrylonitrile with a geometric mean of 0.24 mg/m^3 (0.11 ppm) and range of 0–3.74 mg/m^3 (0-1.7 ppm). 570 samples were concerned with fibre production, giving a geomean concentration of 2.00 mg/m^3 (0.91 ppm) and range of 0–18.3 mg/m^3 (0-8.34 ppm).

Exposure to acrylonitrile and other volatile organic compounds can also occur during plastics recycling (He *et al*., 2015), specifically during the melting extrusion procedure. In a study of plastic recycling workshops in China acrylonitrile was measured at 25 ± 13 mg/m^3 during the extrusion of solid plastic waste (this process takes place at 200-300 °C for ABS plastics[15]).

The principal data from these studies are summarised in Table 5.

[14] LoD = 0.005-0.46 ppm (0.011-1.0 mg/m^3)

[15] Decomposition of ABS occurs at 290 °C (He *et al*., 2015)

Table 5: Summary of occupational exposure concentrations for acrylonitrile taken from Chemical Safety Report and recent literature

Use	EU Tonnage	Description; RMMs	Exposure concentrations detailed in CSR (value in bold taken as exposure concentration for risk characterisation in CSR)	Comments (CSR)	Additional values
ES1 Production of acrylic and modacrylic textile fibres	314,700 tpa	'Closed process' (containment); LEV, PPE	Inhalation exposure ranges from 0.1-0.5 ppm for various tasks (0.22-1.08 mg/m^3) Long term inhalation exposure: **1.08** mg/m^3 (converted from 0.5 ppm) Long term dermal exposure: up to **0.686 mg/kg bw/d**	Measured data from EU RAR for inhalation exposure Dermal exposure is TRA modelled	Mean exposure of 0.2 ppm (0.44 mg/m^3) reported in EU RAR (EC, 2004) for a European fibre processing plant during the 1990s (n=270) Rongzhu *et al.* (2005) reported geomean concentration of 2.00 mg/m^3 (0.91 ppm) and range of 0–18.3 mg/m^3 (0-8.34 ppm) during use of acrylonitrile in fibre production in China based on short-term 'area sampling' between 1997 and 1999.
ES 2 Production of acrylonitrile-butadiene-styrene (ABS) and styrene-acrylonitrile (SAN) plastics	179,300 tpa	'Closed process' (containment); LEV, PPE	Inhalation exposure ranges from 0.05-0.3 ppm for various tasks/PROCs (0.1-0.65 mg/m^3) Long term inhalation exposure: **1.72 mg/m^3** Long term dermal exposure up to **0.686 mg/kg bw/d**	Measured data from EU RAR (not used in risk characterisation) TRA modelled exposure data for inhalation and dermal exposure	(NICNAS, 2000), Australia: <0.0044–0.37 mg/m^3 during normal operation of SAN plant in Australia in 1990s <0.0066–700 mg/m^3 during maintenance when RPE is worn. <0.02–2.6 mg/m^3 in SAN/ABS pellet extrusion plant

				used in risk characterisation	
ES 3 Monomer for production of nitrile rubbers	53,000 tpa	'Closed process' (containment); LEV, PPE	Long term inhalation exposure: **1.72 mg/m³** Long term dermal exposure up to **0.686 mg/kg bw/d**	TRA modelled exposure data for inhalation and dermal exposure used in risk characterisation	No measured data identified
ES 4 Intermediate for the production of bulk chemicals, resins and adiponitrile/acrylamide synthesis	136,000 tpa	'Closed process' (containment); LEV, PPE	Long term inhalation exposure: **1.72 mg/m³** Long term dermal exposure up to **0.686 mg/kg bw/d**	TRA modelled exposure data for inhalation and dermal exposure used in risk characterisation	8 hr TWA of 0.44 mg/m³ for workers in acrylomide production in European countries (EC, 2004)
ES 5 Lab reagent	<1 tpa	LEV, PPE	Long term inhalation exposure: **0.344 mg/m³** Long term dermal exposure up to **0.343 mg/kg bw/d**	TRA modelled exposure data for inhalation and dermal exposure used in risk characterisation	<0.11–2.6 mg/m³ during quality control sampling and laboratory use in Australia (NICNAS, 2000)
ES 6 Manufacture of Acrylonitrile	750,000 tpa	'Closed process'	Long term inhalation exposure: **1.72 mg/m³**	TRA modelled exposure data for inhalation and	Mean exposure of 0.26-1.06 mg/m³ reported in EU RAR (EC, 2004) for six European

			Long term dermal exposure up to **0.686 mg/kg bw/d**	dermal exposure used in risk characterisation	production plants (overall range of <0.22-4.86 mg/m^3) Swaen *et al.* (1998) gives average pre-1980 exposures as 1.1 mg/m^3 for acrylonitrile plant in Netherlands Mean 8-hour TWA of 2.4 mg/m^3 or less from 1978-86 at four US production plants with maximum of 82 mg/m^3 (WHO 2002) Rongzhu *et al.* (2005) reported geomean concentration of 0.24 mg/m^3 (0.11 ppm) and range of 0–3.74 mg/m^3 (0-1.7 ppm) during acrylonitrile monomer production in China

5.4 Routes of exposure and uptake

5.4.1 Worker exposure

The primary route of exposure to acrylonitrile for the worker population is through inhalation, although exposure can also occur through dermal contact (Scelo *et al.*, 2004; EC, 2004). Indeed, SCOEL (2003) note that the high potential for acrylonitrile to penetrate skin can lead to a high risk of accidents. Strict controls for the handling of the compound in the workplace are therefore required. The effects following exposure to acrylonitrile may be local at site of contact or systemic following exposure via inhalation or dermal routes.

5.4.2 General population

The general population may be exposed to acrylonitrile in the air within the close vicinity of industrial plants producing or using the substance as it is readily volatile. WHO (2000) reports that air concentrations near industrial sites can exceed 100 µg/m^3 but are usually less than 10 µg/m^3 at a distance of about 1 km. ATSDR (1990) state that the concentrations in air near such facilities average less than 1 ppb (2.2 µg/m^3).

In the Netherlands, the average acrylonitrile concentration in the ambient air of unpolluted areas was estimated to be 0.01 µg/m^3 based on large-scale calculations using dispersion models (WHO 2000). The State of California Air Resources Board, ARB MLD (2011), measured an average concentration of 2.4 ppt (0.0052 µg/m^3) from forty-six 24 hr ambient air samples in July 2010 with a maximum value of 8.2 ppt (0.0176 µg/m^3).

It has been considered that the general population can also be exposed to acrylonitrile from smoking and environmental tobacco smoke. ATSDR (1990) considered that this may have been due to the use of acrylonitrile as a fumigant for stored tobacco, a practice that has now been discontinued. However, studies still appear to find that tobacco smoke can lead to acrylonitrile exposure. Research in German hospitality venues in 2008/2009, prior to the introduction of a smoking ban, IFA (2010), found acrylonitrile in all air samples at concentrations of 0.1–8.2 µg/m^3. Jain (2015a; 2015b) reported metabolites of acrylonitrile in urine of children (1.3-2 ng/mL) and adults (Male: 11.7, 10.3-13.2; Female: 12.4, 10.5-14.8 ng/mL) living in homes of smokers in the US NHANES study of 2011-2012. Indoor air concentrations of acrylonitrile in residences of smokers were estimated at 0.5 to 1.2 µg/m^3 (Nazaroff & Singer, 2004). The acrylonitrile-haemoglobin adduct CEV has been observed in smokers (Fennell *et al.*, 2000) and in infants born to mothers who smoke (Tavares *et al.*, 1996; Schettgen *et al.*, 2004). Tavares *et al.* (1996) measured average CEV levels in smokers of 217 pmol/g globin compared to undetectable levels in non-smokers.

Acrylonitrile monomers are found at very low levels in textiles and plastics produced from it and these are generally considered to be bound within the polymer matrix. The general population may though be exposed to very low levels of acrylonitrile in materials such as acrylic carpeting and plastic food containers. However, only foods in direct contact with acrylonitrile-based plastics are subject to potential contamination, and then only at very low levels (ATSDR, 1990). In the US there is a regulated maximum permitted leaching of 0.17 ppb acrylonitrile to food from packaging meaning that overall intake via this pathway would be extremely low. The European Commission (1983) reported that the levels of acrylonitrile in contaminated foods are generally about 1 µg/kg but it is considered that there is little migration of the monomer from current packaging materials due to the use of different and improved resins (ATSDR, 1990).

6. Monitoring Exposure

6.1 External exposure

Acrylonitrile is a relatively volatile, organic compound, so analytical techniques are focussed on measurement of the vapour phase in air. The most common method for analysis of acrylonitrile is by gas chromatography (GC). Method 1604 of the US National Institute for Occupational Safety and Health (NIOSH, 1994) specifies sampling with activated charcoal sorption tubes, desorption with acetone in carbon disulphide, and subsequent analysis by gas chromatography with a flame ionisation detector (FID)[16]. The working range of this method is 0.7–46 ppm (1.5–100 mg/m^3) for a 10 litre sample. This method is stated as being applicable to 15-minute ceiling measurements.

The US Occupational Safety and Health Administration (OSHA) specifies a similar method (OSDH Method ORG-37, 1982) of sampling with charcoal tubes, desorption with acetone, and subsequent analysis by gas chromatography using a nitrogen–phosphorus detector (NPD). The detection limit for this method is 0.01 ppm (0.026 mg/m^3) with a reliable quantitation limit of 0.3 ppm (0.66 mg/m^3), based on a 20 L air sample and 1 ml desorption volume (OSHA 1982, 1990).

The UK Health and Safety Executive (HSE) recommend several 'compendium methods' that are applicable to the measurement of volatile organic compounds (VOCs) in air, including acrylonitrile. Method MDHS72 (HSE, 1993) is a laboratory method using pumped solid sorbent tubes, thermal desorption and gas chromatography and has a working range of 0.2-100 mg/m^3 for samples of 2.5 litres of air. Method MDHS80 (HSE, 1995) uses diffusive solid sorbent tubes, thermal desorption and gas chromatography. Method MSHS88 (HSE, 1997) uses diffusive samplers, solvent desorption and gas chromatography. Methods MDHS80 and MDHS88 have a working range of 1 to 1 000 mg/m^3. All three methods are recommended for the determination of time-weighted average concentrations of VOCs in workplace air for exposure times between 30 min and eight hours.

The EU RAR for acrylonitrile (EC, 2004) states that *"Exposure measurements in workplace atmospheres are made in compliance with both the requirements on measurement strategy as laid down in (DIN) EN 689 and the requirements on measurement methods as laid down in (DIN) EN 482. For this purpose, a defined volume of air is drawn through a silica gel tube by means of a sampling pump with tube holder. After extraction with diethyl ether, the quantitative determination is carried out gas-chromatographically using a flame ionisation detector (FID). The analytical detection limit for a two-hour sampling period is 0.05 mg/m^3."*

In Germany, the DGUV (DGVU, 2008)(IFA, 2010) undertook sampling of acrylonitrile when measuring indoor air pollution on Type B activated carbon tubes for a duration of five hours with a volumetric flow rate of 0.66 l/min. Analysis is performed by gas chromatography utilising a nitrogen phosphorous detector. The method has a limit of detection of 0.1 µg/m^3.

The State of California Air Resources Board (ARB) Monitoring and Laboratory Division (MLD) details a methodology for measurement of acrylonitrile in ambient air using sorbent tubes with thermal desorption[17] and quantification by GC coupled with mass spectrometry

[16] NIOSH (1994) states that a nitrogen selective detector (NPD) increases the sensitivity and specificity of the analysis

[17] https://arb.ca.gov/airwebmanual/owl-ultralite/Documents/Draft/AMTAC/media/AWMAAcrylonitrileExtendedAbstract.pdf

(GC-MS). This methodology was used to measure concentrations in the parts per trillion range.

Table 6: Analytical methods for determining acrylonitrile in air samples

Sample Matrix	Assay procedure	Limits of Quantification / Detection	References
Air (NIOSH Method 1604)	GC-FID	Limit of detection: 0.05 mg/m^3 (0.02 ppm) Working range: 1.5 to 1000 mg/m^3	NIOSH (1994)
Air (OSHA Method ORG-37)	GC-NPD	Limit of detection: 0.026 mg/m3 Limit of quantification: 0.66 mg/m^3	OSHA 1982[18], 1990
Air (HSE Method MDHS72)	GC-FID	Working range: 0.2 to 100 mg/m^3	HSE 1993
Air (HSE Methods MDHS80 and MDHS88)	GC-FID	Working range: 1 to 1000 mg/m^3	HSE 1995 HSE 1997
Air EN 689 (DIN) and EN 482 (DIN)	GC-FID	Limit of detection: 0.05 mg/m^3	EU RAR (EC 2004)
Air (DGUV study on environmental tobacco smoke in the workplace)	GC-NPD	Limit of detection: 0.1 µg/m^3	DGUV 2008
Air (using solid sorbent tubes and thermal desorption)	GC-MS	Limit of detection: <0.002 µg/m^3	Bricarello JR, State of California Air Resources Board[16]

6.2 Biomonitoring of exposure (internal exposure)

N-(2-Cyanoethyl)valine (CEV), thiocyanate (SCN) and cyanide (CN) can all be used as biomarkers for the assessment of acrylonitrile exposure and toxicity (Colenbie *et al.*, 2017). Minet *et al.* (2011) has also recommended using urinary 2-cyanoethylmercapturic acid (CEMA), an acrylonitrile metabolite, as a biomarker for specifically assessing smoking-related exposure to acrylonitrile. CEV is by far the most important as it is the only one of these biomarkers that is specific to acrylonitrile and the analytical methodology is also extremely sensitive.

CEV is a protein adduct formed by reaction of acrylonitrile with the N-terminal valine in haemoglobin and is specific to acrylonitrile exposure. The level of CEV reflects exposure during a 4-month period (i.e. the life-span of erythrocytes) prior to a blood sample being taken. CEV itself has a relatively long half-life corresponding to half the life-span of the erythrocytes (i.e. approximately 60 days; Granath *et al*. 1992). Significantly, a positive correlation has been observed between the concentration of acrylonitrile in ambient air and the N-2-cyanoethylvaline level in the globin of erythrocytes (Bader & Wrbitzky,2006 and IFA Report 1/216 – see Table 8); this linear dose-response relationship means that

[18] https://www.osha.gov/dts/sltc/methods/organic/org037/org037.html

concentrations of acrylonitrile in air may be directly translated to internal exposure (as indicated by CEV concentration). However, use of this correlation may be complicated at low levels of acrylonitrile by background levels of CEV (e.g. in smokers). It should also be noted that this correlation applies to long-term and not short-term exposure.

Based on the linear dose-response relationship established, Germany[19] recommends a bioequivalence value of 650 pmol CEV/g globin (blood erythrocytes); sampling after at least 3 months of exposure as an 'acceptable concentration' (equivalent to a statistical risk level of 4:10 000 for developing cancer) and of 6 500 pmol CEV/g globin as a 'tolerable concentration' (risk level of 4:1 000). This recommendation is based on the EKA correlation (Exposure Equivalent for Carcinogens) as established for acrylonitrile, see Table 8 (DGUV, 2016). According to the EKA correlation the tolerable risk of a long-term air concentration of 2.6 mg/m^3 (1.2 ppm) corresponds to an internal exposure of 156 µg CEV/L blood (erythrocyte)[20]. For adult non-smokers the MAK Commission (DFG, 2016) has established a biological reference value (BAR)[21] of 0.3 µg/L blood (erythrocyte).

The method for measurement of CEV is based on a modified Edman procedure and detection with selected ion monitoring by gas chromatography–mass spectrometry. The limit of detection is about 0.1–1 pmol/g globin (WHO, 2002) and Colenbie *et al.* (2017) report a limit of quantification in the range of 0.5-4.0 pmol/g globin. Adduct levels ranging from 20 to 66 000 pmol/g have been observed in occupationally exposed workers (WHO, 2002).

Cyanide is a metabolite of acrylonitrile and thiocyanate is formed in the body during cyanide detoxification formed by mitochondrial rhodanese. When cyanide is used as a biomarker it directly measures the presence of both exogenous and endogenously formed cyanide. Lactate is formed due to anaerobic metabolism following inhibition of the electronic transport chain by cyanide and thiocyanate and can be used as an indicator of this type of toxicity. A study by Colenbie *et al*. (2017) compared levels of CEV with lactate and SCN in patients admitted to emergency care following a railway accident and fire in Belgium in 2014 in which large amounts of acrylonitrile were released.

[19] GMBl-Bek.-TRGS 910 (Fassung 8.6.2017)

[20] With 1 litre blood containing 144 g globin, 156 µg CEV/L blood converts to (rounded up) 6500 pmol CEV/g globin (mw CEV = 170.21 g)

[21] A BAR describes the background level of a substance which is present concurrently at a particular time in a reference population of persons of working age who are not occupationally exposed to this substance. The BAR are based on the 95th percentile without regarding effects on health. It must be taken into account that the reference level of the background exposure can be influenced by such factors as age, sex, social status, residential environment, life style and geographical region.

Table 7: Analytical methods for determining acrylonitrile in biological samples

Sample Matrix	Assay procedure	Limits of Quantification / Detection	References
CEV in Blood (erythrocytes)	Modified Edman degradation followed by GC-MS	Limit of detection: 0.1-1 pmol/g globin Limit of quantification: 0.5-4.0 pmol/g globin	Tavares *et al.* (1996) Licea Perez *et al.* (1999) Cited in WHO 2002 Colenbie *et al.* 2017
SCN in Blood (serum or plasma)	Colorimetry	Limit of quantification: 0.5 mg/L	Colenbie *et al.* 2017
CN in Blood	Liberation of hydrogen cyanide into alkaline solution and reaction with reagents	Limit of quantification: 0.1 µg/L	Colenbie *et al.* 2017 (after Lambert *et al.* 1995)

Table 8: Correlation of concentration of acrylonitrile in air (long-term exposure) with the biomarker CEV (N-(2-Cyanoethyl)valine) in the erythrocyte fraction of whole blood (from DGUV, 2016)

Acrylonitrile concentration in air (estimated) mg/m³ (ppm)	µg CEV/L blood (erythrocyte)
0.3 (0.14)	16
0.5 (0.23)	35
1.0 (0.45)	60
7.0 (3.0)	420

7. Health Effects

7.1 Toxicokinetics (Absorption, distribution, metabolism and excretion - ADME)

7.1.1 Human data

Human volunteer studies *in vivo* and the outcomes of accidental poisoning cases all indicate that the dermal absorption of acrylonitrile is high, such that a dermal absorption level approaching 100% (comparable to oral absorption) is assumed for the purposes of risk assessment.

A small group of human volunteers (6 males exposed for eight hours to acrylonitrile at concentrations of 5 or 10 mg/m^3) comprised a study of inhalation kinetics (Jakubowski *et al*., 1987) in which retention of acrylonitrile in the lungs averaged approximately 52%, which is reported to be lower than that seen in the rat, and approximately 22% of the retained acrylonitrile was metabolised to 2-cyanoethylmercapturic acid (CEMA).

There are limited data on the toxicokinetics of acrylonitrile in humans, but the available data indicate that the metabolic pathway via cyanoethylene oxide (CEO) seen in the rat, also exists in humans (Kedderis & Batra, 1991; 1993a).

7.1.2 Animal data

A large number of non-standard investigative studies have been conducted examining the toxicokinetics of acrylonitrile in a variety of experimental species (predominantly rats), at a wide range of doses and administered by either inhalation or, more usually, oral routes. The large majority of these data is summarised in the EU RAR (EC, 2004) and The Sapphire Group Inc. (2004). Also the latter has been the subject of an independent, external peer review (Haber and Patterson, 2005). Oral gavage studies in rats have shown that acrylonitrile is extensively absorbed. This is also the case following inhalation exposure.

It has been shown in rodents that acrylonitrile is extensively absorbed by all the major routes of exposure, undergoes significant first-pass metabolism and is initially metabolised by two pathways (see Figure 1), a detoxification step (Pathway 1) and an activation step (Pathway 2).

Pathway 1 occurs via conjugation with glutathione, either through catalysis with a cytosolic glutathione-S-transferase (GST) or non-enzymatically and, consequent to extensive absorption and first-pass metabolism, could be considered as the dominating pathway following oral administration. Based on urinary metabolite data, following conjugation with glutathione, the primary metabolite of acrylonitrile following oral administration is N-acetyl-S-(2-cyanoethyl)cysteine. Further metabolism via this pathway results in a number of major metabolites in rodent urine including thiocyanate, N-acetyl-S-2-(2-cyanoethyl)cysteine and 4-acetyl-5-cyanotetrahydro-1,4-2H-thiazine-3-carboxylic acid. The glutathione conjugate (GSH) of acrylonitrile can also be converted to a mercapturic acid and then also excreted in urine.

Pathway 2 is via epoxidation of acrylonitrile by microsomal cytochrome (CYP) P450 2E1 forming 2-cyanoethylene oxide (CEO) and it has been demonstrated in rodents that this metabolic pathway can be induced by known hepatic enzyme-inducing substances (Kedderis & Batra, 1993a). However, also other enzyme systems may play a role in acrylonitrile oxidation as well (e.g., lactoperoxidase and lung lipoxygenase) (Nasralla et al., 2009; Roy and Kulkarni, 1999). CEO has a half-life of approximately 1.5 h (Kedderis and Batra, 1993a). CEO is metabolised by two pathways: Pathway 2a via conjugation with glutathione, either through catalysis with cytosolic GST or non-enzymatically, forming

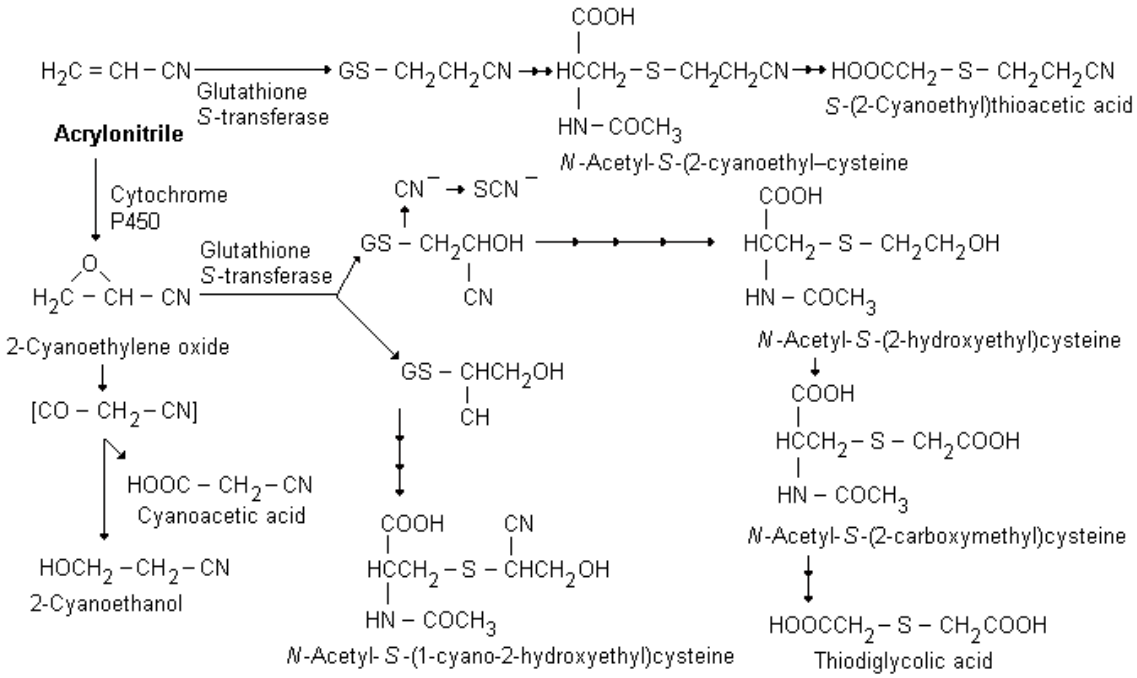
conjugates on the second or third carbon; and Pathway 2b via hydrolysis by microsomal epoxide hydrolase. The secondary metabolites of CEO can undergo further metabolism/decomposition. For example, cyanide can be released from the CEO metabolite generated by the epoxide hydrolase pathway and from the GSH conjugate formed on the third carbon. Cyanide is detoxified by the mitochondrial enzyme, rhodanese, which uses thiosulphate to form thiocyanate. Thiocyanate has been measured in the blood and brain of rats and mice exposed to acrylonitrile by oral gavage, and hydrogen cyanide detected in the exhaled breath of rats exposed to acrylonitrile by the oral route, accounting for 0.5% of the administered dose (Ahmed *et al*., 1982). The authors reported that the release of cyanide appeared to require CYP2E1 activity suggesting that the primary site of acrylonitrile metabolism is the liver. It is logical to assume that, due to the extensive absorption of acrylonitrile via the main exposure routes, the liver would be the primary site of metabolism in most cases. As for Pathway 1, in rats, the oxidation of acrylonitrile is also indicated by urinary excretion of thiocyanate. The appearance of thiocyanate in the urine via this pathway was seen to be greater following either oral or inhalation exposure when compared to parenteral routes of exposure.

In rats administered acrylonitrile by oral gavage, approximately 5% of the dose is excreted in exhaled air, with peak excretion reported at 30 minutes after dosing. Acrylonitrile is also excreted as other volatile metabolites including hydrogen cyanide (0.5%) and CO_2 (9%); it has been demonstrated that the CO_2 is derived from the cyano (-CN) group on the acrylonitrile molecule. The predominant route of excretion is urinary excretion; a small proportion (3-8%) is excreted in the faeces. Excretion is rapid and occurs mainly within 24 hours, although approximately 25% of the administered radioactivity is retained in the body beyond 10 days, possibly due to the binding of metabolites to cellular macromolecules.

Thiocyanate persists longer than acrylonitrile, CEO or cyanide in the blood (half-life of about 1-6 days in humans) and tissue doses may vary significantly from one tissue to another. Long-term exposure to thiocyanate can lead to goiter. Thiocyanate can lead to hypothiocyanite formation which may have important toxicological consequences (Acrylonitrile EU REACH Consortium 2017).

Several metabolic factors contribute to nonlinear kinetics for acrylonitrile, including the presence of a saturable metabolic pathway and the depletion of cofactors required for metabolism. The urinary excretion of N-acetyl-S-(2-cyanoethyl)cysteine and S-(2-cyanoethyl)thioacetic acid across a wide range of oral doses was increased in a non-linear manner. Glutathione depletion has been observed in a number of tissues (brain, lung, liver, kidney, stomach, adrenal gland, erythrocytes) in rats exposed to acrylonitrile and results in an increase in the proportion of acrylonitrile metabolised via the oxidative pathway.

Figure 1: Schematic of acrylonitrile metabolic pathways (after WHO, 2002)



7.1.3 *In vitro* data

In addition to the primary hepatic site of acrylonitrile metabolism, *in vitro* studies in microsomal fractions from a number of rat tissue sites, including testes, kidney, lung, nasal tissue, small intestines and brain, have demonstrated acrylonitrile metabolism. Human lung lipoxygenase also has an appreciable activity for metabolising acrylonitrile to cyanide *in vitro*, suggesting that there may be additional enzymatic pathways leading to the formation of CEO and the release of cyanide.

Existence of additional pathways is supported by a comparative studies using hepatic microsomes from rats, mice and humans (Kedderis & Batra, 1991 and 1993a) showing that human hepatic microsomes significantly increased the rate of hydrolysis of CEO and that this could be potently inhibited by 1,1,1-trichloropropene oxide, indicating the involvement of epoxide hydrolase. It was also observed by these authors that human hepatic microsomes, but not cytosols, significantly increased the rate of CEO hydrolysis and that this could be potently inhibited by 1,1,1-trichloropropene oxide, indicating the involvement of epoxide hydrolase. The authors indicate that this contrasts with no increase in the rate of hydrolysis of CEO when incubation is with hepatic microsomes or cytosols from male rats or mice. Hence a conclusion by these authors that, in humans, this microsomal involvement could be considered as an additional detoxification pathway for CEO which appeared not active (but inducible) in rodents. This interpretation needs to be considered with caution since, although the epoxide hydrolysis reactions might differ between rat and humans, the hydrolytic process has been demonstrated in both species, but the possibility of greater human efficiency in the epoxide hydroxylation pathway cannot be excluded.

7.1.4 Toxicokinetic modelling

See section 8.1.

7.1.5 Biological monitoring

As discussed in Section 6.2, the monitoring of human exposure to acrylonitrile is feasible through measurement of the protein (haemoglobin) adduct N-(2-Cyanoethyl)valine (CEV), and a number of biomonitoring studies are available. Bader & Wrbitzky (2006) reported that the marker was also applicable for monitoring short-term or single exposures where the increase in adduct level can be small which, together with non-linear elimination kinetics, may limit its measurement and validity. The authors measured CEV in the blood of workers 25 days after cleaning-up an accidental spill of acrylonitrile in a train depot, with levels between 566 pmol/g globin and 2020 pmol/g globin. These results significantly exceed both typical non-smoker values (<10 pmol/g globin) and smoker values (>50 pmol/g globin, between 150 pmol/g globin and 300 pmol/g globin for a cigarette consumption of 20–40 per day) (Schettgen *et al*., 2002). Levels decreased following 85, 115 and 175, days post-exposure and a total elimination interval of 148 days was proposed (Bader & Wrbitzky, 2006).

Several biomonitoring studies have been carried out on emergency workers and the general population following the accidental release of acrylonitrile during a train accident in 2013 in Wetteren (Belgium). Van Nieuwenhuyse *et al*. (2014) assessed levels of CEV haemoglobin adducts in 841 emergency responders to the incident. Around 26% of responders showed increased levels of CEV (above 10 pmol/g globulin for non-smokers and above 200 pmol/g globulin for smokers), however, for non-smokers these stayed within the background levels for a smoking population. The authors reported that acrylonitrile exposure was predicted by (1) the distance to the accident, (2) the duration of exposure, and (3) the occupational function.

De Smedt *et al*. (2014) reported on the extent of exposure of 242 local residents close to the site of the train accident through measurement of CEV in blood 14 – 21 days following exposure. In residents within the evacuated zone, 37.3% of non-smokers and 40% of smokers had CEV levels that were higher than background (above 10 pmol/g globulin for non-smokers and above 200 pmol/g globulin for smokers). Exposure of the general population was also found to occur via the sewage system. In a follow-up to this, Simons *et al*. (2016) explored the association between the CEV concentrations measured at 14 – 21 days with self-reported short-term health effects, recorded at the same time as blood measurements. Local symptoms of irritation were most frequently reported with a large proportion of non-smokers with CEV levels >100 pg/g globulin reporting this. In non-smokers, the authors described a dose-response relationship between CEV levels and the reporting of short-term health effects and recommended that biomarkers allow an objective assessment of exposure as opposed to self-reported symptoms which may be subject to recall bias.

7.1.6 Summary

Acrylonitrile is well absorbed in humans and animals via all routes of exposure. There are limited data on the toxicokinetics of acrylonitrile in humans, however available evidence is supportive of the same, or very similar, metabolic pathways as seen in the rat. Following absorption, acrylonitrile is metabolised in the liver by two pathways, a detoxification step (Pathway 1) via conjugation with glutathione, and an activation step (Pathway 2) via epoxidation forming 2-cyanoethylene oxide (CEO). This epoxide intermediate could be the origin of some mutagenic-induced carcinogenic effects of acrylonitrile in the rat as epoxides are well known to be highly reactive with DNA. Further metabolism of CEO, via one or more hydrolysis reactions, leads to the production of various metabolites including

cyanoacetic acid, 2-cyanoethanol and cyanide (thereafter excreted as thiocyanate). The metabolism of acrylonitrile exhibits a strong first-pass effect, and as such, the relative importance of each pathway depends upon the route of exposure. The predominant route of excretion is urinary excretion, with only a small proportion (3-8%) being excreted via faeces. Excretion of the absorbed dose is rapid, with 75% occurring within 24 hours, however, some is retained beyond 10 days, possibly due macromolecular binding. Several metabolic factors contribute to nonlinear kinetics for acrylonitrile, including the presence of a saturable metabolic pathway and the depletion of cofactors required for metabolism. *In vitro* studies support the liver as the primary site of metabolism and suggests additional sites of metabolism including testes, kidney, lung, nasal tissue, small intestines and brain. An additional detoxification enzymatic pathway for CEO has been proposed in humans via human lung lipoxygenase, which is not active in rats but can be induced.

PBPK models have been developed for acrylonitrile in rats and humans and have been applied to cancer risk assessment. A number of biomonitoring studies utilising measurement of CEV in blood has shown the utility of the biomarker for assessing exposure, including single and short-term exposures, to acrylonitrile in workers and the general population. Local symptoms of irritation have been reported in non-smokers with CEV levels >100 pg/g globulin.

7.2 Acute toxicity

7.2.1 Human data

A single 8-hour experimental inhalational exposure of volunteers to acrylonitrile at concentrations of 5-10 mg/m^3 produced no subjective symptoms such as headache, nausea, or general weakness described at a similar level of industrial exposure (Babanov, 1959 – cited in Jacubowski *et al*., 1987).

Baxter (1979) summarised the sequence of symptoms of acrylonitrile poisoning in man as: irritation of eyes and nose, limb weakness, laboured breathing, dizziness, impaired judgement, cyanosis and nausea, collapse, irregular breathing and convulsions, based on a number of early case studies (prior to 1980). These reported symptoms of nasal irritation, an oppressive feeling in the upper respiratory passages, dull headaches, nausea, apprehension and nervous irritability in workers exposed acrylonitrile at levels between 16-100 ppm (35 -219 mg/m^3) for 20 to 45 minutes (Wilson, 1944; Wilson *et al*., 1948). Low-grade anaemia, leucocytosis, kidney irritation and mild jaundice were also apparent but subsided with cessation of exposure. Other case studies report similar symptoms within 5-15 mins of exposure (Sartorelli, 1966; Zeller *et al*., 1969) and even death (Davis et al., 1973; Radimer *et al*., 1974; WHO, 1983) following exposure, however, levels are not defined.

There are a number of more recent studies reporting adverse effects following acute acrylonitrile exposure in humans, mainly associated with accidental occupational exposures via inhalation or, occasionally, the dermal route. In one such incident involving 144 subjects, inhalation exposure to a low concentration (in terms of accidental release) of 40-79 mg/m^3 resulted in slight changes to liver function tests in a small number of subjects and leucocytosis in a large proportion of subjects. All subjects recovered following treatment (Chen *et al*., 1987).

In a second industrial incident (Thier *et al*., 2000), five subjects were accidently exposed by inhalation to acrylonitrile vapours and three others exposed through the dermal route. Presenting clinical symptoms ranged from none to eye and throat irritation and headache and vomiting with high blood levels of cyanide. This pattern of effect was noted for both groups of subjects exposed either via inhalation or via dermal exposure.

Jakubowski *et al*. (1987 as cited in EC 2004) assessed the effects of exposure of human volunteers exposed acutely (8-hour duration) to acrylonitrile levels between 2.4 and 5.0 ppm (5.4 to 10.9 mg/m^3). No deleterious effects were exhibited by volunteers, indicating low respiratory tract irritant effects at these levels of exposure (Jakubowski *et al*., 1987). Local effects were similar to those observed in other incidents but low concentration exposure was also associated with slight liver enlargement, jaundice, low grade anaemia and leucocytosis.

The death of a 10-year old girl was attributed to increased dermal absorption of acrylonitrile following application of an insecticide formulation containing acrylonitrile to damaged skin of the scalp as a treatment for head lice, but no exposure level is indicated (Lorz 1950 as cited in WHO 1983).

7.2.2 Animal data

A number of regulatory compliant acute toxicity studies have been conducted in a variety of species using a number of routes of exposure. The corresponding LD_{50} or LC_{50} data are presented in Table 9.

Table 9: Acute toxicity data for acrylonitrile

Species	Route of administration					
	Oral (LD_{50} mg/kg)	Inhalation (LC_{50} mg/L)	Dermal[a] (LD_{50} mg/kg)	ip (LD_{50} mg/kg)	iv (LD_{50} mg/kg)	sc (LD_{50} mg/kg)
Rat	72-186 (Smyth & Carpenter, 1948; Paulet & Vidal, 1975; Zeller *et al*. 1969; Tullar, 1947; Benesh & Cherna, 1959; Monsanto, 1975)	0.5hr: 7.88 1hr: 2.19-4.00 2hr: 2.03 4hr: 0.47-2.09 6hr: 0.69 (Dudley *et al*, 1942a)	148-282 (Zotova, 1976)	65-100 (Knobloch *et al*. 1971; Paulet & Vidal, 1975)	-	80-96 (Knobloch *et al*. 1971; Magos, 1962)
Mouse	25-48 (Tullar, 1947; Benesh & Cherna, 1949; American Cyanamid, 1951)	4hr: 0.3 (Knobloch *et al*. 1971)	-	47-50 (Tullar, 1947; Zeller *et al*. 1969; Yoshikawa, 1968)	-	25-50 (Graham, 1965; Knobloch *et al*. 1971)
Rabbit	93 (Lefaux, 1996)	-	24hr: 200-226 (Vernon *et al*. 1969)	-	69 (Paulet & Desnos, 1961)	-

Guinea pig	50-85 (Carpenter *et al*. 1949; Tullar, 1947; Jedlicka *et al*. 1958)	4hr: 0.99 (Knobloch *et al*. 1971)	260-690 (BUA, 1995)	-	72 (Tullar, 1947)	130 (Ghiringhelli, 1954)
Dog	-	4hr: 0.24 (Dudley *et al*, 1942a)	-	-	-	-

[a] Duration given where stated

Source: European Chemicals Bureau, 2004; WHO 1983.

The clinical symptoms associated with the acute toxicity of acrylonitrile appears to be phasic based on duration of exposure and/or dose concentration and are indicative of the CNS being a target organ. Immediately after administration the animal goes through an excitatory phase, with agitation and lacrimation. A tranquil phase follows and cholinergic symptoms, such as salivation, lacrimation, urination and defecation occur. Next there is a convulsive phase in which the animal undergoes clonic seizures. The terminal stage preceding death is a paralytic phase in which the animal is immobile. The clinical signs indicate that the action of acrylonitrile is that of a typical nitrile, with a toxic action probably due to a cleavage of the molecule to produce hydrogen cyanide, which is one of the key mediators of the toxicity.

The sensitivity of various species to orally or inhalatory administered acrylonitrile is noted to decrease in the order mouse/dog>guinea pig/rabbit>rat. The mouse is also noted as the most sensitive species to subcutaneous administration of acrylonitrile in comparison with rats and guinea pigs. For dermally exposed rats, rabbits and guinea pigs the determined LD_{50} concentrations were all of similar ranges.

7.2.3 *In vitro* data

No data available

7.2.4 Summary

Acrylonitrile is classified under the CLP Regulation (1272/2008) as for acute toxicity Category 3 (H301, H311 and H331): Toxic if swallowed, Toxic in contact with skin, Toxic if inhaled.

In humans, acrylonitrile shows acute toxicity through all routes at concentrations > 5 ppm (10.9 mg/m^3). Effects range from irritation of eyes and nose, limb weakness, laboured breathing, dizziness, impaired judgement, cyanosis and nausea, through to collapse, irregular breathing and convulsions, depending on dose and duration of exposure. Findings in humans are consistent with those in experimental species.

For acute oral toxicity, the sensitivity of various species to acrylonitrile is noted to decrease in the order mouse>guinea pig>rabbit and rat with acute oral LD_{50} values ranging from 25-186 mg/kg. The sensitivity of various species for acute inhalation toxicity of acrylonitrile followed a similar pattern with the addition of the dog as most sensitive alongside the mouse. As shown in Table 9, acute inhalation LC_{50} values in the range of 0.2-2.9 mg/L for a four-hour exposure were reported. The increased sensitivity of dogs to acrylonitrile is considered to be due to the release of cyanide during metabolism as dogs are more susceptible to the toxicity of cyanide due to lower levels of the detoxifying enzyme, rhodanase in the liver than other mammals.

7.3 Specific target organ toxicity / Repeated dose toxicity

7.3.1 Human data

Human repeat-dose exposure data may be derived from three major cross-sectional medical questionnaire studies of workers exposed to acrylonitrile in acrylic fibre factories in Japan (Sakurai *et al*. 1978; Kaneko & Omae, 1992; Muto *et al*. 1992). However, data from these data are difficult to assess in relation to the establishment of a dose-response relationship because of variation in levels of individual exposure. It is noted that the median concentration for the highly exposed population of workers in 1978 was reported to be 5 ppm (10.85 mg/m^3) and the time-weighted average concentrations reported for the two groups of factories in 1992 were 0.27 and 0.84 ppm (0.59 and 1.82 mg/m^3) respectively. Self-reported prevalence of reddening of conjunctiva was statistically

significantly higher (compared to unexposed) in the factories with a reported average concentration of 0.84 ppm (21.2 % vs. 11.7 %) but not in the factories with a reported average concentration of 0.27 ppm (11.6 % vs 10.4%) (Muto *et al.* 1992). The self-reported prevalence of eye pain or lacrimation was also statistically significantly increased in the former factories (32.4% vs. 19.2%) but not in the latter (18.6% vs. 21.5%). The higher prevalence in the higher exposure factories was reported to be due to one specific factory with a maximum exposure concentration of 1.4 ppm by personal sampling which made the authors suspect that other exposures than acrylonitrile could have caused the increase in the factory. In a medical examination, no cases of reddening of conjunctiva was observed either in the exposed or the unexposed of the lower exposure factories, and in the high exposure factories the difference was not statistically significant and based on very few cases (1% in the exposed vs 0% in unexposed). This was a cross-sectional study reporting only group level exposures, with no quantitative information on other exposures, and the outcomes were based on symptoms recorded in self-report questionnaires using rather unspecific symptoms as regards irritation effects. Consequently, it is difficult to draw firm conclusions from these data.

Cave *et al.* (2011) measured cytokeratin 18 (CK18), a biomarker for liver disease, in male elastomer/polymer workers exposed to acrylonitrile, 1,3-butadiene and styrene (ABS). A total of 39% had elevated CK18 levels which were not explained by other exposures, and suggestive of occupational liver disease. CK18 patterns were consistent with toxicant-associated steatohepatitis (TASH). TNFα, IL-16, IL-8, MCP-1 and PAI-1 (pro-inflammation cytokines) were increased in workers with elevated CK18 levels compared to those with normal levels. Limitation include imaging and liver biopsies not taken to confirm presence of liver disease, serum CK18 may be increased by wide variety of diseases affecting epithelial cells.

Neurotoxicity

At levels of ≤ 0.53 ppm no subjective symptoms were seen in Muto *et al.* (1992). When the population was divided in two exposure groups, statistically significantly increased prevalence of subjective symptoms (e.g., heaviness of stomach, poor memory, irritability) were observed in group B with exposure of 0.84 ppm (mean TWA) or 1.13 ppm (personal air concentrations). However, as pointed out in the above paragraph for irritation effects, this study was cross-sectional reporting only group level exposures, with no quantitative information on other exposures, and the outcomes were based on symptoms recorded in self-report questionnaires using rather unspecific symptoms. Consequently, it is difficult to draw firm conclusions from these data.

Rongzhu *et al.* (2005) conducted a cross-sectional study of neurobehavioral performance in Chinese workers (81 workers in the acrylonitrile monomer plant, 94 workers in the acrylic fibers plant and 174 workers in the departments with no acrylonitrile exposure). The geometric means of exposure from periodic short-term area sampling (1997 – 1999) were 0.11 ppm (range 0.00-1.70 ppm for 390 samples) in the monomer plant and 0.91 ppm (range 0.00-8.34 ppm for 570 samples) in the acrylic fiber plant. No personal sampling data were collected. The WHO-recommended neurobehavioral core test battery was used to evaluate neurobehavioral functions. Exposure to acrylonitrile was associated with increases in negative mood states (increased tension, depression, anger, fatigue and confusion) and poorer performance in the Simple Reaction Time, Digit Span, Benton Visual Retention and Pursuit Aiming II. However, there are several important limitations of the study (e.g., for some tests there were indications for better performance associated to acrylonitrile exposure, crude exposure assessment, co-exposure of the monomer workers to cyanide and of the fiber workers to methyl methacrylate and heat, potential selection bias). The study can therefore not be used quantitatively in risk assessment.

A conference abstract by Gincheva *et al.* (1977) reported no symptoms following 3-5 years of occupational exposure to acrylonitrile at 1.9-3.3 ppm (n=23), however the reliability of these data is considered "not assignable" (Klimisch score 4).

Russian workers exposed to acrylonitrile concentrations of 0.6-6 mg/m^3 (0.27-2.7 ppm) for about three years suffered from headache, insomnia, pains in the heart region, general weakness, decreased working capacity, increased irritability, reduced blood pressure, inflamed vocal cords, non-specific changes in the vestibular apparatus and pale mucous membranes and skin (Babanov *et al.* 1959 as cited in IARC 1999).

Stamova *et al.* (1976 as reported in IARC 1999) found an increased incidence of skin diseases and various 'neurasthenic' complaints and diseases in workers of a polyacrylic fibre plant in which acrylonitrile exposure levels were around 10 mg/m^3. Workers were also exposed to other substances.

Ageeva (1970 as reported in IARC 1999) reported depression, lability of autonomic functions (lowered arterial pressure, labile pulse, diffuse dermographia, increased sweating, change in orthostatic reflex) in workers involved in acrylonitrile production.

Anecdotal evidence of a neurotoxic effect was reported by Bakker *et al*. (1991) in a worker with contact dermatitis undergoing a patch-test using 0.1% solutions of acrylonitrile (99.6% purity). After 2-3 days the worker presented with paresthesiae.

Although human data starts to show some effects at around 1 ppm and above, the health outcome definitions used in the above human data were often not specific to a given neurological effect, individual exposure estimation was often based on relatively coarse group level information and exposures other than acrylonitrile were often not addressed. Therefore, the human data are not considered sufficiently robust to use as a point of departure in risk assessment.

7.3.2 Animal data

A number of regulatory repeat-dose toxicity studies have been conducted in a variety of species by either oral or inhalation routes of exposure. The oral studies were conducted mainly via the drinking water and occasionally by oral gavage and the exposure period for inhalation was predominantly 4 hours per day. The corresponding no effect levels (NOAEL or NOAEC) are presented in Table 10.

Table 10: Repeat-dose toxicity data for acrylonitrile (Gagnaire et al. 1998)

Species	Route	Duration				
		Sub-acute	Sub-chronic	Chronic		
		up to 4 wks	up to 13 wks	up to 26 wks	up to 52 wks	up to 104 wks
Rat	**Oral (mg/kg bw/day)**	NOAEL (2 wks): 75 NOAEL (5 days): 45 LOAEL: <60 oral gavage [Klimisch 2] (Working *et al.* 1987) NOAEL (up to 60 days): approx. 4 LOAEL: <60 drinking water & oral gavage [Klimisch 2] (Szabo *et al*., 1984)	NOAEL: <10 LOAEL: 10 drinking water [Klimisch 2] (Humiston & Frauson, 1975) NOAEL: 25 LOAEL: 50 gavage [Klimisch 2] (Gagnaire *et al.* 1998)	No data	No data	**NOAEL: <3.4 (male); <4.4 (female)** **LOAEL: 3.4 (male); 4.4 (female)** **drinking water** **[Klimisch 2]** **(Quast JF, 2002)** **NOAEL: 0.25 (male); 0.36 (female)** **LOAEL: 0.84 (male); 1.25 (female)** **drinking water** **[Klimisch 2]** **(Johannsen & Levinskas, 2002)**
	Inhalation (ppm)	NOAEC: 130 4 hours/day;5 days/week [Klimisch 2] (Dudley *et al*., 1942b)	**NOAEC: <5 (F1) ; 15 (F0)** **LOAEC: 5 (F1); 45 (F0)** **whole body** **6 hours/day; 7 days/week;10 week** **[Klimisch 1]** **(Nemec *et al*., 2008)** **NOAEC: 25** **LOAEC: 50** **whole body** **6 h/d, 5 d per week,**	No data	NOAEC: 5-10 LOAEC: 5 (female); 10 (male) whole body 4 hours/day; 5 days/week; 12 months [Klimisch 2] (Maltoni *et al*., 1977, 1988)	NOAEC: 4 (estimated from 20 ppm LOAEC) whole body 6 hours/day; 5 days/week; 2 years [Klimisch 2] (Quast *et al*., 1980a)

			for 24 weeks [Klimisch 2] (Gagnaire *et al.* 1998)			
Mouse	**Oral (mg/kg bw/day)**	No data	NOAEL:5 LOAEL: >10 oral gavge Klimisch 2 (NTP, 2001) NOAEL: 12 LOAEL: >12 oral gavage Klimisch 2 (Serota *et al*., 1996)	No data	No data	NOAEL: <2.5 (male); 2.5 (female) LOAEL: 2.5 oral gavage Klimisch 2 (NTP, 2001)
	Inhalation (ppm)	No data	No data	No data	No data	No data
Dog	**Oral (mg/kg bw/day)**	No data	No data	NOAEL: <8-10 LOAEL: 8-10 drinking water Klimisch 2 (Quast *et al*. 1975)	No data	No data
	Inhalation (ppm)	No data	NOAEC:<24 Klimisch 2 (Dudley *et al*., 1942b)	No data	No data	No data
Monkey	**Inhalation (ppm)**	NOAEC: 56 Klimisch 2 (Dudley *et al*., 1942b)	No data	No data	No data	No data

The studies indicated in **bold type** are those considered to be key studies in this assessment

7.3.2.1 Oral administration

Sub-chronic oral studies in mice (NTP, 2001; Serota *et al*., 1996) showed treatment-related effects at dose levels of 10 mg/kg/day and above by oral gavage and >12.0 mg/kg/day when administered via drinking water. Principal toxicities were demonstrated in the gavage study as lethargy and abnormal breathing. Local effects were also seen in the forestomach (characterised by histopathological observations of inflammation and hyperplasia) and haemolytic anaemia was recorded at the higher dose levels (>20 mg/kg/day). No specific target organ for toxicity was identified and, although some haematology parameters showed some differences from control animals in the drinking water study, no specific toxicity was identified. In the chronic toxicity drinking water study in mice, over a two-year period, adverse effects on survival were recorded at 20 mg/kg/day and significantly increased incidences of neoplastic pathologies (including squamous cell papilloma and carcinoma in the forestomach, Harderian gland adenoma and carcinoma, benign and malignant ovarian granulosa cell tumours and alveolar/bronchiolar adenoma or carcinoma) were seen at 10 mg/kg/day and above.

In rats (Szabo *et al*., 1984), sub-acute and sub-chronic administration of acrylonitrile in drinking water or via oral gavage induced local changes in the stomach (hyperplasia in regions of the gastric mucosa) but also demonstrated the adrenal to be a target organ. At drinking water inclusion levels of 500 ppm and above or oral gavage dose levels of 60 mg/kg/day and above, enlargement of the adrenal accompanied by polyuria was noted. Histopathological findings in the adrenal gland were variable ranging from atrophy of the adrenal cortex to cellular hyperplasia and shrunken cells. These findings were often associated with decreases in plasma corticosterone levels.

A number of other short-term toxicity studies have been conducted (EC, 2004), most notably a 5-day repeat dose study in rats by gavage administration (Working *et al*., 1987) in which deaths occurred at dose levels above 60 mg/kg/day.

Two chronic studies in rats via the drinking water (Quast *et al*. 1980b, 2002; Johannsen and Levinskas, 2002a) are considered to be the key studies via the oral route of exposure since, although they are both Klimisch 2 studies (reliable with restrictions), they are both long-term regulatory studies and show clear dose response and toxicity at the higher dose levels. At inclusion levels ranging from 1 to 300 ppm both studies showed clear adverse effects on body weight, clinical observations and mortality at inclusion levels of 10 ppm and above. The principal pathological effects continued to be forestomach lesions (hyperplasia and/or hyperkeratosis suggestive of chronic irritation) and central nervous system (brain) changes (gliosis with or without perivascular cuffing). The LOAEL for these changes was estimated to be in the region of 3.4-4.4 mg/kg/day.

A subchronic study in male SD rats assessed motor and sensory conduction velocities (MCV and SCV) and amplitudes of the sensory and motor action potentials (ASAP and AMAP) of the tail nerve (Gagnaire *et al.* 1998). Groups of rats (n=12/group) were administered 12.5, 25 and 50 mg/kg bw/day of acrylonitrile for 5 days per week, for 12 weeks. Dose dependent salivation, locomotor hyperactivity and moderately intense stereotypies associated with fur wetting were observed in all dose groups. They developed just after each gavage and were transient in nature, and were consistent with general central nervous system excitation. The effects showed some similarity with acute toxicity, which may result from gavage, but interestingly, the effects increased over time. In the high dose group rats showed weakness in hindlimbs and could not rear which was associated with decreases in SCV and ASAP. A NOAEL of 25 mg/kg bw/day could be supported based on the weakness in hindlimbs and could not rear which was associated with decreases in SCV and ASAP at 50 mg/kg bw/day.

In the 6-month dog study with acrylonitrile administered via the drinking water, deaths were recorded at the high and intermediate inclusion level equivalent to achieved doses of approximately 16 mg/kg/day and above. Adverse clinical signs were also observed at the lowest dose level equivalent to 8-10 mg/kg/day.

7.3.2.2 Inhalation

Dudley *et al.* (1942b) investigated the effects of repeated inhalation exposure to acrylonitrile in a series of studies in various species (rats, rabbits, guinea pigs, dogs, cats and rhesus monkeys). The experiment was split into three studies: Study 1: 4 weeks exposure (4 hours/day, 5 days/week) to 56 ppm acrylonitrile in air, dogs and monkeys; Study 2: 8 weeks exposure (4 hours/day 5 days/week) to 100 ppm acrylonitrile in air, rats, guinea pigs, rabbits and cats; Study 3: 8 weeks exposure (4 hours/day 5 days/week) to 153 ppm acrylonitrile in air, rats, guinea pigs, rabbits, cats and monkeys. On the basis of mortality rates among the various species in the various dose regimes, the experiments demonstrated that dogs were more susceptible to acrylonitrile than monkeys. Cats were shown to be more sensitive to acrylonitrile than rodents and rabbits. Repeated exposures to 153 ppm were toxic to guinea pigs, rats, rabbits, and were less toxic to monkeys and cats. Exposure to 153 ppm resulted in irritation of the eyes and nose, loss of appetite, gastro-intestinal disturbances, and an incapacitating weakness of the hind legs from which the animals recovered relatively rapidly. Target organs identified were the nervous system (transitory limb weakness and/or paralysis in dogs and cats), the kidney (histopathological changes in rats and rabbits), the upper respiratory tract (nasal irritation in all species studied) and the lung (bronchopneumonia in all species except cats).

More contemporary inhalatory studies utilise long-term exposures to acrylonitrile and include a two-generation reproductive toxicity study in rats (Nemec *et al.* 2008), a two-year carcinogenicity study in rats (Quast *et al*., 1980a) and a 12-month rat study (Maltoni *et al*., 1977).

The two-generation reproductive study in the rat (Nemec *et al.* 2008) is selected as one of the key studies for inhalation exposure since it is a Klimisch 1 (reliable without restrictions) regulatory study in which a dose response was observed and toxicity at the high dose levels. Whole-body inhalation was employed at concentrations of acrylonitrile of 0, 5, 15, 45 and 90 ppm, 6 hours daily, seven days per week for 10 weeks prior to mating, during mating and during gestation and lactation for parent females. Parental systemic toxicity was evident in F0 and F1 generations, with reduced body weights and/or food consumption in both sexes at exposures of 45 and/or 90 ppm. F1 offspring body weights were also reduced during PND 14 to 28 at an exposure of 90 ppm, which was associated with evident maternal toxicity. In addition, systemic toxicity was evident as an increased absolute and/or relative (to final body weight) liver weights at an exposure level of 90 ppm in F0 males and females and 45 ppm in F1 males. Non-systemic toxicity was also evident and comprised clinical signs of nasal irritation at 90 ppm 1h following exposure, and microscopic changes in nasal tissues. Morphologically similar nasal lesions were described in F0 males and females at 45 ppm, F1 males at all dose levels and F1 females at exposures of 15 and 45 ppm. Although four levels of the nasal cavity were assessed, most lesions occurred in the most rostral section (level 1) examined and showed a dose-response in incidence and severity. Lesions included respiratory/transitional epithelial hyperplasia, sub-acute inflammation, squamous metaplasia, and/or degeneration of the olfactory epithelium, consistent with site-of-contact irritation resulting from exposure to irritant chemicals.

A NOAEC of 15 ppm can be determined for the end-point of nasal irritancy in F0 males and females from this study and a LOAEC of 5 ppm may be derived for F1 males for this endpoint. Both F0 and F1 generations were treated for 18 weeks in total and thus both generations were exposed predominantly during adulthood. However, the LOAEC of 5 ppm in F1 males was not statistically significant, and inhalation exposure was initiated when

pups were approximately 4 weeks old. In the parental generation, the exposure was initiated when rats were approximately 8 weeks old. The Acrylonitrile EU REACH Consortium (2017) commented that the F1 rats were exposed at an immature, prepubescent stage which they consider not an appropriate level of maturity for workers. It is indeed plausible that maturation of the airways may play a role in sensitivity[22]. In conclusion, considering that the LOAEC of 5 ppm in F1 males was not statistically significant and that there may be age related sensitivities of the nasal epithelium, the NOAEC of 15 ppm for the parental generation is chosen as a point of departure.

The two-year carcinogenicity study in the rat (Quast *et al*., 1980a) employed a similar dosing regimen but at concentrations of 20 and 80 ppm. As a result of irritation, inflammatory and degenerative changes (hyperplasia and metaplasia of the respiratory epithelium) were present in the nasal turbinates of both exposure groups. A significantly increased number of rats exposed to 80 ppm also showed focal gliosis and perivascular cuffing in the brain. The key toxicological findings in this study were considered to be local irritant effects in the nasal epithelium comprising suppurative rhinitis, hyperplasia, focal erosions, and squamous metaplasia of the respiratory epithelium, with hyperplasia of the mucus-secreting cells. Effects were seen at the lowest exposure level of 20 ppm. When determining the starting point from this study the EU RAR (EC, 2004) applied an uncertainty factor of five to the LOAEC of 20 ppm giving a suggested NAEC of 4 ppm (9 mg/m^3).

A subchronic study in male SD rats assessed motor and sensory conduction velocities (MCV and SCV) and amplitudes of the sensory and motor action potentials (ASAP and AMAP) of the tail nerve (Gagnaire *et al.* 1998). Groups of rats (n=12/group) were exposed by inhalation to 0, 25, 50 and 100 ppm acrylonitrile for 6 h/d, 5 d per week, for 24 weeks. Rats exposed to acrylonitrile exhibited time- and concentration-dependent decreases in MCV, SCV and ASAP, which were partially reversible after 8 weeks of recovery. Statistically significant deficits in SCV were observed in all exposure groups. Unlike in the oral experiment in the study by Gagnaire *et al.* (1998), see above, no hindlimb weakness was observed in the inhalation experiment. However, in the high and middle concentrations wet hair and hypersalivation were observed, but without hyperactivity or stereotypies. It is not fully clear whether the reduction in nerve velocity and action potentials are adverse as such, but they may be seen as precusors (Kirman *et al.* 2008). The mode of action of acrylonitrile neurotoxicity appears to involve not only cyanide release during metabolism, but also the parent molecule and the CEO metabolite would be capable of cyanoethylation of essential functional groups which may contribute to the neurotoxicity (Kirman *et al.* 2008). Overall, effects were more consistent at a the mid-dose and a NOAEC of 25 ppm may be derived from Gagnaire *et al.* (1998) based on the observed wet hair and hypersalivation, associated with reduced nerve conduction velocities and action potentials at 50 ppm.

The one-year study (Maltoni *et al*., 1977) only investigated limited chronic toxicity endpoints at dose concentrations up to 40 ppm. There were no effects on mortality or

[22] In a surgical rat model study of lung growth, morphometry and static pulmonary mechanics (Kida & Thurlbeck 1981), it was demonstrated that significant lung development occurred between 4 weeks of age and 8 weeks of age in male rats. This is further supported by Gomes et al. (2001) who studied rats from 10 days to 3 months of age and found by morphometric analysis of the lung that tissue density decreased and total alveolar surface area increased over this age period. They also concluded that the mechanical interdependence between airways and parenchyma is weaker in very young animals compared with mature animals and that this may play a role in the hyperresponsiveness of immaturity.

body weight and it was considered that the study would indicate a NOAEL between 5 and 10 ppm.

A number of other repeat inhalation exposure studies with acrylonitrile have been summarised by EU RAR but these were of non-standard design and of questionable reliability. However, NOAECs of 130 ppm (280 mg/m^3) and 100 ppm (225 mg/m^3) were reported for rats (Gut *et al*., 1985; Bhooma *et al*., 1992) and <24 ppm (54 mg/m^3) in the most sensitive species, the dog (Brewer, 1976).

7.3.3 *In vitro* data

No data available.

7.3.4 Summary

For repeated dose toxicity by the oral route, the key study is the chronic toxicity study in the drinking water with F344 rats (Johannsen & Levinskas, 2002a) from which, due to the lack of a dose-response relationship in the female mortality data, a NOAEL of 3 ppm (equivalent to average daily dose levels of 0.25 mg/kg bw/d in males and 0.36 mg/kg bw/d in females) was derived. For repeated dose inhalation toxicity, a NOAEC of 15 ppm (Nemec *et al.,* 2008) and a LOAEC was 20 ppm (Quast *et al*., 1980a) was determined based on irritant effects on the nasal mucosa. A NOAEC of 25 ppm may be derived from Gagnaire et al. (1998) based on observed wet hair and hypersalivation, associated with reduced nerve conduction velocities and action potentials at the LOAEL.

With regard to the effects of repeated exposure to acrylonitrile in humans, it is concluded that the data are difficult to assess in relation to establishment of a dose-response relationship. This is a consequence of variation in individual exposure and inability to establish accurate exposure levels and a dose-response. However, many of the findings seen in animal studies (notably neurological and irritant effects) reflect the reported findings in workers. It is also noted that the respiratory tract appears to be a key target organ following the inhalation of acrylonitrile, both in humans and in experimental animals.

7.4 Irritancy and corrosivity

7.4.1 Human data

There is limited information concerning the irritancy and/or corrosivity in humans following exposure via any route. A small number of case-studies have reported adverse effects following acute exposure to acrylonitrile.

7.4.1.1 Dermal route

Dudley *et al.* (1942a) described irritation and diffuse erythema on the hands of a male laboratory worker within 24 h of contact with ‘small quantities’ of liquid acrylonitrile. This progressed to blistering by day 3, with symptoms still apparent at day 10. Wilson et al. (1948) also noted that direct skin contact in humans with acrylonitrile resulted in irritation, erythema and scab formation, with slow healing. In workers at an acrylonitrile processing and manufacturing plant, Bakker *et al.* (1991) reported 5 cases of irritant dermatitis and 5 subjects with a positive patch test to acrylonitrile, with one subject also having paresthesia (tingling/prickling sensation). Further details of these case studies are unavailable.

7.4.1.2 Inhalation route - acute

The EU RAR (EC, 2004) reports a re-evaluation of the findings reported by Sakurai et al. (1978) (described in section 7.3.1) in Japanese acrylic fibre factory workers, concluding that workers experienced ocular and upper respiratory tract irritation at inhalation levels > 10 ppm. Although the findings from this study are not considered of use for establishing a NOAEC, the findings may be used as supporting evidence for additional studies.

Acute inhalation exposure to liquid or vapour forms of acrylonitrile (often occurring as a result of accidental release) has been associated with a range of effects including irritation of the mucous membranes of the nose, eyes and upper respiratory tract. The severity and number of effects is considered to be dose-dependent, (Grahl, 1970; Davis *et al.*, 1973; Vogel and Kirkendall, 1984; Simons *et al.*, 2016).

The EU RAR (EC, 2004) reports a case study by Grahl (1970) in which one volunteer exposed to acrylonitrile for 70 seconds at levels of 370-460 ppm (800-1 000 mg/m^3) did not show adverse effects. In addition, Davis *et al.* (1973) reported lachrymation and respiratory tract irritation, resulting in coughing and sneezing, in non-fatal cases of exposure to high levels (not defined) of acrylonitrile. Mild skin irritation and conjunctivitis were apparent in a ship-yard worker following accidental exposure of the face, eyes and body to acrylonitrile (Vogel and Kirkendall, 1984). As previously discussed (section 7.3.1), Simons *et al.* (2016) reported local symptoms of irritation associated with CEV levels of > 100 pg/g globulin in the blood of non-smoking residents exposed to acrylonitrile following accidental release following a train accident.

No deleterious effects were exhibited by volunteers exposed acutely (8-hour duration) to acrylonitrile levels between 2.4 and 5.0 ppm (5.4 to 10.9 mg/m^3) (Jakubowski *et al*. 1987).

7.4.1.3 Inhalation route - chronic

The EU RAR (EC, 2004) reports a re-evaluation of the findings reported by Sakurai *et al.* (1978) (described in section 7.3.1) in Japanese acrylic fibre factory workers (n=102 exposed, 62 matched controls), concluding that workers experienced ocular and upper respiratory tract irritation at inhalation levels > 10 ppm. Although the findings from this study are not considered of use for sufficiently reliable for establishing a NOAEC, the findings they may be used as supporting evidence for additional studies.

A conference abstract by Gincheva *et al.* (1977) reported no symptoms following 3-5 years of occupational exposure to acrylonitrile at 1.9-3.3 ppm (n=23), however the reliability of these data is considered “not assignable” (Klimisch score 4).

Cheng *et al.* (2004) noted in a group of 52 injection-moulding workers exposed repeatedly to ABS thermal decomposition (mixed exposure, levels not defined) significant loss of olfactory function, notably mean composite scores and larger decreases in composite score in both nostrils when compared to 72 unexposed workers from other departments of the same plant. The authors suggest that ABS injection-moulding may have a deleterious impact on worker olfactory function when compared to a control group of other workers at the plants. Although the olfactory function recovered after one night of rest, the long-term effects of chronic exposure are unknown. Due to the lack of exposure measurements, and the presence of additional chemicals (butadiene and styrene), it is not possible to derive a NOAEC from this study.

7.4.2 Animal data

7.4.2.1 Skin irritation

Acrylonitrile has been shown to be strongly irritating when applied (0.5 mL) to the shaved skin of New Zealand White rabbits and occluded for 24 hours, with higher irritancy potential on abraded skin (Vernon *et al*., 1969). Zeller *et al*. (1969) also reported oedema following 15 min exposure of shaved rabbit skin to acrylonitrile, progressing to necrosis of the tissue after 20 hours exposure.

7.4.2.2 Eye irritation

The eye irritancy potential of acrylonitrile has been investigated in several studies in rabbits. As reported in the EU RAR (EC, 2004) in a study carried out by BASF (BASF, 1963) administration of undiluted liquid acrylonitrile (0.05 ml) caused conjunctival redness, diffuse corneal opacity and oedema from one hour after exposure, returning to normal between 72 hours and seven days post-exposure.

Vernon *et al*. (1969) determined Draize scores (for intensity and area of involvement; maximum score of 110) following instillation of undiluted liquid acrylonitrile (0.1 ml) at 24, 48 and 72 hours. A maximum Draize score of 35 was seen following 24 hours, falling to 31 and 22 after 48 and 72 hours respectively. Iritis had a mean score of 1.0 between 24-72 hours, and corneal opacity in the range 1–2 over the same time period, however corneal opacity damage did not reverse by 72 hours. Conjunctival redness and chemosis scored in the range 2–3, with some reversibility of damage by 72 hours.

Additional studies have also shown development of mild conjunctivitis within one hour of exposure to undiluted acrylonitrile (0.05 ml) (McOmie *et al*., 1949), burning of the cornea following application of undiluted acrylonitrile (0.02 ml) (VROM, 1984) and moderate corneal opacity, moderate iritis and severe conjunctival irritation following administration of undiluted acrylonitrile (0.1 ml) into the conjunctival sac (DuPont, 1975, cited in EC, 2004).

7.4.2.3 Respiratory tract irritancy

The potential for acrylonitrile to act as a respiratory irritant has not been directly assessed however, long- and short-term experimental studies in a range of species have shown delayed irritant effects of acrylonitrile on the upper respiratory tract. These included rhinitis, nasal discharge and hyperplastic changes in the nasal mucosa (EC, 2004).

In a recent two-generation reproductive study in Sprague-Dawley rats, whole-body inhalation of acrylonitrile at levels between 5 and 45 ppm resulted in microscopic lesions of the rostral nasal epithelium in adult rats, representing local site-of-contact irritation (Nemec *et al*., 2008). In the two-year carcinogenicity study by Quast *et al*. (1980a) irritation due to acrylonitrile exposure was observed as well as inflammatory and degenerative changes (hyperplasia and metaplasia of the respiratory epithelium) in the nasal turbinates. These effects were seen in both dose groups (20 and 80 ppm). Both studies are described in more detail in section 7.3.2.2.

From Nemec *et al.* (2008) a NOAEC for the F0 generation of 15 ppm was determined based on irritant effects on the nasal mucosa, and from Quast *et al*. (1980a) a LOAEC of 20 ppm can be derived.

7.4.2.4 Mode of action of irritancy

The mode of action of acrylonitrile irritancy is unknown. Irritation may result from binding of acrylonitrile or CEO to cellular macromolecules or through GSH depletion

(Kirman *et al.,* 2008). GSH depletion may result from acrylonitrile and CEO metabolism (Kirman *et al.,* 2008). Nasal tissue in rats has shown significant metabolism of acrylonitrile to cyanide (Kirman *et al.,* 2008).

7.4.3 *In vitro* data

In vitro studies to assess the potential irritant properties of acrylonitrile could not be identified.

7.4.4 Summary

Acrylonitrile can be considered an irritant to the skin and respiratory tract and a severe irritant to eyes. It cannot be considered as corrosive.

7.5 Sensitisation

7.5.1 Human data

Skin sensitisation

Acrylonitrile has been classified as a sensitising agent under CLP (Skin Sen cat. 1; H317, may cause an allergic skin reaction). There appears to be limited evidence of sensitisation in acrylonitrile workers following dermal contact, from a very small number of studies, despite the many thousands of workers potentially exposed (EC, 2004).

Hashimoto & Kobayashi (1961) reported an allergic reaction to acrylonitrile, leading to the rapid appearance of lesions over the entire body several days following initial contact with the skin. The authors concluded that the later lesions resulted from an allergic reaction to the initial exposure to acrylonitrile (further details are not available). The study has inherent limitations and does not provide robust findings that can be used for risk assessment purposes.

Bakker *et al*. (1991) also described an allergic response to acrylonitrile in 5 workers at an acrylonitrile processing and production plant who had presented with contact dermatitis and subjected to patch tests using 0.1% solutions of acrylonitrile (99.6% purity) were performed; these were read after 2-3 days.

Respiratory sensitisation

Although no evidence relating to respiratory sensitisation in humans following exposure to acrylonitrile could be identified, there is no indication that acrylonitrile is associated with development of respiratory sensitisation in workers (presenting as occupational asthma).

7.5.2 Animal data

Skin sensitisation

In a regulatory Guinea Pig Maximisation test, carried out to EC and OECD guidelines, Koopmans & Daamen (1989) reported induction of sensitisation through intradermal injections of 2.5% acrylonitrile followed by an epidermal application of 2% acrylonitrile seven days later. Sensitisation was also achieved using acrylonitrile at concentrations of 1% (95% positive), 0.5% (95% positive) and 0.2% (80% positive).

Respiratory sensitisation

No evidence relating to respiratory sensitisation in animals following exposure to acrylonitrile could be identified from the available inhalation studies.

7.5.3 *In vitro* data

In vitro studies to assess the potential skin sensitising properties and/or corrosivity of acrylonitrile could not be identified.

7.5.4 Summary

Acrylonitrile has been classified as a sensitising agent under CLP (Skin Sen cat. 1; H317, may cause an allergic skin reaction). The evidence of skin sensitisation in humans following dermal exposure to acrylonitrile in occupational settings is limited, with only a few cases reported from the large (many thousands) worker population. Animal data provide clear evidence of skin sensitisation following dermal exposure to acrylonitrile.

There is no directly relevant data data for humans or animals relating to respiratory sensitisation effects for acrylonitrile. Indirect observations from occupational and animal inhalation studies suggest acrylonitrile to be a non-respiratory sensitising agent.

7.6 Genotoxicity

7.6.1 Human data

There have been a number of molecular studies in acrylonitrile-exposed workers using the binding of CEV to haemoglobin as a marker of exposure. Effects on a number of genotoxic and other parameters have been investigated: chromosomal aberrations, micronuclei induction, DNA strand breaks, sex chromosome aneuploidy and sperm parameters (reviewed in EC, 2004). The results of these studies have been inconsistent with some positive results confounded by other chemical exposures or with a response pattern not indicating acrylonitrile as a causative agent.

Two studies conducted by Sram *et al*. (2004) and Beskid *et al.* (2006), investigated chromosomal aberration in workers exposed to acrylonitrile. Changes to chromosomes 1 and 4 were analysed using fluorescence *in situ* hybridisation (FISH) or conventional cytogenetic methods. These results showed no increase in chromosomal aberrations at exposure levels less than 0.3 mg/m^3. Etebari *et al.* (2014) assessed the DNA damage, using comet assay, caused by occupational exposure in workers of a company that produced polyacrylamide. The amount of DNA damage in production line workers was significantly higher than in office workers.

Among 60 employees of a petrochemical plant where acrylonitrile is produced and polymerised, with an average exposure of 0.05-0.7mg/m^3, blood samples were analysed for the chromosomal aberrations by conventional cytogenetic analysis (Beskid *et al.*, 2006). A significant increase in percentage of aberrant cells (%ABC) was observed in exposed group (3.27±1.91 %ABC) in comparison with controls (2.05±1.53 %ABC; $p<0.01$). However, there was no difference between the two groups in genomic frequency of translocations per 100 cells. Smoking had no effect on the number of aberrations.

7.6.2 Animal data

The *in vivo* genotoxicity studies are summarised in Table 11. It should be noted that the EU review (EC, 2004) does not rate each study by Klimisch ratings. Therefore the Klimisch ratings in Table 11 are taken primarily from the CSR submitted by the Lead Registrant for REACH registration. Where further information has been obtained leading to a change in rating, this is indicated.

Table 11: In vivo genotoxicity studies

Test	Assay details	Klimisch	Result	Reference
Studies in *Drosophila melanogaster*	Variety of mutational and genotoxic endpoints	2	Positive in mitotic recombination and somatic mutation (SRM) assay	Vogel, 1985 cited in EC, 2004
			Weakly positive in SRM assay	Wurgler, 1985 cited in EC, 2004
			Somatic mutation and chromosome loss	Fujikawa *et al*., 1985; Osgood *et al.,* 1991 cited in EC, 2004
MN in NMRI male mice	IP, mouse bone marrow	2	Negative	Leonard *et al.,* 1981
MN and strand break assay in mice	IP, mouse bone marrow	3*	Negative	Hachiya *et al*., 1984, 1986, 1987
MN in B6C3F1, male and female mice	Oral gavage, mouse bone marrow	2	Negative	NTP, 2001

CA in male mice (strain unknown)	Oral gavage	3	Positive in spermatocytes, bone marrow and spleen. Effects follow a dose-response.	Fahmy 1999
CA in NMRI mice	IP	2	Negative in bone marrow	Leonard *et al.,* 1981 as cited in EC, 2004
CA in C57B1/6 male mice	IP	3	Negative in bone marrow	Sharief *et al.*, 1986 as cited in EC (2004)
CA in Swiss mice	Oral IP	2	Negative in bone marrow	Rabello-Gay & Ahmed, 1980
CA in mice	Inhalation	2	Negative in bone marrow	Zhurkov *et al.*, 1983
CA in CD1 mice and Sprague Dawley rats	Oral gavage, IP & IV	2	CD1 mice: Negative in bone marrow and peripheral blood Sprague Dawley: equivocal in bone marrow, negative in peripheral blood	Morita *et al.*, 1997; Wakata *et al.*, 1998
CA in Sprague Dawley rats	IV	2	Positive in bone marrow, negative in peripheral blood	Morita *et al.*, 1997; Wakata *et al.*, 1998
Dominant lethal in F344 male rats	Oral gavage for 5 days	2	Negative (only one dose used but considered valid for risk assessment, EC 2004) but results in contrast to acrylamide	Working *et al.,* 1987

UDS in male rats	Oral gavage, autoradiography	2	Negative acrylonitrile in rat spermatocytes Positive cyanoethylene oxide	Butterworth *et al.,* 1992
UDS in F344 male rats	Oral gavage	2	Positive liver Negative brain	Hogy & Guengerich, 1986
UDS in Sprague-Dawley male rats	Oral	2	Positive gastric mucosal cells, dose dependent. When P450 inhibitor SKF 525-A is present which slows oxidation to CEO then UDS is decreased suggesting important role for CEO	Ahmed *et al.,* 1996
UDS in rats	Single oral dose	2	Positive in gastric tissue	Abdel-Rahman *et al.*, 1994
UDS in Sprague-Dawley male rats	Oral gavage Single dose Measurement by liquid scintillation. UDS for lung as target tissue not validated	2/3	Positive (with some toxicity) in the lung.	Ahmed *et al.,* 1992a
UDS in male rats	Single oral dose	2	Positive in testes	Ahmed *et al.,* 1992b
SCE in C57B1/6 male mice	IP	3	Ambiguous, weakly + at 1 out of 3 doses	Sharief *et al.*, 1986 as cited in EC (2004)
SCE in male mice (strain unknown)	IP	3	Positive	Fahmy 1999

Hprt in splenic T-cells wild-type and CYP2E1 knock-out B6C3F1 mice for 6 weeks	Gavage	4*	Positive - Increased mutation frequency with dose-response	Walker & Ghanayem, 2003
Hprt in thymic and splenic T-cells in female F344 rats treated for 4 weeks up to 500 ppm	Drinking water	4*	Positive - Increased mutation frequency with dose-response	Walker, 1994 as cited in Acrylonitrile EU REACH Consortium (2017)
Transgenic animal mutagenicity assays in CD2F1 (BALB/C-DBA2 male mice	Oral drinking water	2	Negative in splenic lymphocytes, lung, seminiferous tubules, brain, bone marrow	Lambert *et al*., 2005
Comet assay in Sprague-Dawley rats 48 h treatment	Oral gavage	2	Weakly Positive (2-5% increase at highest dose 62.5 mg.kg bw/day. Positive control, EMS 50%+)	Nakagawa *et al*., 2015

* This study has been rated 2 (reliable with restrictions) in the Lead Registrant CSR. However, in the EU RAR (EC, 2004) there is a comment suggesting that there is a lack of available experimental detail (as the study is only available in English as an abstract). This makes these studies of limited value in risk assessment. This would make this study Klimisch rating 3.

**This study has been published only in abstract form

Taken as a whole, the studies on genotoxicity *in vivo* are much more inconclusive than the results *in vitro* (see section 7.6.3).

One of the three negative micronucleus results in the mouse (Hachiya *et al.* 1984, 1986, 1987) is of limited value for risk assessment resulting from a lack of experimental detail available.

Chromosome aberration is predominantly negative in mice and peripheral blood in rats, but positive in bone marrow of rats.

One sister chromatid exchange studies in mice was positive (Fahmy 1999), with ambiguous positive results in a second study (Sharief *et al.*, 1986).

Acrylonitrile appeared to be negative in a dominant lethal assay in rats (Working et al. 1987). The study can be used for risk assessment, albeit a limitation is that only one dose level was used in the study (EC 2004).

Conflicting results have been obtained in studies of unscheduled DNA synthesis (UDS) in the rat. Positive in vivo results have been reported in rat lung, testes and stomach in vivo (Ahmed *et al.*, 1992a,b, 1996; Abdel-Rahman *et al.*, 1994; Hogy & Guengerich, 1986). Negative in vivo results have been obtained in rat brain and spermatocytes (Hogy & Guengerich, 1986; Butterworth *et al.*, 1992). Several of these studies used the methodological approach of determination of radioactivity associated with the nucleic acid cell fraction by liquid scintillation counting, which is regarded as being less reliable than autoradiography.

Mutagenicity studies in transgenic mice were negative in many organs while the Comet assay was weakly positive at high doses in rats.

There are positive results in standard drosophila assays, using a range of genetic markers. The Acrylonitrile EU REACH Consortium (2017) states that other nitrile compounds such as acetonitrile, which are not mutagenic or carcinogenic have been shown to induce aneuploidy and that the acrylonitrile concentrations were high in the drosophila assays. The industry does not consider these studies instructive. However, the statements were not supported with references or with information from the CSRs of the acrylonitrile registrations.

The results of these many studies on the mutagenicity of acrylonitrile (and CEO) in vivo can be considered in conjunction with those recently conducted on the effects of these compounds on oxidative stress which are considered in the next section.

7.6.2.1 Studies on exposure to acrylonitrile and oxidative stress

Further studies have shown that oxidative stress may also be involved indirectly in the DNA damage that may lead to genotoxicity and carcinogenicity.

There have been a number of *in vitro* studies on the induction of oxidative stress after exposure of brain cells to acrylonitrile. Kamendulis *et al*. (1999) examined the induction of oxidative stress in a rat glial cell line, considered a target tissue, after acrylonitrile exposure and compared the results with exposure in rat hepatocytes, a non-target tissue. An increase in oxidative damage was observed as evidenced by increased 8-hydroxy-2'-deoxyguanosine (8-OH-dG) and hydroxyl radical formation in glial cells but not hepatocytes. There was a decrease in antioxidant defences, catalase, superoxide dismutase and glutathione, while lipid peroxidation and glutathione peroxidase were not affected. None of these markers were changed in hepatocytes. In rat primary glial cells,

lipid peroxidation was increased by acrylonitrile exposure (Esmat *et al*., 2007) and depleted reduced glutathione but not glutathione. Prior treatment with the antioxidant, n-acetylcysteine (NAC), prevented these effects and decreased cyanide formation.

Similar experiments were conducted with rat and human astrocytes (Pu *et al*., 2006; Jacob & Ahmed, 2003). Using the alkaline Comet assay and the modified technique for measuring oxidative damage, no direct DNA was observed in the first 24 hours; however, oxidative DNA damage was observed after 24 hours. This damage was reduced by addition of anti-oxidants such as α-tocopherol or a precursor of glutathione. Co-treatment with 1-aminobenzotriazole, a suicide inhibitor of P450, also prevented oxidative damage. This suggested that oxidative DNA damage occurring after acrylonitrile exposure appears to act through a P450 metabolic pathway with glutathione depletion possibly playing a role. Experiments with human astrocytes exposed to acrylonitrile also showed an increase in the formation of reactive oxygen species and a decrease in antioxidant defence mechanisms such as a depletion of reduced glutathione.

El-Sayed *et al*. (2008) treated albino rats orally with 50 mg acrylonitrile/kg bw/day for 28 days. This produced a significant elevation in brain lipid peroxidation measured by malondialdehyde and a decreased in reduced glutathione and other antioxidant parameters such as superoxide dismutase, catalase and glutathione peroxidase. Pre-treatment with the antioxidant flavonoid, hesperidin, prevented the alterations in brain lipid peroxidation.

Jiang *et al*. (1998) examined the ability of acrylonitrile to induce oxidative stress in male Sprague-Dawley rats. Rats were administered acrylonitrile in drinking water at concentrations of 0, 5, 10, 100 and 200 ppm and sampled after 14, 28 or 90 days of treatment. Increased levels of 8-OH-dG, lipid peroxidation and reactive oxygen species were observed in the brains of treated rats, with a dose-response for 8-OH-dG formation but lipid peroxidation only seen at the highest dose. Decreased levels of reduced glutathione, catalase and superoxide dismutase were also found in the brain. No changes in these indicators of oxidative stress were found in the livers of treated animals, a non-target tissue.

Whysner *et al.* (1998) treated male Sprague-Dawley rats with 0, 3, 30 and 300 ppm acrylonitrile in drinking water for 21 days, these doses included those associated with the formation of brain tumours with long-term treatment. In the 30 and 300 ppm groups, oxidative damage in the brain was observed in the form of 8-oxodeoxyguanosine (8-OH-dG) levels two-fold greater than in the controls. However, no changes were found in other markers of oxidative damage such as lipid peroxidation, and levels of glutathione levels, glutathione peroxidase, catalase and cytochrome oxidase activity, which would indicate cellular antioxidant defence. In a further longer-term experiment, rats were exposed to 0 or 100 ppm acrylonitrile in drinking water for 94 days with interim kills at 3, 10 and 31 days. 8-OH-dG in brain nuclear DNA was significantly increased compared with control.

No increases in 8-OH-dG were observed when rats were treated with weekly intravenous injections of the mutagenic carcinogen, methylnitrosourea. This experiment suggested that the mechanism of action in acrylonitrile-induced carcinogenicity may involve the formation of 8-OH-dG, although this formation is not understood and, in some cases, does not appear to include other oxidative damage such as lipid peroxidation or disruption of cellular antioxidant defence.

Pu *et al*. (2009) treated male Sprague-Dawley rats with 0, 3, 30, 100, and 200 ppm acrylonitrile in drinking water for 28 days. One group of rats were also co-administered N-acetyl cysteine (NAC) (0.3% in diet) with acrylonitrile (200 ppm in drinking water) to examine whether antioxidant supplementation was protective against acrylonitrile-induced oxidative stress. Direct DNA strand breakage and oxidative DNA damage in white blood cells (WBC) and brain was measured using a modified Comet assay. Oxidative DNA

damage in WBC and brain was evaluated using a modified Comet assay. No significant increase in direct DNA strand breaks was observed in brain and WBC from acrylonitrile-treated rats. However, oxidative DNA damage (8-OH-dG) in brain and WBC was increased in a dose-dependent manner. In addition, plasma levels of reactive oxygen species increased in rats administered acrylonitrile. This damage was prevented by dietary NAC. There was a slight, but significant, decrease in the GSH:GSSG ratio in the brain at acrylonitrile doses >30 ppm. These results suggest a mode of action for acrylonitrile-induced astrocytomas involving the induction of oxidative stress and damage.

A number of recently published studies have sought to investigate further the role of oxidative stress in acrylonitrile toxicity.

Female F344 rats were administered 0 and 100 ppm acrylonitrile in drinking water and fed diets supplemented with a range of antioxidants, vitamin E, green tea polyphenols, NAC, sodium selenite and taurine (Pu *et al*., 2016). Oxidative damage in the brain was observed with acrylonitrile treatment (elevated 8-OH-dG) which was reduced by all the supplemented diets except selenite and taurine. Oxidative damage (measured by the modified Comet assay) was seen in white blood cells which was reduced by all supplementation. Acrylonitrile also induced inflammatory cytokines and growth stimulating cyclins which were down-regulated by antioxidants, while the antioxidants stimulated apoptotic and DNA repair genes. The authors concluded that the study supported the involvement of oxidative stress in the development of acrylonitrile-induced astrocytomas.

Caito *et al.* (2017) investigated the effects of acrylonitrile on primary mouse glia and astrocytes as the long-term mouse study indicated that acrylonitrile did not cause gliomas in mice. Mouse glial cells accumulated acrylonitrile but were resistant to acrylonitrile-induced oxidative stress (measured by nrf2 and glutathione levels).

Williams *et al*. (2017) investigated possible mechanisms of tumour formation in two target tissues (brain and Zymbal gland) of female F344 and Sprague-Dawley rats. Rats received 100 ppm acrylonitrile in drinking water for 28 days while one group also received ^{14}C-acrylonitrile by gavage on day 28. No evidence of association of labelled acrylonitrile with brain DNA, was detected, while binding to protein may have been present. No DNA adducts were detected or DNA strand breaks (measured by the Comet assay) in the brain or the Zymbal gland. Oxidative DNA damage (measured by modified Comet assay) was detected in the brain but not the Zymbal gland. These results suggested a role for oxidative stress in the induction of brain tumours but not in those of the Zymbal gland.

Dang *et al*. (2017a,b) investigated the role of oxidative stress in sperm and testes toxicity in Sprague-Dawley rats. In their first study, Dang et al. (2017a) Treated Sprague-Dawley rats with 50 mg/kg bw/day by gavage for 90 days. A further group was administered with the antioxidant, NAC (300 mg/kg bw) 30 minutes prior to the acrylonitrile gavage. Acrylonitrile increased markers of oxidative stress (malondialdehyde, superoxide dismutase, glutathione peroxidase) and decreased glutathione with these effects being blocked by NAC. Acrylonitrile treatment increased the expression of the transcription factor, NFκβ and its signalling pathway with upregulation of the apoptotic protein, Bax (this appears to be a different mechanism than that described for the rat brain above (Pu et al., 2016) when antioxidant stimulated apoptosis).

In a second study using a similar treatment regime for acrylonitrile, the antioxidant apigenin was also administered (Dang *et al*., 2017b). Acrylonitrile treatment decreased sperm concentration, motility and mitochondrial membrane potential and this was reversed by apigenin. The markers of oxidative stress as described above increased by acrylonitrile treatment were decreased in the apigenin-treated animals.

These two studies suggest that oxidative stress induced by acrylonitrile plays a role in testicular toxicity. The testes are not a target for acrylonitrile carcinogenicity in rats.

7.6.3 *In vitro* data

The in vivo genotoxicity studies are summarised in Table 11. The EU RAR (EC, 2004) does not rate each study by Klimisch ratings. Therefore, the Klimisch ratings in Table 11 are taken primarily from the CSR submitted by the Lead Registrant for REACH registration.

Table 12: *In vitro* Genotoxicity studies

Test	Assay details	Klimisch	Result	Reference
Bacterial Mutation Assay *S. typhimurium*	TA1535, 1978, 1538	1	Positive with metabolic activation Negative without activation Base substitution and frameshift mutations	Milvy & Wolff, 1977 cited in EC, 2004
Bacterial Mutation Assay *S. typhimurium*	TA1530, 1535, 1537, 1538, 1950, 1978, 98, 100	2	Positive with metabolic activation Mainly base substitution TA1530, 1535, 1950, less pronounced frameshift, TA98, 100, 1978	De Meester *et al.* 1978 cited in EC, 2004
Bacterial Mutation Assay *S. typhimurium*	TA1535, 1537, 1538, 98, 100	2	Weakly positive with metabolic activation, only at higher concentrations in TA1535	Lijinsky & Andrews, 1980 cited in EC 2004
Bacterial Mutation assay *S. typhimurium*	TA97, 98, 100, 1535	2	Positive in TA1535, 100 with metabolic activation	Zeiger & Haworth, 1985 cited in EC, 2004
Bacterial Mutation assay	8-azaguanine- resistant mutations	2	Equivocal with some evidence of weak mutagen in this system	Liber, 1985 cited in EC, 2004

Test	Assay details	Klimisch	Result	Reference
S. typhimurium				
Bacterial Mutation assay *S. typhimurium*	TA1535, 1537, 1538, 98, 100	2	Negative with and without metabolic activation	Rexroat & Probst, 1985 cited in EC, 2004
Bacterial Mutation assay *S. typhimurium*	TA98, 100, 97, 102 SOS chromotest	2	Negative with and without metabolic activation	Mitsushima *et al.,* 1985 cited in EC, 2004
Bacterial Mutation assay *S. typhimurium*	TA97, 98, 100, 102	2	Weakly positive in TA102	Baker & Bonin, 1985 cited in EC, 2004
Bacterial Mutation assay *S. typhimurium*	TA102	2	Negative with and without metabolic activation	Jung, 1986 cited in EC, 2004
Bacterial Mutation assay *S. typhimurium*	Urine collected from rats and mice treated with acrylonitrile tested in TA1530	2	Positive in absence of metabolic activation Pre-treatment of animals with enzyme inducers makes this study difficult to interpret	Lambotte-Vandepaer *et al.*, 1980 cited in EC, 2004
Bacterial Mutation assay *E. coli*	WP2, *uvr*A, *uvr*A *polA, lexA*	2	Positive (except WP2*lexA)* without metabolic activation Suggestion that acrylonitrile causes non-excisable mis-repair DNA damage	Venitt *et al*., 1977

Test	Assay details	Klimisch	Result	Reference
			thought to be associated with DNA strand breaks	
Yeast Mutation assay *S. cerevisiae*	Multiple strains	2	Positive with and without metabolic activation Mitotic recombination and mutations Positive with metabolic activation	Parry & Eckardt, 1985; Mehta & von Borstel, 1985; Arni, 1985; Ferguson, 1985 cited in EC, 2004; Brooks *et al*., 1985 cited in EC, 2004
Yeast mutation assay *S. Pombe*	Forward mutation assay	2	Negative Positive with metabolic activation	Loprieno *et al*., 1985 cited in EC, 2004 Rizzi *et al*., 1984 cited in Ec, 2004
Mutation study *Aspergillus nidulans*	Detection of somatic segregation	2	Increase in mitotic cross-overs and induction of haploid and diploid segregants	Carere *et al*., 1985 cited in EC, 2004
Mouse lymphoma L5178Y	Ouabain and 6-thioguanine resistance	1	Positive with and without metabolic activation Positive with metabolic activation	Garner & Campbell, 1985; Lee & Webber, 1985; Amacher & Turner, 1985; Myhr *et al*., 1985 cited in EC, 2004

Test	Assay details	Klimisch	Result	Reference
				Anderson & Cross, 1985 cited in EC, 2004
Mouse lymphoma L5178Y $TK^{+/-}$		2	Weakly positive with and without metabolic activation	Oberly *et al.*, 1985 cited in EC, 2004
Mouse lymphoma L5178Y	6-thioguanine and ouabain resistance	2	Negative at both loci	Styles & Clay, 1985 cited in EC, 2004
TK Human lymphoblastic cells	Tk locus	2	Positive with and without metabolic activation Acrylonitrile only weakly positive with metabolic activation (4-fold increase) while CEO is strongly positive (17-fold)	Crespi *et al.*, 1985 cited in EC, 2004 Recio & Skopek, 1988 cited in EC, 2004
Sister chromatid exchange (SCE)	CHO cells	1	Positive with and without metabolic activation SCE and CA positive with and without metabolic activation	NTP, 2001 Gulati *et al.*, 1985 cited in EC, 2004
	Human bronchial epithelial cells from autopsy. No added metabolic activation.		Positive for SCE and DNA strand breaks	Chang *et al.*, 1990 cited in EC, 2004
	Human lymphocytes		Positive SCE	Perocco *et al.*, 1982 cited in EC, 2004
			Negative SCE	Obe *et al.*, 1985

Test	Assay details	Klimisch	Result	Reference
Chromosomal aberrations	Chinese Hamster Livers	2	Positive without metabolic activation	Danford, 1985 cited in EC, 2004
Various mammalian cells	Chinese Hamster Lung		Positive without metabolic activation	Ishadite & Sofuni, 1985 cited in EC, 2004
CA CHL cells		1	Positive with and without activation	Asakura *et al*., 1994
DNA damage and repair	Isolated rat hepatocytes	2	Increased single strand DNA breaks	Bradley, 1985 cited in EC, 2004
			Negative for single strand DNA breaks	Lakhanisky & Hendricks, 1985 cited in EC, 2004
DNA damage and repair	HeLa cells	2	Mutagenic and genotoxic at low doses	Rizzi *et al*., 1984 cited in EC, 2004

Test	Assay details	Klimisch	Result	Reference
			Negative in HeLa cells for UDS	Martin & Campbell, 1985 cited in EC, 2004
DNA damage and repair	Rat hepatocyte cultures	2	Positive UDS	Glauert *et al*., 19985 cited in EC, 2004
			Negative for DNA repair response for acrylonitrile and CEO	Butterworth *et al.*, 1992 cited in EC, 2004
DNA damage and repair	HMEC (human mammary epithelial cells) DNA repair assay	2	Negative UDS for acrylonitrile but positive for CEO	Eldridge *et al*., 1992 cited by EC, 2004

There has been a large number of bacterial mutagenicity assays conducted using a range of strains of *Salmonella typhimurium* with and without metabolic activation. These are summarised in Table 12 above and considered in further depth in the EU RAR (EC, 2004).

The *in vitro* assays indicate that acrylonitrile is genotoxic. Although there are several bacterial assays which are negative both with and without metabolic activation, there is sufficient evidence from both standard bacterial *(Salmonella* and *E.coli*) and mammalian cell assays (mouse lymphoma and human lymphoblastic cells, SCE and CA) to show that acrylonitrile is mutagenic and has genotoxic potential *in vitro*. The bacterial assays are positive both with and without metabolic activation and others show a requirement for metabolic activation. Therefore, no conclusion can be made on the need for metabolic activation *in vitro*, although there is some suggestion that metabolic activation may be required in some assays. Some variability in the results of mutagenicity studies is not uncommon and a weight-of-evidence approach is usual. It should be noted that the negative results in the Ames tests include use of the strains TA1537 and TA 1538 which detect frameshift mutations, although there are also positive results with these strains. However, the tests using strains TA1530, TA1535 and TA1950 are positive and these detect GC to AT mutations and these mutations could be due to oxidative stress.

There is evidence that the metabolite, CEO, is positive in a *Salmonella typhimurium* HisG46 base *oua* and *E.coli* assays (Venitt *et al*., 1977; Lijinsky and Andrews, 1980; Zeiger and Haworth, 1985). Adducts affecting base pairing have formed in isolated DNA exposed *in vitro* to CEO (Whysner *et al*., 1998) and binds to DNA with greater affinity than acrylonitrile. These results suggest that it is a directly acting mutagen *in vitro*.

This conclusion is in agreement with those of authoritative reviews that acrylonitrile is a genotoxic chemical *in vitro* (EC, 2004), although it is considered to be only weakly directly mutagenic (many of the assays indicate mutagenicity at higher concentrations) while the metabolite, CEO, is a more potent directly-acting mutagen (EC, 2004; Bolt *et al.,* 2003; Environment Canada, 1999).

7.6.4 Summary of Genotoxicity

The weight-of-evidence of the results for acrylonitrile *in vitro* indicates that it has genotoxic potential, but is only weakly mutagenic in *Salmonella typhimurium* and *E. coli* strains. The positive effect generally requires the presence of metabolic activation. There have been a number of studies reporting negative results in *S. typhimurium*. Positive results have also been observed in mutagenicity assays with yeast and aspergillus and in one of the regulatory mammalian cell assays, mouse lymphoma cells (at both the $TK^{+/-}$and the *oua* loci) and the human lymphoblast cell line, TK6; again, this is usually in the presence of metabolic activation and frequently at cytotoxic concentrations. Acrylonitrile induced sister chromatid exchange and chromosomal aberrations *in vitro,* but was generally negative in DNA repair assays in rat and human cell lines.

A number of studies indicate that the metabolic epoxide, CEO appears to be a directly acting mutagen. This observation, taken together with the need for metabolic activation for a positive effect in a number of mutation assays *in vitro,* indicates that the DNA active compound may be CEO and that acrylonitrile itself has low reactivity with DNA. CEO has a greater affinity for DNA then acrylonitrile.

The results in *in vivo* assays are much more equivocal. In rats, acrylonitrile appears to be negative in dominant lethal assays and weakly positive at high doses in the Comet assay. Chromosome aberration was positive in bone marrow and negative in peripheral blood and conflicting results have been obtained in UDS studies in the rat. In mice, micronucleus tests, mutagenicity tests in transgenic mice, and all but one chromosome aberration tests

were negative, whereas sister chromatid exchange studies in mice were on the positive side. A number of studies in *Drosophila* have given positive results but the relevance of the results for genotoxicity in mammals is difficult to interpret.

More recent studies including those by Pu *et al*. (2009; 2016), Nakagawa *et al*. (2015), Williams *et al*. (2017) and Dang *et al*. (2017a,b) yield further information on the mechanism of action of acrylonitrile confirming genotoxic damage and suggesting oxidative DNA damage in a number of studies, particularly in the target tissue, the rat brain but not affecting the overall conclusion. Currently, a non-genotoxic mechanism of action has not been clearly proven as the only mechanism of action.

In summary, acrylonitrile is clearly genotoxic *in vitro*, indicative of a genotoxic potential. However, this conclusion is far less clear from *in vivo* studies suggesting that acrylonitrile or the active metabolite, CEO, may not reach target tissues *in vivo,* perhaps due to detoxification of CEO by glutathione conjugation pathways not present *in vitro*. Although a number of recent studies both *in vitro* and *in vivo* have suggested that oxidative damage may be a non-genotoxic mechanism of action, there is insufficient evidence that genotoxic effects have no role in the toxicity of acrylonitrile (Kirman *et al*., 2005).

7.7 Carcinogenicity

7.7.1 Human data

7.7.1.1 Early reports

The early reports of a four-fold excess lung cancer risk in workers exposed to acrylonitrile in a US textile factory (US Dept. of Labour, 1978), in conjunction with experimental studies, led to the International Agency for Research on Cancer (IARC) concluding acrylonitrile should be regarded as if it were probably carcinogenic to humans, Group 2A (IARC, 1979). Early epidemiological studies indicated an increase in respiratory, particularly lung cancer, but also colorectal and other cancers (Thiess *et al.*, 1980; Zhou & Wang, 1991; Mastrangelo *et al.*, 1993). But, for example, although Mastrangelo *et al.* (1993) observed a significant overall excess (and in those involved in fibre manufacture and maintenance) for intestinal and colonic cancers, these were restricted to sub-groups with one to four years exposure (no excesses observed with five years or more exposure) or one to nine years since first exposure (none observed more than ten years since first exposure). Also, in this study, the lung cancer excess was restricted to maintenance workers who were also exposed to dimethylacetamide. However, in this study, and the others, the results were based on a small number of cases (and could have happened by chance), exposure levels were not given, and other confounding exposures (including smoking) were not considered. An early meta-analysis of these early reports observed a summary SMR for all cancers of 1.03 (95%CI=0.90-1.17; based on 224 cancers) and for respiratory cancers of 1.07 (95%CI=0.86-1.32; based on 85 cancers) (Rothman, 1994). Rothman concluded that there is insufficient information to support confidence about a lack of carcinogenicity at all sites, but that despite the flaws in some of the individual studies, the summarised findings offer reassurance that workers exposed to acrylonitrile face no striking increases in mortality for all cancers or for respiratory cancer.

7.7.1.2 International Conference

When it was realised that acrylonitrile could cause cancer in animals, and that large numbers of people were occupationally exposed to this industrial chemical, it was felt that this weak evidence for it as a human carcinogen was unsatisfactory (Doll, 1998). Doll suggested that the risk to which workers had been exposed cannot be large, and that it was unlikely that any one study could be large enough to provide a clear answer. Therefore, when it was realised that four cohort studies of occupationally exposed workers

were nearing completion, it was suggested a meeting be convened to discuss the results of them and consider what may be required to answer any questions that may arise. Therefore, in 1997, new data from The Netherlands (Swaen *et al.*, 1998), the UK (Benn & Osborne, 1998), the National Cancer Institute in USA (Blair *et al.*, 1998) and the DuPOnt Company in USA (Wood *et al.*, 1998) were presented at an international conference held in Oxford. The conference was funded by the Acrylonitrile Group in the USA and the CEFIC Acrylonitrile Producers Association, Sector Group within the European Chemical Industry Council in Europe. It was attended by principal investigators, experts in the field of occupational medicine, as well as representatives of regulatory bodies, industry groups and labour unions. These studies were significantly larger than previous ones, had longer follow-up and assessed exposure levels. At the meeting papers were also presented that considered the toxicological profile of acrylonitrile (Woutersen, 1998), assessed exposure assessment in the cohorts (Stewart *et al.*, 1998; Esmen, 1998) and a review/meta-analysis (Collins & Acquavella, 1998).

Blair *et al.* (1998) studied 25 460 workers from eight US acrylonitrile-producing and -processing plants in the US to evaluate the potential cancer risk from exposure to acrylonitrile. Part of this cohort had already been studied by Collins *et al.* (1989). A total of 25 460 workers, employed from the 1950s through 1983, were studied and followed through 1989 (348 642 person-years [py] of follow-up in exposed workers, 196 727py in unexposed). This study also included a well-documented procedure to develop quantitative estimated of historical inhalation and dermal exposures, which allowed exposure-response relationships to be evaluated (Stewart *et al.*, 1998). The overall SMRs among exposed workers were 0.90 (95%CI=0.80–1.10; observed cases [Obs]=134) for lung cancer; 0.8 (95%CI=0.40–1.80; Obs=6) for bladder cancer; 0.7 (95%CI=0.40-1.30; Obs=12) for CNS/brain cancer; and 0.9 (95%CI=0.6-1.5; Obs=16) for prostate cancer. These were similar or lower to the SMRs observed in the unexposed. Cumulative exposure categories were defined as 0, <0.13, >0.13–0.57, >0.57–1.50, >1.5–8.0 and >8.0 ppm-years; no exposure-response trend was observed for lung cancer (p-trend=0.65), the respective RRs were 1.10 (95%CI=0.70–1.70), 1.30 (95%CI=0.80–2.10), 1.20 (95%CI=0.70–1.90), 1.00 (95%CI=0.60–1.60) and 1.50 (95%CI=0.90–2.40). Similarly, cancer of the bladder, prostate and CNS/brain showed no indication of rising risk with increasing level of cumulative exposure. Similar, non-significant, patterns were also observed for lung cancer in the RR analyses by average exposure, exposure duration, and lagged exposure. Adjustment of cigarette use, obtained from a sample of workers to assess the potential for confounding, did not change the RR for lung cancer significantly. However, the rate ratio for lung cancer (RR=3.6) of ever cigarette smokers as compared with never smokers was “surprisingly low”, a finding that makes it harder to dismiss the possibility of residual confounding (Coggon & Cole, 1998).

Wood *et al.* (1998) investigated exposure to acrylonitrile in the production of Orlon by 2559 male employees at two Du Pont plants in USA. This was a follow-up of previous studies (O’Berg, 1980; O’Berg *et al.*, 1985; Chen *et al.*, 1987) as well as new workers exposed to acrylonitrile at these plants from 1944 to 1991 (Person-Years: mortality analysis – 71 763; morbidity analysis – 49 577). There were 46 lung cancer deaths and 17 additional lung cancer incidences, giving an overall SMR for lung cancer of 0.76 (95%CI=0.56–1.02) based on the general US population mortality rate and 0.89 (95%CI=0.65-1.81) based on plant-specific mortality rates. The SIR based on plant-specific cancer rates was 0.81 (95% CI 0.48–1.28). Cumulative exposure was assessed using a developed job-exposure matrix (that included air sampling measurements), and classified into four categories, i.e. <10, 10–50, >50-100, ≥100 ppm-years. The average duration of exposure for the workers was 7.6 years with an average cumulative exposure of 57.6 ppm-years. All SMRs and SIRs for lung cancer were below 1.00 and no trend of increased rates was observed with respect to any of the measures of exposure (latency, duration, highest, or cumulative). For bladder cancer the SMR was 1.15 (95%CI=0.31-2.95; Obs=4) and SIR was 0.69 (95%CI=0.19-1.77; Obs=4). For CNS/brain cancers the

SMR was 1.13 (95%CI=0.41-2.47; Obs=6) and SIR was 1.11 (95%CI=0.30-2.85; Obs=4). For prostate cancer the SMR was 1.29 (95%CI=0.64-2.80; Obs=11) and SIR was 1.58 (95%CI=0.82-2.76; Obs=12); no dose-response relationship was observed for SMR, however, SIR values increased in the analyses of latency, duration of exposure, highest exposure levels, and cumulative exposure dose but the 95% CI values included unity.

Benn & Osborne (1998) investigated exposure in 2763 male workers of UK plants involved in the polymerization of acrylonitrile and the spinning of acrylic fibres employed between 1950 and 1978, and followed up through 1991; a follow-up of a previous study (Werner & Carter, 1981). The authors stated there was a downward trend of exposure level to a limit of 2 ppm, although exposure levels were not specified. The overall SMR for lung cancer was 1.03 (95%CI=0.77–1.35) based on 53 observed deaths. No excess lung cancer death rate among the workers holding high-exposure jobs was found; there was no clear relation of lung cancer mortality either with duration of exposure or with time since first exposure. Results were not presented for any other specific cancer site apart from stomach. There was a lack of information on smoking habits and limited estimates of acrylonitrile exposure in this study.

Swaen *et al.* (1998) investigated mortality in 2842 workers exposed to acrylonitrile for at least 6-months before 1979 in The Netherlands, a follow-up of the Swaen et al. (1992) analysis. Past exposure to acrylonitrile and other potential occupational carcinogens was assessed by industrial hygienists. An unexposed group of 3 961 workers at a nitrogen fixation plant was assembled for comparison. Follow-up was to 1996, with 65 515 person-years-at risk (PYAR) in exposed workers and 120 976 pyar in unexposed. Overall, no statistically significant excess of any specific cancer was observed (Lung: SMR=1.10, 95%CI=0.81-1.46, Obs=47; Prostate: SMR=0.83, 95%CI=0.22-2.13, Obs=4; Bladder: SMR=0.98, 95%CI=0.20-2.86, Obs=3; Brain: SMR=1.74, 95%CI=0.64-3.78, Obs=6). No dose-response relationship was observed for cancer mortality, and specifically for cancers of the lung, prostate or brain. However, four out of five deaths from leukaemia (one lymphatic, 3 myeloid) occurred in the highest exposure category (SMR=4.421, 95%CI=1.19-11.30). No relationship was observed with peak exposure. What was interesting was that when they examined lung cancer mortality by whether there was exposure to other occupational carcinogens, the SMR in those with no exposure was 1.23 (95%CI=0.81-1.77) compared to 0.95 (95%CI=0.57-1.49) with exposure, giving a rate ratio of 1.29; however, smoking was not considered as a potential confounder.

These four studies, along with 21 previously published, were considered in the meta-analysis of Collins & Acquavella (1998), that examined the risk of ten cancers. In these studies, the use of acrylonitrile included monomer and resin, acrylic fibres, styrene, nitrile rubbers, and production of plastics in North America (n=17), Europe (n=7) and China (n=1). The meta-Relative Risk (mRR) for lung cancer for all studies was 0.9 (95%CI=0.9-1.1), based on 315 cases. Cumulative RR (CRR) by date of the study showed that before 1992 the CRR for lung cancer mortality was greater than 1.0, but with a large confidence interval (CI). After the completion of the large studies described above the CRR was below 1.0 and CI narrower. Examination of the studies that considered latency and exposure levels showed no dose-response relationship with lung cancer risk that could be considered as causal. The mRRs for prostate cancer (1.0, 95%CI=0.7-1.5) and brain cancer (1.1, 95%CI=0.8-1.5) indicated no significant excess. Bladder cancer was elevated (mRR=1.4, 95%CI=0.9-2.04), however, the excess was not dose-related and was restricted to plants with potential exposure to aromatic amines, and therefore, probably unrelated to acrylonitrile exposure. An analysis of the data considering the quality of individual studies suggested there was little difference between published and unpublished studies in completeness of vital status follow-up, completeness of death ascertainment or mean duration of exposure. There was some evidence of publication bias although it didn't affect individual cancer risk estimates. The authors did comment there was some evidence of failing to report RRs of less than 1.0 in published studies for some cancer, notably prostate and brain. The finding for brain cancer was confirmed in a more detailed meta-analysis of CNS tumours by Collins and Strother (1999). Collins & Acquavella (1998) noted that there

was little evidence that acrylonitrile workers have increased cancer rates even though exposures in some groups of workers were at levels which have caused tumours in rats. Even though older studies recorded higher exposure levels compared to current levels no cancer excess was observed.

In their overview of the conference, Coggon and Cole (1998) asked "How confident can we be that acrylonitrile is not a human carcinogen?". They stated that it is difficult to exclude an agent as a human carcinogen by epidemiologic studies, however, the weight of evidence available suggests either that acrylonitrile is not a human carcinogen or that it produces only small increases in cancer risk, at least at the exposure levels that have occurred in North American and Western Europe. They also compared the cancer risk in high exposure groups in the Dutch (≥10 ppm-years) and US (NCI: ≥8 ppm-years; DuPont: ≥10 ppm-years) studies. Apart from a small excess of leukaemia in the Dutch study which contrasted to a deficit in the NCI study, there was an overall paucity of cases (cancer of lung, prostate and brain), concluding there seems little cause for concern. Also, they state that given the uncertain impact of smoking on the risk of lung cancer in the various cohorts, any suspicions about a hazard of lung cancer from acrylonitrile can only be weak. They ended that to assess cancer risk any future epidemiologic research should focus on workers with cumulative exposure in excess of 10 ppm-years.

Following publication of the studies in 1998, IARC reviewed the updated evidence, and in 1999 a working group modified the classification for acrylonitrile from "2A (probable carcinogen)" to "2B (possible carcinogen)" (IARC, 1999). The re-evaluation of human data were summarised: "*the earlier indications of an increased risk among workers exposed to acrylonitrile were not confirmed by the recent, more informative studies*". The overall evaluation states that there is *inadequate evidence* in humans for the carcinogenicity of acrylonitrile, and *sufficient evidence* in experimental animals, and concluded it is possibly carcinogenic to humans.

7.7.1.3 Latest Reports

Although epidemiological studies have been key to the identification and quantification of cancer risks associated with a number of factors, including occupational exposures, the reporting of associations that are not replicated is also a common occurrence (Boffetta *et al.*, 2008). This is because in many studies large numbers of comparisons are made and large sets of results produced that some may occur by chance. A cumulative meta-analysis of the initial 1978 findings and of 15 subsequent studies of acrylonitrile and lung cancer published in 1980-1998 found a steady decrease over time in the overall RR estimate (Boffetta *et al.*, 2008). The final pooled estimate of RR was 1.1 (95%CI=0.9-1.4). The authors concluded that the declining trend in the summary RR estimate as further data accumulated provided evidence that the initial finding of an increased lung cancer risk was a false-positive.

A more recent meta-analysis of occupational acrylonitrile exposure and lung cancer identified 11 cohort studies published up to March 2005 (Sponsiello-Wang *et al.*, 2006). This included updated analyses of previous studies, including a cohort of chemical plant workers (Marsh *et al.*, 2001) included in the study of Blair *et al.* (1998), an update of the Netherlands cohort (Swaen *et al.*, 2004), and a European case-control study (Scelo *et al.*, 2004). The study estimated the overall SMR, based on both national and regional reference rates, was 0.95 (95%CI=0.86 to 1.06). After aggregation of rate ratios based on regression analyses and ratios of SMRs, adjusting for the healthy worker effect, an overall SMR of 1.25 (95%CI=1.10-1.43) was obtained. However, this estimate, and also those obtained by Collins and Acquavella (1998), could be affected by significant residual confounding because no consideration was given to other potential occupational exposures. More importantly, potential confounding by smoking could not be ruled out, and in three of the studies included in the analysis almost all the exposed workers who

developed lung cancer were smokers. A sensitivity analysis showed that less than 10% excess smoking prevalence in the exposed group could fully explain the elevated lung cancer risk. In addition, the analysis was not able to carry out an exposure-response analysis. Also, one study that did evaluate smoking levels of workers, found that the proportion of smokers increased with increasing cumulative acrylonitrile exposure category (Blair *et al.*, 1998). This finding, if reflected across all other studies, may indicate smoking is an important confounder to be considered.

A more recent review by Cole *et al.* (2008) identified 28 studies that examined cancer mortality and/or incidence among individuals with acrylonitrile exposure. However, they considered only four studies that primarily included workers with high acrylonitrile exposures (Blair *et al.*, 1998; Benn and Osborne, 1998; Swaen *et al.*, 2004; Symons *et al.*, 2008). They also concentrated on four cancers (lung, bladder, prostate and central nervous system), which they stated had received the most attention and for which some positive results have been reported. Table 13 summarises those presented in the review. The authors concluded that the results from these four studies do not support a causal relationship between high exposure to acrylonitrile and all cancers or any specific type of cancer (lung, bladder, CNS, prostate).

A summary of the findings from these studies can be seen in Appendix 2.

Table 13: Summary of observed and expected numbers of specified cancer deaths and SMRs for exposed subjects in four major follow-up studies (source: Cole et al., 2008)

Cancer	Study				Total
	DuPont[1]	UK[2]	NCI[3]	Dutch[4]	
Lung:					
Observed	88	53	134	67	342
Expected	95.6	51.5	141.0	62.5	350.6
SMR	0.92	1.03	0.95	1.07	0.98
95%CI	0.74-1.14	0.78-1.34	0.80-1.31	0.83-1.36	0.88-1.09
Bladder:					
Observed	16	12	6	5	39
Expected	12.4	14.8	7.5	4.6	39.3
SMR	1.29	0.81	0.80	1.09	0.99
95%CI	0.76-2.05	0.44-1.38	0.32-1.66	0.35-2.52	0.71-1.37
Brain & CNS:					
Observed	6	Not	12	6	24
Expected	8.1	reported	17.1	4.8	30.0
SMR	0.74		0.70	1.25	0.80
95%CI	0.30-1.54		0.39-1.19	0.46-2.71	0.51-1.19
Prostate:					
Observed	25	12	16	8	61
Expected	24.5	14.9	17.8	8.7	65.9
SMR	1.02	0.81	0.90	0.92	0.93
95%CI	0.68-1.48	0.44-1.37	0.53-1.43	0.40-1.81	0.72-1.20

[1] Symons *et al.* (2008); [2] Benn & Osborne (1998); [3] Blair *et al.* (1998); [4] Swaen *et al.* (2004)

NB: Lung cancer - UK study includes 19 cases with little/no exposure;
Bladder cancer – UK study numbers are all genitourinary cancer, and DuPont study numbers are for urinary organs;
Brain/CNS cancers – DuPont and NCI studies reported all CNS cancers; Swaen *et al.* (2004) reported only cancers of the brain;
Prostate cancer –UK numbers are all genitourinary cancers.

Since this last review, a number of studies included in the previous meta-analyses (Cole *et al.* 2008), have been updated. Marsh and Zimmerman (2015) updated the US study of chemical workers in Lima, Ohio (Marsh *et al.*, 1999); extending the follow-up through 2011 and resulting in over 46 000-PYAR (Note: this study is a sub-cohort of workers in one of the eight plants included in the US-NCI study by Blair *et al.,* 1998). However, no statistically significant excess mortality risks for any cause of death in exposed workers, including lung cancer (SMR=0.73, 95%CI=0.41-1.20) and other cancer sites of interest (prostate: SMR=1.32, 95%CI=0.43-3.09; bladder: SMR=2.27, 95%CI=0.62-5.80; No case of brain cancer reported), were observed. Dose-response analysis did not show any increase in relative risk for lung cancer, irrespective of the dose metric (Duration of exposure: mean=11.39y, 0.003-39.33y; cumulative exposure: mean=39.75, 0.002-609.28y; average intensity: mean=3.69y, 0.04-26.3y). The original excess risk for bladder cancer in this cohort had decreased over time to a non-statistically significant level, suggesting the original findings may have been by chance.

In addition, with the understanding that smoking may confound the results for lung cancer risk, Zimmerman *et al.* (2015) undertook a sensitivity analysis of a sub-cohort of this study. Monte Carlo modelling of data from 992 men that accounted for the relationship

between smoking and acrylonitrile exposure showed that mean RRs for lung cancer mortality decreased significantly, and that there was even less evidence for an exposure-response relationship.

An update of the DuPont cohort study of workers of two facilities responsible for developing and manufacturing acrylic fibre (Wood et al., 1998), extended the follow-up to 55-years and giving over 95 000-PYAR (Symons *et al.*, 2008). The mean exposure duration was 9.2-years, the median intensity exposure was 10.0 ppm, and median cumulative exposure (CE) was 31.5ppm-years. Mortality analysis indicated no statistically significant increase in SMR for any cause of death. Hazard ratio estimates (HR) for a 100ppm-year increase in CE was estimated as 0.95 for lung cancer (95%CI=0.73-1.23), 0.96 for respiratory system cancer (95%CI= 0.74-1.25) and 0.78 for prostate cancer (95%CI=0.46-1.32). Lagging exposures had no effect on HR estimation. Analysis of only highly exposed workers with CE exposures >10ppm-years, suggested no significant increase for any malignant cancer.

In a recent study of the risk of renal cell carcinoma (RCC) in a case-control study from Central and Eastern Europe, there was a suggestion of a positive trend for cumulative occupational exposure to acrylonitrile and RCC risk (<median: OR=1.6, 95%CI=0.4-6.4; ≥median: OR=4.3, 95%CI=0.9-22.1; p-trend=0.06), after adjusting for significant co-exposures (although these were not specified) (Karami *et al.,* 2011). Stratifying the analyses by gender, BMI, self-reported hypertension and smoking status had no effect on the results. Occupations held by subjects included manufacturers of acrylonitrile or acrylic fibres, manufacturers of plastic shoes that processed polymers, and workers who cut acrylic fibres. However, care must be taken as exposure was assessed using an occupational history questionnaire and occupations categorised as low, medium or high exposure by an expert. Some possibility of non-differential, inaccurate or incomplete recall of occupational history, and therefore, exposure misclassification. There is also the possibility of residual confounding from other potential exposures such as diet and non-occupational exposures.

ECHA has been informed that an update of the US-NCI cohort earlier published by Blair et al (1998) is anticipated in 2018.

7.7.1.4 Discussion and conclusion

As described in the previous section, several limitations have been identified in many of the earlier studies. The quantification of exposure to acrylonitrile was not possible, lacking or limited, for many of the cohorts studied, and in some cases, it is likely that a proportion of the exposed cohort were not, in fact, exposed. Some of the studies acknowledged that subjects had been exposed to multiple chemicals, in addition to acrylonitrile, some of which are known or suspected carcinogens (e.g. bladder cancer and aromatic amines; see meta-analysis of Collins and Acquavella, 1998 above). However, confounding by concomitant exposure to other chemicals could only have occurred if an excess cancer risk was observed. There is also the potential confounding from exposure to cigarette smoke in the case of lung and bladder cancers, which most of the studies were not able to adjust results for. Nevertheless, although both negative and positive confounding occur, in a blue collar worker cohort with likely a higher prevalence of smoking than in white collar workers, the concern on such confounding by smoking seems to be more pertinent if an excess cancer risk was observed. In addition, as stated above Zimmerman et al. (2015) noted that workers exposed to acrylonitrile also had a higher prevalence of smoking than the non-exposed workers, meaning that if an excess lung cancer was reported, this to an extent should be attributed to this difference in smoking. Many of the individual studies, especially the early ones, did not provide detailed information on how subjects were recruited. There was also incomplete follow-up in some of the earlier studies.

There is an inconsistency of reporting all causes of death in some studies (e.g. Benn and Osborne, 1998 reported the risk for all genitourinary cancers but not bladder cancers). For some studies, results for every specific cancer site were not reported. There is a concern

that when a study does not report on a given cancer site, it could be either because there were no cases for that cancer site or that there was no excess for that site. Not reporting such results would create a "publication bias" as some of the studies may have had data that would balance the other individual reports of positive findings for specific sites, but simply fail to show it. This would lead to overestimation of risks in a meta-analysis.

However, it is to be noted that the above mentioned large cohort studies are not subject to the above problems and are considered of high quality. With the exception of Benn and Osborne 1998 reporting genitourinary cancer overall and not separating bladder cancer.

The most recent follow-ups of these cohorts, individually or combined, did not identify increased risks for cancer of the lung, brain, prostate and bladder. The published reports allow for the estimation of total cumulative exposure for them (Symons *et al.*, 2008; Blair *et al.*, 1998/Stewart *et al.*, 1998; Marsh *et al.*, 1999, 2015; Swaen *et al.*, 1992, 2004). Using the average of the lower and upper limit of each cumulative exposure category reported and the number of workers included indicate that over 361,291 ppm-years were accrued by 16,503 exposed workers. A conservative assumption that this was accumulated during 40-years of exposure would result in an average exposure level of 0.55 ppm (361,291/16,503/40). As Marsh *et al.* (1999) and Marsh and Zimmerman (2015) contain partly the same workers as one of the eight sub-cohorts of Blair et al (1998) but extended the exposure assessment and cancer follow-up to later years, this would result in double counting those workers for the earlier years (less than 10% of the Blair et al 1998 cohort). Excluding that study (Marsh *et al.*, 2015) results in an estimate of 0.52 ppm (329,360/15714/40). As the above estimation relies on the upper and lower limits of each exposure category without knowing the distribution of exposures inside that range, the estimates should rather be rounded down, i.e. to 0.5 ppm. Yet many of the workers were exposed to much higher concentrations than the above-calculated averages, e.g. the median exposure in the study of Symons *et al.* (2008) was reported to be 10 ppm.

In 2005 a peer review expert panel (Haber and Patterson, 2005) firstly observed for epidemiological data that "*The acrylonitrile database contains unusually extensive data. There are several large, well-conducted epidemiological studies, several including good exposure data and a long and complete follow-up*". After having reviewed also the animal data and mode of action data, the panel concluded in a weight of evidence statement that "*Epidemiology data do not support an increased cancer risk from acrylonitrile exposure in exposed workers*". This conclusion was because "*No increased cancer risk has been consistently observed in several different large, well-conducted epidemiology studies using several different occupational cohorts in several different countries*".

It has been questioned whether the epidemiological studies have enough power to be able to detect an increased cancer risk, because of sample size and length of follow-up. Statistical significance and confidence intervals should be used in interpreting the epidemiology data. Although post-hoc power calculations are not recommended some calculations are provided in Appendix 3. If the effect estimate is not statistically significantly different from null, the breadth of the confidence intervals conveys the level of confidence that the true state of nature is close to the null. Additional to the fairly narrow CIs and lack of statistical significance for cancer effects in the good quality cohort studies and their subcohorts individually (i.e., Symons et al. 2008; Benn & Osborne 1998; Blair et al. 1998, Marsh and Zimmerman 2015; Swaen et al. 2004) and combined (Table 13), one should also consider the consistency of the negative findings in the interpretation of the epidemiology as a whole (see e.g., the meta-analysis of Collins & Acquavella (1998) which considered 21 additional studies).

One could even consider that the unusually large (negative) data accumulated so far is likely to result in a low interest to conduct new epidemiological studies or even to fund further follow-ups of the existing cohorts to ensure even more narrow confidence limits around unity. Hagmar (2001), concluded in an editorial article that "*results from*

epidemiology and the bioassay model make it obvious that the excess risk for the acrylonitrile-exposed workers cannot have been large". He also pointed out that *"it is extremely difficult to verify or falsify low risk increases for rare diseases in occupational cohort studies*" and therefore "*it is not realistic that an excess risk for CNS cancer deaths due to occupational acrylonitrile exposure, predicted from the rat inhalation bioassay-model, can ever be evaluated against empirical data from epidemiologic studies*".

In summary, the additional evidence provided by the updates of the various cohorts reiterates the conclusion made by Coggon and Cole (1998) that, at the exposure levels experienced in Western Europe and North America, the weight of evidence from epidemiological studies suggests that acrylonitrile is not a human carcinogen or that it produces only small increases in cancer risk.

7.7.2 Animal data

There have been a number of two-year carcinogenicity studies, mainly in rats, but also in mice.

7.7.2.1 Inhalation studies

Occupational exposure to acrylonitrile is predominantly by inhalation, and dermal and oral exposure is negligible. Therefore, long-term animal studies with inhalation exposure are the most relevant and represent key studies for the risk assessment of acrylonitrile.

In an inhalation study in Sprague-Dawley rats (100/sex/concentration) animals were exposed 6 hours/day, 5 days/week for 2 years to concentrations of 0, 20 or 80 ppm acrylonitrile (duration-adjusted concentrations of 0, 7.7 and 31 mg/m^3) (Quast *et al.,* 1980a), see also section 7.3.2.2. The control group was exposed only to air. Additional groups of rats were also included for interim post-mortem at both 6 (7/sex/dose) and 12 months (13/sex/dose).

There was a statistically significant increase in mortality ($p<0.05$) within the first year in both male and female rats administered 80 ppm and in the 20 ppm females during the last 10 weeks of the study (the increase in mortality for the 20 ppm females was mainly due to early killing of rats with large, benign, mammary gland tumours; Quast, 1980a). In Sprague-Dawley rats, these tumours occur spontaneously at a high rate, but in this experiment the tumours were observed earlier and more frequently, and became larger in exposed animals.

Histopathology of the animals indicated treatment-based toxicity in two tissues: the nasal respiratory epithelium and the brain.

There were significant degenerative and inflammatory changes in the respiratory epithelium of the nasal turbinates at both exposure concentrations (20 and 80 ppm) which were considered to be treatment-related irritation of the nasal mucosa. These effects were more serious at the higher dose level. No treatment-related effects in the olfactory epithelium, trachea, or lower respiratory system were observed in either males or females at either concentration. In this study, 20 ppm was considered to be the LOAEL for pathological alterations in the respiratory epithelium of the extrathoracic region of the respiratory system. NOAEL and LOAEL for non-carcinogenic, extra-respiratory effects were considered to be 20 ppm and 80 ppm, respectively.

In the following summary, the number of animals with tumours has been corrected for mortality during the study as outlined in EU RAR (EC, 2004).

In the brain, a significant increase ($p<0.05$) in focal gliosis and perivascular cuffing was observed in the brains of higher concentration males (1/97; 7/83 exposed) and females

(0/99 controls; 8/99 exposed), but not in low concentration rats. Glial cell proliferation and the presence of astrocytomas were also detected in this study and the incidence for this early proliferation and tumours are given in section 8.1.1., Table 17. Reanalysis using more advanced methods of specific staining has identified these tumours as malignant microglial tumours, while most spontaneous brain tumours were oligodendrogliomas (Kolenda-Roberts *et al*., 2013). This study is considered the most complete long-term animal study with exposure by inhalation and the results have been extensively used for the risk assessment of acrylonitrile. The results of this study have been used in the estimation of cancer risk in Section 8.

At the higher dose, there was an increase in benign and malignant tumours of the Zymbal gland in both sexes and, in males the small intestine and tongue. In higher dose females there was an increased incidence of mammary tumours.

Acrylonitrile has also been administered by inhalation at lower doses of 0, 5, 10, 20, and 40 ppm, 4 hours/day, 5 days/week for 12 months to 30 Sprague-Dawley rats/sex/group by Maltoni *et al*. (1977). This resulted in a statistically significant increase in mammary and forestomach tumours in males and skin carcinomas in females. In a follow-up study, female rats and male and female offspring were similarly administered 60 ppm, some for 104 weeks, others only 15 weeks (Maltoni et al., 1988). Non-neoplastic changes included slight but significant increases in glial cell hyperplasia. Increased incidence in tumours included: mammary tumours in females, Zymbal gland tumours in males and extrahepatic angiosarcomas in both males and females.

7.7.2.2 Oral studies

Quast *et al*. (1980b) administered acrylonitrile in drinking water to Sprague-Dawley rats (48 rats/sex/group) for two years at dose levels of 35, 100, and 300 ppm. A statistically significant increase in tumours was observed in the CNS (astrocytomas, now identified as microgliomas), Zymbal gland, stomach, tongue, and small intestine for both sexes and in the mammary gland of female rats. In general, the increase was dose dependent.

Biodynamics (1980a) administered acrylonitrile in drinking water to Sprague-Dawley rats at doses of 0, 1, and 100 ppm (100 rats/sex/group). Interim necropsies were performed at 6, 12, and 18 months (10/sex/group). The study was terminated early because of low survival rates. There was increased incidence of astrocytomas of the brain and spinal cord, carcinomas and adenomas of the Zymbal gland or ear canal, and squamous cell carcinomas and papillomas of the forestomach in higher dose animals.

In a second study (Biodynamics,1980b) acrylonitrile was administered in drinking water to Fischer 344 rats (100 rats/sex/group; control group 200 rats/sex) at dose levels of 0, 1, 3, 10, 30, and 100 ppm. Interim necropsies were performed at 6, 12, and 18 months (10/sex/group and 20/sex/control group). The study was terminated early because of the low survival rate. Increased dose-dependent incidence of tumours (astrocytomas of the brain and spinal cord, and carcinomas of the Zymbal gland) was seen in dose groups of 3 ppm or higher. An increased incidence of mammary gland tumours was seen in females in the 100 ppm dose group.

In a three-generation reproductive study in Sprague-Dawley rats exposed to acrylonitrile in drinking water, the second generation showed an increased incidence of tumours (astrocytoma and Zymbal gland) at a dose of 500 ppm (Beliles, 1980).

Maltoni *et al*. (1977) administered acrylonitrile in olive oil 3 times/week for 52 weeks to Sprague-Dawley rats at doses of 0 ppm (75 rats/sex) and 5 ppm (40 rats/sex). An increased incidence of tumours of the mammary gland and forestomach was observed in

female rats. This strain of rat had a high spontaneous incidence of mammary tumours and the short duration and single dose level makes this study inadequate for the purposes of risk assessment (Environment Canada, 1999).

In another study (Biodynamics, 1980c), acrylonitrile was administered at doses of 0, 0.10, and 10.0 mg/kg/day for 5 days to Sprague-Dawley (70 rats/sex/group). The study was terminated at 20 months. Statistically significant increased incidences of brain (astrocytoma) and Zymbal gland tumours were observed in the higher dose group. A statistically significant increased incidence of stomach and intestinal tumours was observed in males and of tumours of the mammary gland in females.

In an NTP study, B6C3F1 mice (50/dose group) were administered 0, 2.5, 10 or 20 mg/kg bw/day by gavage for two years (NTP, 2001). Tumours were observed in both males and females in the forestomach at the two higher doses and in the Harderian gland in males at all doses and in females at the two higher doses. An increase in lung and ovary glands in female mice was considered equivocal, but may be associated with administration of acrylonitrile.

Table 14: Summary of oral and inhalation animal studies

Oral studies:				
Method	**Delivery**	**Dose**	**Tumours**	**Reference**
Mouse B6C3F1	Oral gavage	0, 2.5, 10, 20 mg/kg for 2 years (5 days/week for 104-105 weeks	Harderian gland, forestomach, equivocal lung and ovary	NTP (2001)
Rat Sprague-Dawley	Oral drinking water	0, 35, 85, 210 ppm (1st 21 days) 0, 35, 100, 300 ppm (remainder of duration) for 2 years daily *ad libitum*	Astrocytomas, Zymbal gland, stomach, tongue, small intestine, mammary gland	Quast *et al*. (1980b)
Rat Fischer 344	Oral drinking water	0, 100, 500 ppm for 18 months daily *ad libitum*	Brain, skin, stomach, Zymbal gland	Bigner *et al*., 1986
Rat Sprague-Dawley	Oral drinking water	0, 20, 100, 500 ppm for 2 years daily *ad libitum*	Zymbal gland, forestomach (papillomas)	Gallagher *et al.*, 1988
Rat Fischer 344	Oral drinking water	0, 1, 3, 10, 30, 100 ppm intended duration 2 years but terminated at 23 months (females), 26 months due to low survival rates, daily *ad libitum*	Mammary gland carcinomas, astrocytomas, forestomach, Zymbal gland	Biodynamics, 1980b Johannsen & Levinskas, 2002a
Rat Sprague-Dawley	Oral drinking water	0, 1, 100 ppm intended duration 2 years but terminated at 19 months (females), 22 months (males) due to low	Astrocytomas, Zymbal gland, mammary gland	Biodynamics, 1980a Johannsen & Levinskas, 2002b

		survival rates, daily *ad libitum*		
Rat Sprague-Dawley	Oral gavage	0, 0.1, 10 mg/kg bw/day intended duration 2 years but terminated at 19 months (females), 22 months (males) due to low survival rates	Astrocytomas, mammary gland, Zymbal gland, gastrointestinal, forestomach	Biodynamics, 1980c Johannsen & Levinskas, 2002b
Rat Sprague-Dawley	Oral gavage	5 mg/kg bw 3 times weekly for 52 weeks	Mammary gland, forestomach	Maltoni *et al.*, 1977, 1988
Rat Sprague-Dawley 3-generation study	Oral drinking water	0, 100, 500 ppm 100 days prior to mating and subsequent gestation and lactation phases in females	Astrocytomas, Zymbal gland, low level in all generations	Beliles *et al.*, 1980
Inhalation studies:				
Method	**Delivery**	**Dose**	**Tumours**	**Reference**
Rat Sprague-Dawley	Inhalation, whole body	0, 20, 80 ppm (0, 44, 176 mg/m^3), 2 years, 6 hours/day, 5 days/week	Astrocytomas, gliomas, Zymbal gland, small intestine, tongue, mammary gland,	Quast *et al.*, 1980a
Rat Sprague-Dawley	Inhalation vapour, whole body	5, 10, 20, 40 ppm, 12 months, 4 hours/day, 5 days/week	Mammary gland, forestomach, Zymbal gland, forestomach, skin, extrahepatic angiosarcoma	Maltoni *et al.*, 1977, 1988

7.7.3 Summary

The results of the long-term toxicity and carcinogenicity studies in rats indicate conclusively that acrylonitrile is carcinogenic via both the oral (Quast *et al*., 1980b and others), and inhalation routes (Quast *et al*., 1980a) see Table 14. The inhalation study in rats (Quast *et al*., 1980a) is considered the key study for risk assessment. The animals developed tumours of the central nervous system, forestomach, intestines (including gastrointestinal tract, tongue, non-glandular stomach and small intestine), Zymbal gland (a sebaceous tissue associated with the ear duct of rodent species) and the mammary glands.

The mode of action of acrylonitrile tumour formation is likely complex and could include multiple mechanisms. Although it cannot be excluded that acrylonitrile may have genotoxic potential, and therefore could be considered a genotoxic carcinogen, the evidence suggests that indirect genotoxicity via oxidative stress may be the main mode of action.

The epidemiology data on acrylonitrile include several large, high quality studies using different occupational cohorts in several different countries and several meta-analyses. These studies were not able to confirm a causal association between acrylonitrile exposure in workers and increased cancer. Negative epidemiology data do not allow to reach absolute conclusions that a substance is not a human carcinogen: it is extremely difficult to verify or falsify low risk increases for rare diseases (such as brain tumours) in occupational cohort studies. Yet, the weight of evidence from good quality epidemiology data on current and past workplace exposures suggests that acrylonitrile is either not a human carcinogen or that it produces only small increases in cancer risk.

7.8 Reproductive toxicity

7.8.1 Human data

7.8.1.1 Reproductive and developmental toxicity

A review of four epidemiology studies performed on Chinese workers exposed to acrylonitrile (Collins *et al*., 2003) reported reproductive and developmental effects following maternal and/or paternal exposure. The results of the studies were consistent for indicating an increased risk of stillbirth, birth defects, miscarriage, infertility and low birthweight. A weight of evidence evaluation of the human and animal database for acrylonitrile (Neal *et al*., 2009) concluded that there was no evidence to support developmental effects from exposures below those producing overt maternal toxicity. In addition, the authors stated that their findings did not show acrylonitrile to be a reproductive or foeto-toxicant.

7.8.2 Animal data

7.8.2.1 Reproductive toxicity

The two drinking-water reproductive toxicity studies of acrylonitrile (Beliles *et al.,* 1980; Schwetz *et al*., 1975) were both pre-GLP studies that evaluated a limited number of reproductive parameters, compared to current study design guidelines. The two-generation inhalation reproductive toxicity study (Nemec *et al.,* 2008) was conducted in compliance with GLP requirements and with current EPA regulatory testing guidelines and was, therefore, considered to provide data of the highest confidence for reproductive toxicity hazard assessment by a relevant route of exposure, and provides NOAELs that are appropriate points of departure for risk assessment.

No obvious compound-related effects on reproductive success were noted in any of the reproductive toxicity studies, even at exposure levels producing toxicity to the parent animals (Table 15). Both the drinking-water studies and the inhalation study showed decreased pup weight gain at maternally toxic doses. However, the severe decrease in maternal water consumption at 500 ppm in the study reported by Beliles *et al.* (1980) was considered to have contributed to the reduced pup growth reported in both drinking-water studies since lactation may have been affected by maternal dehydration, resulting in a change in quantity or quality of the milk. The decreased weight gain in the pups was noted at a younger age in the drinking-water studies than was the decreased pup weight gain in the inhalation study, which would indicate a potential influence of the drinking-water deficit because of the sole reliance on milk for nutrition in pups early in the lactation period. However, the decreased pup growth in the inhalation study suggests that dehydration may not have been the only mechanism in the drinking-water studies delaying growth at maternally toxic doses. Delayed growth, a possible treatment-related adverse effect on pup survival, was noted in the 500 ppm F1a pups in the 3-generation drinking water study, however it was not replicated in other generations/litters (Friedman & Beliles, 2002).

Additionally, the 1-generation drinking-water study (Schwetz *et al.,* 1975) showed no exposure-related effects on pup viability at the same high exposure level, despite commensurate decreases in maternal water consumption, and the inhalation study showed no effect on pup survival at a maternally toxic dose.

A dominant lethal study performed with acrylonitrile was negative (Working *et al*., 1987), demonstrating a lack of male-mediated reproductive toxicity.

Data from short-term gavage studies such as Tandon *et al.,* (1988) suggest that some effects on sperm quality resulting from testicular damage (tubular atrophy and degeneration of seminiferous tubules, cytolysis and nuclear pyknosis of spermatids, formation of multinucleate giant cells and interstitial oedema) and a decrease in epididymal spermatazoa might result from high-dose (10 mg/kg/day) gavage exposure in mice. Similar effects on sperm count and motility were observed in rats by oral gavage administration (Abdel Naim *et al*. 1994) and inhalation exposure (Wang *et al*. 1995) at similar doses (11.5 mg/kg/day and >60 mg/m^3 respectively). However, the absence of functional effects on reproductive success in the drinking-water and other inhalation reproductive toxicity studies, biologically significant effects on andrology, or male reproductive organ histopathological findings in the inhalation reproductive toxicity study (Nemec *et al*., 2008) does not support any concerns regarding these endpoints.

It is noted that the NTP (2001) chronic study in mice showed an increased incidence of ovarian atrophy in reproductively senescent mice exposed to acrylonitrile; the biological significance of this finding is unclear. There were no similar findings in the inhalation two-generation rat reproductive toxicity study by Nemec et al. (2008). No data were seen in animal studies supporting an increased incidence of stillbirths, pre-or post-term deliveries or maternal mortality following exposure to acrylonitrile at dose levels producing other evidence of systemic toxicity. There was very weak support in the animal data for increased infant mortality, with pup deaths increased only at the high dose level in a single generation of a three-generation reproductive toxicity study. As discussed previously, the pup deaths may have been contributed to by decreased water intake of the dams. No evidence of increased pup mortality was seen in the two-generation inhalation reproductive toxicity study, considered to have the highest confidence level.

Table 15: Reproductive toxicity studies

Species / Ref	Route / dose levels	Study type	Klimisch	NOAEC or NOAEL	Findings
Rat Male/female (Nemec *et al*., 2008)	Inhalation/ 0, 5, 15, 45 and 90 ppm	2-generation study	1	Reproductive toxicity (P): 90 ppm Reproductive toxicity (F1): 45 ppm Systemic toxicity (F0): 15 ppm	Excessive toxicity reported at 90ppm including body weight and food consumption effects. Histopathology of nasal epithelium at all dose levels
Rat Male/female (Beliles *et al*., 1980)	Oral: drinking water/ 0, 100 and 500 ppm	3-generation study	2	100 ppm	Decreased body weight and food consumption in adults. Pup toxicity at 500 ppm including reduced pup survival and viability
Rat Male/female (Schwetz *et al*., 1975)	Oral: drinking water/ 0, 35, 210 and 500 ppm	1-generation study	2	Reproductive toxicity (P): 500 ppm	No effects on reproduction were observed
Rat Male	Oral:gavage/	Fertility study (exposure for 2 and 4 weeks)	4	LOAEL (P): 11.5 mg/kg/day	Effects on organ weights, sperm count and sperm motility

Species / Ref	Route / dose levels	Study type	Klimisch	NOAEC or NOAEL	Findings
(Abdel Naim *et al.*, 1994, 1995)	0, 11.5, 23 and 46 mg/kg/day				were recorded at the low dose level
Rat Male (Wang *et al.*, 1995)	Inhalation/ 0, 60, 90 and 120 mg/m^3	Investigative (exposure for 7, 14 or 28 days)	3	No NOAEC identified	Effects reported on sperm aberrations at all dose levels
Mouse Male (Tandon *et al.*, 1988)	Oral:gavage/ 0, 1 and 10 mg/kg/day	Investigative (exposure for 60 days)	2	1 mg/kg/day	10 mg/kg/day caused testicular effects in the absence of overt toxicity
Mouse Male/Female (NTP, 2001)[a]	Oral: drinking water 0, 2.5, 10, or 20 mg/kg, 5 days per week	Chronic study (exposure for 105 weeks)	2	NOAEL of 2.5 mg/kg	Treatment related increase in the incidences of combined benign or malignant ovarian granulosa cell neoplasms in dosed female mice at 10 and 20 mg/kg groups.

[a] This study is a carcinogenicity study but has relevant reproductive endpoints

7.8.2.2 Developmental toxicity

Acrylonitrile has been assessed thoroughly in one species (rat) for developmental toxicity and this assessment included several well-conducted studies with generally concordant NOAELs for maternal and developmental toxicity (Table 16). It should be noted that all three principal developmental toxicity studies were conducted at high dose levels which induced dose-dependent maternal toxicity. No unique foetal susceptibility was identified in any of these studies with effects seen only at high and overtly maternally toxic doses.

The two inhalation studies (Murray *et al*., 1978; Saillenfait *et al*., 1993) were both conducted at similar exposure levels which caused maternal toxicity evidenced by reduced weight gain and food intake. The main difference between these two studies was that an increase in malformations was observed at the 80 ppm exposure level (Murray *et al*., 1978) but not at 100 ppm (Saillenfait *et al*., 1993). The findings in the Murray *et al.* (1978) study were similar to those observed in the oral gavage study in rats by the same authors (Murray *et al*., 1978).

The principal malformation reported in the rat oral gavage developmental toxicity study (Murray *et al*., 1978) was an increased incidence of tailless or short-tailed foetuses. This is not a common finding and so incidences in relevant historical control data were not available. However, mean foetal control incidence of agenesis of caudal vertebrae has been reported from 222 developmental toxicity studies with Sprague-Dawley rats to be 0.014%, with a maximum incidence of 0.58% (Hood, 1996). Higher foetal incidences were reported in both the gavage and inhalation studies discussed. A review of the reproductive toxicity studies revealed no clear other exposure-related developmental malformations in these studies and, based on litter size, there was no evidence of increased post-implantation loss or resorptions up to an exposure level of 25 mg/kg bw/day, that might have obscured such a finding. Overall, the incidence of tailless pups in the reproductive toxicity studies was considered to be too low and sporadic to make a definitive assessment of potential relationship to treatment with acrylonitrile.

The malformations observed in the Murray gavage study (Murray *et al*., 1978), however, were not considered to be characteristic of foetal findings due to stress-induced teratogenicity. There was no apparent correlation between the affected litters and the degree of toxicity for individual dams. Maternal toxicity in this study was most evident at the start of gavage dosing (GD 6–9), whereas the affected structures (posterior portion of the axial skeleton) would be forming from approximately GD 10–11 onward, which shows some temporal correspondence. An outbreak of sialodacryadenitis (SDA) complicates the interpretation of the results.

The findings in this study show some similarity to findings in the *in vitro* embryotoxicity study (Saillenfait & Sabate, 2000), although the characterization of the *in vitro* findings in the Saillenfait & Sabate study is very limited. The very high gavage dose also reported by Saillenfait & Sabate (2000), which evaluated a single dose administered on GD10, also showed malformations in the presence of very severe maternal toxicity, showing a temporal correspondence between the occurrence of malformations and the dosing gestational interval.

All of these factors support that there may have been direct developmental toxicity to the foetus in the Murray gavage study (Murray *et al*., 1978). It should be noted, however, that this study was compromised by concurrent SDA infection, which could have increased both maternal and foetal susceptibility.

The Murray inhalation study (Murray *et al*., 1978) showed a very slight response (not statistically significant); the difference in response from that seen in the gavage study

may be due to differences in kinetics or may have been influenced by the concurrent infection present in the first study.

The most contemporary of the developmental toxicity studies (Saillenfait *et al.* 1993), by the most relevant route of exposure (inhalation), did not show any evidence of exposure-related malformations, even though maternal and foetotoxicity were both evident, and a higher exposure was tested than in the Murray inhalation study (Murray *et al*., 1978).

The Nemec *et al.* (2008) rat inhalation reproductive toxicity study showed only a single high-dose malformation considered, at most, equivocally related to treatment.

Table 16: Developmental toxicity studies

Species / Ref	Route	Study type	NOAEC / NOAEL	Findings
Rat (Murray *et al*., 1978)	Inhalation 0, 40 and 80 ppm	Developmental tox (GD 6-15)	Developmental tox: 40 ppm or 80 ppm Maternal tox: 40 ppm	Maternal toxicity as reduced body weight gain and food intake; total malformations very slightly increased at 80 ppm but was not significant
Rat (Saillenfait *et al*., 1993)	Inhalation 0,12,25,50 and 100 ppm 6 hours/day	Developmental tox (GD 6-20)	Developmental tox: 100ppm Foetal and maternal toxicity: 12 ppm (26 mg/m^3)	Maternal toxicity as reduced body weight gain at ≥25 ppm and reduced foetal weight at ≥25 ppm (5% decrease at 25 ppm, reaching 13-15% at 100 ppm). No evidence of a developmental effect.
Rat (Murray *et al*., 1978)	Oral: gavage 0, 10, 25 and 65 mg/kg/day	Developmental tox (GD 6-15)	Developmental tox: 25 mg/kg/day Maternal tox: 10 mg/kg/day	Gastric irritation in dams at 65 and 25 mg/kg/day; significantly decreased BW and one mortality at 65 mg/kg/day in

Species / Ref	Route	Study type	NOAEC / NOAEL	Findings
				dams; increased post implantation loss at 65 mg/kg/day; increased incidence of skeletal and visceral abnormalities and short-tailed foetusses and foetotoxicity (7.4% BW decrease) at 65 mg/kg/day
Rat (Mehrotra *et al*., 1988)	Oral: gavage 0 and 5 mg/kg/day	Developmental tox (GD 5-21)	Maternal and developmental tox: 5 mg/kg/day	Increased CNS levels of 5HT and Noradrenaline and decreased levels of MAO in pups

7.8.3 Summary

Based on the weight of evidence, there were no obvious compound-related effects on fertility in any of the reproductive toxicity studies, even at exposure levels producing toxicity to the parent animals.

The evaluation of developmental toxicity and malformations in the animal studies discussed above leads to the conclusion that very high, maternally toxic, exposures to acrylonitrile results in foetotoxicity, and may result in teratogenicity. The inhalation route is the most relevant to worker exposure. The inhalation studies (Saillenfait *et al.,* 1993 and Murray *et al*., 1978) do not show clear evidence of teratogenicity, despite maternal toxicity. The most recent inhalation developmental toxicity study in the rat (Saillenfait *et al.* 1993), which tested to the highest inhalation concentration, showed decreased foetal body weights at a maternally toxic dose, but no evidence for exposure-related malformations. There was no clear evidence of developmental toxicity in any study in the absence of maternal toxicity.

Based on foetotoxicity (limited reduction in foetal weight) at maternally toxic exposure levels, a conservative NO(A)EC of 12 ppm (26 mg/m^3) from Saillenfait *et al.* (1993) might be considered. The adversity of a 5% decrease in foetal weight at 25 ppm, accompanied with maternal toxicity, is debatable.

7.9 Health-based values based on non-carcinogenic endpoints

Although carcinogenicity is considered to be a critical endpoint for establishing an OEL, the OEL should also be sufficiently protective for non-cancer effects of acrylonitrile, in particular neurotoxicity and nasal irritation.

The non-carcinogenic effect of acrylonitrile is determined by its high acute toxicity by inhalation and skin contact in humans and animals, considered to be due primarily to the release of cyanide from the metabolite CEO. Local irritation, headaches, vertigo and weakness are typical at levels of >5 ppm, with more severe symptoms of tremor, convulsions, unconsciousness and respiratory and circulatory arrest at higher levels of exposure.

Repeated exposure in workers is also associated with neurological and irritant effects, mainly manifesting as irritancy to the nose and throat, coughing and breathing difficulties, headaches and general sickness together with a variety of non-specific clinical observations. Following repeated exposure, some subjective effects seem to start around 1-10 ppm and above, but the data are difficult to assess in relation to dose-response and thus not considered sufficiently robust to use as point of departure for setting a limit value for the non-cancer effects (see section 7.4.1).

Irritation

In rats chronically exposed by inhalation, degenerative and inflammatory changes in the respiratory epithelium of the nasal turbinates and hyperplasia of mucous secreting cells has been observed at levels ≤20 ppm. As starting points to derive a protective level for nasal irritation, a NOAEC of 15 ppm from a two-generation reproductive study in the rat (Nemec *et al.* 2008) and a LOAEC of 20 ppm from a two-year carcinogenicity study in the rat (Quast *et al*., 1980a) can be considered (see section 7.3.2.2). The resulting protective levels are as follows:

1. PoD of 15 ppm for F0 parental generation in Nemec *et al*. (2008)
   - PoD = a NOAEC of 15 ppm from a 2-generation rat inhalation study (6h/d, 7d/wk). This PoD is the lowest level resulting in no treatment-related local irritant effects in the nasal epithelium in F0 males or females. Exposure was initiated when rats were approximately 8 weeks old for 18 weeks with necropsy when rats were 26 weeks old.
   - For workers this PoD converts into a HEC of 10.6 ppm (= 15 ppm x 6/8 x 7/5 x 6.7/10).
   - Subsequently applying a total AF of 10 (1 for remaining interspecies differences[23], 5 for worker intraspecies differences, 2 for extrapolation from sub-chronic to chronic[24]) results in a level of 1.1 ppm.

[23] According to the ECHA guidance Chapter 8 the default factor of 2.5 should be applied where tissue metabolism is a factor as it is prudent to assume that humans would be more sensitive than animals to effects on the respiratory tract irritation. The available information on the mode of action for irritancy suggests indeed that tissue metabolism is a significant factor. However, since the limited available human data indicates that below 1-10 ppm no local irritation is observed in humans, the interspecies factor of 2.5 is not considered justified.

[24] According to the ECHA guidance Chapter 8, a correction for exposure duration of 2 is recommended by default for sub-chronic to chronic extrapolation. The guidance states that the default assessment factor should be used for systemic effects and, in case of toxicity testing by inhalation, for local tissue damage in the respiratory tract. There is no

2. PoD of 20 ppm from Quast et al. (1980a)
   - PoD = a LOAEC of 20 ppm from a two-year inhalation study in rats (6h/d, 5d/wk).
   - For workers this PoD converts into a HEC of 10.1 ppm (= 20 ppm x 6/8 x 5/5 x 6.7/10).
   - Subsequently applying a total AF of 15 (1 for remaining interspecies differences, 5 for worker intraspecies differences, 3 for LOAEC-NAEC extrapolation, 1 for study duration) results in a level of 0.67 ppm.

Since the level derived from Quast et al. (1980a) is from a good quality chronic study, the forward is **0.67 ppm** (1.5 mg/m^3).

In comparison, TCEQ (2013) derived a chronic reference value of 0.0071 mg/m^3 and Kirman *et al*. (2008) subchronic and chronic reference values of 0.1 and 0.06 mg/m^3, respectively for continuous lifetime exposure. The chronic reference values from Kirman *et al*. (2008) and TCEQ (2013) would correspond to an occupational reference value of 0.17 mg/m^3 (0.078 ppm)[25] and 0.02 mg/m^3 (0.009 ppm), respectively.

Neurotoxicity

A NOAEC of 25 ppm may be derived from Gagnaire et al. (1998) based on observed wet hair and hypersalivation in rats, associated with reduced nerve conduction velocities and action potentials at the LOAEL, see section 7.3.2.2. The resulting protective level can be derived as follows:

- PoD = a NOAEC of 25 ppm from a rat inhalation study (6h/d, 5d/wk, 24 weeks).
- For workers this PoD converts into a HEC of 12.6 ppm (= 25 ppm x 6/8 x 5/5 x 6.7/10).
- Subsequently applying a total AF of 10 (1 for remaining interspecies differences[26], 5 for worker intraspecies differences, 2 for extrapolation from sub-chronic to chronic) results in a level of **1.3 ppm** (2.8 mg/m^3).

Foetotoxicity

Based on foetotoxicity at maternally toxic exposure levels, a *conservative* NO(A)EC of 12 ppm (26 mg/m^3) from Saillenfait *et al.* (1993) might be considered. The resulting protective level can be derived as follows:

- PoD = a NO(A)EC of 12 ppm from a rat inhalation developmental toxicity study (6h/d, GD 6-20).
- For workers this PoD converts into a HEC of 8.44 ppm (= 12 ppm x 6/8 x 7/5 x 6.7/10).
- Subsequently applying a total AF of 12.5 (2.5 for remaining interspecies differences, 5 for worker intraspecies differences, 1 for duration of exposure) results in a level of **0.68 ppm** (1.5 mg/m^3).

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substance-specific evidence available that increasing exposure duration does not increase the severity of irritation and thus the default AF is considered justified. Applying the AF is also consistent with the correction of the POD to HEC which has an element of correcting for duration. See also section 7.4.2.4 regarding the mode of action for irritancy.

[25] 0.06 mg/m^3 * 24/8 * 7/5 * 6.7/10 = 0.17 mg/m^3

[26] According to the ECHA guidance Chapter 8 the default factor of 2.5 should be applied. However, since the adversity of the effects observed at the LOAEC in Gagnaire et al. (1998) is unclear and the limited available human data suggests that below 1 ppm no neurotoxicity in humans is observed, the interspecies factor of 2.5 is not considered necessary.

Conclusion

Levels of **0.67 ppm** (1.47 mg/m^3) and **1.3 ppm** (2.8 mg/m^3) may be derived as 8-hour TWA levels appropriately protecting for nasal irritation and neurotoxicity, respectively[27]. Current OELs in the range of 1-2 ppm may not sufficiently protect against non-carcinogenic effects.

7.10 Mode of action (MoA) and Adverse Outcome Pathways (AoP) considerations

7.10.1 Carcinogenic endpoints

Many studies show that acrylonitrile is genotoxic *in vitro* with generally positive results mainly requiring metabolic activation, although, it is considered only weakly mutagenic. The results in tests carried out *in vivo* are more mixed with a number of negative results. It has been suggested that the genotoxic agent is the metabolite, CEO and that the negative results *in vivo* are due to a CEO detoxification mechanism by glutathione not present *in vitro*, but this remains unproven. It is clear that CEO does react directly with DNA, while acrylonitrile does so only very weakly if at all. Experimental animal data provide evidence that acrylonitrile is a multi-organ carcinogen, with the most consistent endpoint being tumours of the CNS following inhalation or oral exposure in rats and orally in mice. Quast *et al*. (1980a) reported a high incidence of astrocytomas in the brain (now identified as microgliomas; Kolenda-Roberts *et al*., 2013) and spinal cord following inhalation exposure of rats to acrylonitrile for two-years (6h/d, 5d/wk)

The potential carcinogenicity of acrylonitrile in occupationally exposed populations has been investigated in several epidemiological studies. Early studies carried out in the 1970s and 1980s suggested a possible increased risk of lung cancer and some other cancer types among workers exposed to acrylonitrile. Later on four large, high quality occupational cohort studies were initiated to further explore these preliminary observations. These studies, including their latest follow-ups, have not been able to confirm an increased risk of cancer in acrylonitrile exposed workers. However, it is extremely difficult to verify or falsify low risk increases for rare diseases in occupational cohort studies. Consequently, it is difficult to exclude an agent as a human carcinogen by epidemiologic studies. However, the weight of evidence available suggests either that acrylonitrile is not a human carcinogen or that it produces only small increases in cancer risk, at least at the exposure levels that have occurred in North American and Western Europe.

The mode of action leading to brain tumour formation following exposure to acrylonitrile is not fully understood. Acrylonitrile, CEO and cyanide are able to cross the blood-brain barrier and could therefore act via direct- or indirect genotoxicity, or through a non-genotoxic mechanism. However, a lack of DNA adducts and an absence of induction of DNA repair mechanisms following exposure of brain tissue (*in vivo* and *in vitro*) to acrylonitrile suggest indirect and/or non-genotoxic mechanisms are involved. This is further supported by evidence showing a threshold for oxidative damage in astrocytes exposed to acrylonitrile, and a reversible loss of gap junction intercellular communication in astrocytes exposed to acrylonitrile. However, the oxidative DNA seen in the brain is not accompanied by other markers of oxidative stress such as lipid peroxidation or cytochrome induction. Suggestion has been made that difference in metabolism between rodents and

[27] Conservatively, a level of 0.68 ppm for foetotoxicity might be derived as well.

humans may contribute to an observed lack of cancers in exposed workers in comparison to the multi-organ carcinogenicity seen in rodents.

It does appear that oxidative damage to DNA may play a major role in the carcinogenicity of acrylonitrile, perhaps through its metabolite, CEO. As techniques have developed, this mechanism has been shown in a number of chemicals which also have genotoxic mechanisms, such as benzene (e.g. Fenga *et al*., 2016). There have been animal carcinogens such as peroxisome proliferators and chemicals which interfere with α-2-microglobulin, where the mechanism of carcinogenicity has been proven to be irrelevant to humans. At present, there is no clear proven mechanism of action for the carcinogenicity (and positive genotoxicity *in vitro*) of acrylonitrile which has been shown to be irrelevant to humans.

The study of Kirman *et al*. (2005), which has been subjected to an independent, external peer review (Haber and Patterson, 2005), concluded that the weight-of-evidence for indirect genotoxic mechanisms, mainly oxidative damage, caused by CEO, was such that risk assessment based on non-linear extrapolation was the most relevant. They further concluded that the data were insufficient to rule out any contribution due to direct DNA reactivity, but that the weight of evidence did not support such a mechanism as a major contributor to rodent carcinogenesis (see section 8.1 for further details).

In weighing all the available evidence, a 'mode of action-based threshold'[28] for the carcinogenic effects is considered plausible and a limit value was derived for acrylonitrile.

7.10.2 Irritation and neurotoxicity

The most sensitive effects non-cancer effects for risk assessment are local irritant effects in the nasal epithelium and neurotoxicity. The mode of action for irritation and neurotoxicity are briefly described in sections 7.4.2.4 and 7.3.2.2.

7.11 Lack of specific scientific information

To date, the carcinogenic mode of action of acrylonitrile is not fully resolved (see section 7.10.1).

At present, there is no information on the mechanisms involved in the formation of tumours in rodents exposed to acrylonitrile at sites other than the brain and CNS, some of which may involve genotoxicity. A recent paper did not find oxidative damage nor DNA reactivity in the Zymbal gland after short-term acrylonitrile treatment (Williams *et al*., 2017).

In addition, the body of evidence for the involvement of oxidative stress in the rat brain tumours is from in vitro and oral in vivo studies, but no mechanistic in vivo studies via the inhalation route are available.

Studies so far suggest that metabolism of acrylonitrile may well play a role in its toxicity, including the production of CEO and its possible carcinogenic effect. Further information on the pathways involved and the possible threshold of acrylonitrile concentrations needed for their involvement would inform the risk assessment process.

There is little information on why acrylonitrile is genotoxic *in vitro*, while its effects and that of its metabolite, CEO, are much less convincing *in vivo*.

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[28] Regarding the term "mode of action-based threshold" see RAC and SCOEL (2017).

There appear to be differences in both metabolism and potential carcinogenic processes involved in the response of animals and humans to exposure to acrylonitrile. Further studies to clarify these differences would improve the accuracy of risk assessment.

It has been pointed out that it is extremely difficult to verify or falsify low risk increases for rare diseases in occupational cohort studies and consequently that epidemiological studies on the health effects of exposure to acrylonitrile may not be sensitive enough to detect rare events which may occur such as brain tumours, a potential target for acrylonitrile exposure. Future follow-ups of the existing large, good quality cohort studies could reduce this uncertainty concerning the human carcinogenicity of acrylonitrile by further narrowing the statistical confidence limits of the calculated effect estimates. ECHA has been informed that an update of the US-NCI cohort earlier published by Blair et al (1998) is anticipated in 2018.

8. Cancer Risk Assessment and exposure limit values

The overall weight of evidence supports to derive an OEL assuming a mode of action-based threshold. At this level of exposure there is no significant residual cancer risk expected. However, as the possibility of an occupational cancer risk cannot totally be excluded, an illustrative dose-response relationship for carcinogenicity is derived by linear extrapolation to estimate the upper boundary of excess risk (if any) at this OEL.

8.1 Non-linear (thresholded) cancer assessment

Kirman *et al*. (2005) derived non-linear and linear risk estimates based on US EPA Benchmark dose software and using the pooled CNS tumour data from 6 studies (3 oral and 3 inhalation studies), for males and females, leading to 12 data sets. They also used data from a number of human epidemiological studies to estimate internal doses (peak CEO in brain). The authors suggested that the weight-of-evidence for mechanisms such as oxidative damage and the effect of metabolites such as CEO, supported the use of non-linear extrapolation. They further stressed that a comparison of the exposure-response data for rats and humans are inconsistent with linear low-dose extrapolation for human cancer risks (for lung and brain tumours).

For their non-linear extrapolation the external exposure levels from 3 oral and 3 inhalation studies with acrylonitrile were converted into internal dose levels (peak CEO in brain[29]) using a rat PBPK model. Validation of the rat model was carried out using *in vivo* data (Gargas *et al.,* 1995; Kedderis *et al.,* 1996) and *in vitro* data (Kedderis *et al.,* 1993; Kedderis & Batra, 1993). The CNS tumour data were then pooled together. Even though none of the benchmark dose models in the USEPA BMDS software provided a statistically acceptable fit, the gamma model was selected since it provided the best overall fit.

The lower confidence limit of 5% extra cancer risk (LED05) of 0.014 mg CEO/l was taken as a PoD, a value corresponding to a concentration region where an increase in brain tumour incidence becomes apparent.

A human PBPK model (Sweeney *et al.* 2003) was subsequently used to derive an equivalent human inhalation level of 21.3 mg/m^3 (9.8 ppm). As no *in vivo* pharmacokinetic data was available for humans, Sweeney et al. (2003) derived a scaling factor for *in vitro* to *in vivo* scaling from the rat data, and applied this to data from human *in vitro* studies.

[29] Based on a mode of action of oxidative stress, the metabolites, CEO and cyanide were estimated with brain CEO concentrations used as a surrogate for cyanide. Estimation of peak concentration or cumulative exposure (AUC) were made. Peak CEO concentration in the brain was considered to provide a better correlation with dose and effect and was chosen as internal dose metric.

This PoD was then used to derive a cancer reference dose by division with an overall uncertainty factor (UF) of 220. This overall UF was composed from individual UFs as follows:

- UF for interspecies variation: the US EPA toxicokinetic component of 3.2 was set to one because the use of the rat and human PBPK models (Kedderis *et al.* 1996; Sweeney *et al.* 2003) improves the confidence in the interspecies extrapolation and only the US EPA toxicodynamic factor of 3.2 was withheld;

- UF for intraspecies variation: the US EPA toxicokinetic component of 3.2 was set to 2.2 based on the model coefficient of variation of 0.72 for CEO AUC in the brain in the human PBPK model (Sweeney *et al.* 2003): assuming a normal distribution, the 95th percentile of CEO AUC in the brain would then be 2.2-fold higher than the mean value (1 + 1.64*0.72). The default toxicodynamic factor according to USEPA guidelines of 3.2 was withheld. The resulting UF thus was 7;

- UF to account for the severity of the response: an UF of 10 was used since it was *"recognised that a 5% response level reflects a fairly significant response, and cannot be treated as a NOAEL for an effect of this severity".* In the review of the methodology of Kirman *et al*. (2005) by 12 expert toxicologists (Haber and Patterson, 2005), 10 of the 12 experts agreed that the 5% extra cancer risk was a better basis for the risk assessment with two suggesting 1%, with just one expert suggesting a lower than 10 severity assessment factor if a 1% extra cancer risk value was used.

- No UFs for study duration or limitations to the database were applied.

Based on the above, a reference dose of 0.1 mg/m^3 (0.045 ppm) was derived. The authors concluded that air concentrations below 0.1 mg/m^3 (about 0.05 ppm) were "not expected to pose an appreciable risk to human populations exposed to AN" (Kirman *et al.* 2005).

The value of 0.1 mg/m^3 (0.045 ppm) was derived for continuous exposure and not for occupational exposure. The corresponding occupational exposure is **0.13 ppm (0.28 mg/m^3)** following multiplication with a factor of 2.814 (24/8 x 7/5 x 6.7/10) as clarified by Kirman (2017) in the public consultation on the draft of the current report.

Strother & Kirman (2011, as reported in the comment by Kirman 2017 in the public consultation on the draft of the current report) further analysed the data in Kirman *et al.,* (2005). Their analysis was presented at the US SOT meeting and is available only as a poster. The analysis contained 3 components:

1. Stepwise addition of 12 nonzero dose groups (covering observations in 846 animals) indicates no evidence of a dose-response relationship below 0.012 mg/L for peak CEO in brain (corresponding to about 24 ppm occupational exposure). It is noted that Figure 1 of the poster uses a logarithmic scale on the x-axis but not on the y-axis which may give a false impression of a threshold. Figure 4 in Kirman *et al.* (2005) is a better representation of the data. Visual inspection of the data indeed shows that effects in the low dose range are within the variation in the controls (which is to be expected at low doses) and that at around 0.012 mg/L incidences start to be above background incidences in the controls.
2. In a second analysis, a threshold term was introduced in a hockey stick model. The model did not result in a statistically significant threshold for the combined or oral studies, but for the inhalation studies, a threshold of 0.0063 mg CEO/L was derived (the corresponding occupational level is not given).
3. The dose-response also correlated with published markers of oxidative stress (8-OH-dG, oxidative stress; Pu *et al.*, 2009), but not with direct DNA damage.

This analysis may be seen to add some further confidence to non-linearity.

It is noted that in most assessments of acrylonitrile so far the non-threshold approach has been taken. Exceptions are Kirman *et al.* (2005) and TCEQ (2013) who preferred a non-

linear approach over the linear approach. Based on the weight of evidence for carcinogenicity in humans, an OEL assuming a mode of action-based threshold may be derived using the assessment in Kirman *et al.* (2005) as in the following.

Derivation of OEL assuming a mode of action-based threshold

Converting the BMDL05 of 21.3 mg/m^3 (9.8 ppm) for continuous exposure to a value for occupational exposure gives a BMDL05 of 60.0 mg/m^3 (27.6 ppm) for workers (correction factor of 24/8 x 7/5 x 6.7/10 = 2.814, see above). This BMDL05, based on pooled CNS tumour data from 3 oral and 3 inhalation studies, is used to derive an OEL for acrylonitrile. Kirman *et al.* (2005) do not report a BMDL05 for the pooled 3 inhalation studies (thus excluding the oral studies) which may have been a more appropriate alternative considering the oral route of exposure is not relevant for workers. However, Kirman *et al.* (2005) do indicate that the data sets are not statistically dissimilar and therefore are appropriate to combine.

By applying a total assessment factor of 62.5 to the BMDL05 of 60.0 mg/m^3 (27.6 ppm), a mode of action-based threshold[30] limit value of 1 mg/m^3 (0.45 ppm) is derived. This overall assessment factor consists of the following individual AFs:

- AF for interspecies differences: 2.5
  As in Kirman *et al.* (2005), for the toxicokinetic differences an AF of 1 was chosen because the use of the rat and human PBPK models improves the confidence in the interspecies extrapolation (see above).
  However, the default AF for toxicodynamic differences of 2.5 (according to ECHA guidance) was considered appropriate because a potentially higher sensitivity of humans cannot be completely excluded, given that the detection of low risk increases for rare tumours such as of the brain would require extremely high numbers of exposed subjects.
- AF for intraspecies differences: 5
  As in Kirman *et al.* (2005), the toxicokinetic component of the AF for intraspecies differences was set to 2.2 based on the variability analysis of the PBPK model (see above).
  The default AF for intraspecies is 5 for workers according to ECHA guidance. The guidance is not explicit on the composition of this AF, but assuming equal contributions of the toxicodynamic and toxicokinetic component, an AF of 2.24 for toxicodynamic differences seems appropriate to retain.
- AF for issues related to dose-response: 5
  Whereas for non-cancer effects a BMDL05 is generally considered comparable to a NOAEL, for cancer 5% may be seen as a fairly significant response for such a severe effect and therefore the BMDL05 level could be seen as an effect level (LOAEL). The ECHA guidance suggests to use an AF of 3 – 10 for extrapolation of the LOAEL to the NAEL. The ECHA guidance foresees also the possibility to apply AF for exceptional cases of serious effects. However, the epidemiology data indicates that the cancer risk to humans is low at 0.5 ppm, if any. Overall, an AF of 5 for issues related to dose-response appears to be justified.

In conclusion, a mode of action-based threshold limit value of 1 mg/m^3 (0.45 ppm) is derived for acrylonitrile (8-hour TWA). This level will also be sufficiently protective against non-cancer effects (see section 7.9).

[30] Regarding the term “mode of action-based threshold” see RAC and SCOEL (2017).

8.2 Linear cancer risk estimates (illustrative)

As mentioned in the previous section, at the limit value of 1 mg/m^3 (0.45 ppm) no significant residual cancer risk expected. However, as the possibility of an occupational cancer risk cannot totally be excluded, an illustrative dose-response relationship for carcinogenicity is derived by linear extrapolation to estimate the upper boundary of excess risk (if any) at this OEL.

A linear extrapolation of the CNS tumour (both benign and malignant; the malignant tumours have now been identified as microgliomas rather than mixed gliomas and astrocytomas; Kolenda-Roberts *et al.,* 2013) incidence observed in a rat inhalation study (Quast *et al.* 1980a) was used to characterise the cancer dose-response relationship for workers exposed to acrylonitrile. The study by Quast *et al.* (1980a) was chosen as a point of departure since it is the most complete study on acrylonitrile administered by the most appropriate route for consideration of occupational exposure. There are a number of good quality oral studies as well but they would require route-to-route extrapolation in order to obtain an estimate for inhalation exposure in humans. Such extrapolation introduces significant uncertainties (e.g. related to first pass metabolism which is prominent with acrylonitrile).

Table 17 shows the results of Quast *et al.* (1980a) with the tumour incidence corrected for mortality as in the European Union risk assessment (EC, 2004).

Table 17: Brain tumours and pre-neoplastic changes in rats following inhalation exposure for up to 2 years (Quast *et al.*, 1980a; EC, 2004)

Brain tumours and pre-neoplastic changes[1]	Acrylonitrile exposure level ppm (mg/m^3)		
	0	20 (44)	80 (176)
Focal glial cell proliferation (Benign)			
Male	0/97	0/93	7/83[2]
Female	0/99	4/99	4/99
Astrocytomas (Malignant)			
Male	0/97	4/93	15/83[2]
Female	0/99	4/99	17/99[2]
Total (Benign + Malignant)			
Male	0/97	4/93	22/83[2]
Female	0/99	8/99[2]	21/99[2]

[1]These animal numbers have been adjusted for mortality (death or euthanasia at 6 months or earlier; EU RAR, 2004)
[2]These incidences are significantly higher than control incidence.

An estimate of the T25 has been derived from the results of the Quast *et al.* (1980a) study and using the incidence of benign and malignant tumours at **20 ppm** of 8/99 (8.1%) which was significant in females. This PoD is preferred over the one taken in the European Union risk assessment (male malignant tumours only, at 80 ppm) as the lowest concentration with significantly increased tumour incidence (EC, 2004). As there is a clear progression from benign to malignant tumours, their incidence can be combined for the risk assessment.

The control incidence in this study was 0, thus the calculation for the rat inhalation T25 for female rats is as follows:

- The rat inhalation T25 for females = 20 x 25/8.1 = 61.7 ppm

The inhalation T25 of 61.7 ppm applies for lifetime exposure, 6 hours/day, 5 days/week for 104 weeks (lifetime exposure).

A T25 for workers' inhalation exposure is calculated as follows:

- Light activity for workers is assumed during an exposure time of 8 h/day, 5 days/week, 48 weeks/year for 40 years out of a lifetime of 75 years.
- Activity driven difference for workers (standard respiratory volume for humans of 6.7 / respiratory volume for workers of 10).
- Inhalation workers' T25 = 61.7 x 6/8 x 5/5 x 52/48 x 75/40 x 6.7/10 = 62.8 ppm (or 136.3 mg/m^3)

Thus, the excess lifetime brain cancer risk corresponding to 1 ppm = 0.25/62.8 = 4 x 10^{-3} ; or **the excess lifetime brain cancer risk corresponding to 1 mg/m^3 = 0.25/136.3 = 1.8 x 10^{-3}.**

Using only one of the two dose level in the dose-response curve has limitations and the estimated T25 (25% tumour incidence level) of 61.7 ppm is clearly conservative given that the observed tumour incidence was 21.2% in females at the highest dose of 80 ppm. The above dose-response relationship therefore should be seen as the **high end** of the illustrative linear dose-response estimation.

Therefore, there is merit in considering other approaches as well. AGS (2010) used female brain tumour incidence data from both dose levels in the Quast *et al.* (1980) study which resulted in an excess lifetime brain cancer risk of 1.4 x 10^{-3} per mg/m^3 (based on 8.2 x 10^{-3} per mg/m^3 for continuous lifetime from Felter & Dollarhide 1997).

Kirman et al. (2005) combined male and female brain tumour incidences from all dose levels in all availabe inhalation and oral carcinogenicity studies. Linear extrapolation below the BMDL05 results in an excess lifetime brain cancer risk of **4 x 10^{-4} per mg/m^3** (based on a continuous lifetime risk value of 2.3 x 10^{-3} per mg/m^3 reported by Kirman et al., 2005[31]). The latter relation dose-response relationship can be seen as the **low end** of the illustrative linear dose-response estimation.

Assuming linearity of response, the cancer risk for lifetime exposure to each unit amount of acrylonitrile will increase in proportion. The excess lifetime brain cancer risk can conservatively also be interpreted as an excess lifetime cancer risk that covers cancers at other sites.

[31] The continuous lifetime risk value is converted to an occupation risk level using a conversion factor of 5.7 (24/8 x 7/5 x 52/48 x 75/40 x 6.7/10), see also Kirman (2017) in the public consultation on the draft of the current report.

The high and low-end cancer estimates of the illustrative linear dose-response relationship are presented in Table 18. Please note however that, based on the weight of evidence of the available data (see sections 7.6, 7.7, and 7.10), also the low estimate is considered as an upper boundary of excess risk (if any).

Table 18 High and low end estimates of excess lifetime brain cancer risk (illustrative)

Acrylonitrile concentration		Excess brain cancer risk	
ppm	mg/m³	low end	high end
1	2.2	8.8×10^{-4}	4×10^{-3}
0.45	**1**	**4×10^{-4}**	**1.8×10^{-3}**
0.25	0.56	2.2×10^{-4}	1×10^{-3}
0.11	0.25	1×10^{-4}	4.5×10^{-4}
0.1	0.22	8.8×10^{-5}	4×10^{-4}
0.045	0.1	4×10^{-5}	1.8×10^{-4}
0.025	0.056	2.2×10^{-5}	1×10^{-4}
0.011	0.025	1×10^{-5}	4.5×10^{-5}
0.01	0.022	8.8×10^{-6}	4×10^{-5}
0.0045	0.01	4×10^{-6}	1.8×10^{-5}
0.0025	0.0056	2.2×10^{-6}	1×10^{-5}

8.3 Short Term Exposure Limits (STELs)

Acrylonitrile is classified for acute inhalation toxicity in CLP Category 3 (H331: Toxic if inhaled). Signs of acute toxicity have also been reported for humans (see section 7.2.1). There may be occupational tasks at industrial sites presenting a short term acute exposure risk. So, a STEL may be warranted, allowing the OEL (8-hour TWA) to be exceeded for a maximum of 4x 15 minutes in 8 hours, with an interval of 60 minutes between two peaks.

In setting the STEL for a carcinogenic substance, the dose-time product is in principal a decisive factor since the total exposure over a shift must remain below the 8-hour TWA. It is important that detoxifying metabolic pathways still obey linear kinetics at the concentration peaks. Assuming this is the case for acrylonitrile, given the major role for an indirect MoA via oxidative stress, and given further that the exposure pattern might be considered more continuous than peak-like, a STEL of 4x the 8-hour TWA may be appropriate. The resulting STEL of 4 mg/m³ (1.8 ppm) is protective against irritation/neurotoxic effects; limited data available for humans seem to indicate that levels below 5 ppm following acute exposure do not appear to result in local irritation and neurotoxicity (see section 7.2.1).

8.4 Biological Limit Value (BLV) and Biological Guidance Value (BGV)

SCOEL (2003), stated: "*A skin notation is supported by reports of severe industrial intoxications following skin contact (Thier et al. 2000). This calls for effective means of biological monitoring. Available methods have been evaluated (DFG 1994). In industrial practice, suitable strategies could reasonably be based on analysis of acrylonitrile adducts to blood proteins (haemoglobin and/or albumin; Thier et al. 1999, 2000, 2002).*".

N-(2-Cyanoethyl)valine (CEV) is a specific biomarker for the assessment of acrylonitrile exposure (Fennell *et al.*, 2000; Colenbie *et al.*, 2017). The analytical methods have a limit

of detection (LoD) of about 0.1–1 pmol CEV/g globin (Tavares *et al.*, 1996, Licea Peres *et al.*, 1999).

The relationship between the air concentration and CEV in blood (erythrocyte) described by DGUV (2016) is presented in Table 8. This correlation is based on measured air concentrations (0.14 ppm (0.3 mg/m^3), 0.23 ppm (0.5 mg/m^3), 0.45 ppm (1 mg/m^3)) and corresponding measured concentrations of CEV in blood. For higher concentrations, the correlation is based on linear extrapolation from the relation found at 1 mg/m^3. As the practical limit value of 1 mg/m^3 (0.45 ppm) equals one of the concentrations underlying the EKA correlation, the corresponding CEV level of 60 µg CEV/L blood (erythrocyte fraction of whole blood) appears to be an appropriate biological limit value (with sampling time after at least 3 months of exposure).

CEV is a marker for long term exposures and is influenced by other sources of acrylonitrile (e.g. smoking). Background levels in smokers are >50 pmol/g globin (>1.2 µg CEV/L blood). Knowing that around 4 (0.8 to 9.2) µg CEV/L blood or 8.5 fmol/mg globin/cigarette/day (Fennell *et al.*, 2000) could be due to smoking, this can be accounted for when evaluating measured CEV concentration in blood. In non-occupationally exposed non-smokers, the CEV level in blood is <10 pmol/g globin (<0.24 µg CEV/L blood).

Since a BLV can be derived, no biological guidance value (BGV) is recommended. It is noted that for adult non-smokers the MAK Commission (DFG, 2016) has established a biological reference value (BAR)[32] of 0.3 µg CEV/L blood (erythrocyte fraction of whole blood)

8.5 Notations

Skin

Acrylonitrile is readily absorbed via the dermal route. SCOEL (2003) suggesting a skin notation because of reports of severe industrial intoxications following skin contact (Thier *et al.,* 2000).

Hence, a 'skin' notation is proposed to be assigned to the OEL[33].

Noise

Four acute animal studies from the same lab were identified studying ototoxicty of acrylonitrile (Fechter *et al.* 2003,2004 and Pouyatos *et al.* 2005,2007). In each of these studies acrylonitrile was administered subcutaneously 50 mg/kg bw/day for 1 to 5 days.

[32] A BAR describes the background level of a substance which is present concurrently at a particular time in a reference population of persons of working age who are not occupationally exposed to this substance. The BAR are based on the 95th percentile without regarding effects on health. It must be taken into account that the reference level of the background exposure can be influenced by such factors as age, sex, social status, residential environment, life style and geographical region.

[33] A 'skin notation' warns of the possible significant contribution of dermal absorption to the total body burden. A skin notation does not relate to and is not intended to give warning of direct effects on the skin such as corrosivity, irritation and sensitisation (there is harmonised classification as skin irritant cat. 2 and skin sensitisation cat. 1A for acrylonitrile).

Overall, it would appear that potentiation of noise-induced hearing loss occurs from high subcutaneously administered doses of acrylonitrile.

Human evidence for ototoxicity is absent and no chronic animal studies are available. The animal experiments were of short duration and used the subcutaneous route, making the results difficult to interpret. Overall, the evidence for ototoxicity is weak and does not warrant a noise notation[34].

[34] Also when attempting to derive a protective level for ototoxicity from these studies a noise notation appears not warranted. Assuming that dermal absorption is 100% in rats following subcutaneous injection and inhalation absorption is 100% in humans, following a correction factor 6.7/10 and dividing by 0.38 m^3/kg bw/day, the exposure level of 50 mg/kg bw/day corresponds to a human equivalent of 88 mg/m^3. Applying an AF of 75 (the default AF of 2.5 for interspecies, 5 for intraspecies and a factor of 6 for duration of exposure), a level of 1 mg/m^3 (0.5 ppm) could be derived which is more or less equal to the proposed OEL and thus no “noise” notation appears to be necessary.

REFERENCES

Abdel Naim AB, Hamada FM, Abdel Aziz AH, Ahmed AE (1994). Acrylonitrile (VCN)-induced testicular toxicity in the rat. The Toxicologist. 14:87.

Abdel Naim AB (1995). Studies on the mechanisms of testicular toxicity induced by acrylonitrile. Ph. D Thesis, Department of Pharmacology and Toxicology, Al-Azhar University, Cairo.

Abdel-Rahman SZ, Nouraldeen AM, Ahmed AE (1994). Molecular interaction of [2,3-14C] acrylonitrile with DNA in gastric tissue of rat. Journal of Biochemical Toxicology. 9(4):191-8.

Acrylonitrile EU REACH Consortium (2017). Comments submitted by the Acrylonitrile EU REACH Consortium in the public consultation on the proposal by ECHA in support of occupational exposure limit values for Acrylonitrile in the workplace. Comment reference number 28. All public comments submitted in the consultation are available at https://echa.europa.eu/echas-executive-director-requests-to-the-committees-previous-consultations/-/substance-rev/17808/term

Ageeva ST (1970). The condition of the metabolism of mediating substances in workers producing acrylonitrile. Trudy Saratov Meditsinskogo Instituta, 78, 10–13 (in Russian), as reported in IARC (1999).

AGS (2010). Exposure-risk relationship for acrylonitrile (CAS 107-13-1). Committee on Hazardous Substances (Ausschuss für Gefahrstoffe, AGS), the German Federal Institute for Occupational Safety and Health (Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, BAuA), Dortmund, 2010. Available at: https://www.baua.de/EN/Service/Legislative-texts-and-technical-rules/Rules/TRGS/pdf/910/910-acrylonitrile.pdf?__blob=publicationFile&v=2

Ahmed AE, Farooqui MYH, Upreti RK, El-Shabrawy O (1982). Distribution and covalent interactions of (1-14C) acrylonitrile in the rat. Toxicology. 23:1159-175.

Ahmed AE, Abdel-Aziz AH, Abdel-Rahman SZ, Haque AK, Nouraldeen AM, Shouman SA (1992a). Pulmonary Toxicity of Acrylonitrile: Covalent Interaction and Effect on Replicative and Unscheduled DNA Synthesis in the Lung. Toxicology. 76(1):1-14.

Ahmed AE, Abdel-Rahman SZ, Nour-Al Deen AM (1992b). Acrylonitrile interaction with testicular DNA in rats. Journal of Biochemical Toxicology. 7:5-11.

Ahmed AE, Nouraldeen AM, Abdel-Rahman SZ, Rajaraman S (1996). Role of glutathione modulation in acrylonitrile-induced gastric DNA damage in rats. Archives of toxicology. 70(10):620-7.

ARB MLD (2011) The Measurement of Acrylonitrile in Ambient Air Using Solid Sorbent Tubes and Thermal Desorption. State of California Air Resources Board, Monitoring and Laboratory, Organic Laboratory Section, 1927 13th Street, Sacramento, CA 95811. Available at: https://arb.ca.gov/airwebmanual/owl-ultralite/Documents/Draft/AMTAC/media/AWMAAcrylonitrileExtendedAbstract.pdf

Asakura M, Noguchi T, Sugiyama Y, Inoue M, Satake H (1994). Research Study on Evaluation of Mutagenicity for Existing Chemical Substances.-Research Study on Industrial Safety and Health in 1994-3) In vitro Chromosomal Aberration Test on Cultured Mammalian Cells. Testing laboratory: Japan Industrial Safety and Health Association:

Japan Bioassay Research Center. Owner company: Japan Industrial Safety and Health Association.

ATSDR [Agency for Toxic Substances and Disease Registry] (1990) Toxicological profile for acrylonitrile. Atlanta, GA, United States Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/toxprofiles/tp125.pdf

Babanov GP, Kljucikov VN, Karajeva ZV (1959). Clinical symptoms of chronic poisoning by acrylonitrile. Vrachebnoe Delo. 8:833–836 (Cited in Jacubowski et al. (1987)).

Bader M and Wrbitzky R (2006) Follow-up biomonitoring after accidental exposure to acrylonitrile - Implications for protein adducts as a dose monitor for short-term exposures. Toxicology Letters. 162(2-3):125-131.

Bakker JG, Jongen SMJ, van Neer FCJ, Neis JM (1991). Occupational contact dermatitis due to acrylonitrile. Contact Dermatitis. 24:50-53.

BAUA (2014). Risk Management Options Analysis Conclusion Document for Acrylonitrile. Member State: Germany (Federal Institute for Occupational Safety and Health).

Baxter R (1979). Evaluation and control of industrial exposure to acrylonitrile. Annals of Occupational Hygiene. 22:429-35.

Beliles RP, Paulin HJ, Makris NG, Weir RJ (1980). Three-generation reproduction study of rats receiving acrylonitrile in drinking water. Litton Bionetics Inc. Project no. 2660, OTS0536313.

Benn T and Osborne K (1998). Mortality of United Kingdom acrylonitrile workers – an extended and updated study. Scandinavian Journal of Work, Environment and Health. 24(2):17-24.

Beskid O, Dulek Z, Solansky I and Sram RJ (2006). The effects of exposure to different clastogens on the pattern of chromosomal aberrations detected by FISH whole chromosome painting in occupationally exposed individuals. Mutation Research. 594:20-29.

Bhooma T, Padmavathi B, Devaraj SN (1992). Effect of acrylonitrile on the procoagulant activity of rat lung. Bulletin of Environmental Contamination and Toxicology. 48:321-326.

Bigner DD, Bigner SH, Burger PC, Shelburne JD, Friedman HS (1986). Primary brain tumours in Fischer 344 rats chronically exposed to acrylonitrile in their drinking-water. Food and Chemical Toxicology. 24(2):129-137.

Biodynamics (1980a). A twenty-four month oral toxicity/carcinogenicity study of acrylonitrile administered in the drinking water to Spartan rats. Prepared by Biodynamics, Inc., Division of Biology and Safety Evaluation, East Millstone, NJ, under project no. 77-1745 for Monsanto Company, St Louis, Mo.

Biodynamics (1980b). A twenty-four month oral toxicity/carcinogenicity study of acrylonitrile administered in the drinking water to Fischer 344 rats. Prepared by Biodynamics, Inc., Division of Biology and Safety Evaluation, East Millstone, NJ, under project no. 77-1746 for Monsanto Company, St Louis, Mo.

Biodynamics (1980c). A twenty-four month oral toxicity/carcinogenicity study of acrylonitrile administered by intubation to Spartan rats, Vol. 1-2. Prepared by Biodynamics Inc., East Millstone, NJ, for Monsanto Company, St. Louis, MO.

Blair A, Stewart PA, Zaebst DD, Pottern L, Zey JN, Bloom TF, Miller B, Ward E, Lubin J (1998). Mortality of industrial workers exposed to acrylonitrile. Scandinavian Journal of Work, Environment and Health. 24(2):25-41.

Boffetta P, McLaughlin JK, La Vecchia C, Tarone RE, Lipworth L, Blot WJ (2008). False-positive results in cancer epidemiology: A plea for epistemological modesty. Journal of the National Cancer Institute. 100(14):988-995.

Bolt HM (2003). Genotoxicity – threshold or not? Introduction of cases of industrial chemical. Toxicology Letters. 140-141:43-51.

Brewer WE (1976). 90-day subacute vapour inhalation toxicity study with acrylonitrile in beagle dogs, albino rats and albino mice (Industrial Biotest Report no. 74-42, prepared for the Monsanto Company).

Butterworth BE, Eldridge SR, Sprankle CS, Working PK, Bentley KS, Hurtt ME (1992). Tissue-specific genotoxic effects of acrylamide and acrylonitrile. Environmental and Molecular Mutagenesis. 20:148-155.

Caito S, Park M, Aschner M (2017) Resistance of mouse primary microglia and astrocytes to acrylonitrile-induced oxidative stress. Neurotoxicology. 63:120-125.

Cave M, Falkner KC, Henry L, Costello B, Gregory B, McClain CJ (2011). Serum cytokeratin 18 and cytokine elevations suggest a high prevalence of occupational liver disease in highly exposed elastomer/polymer workers. Journal of Occupational and Environmental Medicine. 53(10):1128-1133.

Chen JL, Walrath J, O'Berg MT, Burke CA, Pell S (1987). Cancer incidence and mortality among workers exposed to acrylonitrile. American Journal of Industrial Medicine. 11(2):157-63.

Cheng SF, Chen ML, Hung PC, Chen CJ & Mao IF (2004). Olfactory loss in poly (acrylonitrile-butadiene-styrene) plastic injection-moulding workers. Occupational Medicine. 54:469-474.

Coggon DM and Cole P (1998). Acrylonitrile and human cancer--an overview. Scandinavian Journal of Work, Environment and Health. 24(2):81-82.

Cole P, Mandel JS, Collins JJ (2008). Acrylonitrile and cancer: A review of the epidemiology. Regulatory Toxicology and Pharmacology. 52:342-351.

Colenbie S, Buylaert W, Stove C, Deschepper E, Vandewoude K, De Smedt T, Bader M, Göen T, Van Nieuwenhuyse A & De Paepe, P. (2017). Biomarkers in patients admitted to the emergency department after exposure to acrylonitrile in a major railway incident involving bulk chemical material. International Journal of Hygiene and Environmental Health. 220(2):261-270.

Collins JJ, Acquavella JF (1998). Review and meta-analysis of studies of acrylonitrile workers. Scandinavian Journal of Work, Environment and Health. 24(2): 71-80.

Collins JJ, Page LC, caporossi JC, Utidjian HM, Lucas LJ (1989). Mortality patterns among employees exposed to acrylonitrile. Journal of Occupational Medicine. 31:368-371.

Collins JJ and Strother DE (1999). CNS tumors and exposure to acrylonitrile: Inconsistency between experimental and epidemiology studies. Neuro-oncology. 1:221-230.

Collins JJ, Cheng R, Buck GM, Zhang, J, Klebanoff M, Schisterman E, Scheffers T, Ohta H, Takaya K, Miyauchi H, Markowitz M, Divine B, Tsai S (2003). The Feasibility of Conducting a Reproductive Outcome Study of Chinese Acrylonitrile Workers. Journal of Occupational and Environmental Medicine. 1:29-32.

Commission of European Communities (1983) Reports of the Scientific Committee for Food (13th series). Luxembourg. NTIS No. PB83-172759. Cited in ATSDR 1990.

Dang Y, Zhao Q, Luo B, Pan L, Wei Q, Zhang R, Fan Q, Chen J, Chang R, Zhang J & Li Z (2017a) Effects of acrylonitrile-induced oxidative stress on testicular apoptosis through activation of NFκB signaling pathway in male Sprague-Dawley rats. American Journal of Translational Research. 9:4227-4235.

Dang Y, Li Z, Luo B, Pan L, Wei Q, Zhang Y (2017b) Protective effects of apigenin against acrylonitrile-induced subchronic sperm injury in rats. Food and Chemical Toxicology. 109(pt 1):517-525.

Davis JH, Davies JE, Raffonelli A, Reich G (1973). The investigation of fatal acrylonitrile intoxications. In: Pesticides and the Environment: A continuing controversy. Intercontinental Medical Book Corporation. 2:547-556.

De Smedt T, De Cremer K, Vleminckx C, Fierens S (2014). Acrylonitrile exposure in the general population following a major train accident in Belgium: A human biomonitoring study. Toxicology Letters. 231:344–351.

DFG [German Research Foundation] (2016). List of MAK and BAT Values 2016. DFG, Deutsche Forschungsgemeinschaft Copyright 2016 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim ISBN: 978-3-527-80598-3.

DGUV [German Social Accident Insurance] (2008). Environmental Tobacco Smoke (ETS): Measurement of Acrylonitrile and Nicotine in Working Areas. Project No. BGIA 2074. Deutsche Gesetzliche Unfallversicherung 2008. http://www.dguv.de/ifa/forschung/projektverzeichnis/bgia_2074-2.jsp

DGUV [German Social Accident Insurance] (2016). IFA-Report 1/2016, Gefahrstoffliste 2016, Gefahrstoffe am Arbeitsplatz, Deutsche Gesetzliche Unfallversicherung (DGUV), Berlin, Germany. Available at: http://publikationen.dguv.de/dguv/pdf/10002/rep0116.pdf

Doll R (1998). The mortality of acrylonitrile workers--new evidence and a review of the old. Scandinavian Journal of Work, Environment and Health. 24(suppl 2):3-4.

Dos Santos Silva I (1999). Chapter 15: Size of a Study. Cancer epidemiology: Principles and Methods. Lyon: International Agency for Research on Cancer.

Dudley HC and Neal PA (1942a). Toxicology of acrylonitrile (vinyl cyanide). I. Study of the acute toxicity. Journal of Industrial Hygiene and Toxicology. 24(2):27-36.

Dudley HC, Sweeney TR, Miller J W (1942b). Toxicology of acrylonitrile. II. Studies of effects of daily inhalation. Journal of Industrial Hygiene and Toxicology. 24:255-258.

EC [European Commission] (2004). European Union Risk Assessment Report for Acrylonitrile. Final Report. European Chemicals Bureau (Existing Substances, 1st Priority List Volume 32). European Commission, JRC, 2004.

El-Sayed el-SM, Abo-Salem OM, Abd-Ellah MF, Abd-Alla GM (2008) Hesperidin, an antioxidant flavonoid, prevents acrylonitrile-induced oxidative stress in rat brain. Journal of Biochemical and Molecular Toxicology. 22:268-273.

Environment Canada (1999) Acrylonitrile. Priority Substances List Assessment Report. Canadian Environmental Protection Act, 1999.

Etebari M, Jafarian-Dehkordi A, Kahookar A, Moradi S (2014). Assessment of the deoxyribonucleic acid damage caused by occupational exposure to chemical compounds in Isfahan Polyacryl Company. Journal of Research in Medical Sciences. 19(6):542-548.

Esmat A, El-Demerdash E, El-Mesallamy H, Abdel-Naim AB (2007) Toxicity and oxidative stress of acrylonitrile in rat primary glial cells: preventive effects of N-acetylcysteine. Toxicology Letters. 171:111-118.

Esmen NA (1998). Exposure estimation in four major epidemiologic studies in the acrylonitrile industry. Scandinavian Journal of Work, Environment and Health. 63-70.

Fahmy MA (1999). Evaluation of the genotoxicity of acrylonitrile in different tissues of male mice. Cytologia. 64:1-9.

Fechter LD, Gearhart C, Shirwany NA (2004). Acrylonitrile potentiates noise-induced hearing loss in rat. Journal of the Association for Research in Otolaryngology. 5(1):90-8.

Fechter LD, Klis SF, Shirwany NA, Moore TG, Rao DB (2003). Acrylonitrile produces transient cochlear function loss and potentiates permanent noise-induced hearing loss. Toxicological Sciences. 75(1):117-23.

Felter SP and Dollarhide JS (1997). Acrylonitrile: a reevaluation of the database to support an inhalation cancer risk assessment. Regulatory Toxicology and Pharmacology. 26:281-287.

Fennell TR, Macneela JP, Morris RW, Watson M, Thompson CL, Bell DA (2000). Hemoglobin adducts from acrylonitrile and ethylene oxide in cigarette smokers: effects of glutathione S-transferase T1-nul and M1-null genotypes. Cancer Epidemiology and Prevention Biomarkers. 9(7):705-712.

Fenga C, Gangemi S, Costa C (2016) Benzene exposure is associated with epigenetic changes (Review). Molecular Medicine Reports. 13:3401-3405.

Friedman MA and Beliles RP (2002). Three-generation reproduction study of rats receiving acrylonitrile in drinking water. Toxicology Letters. 132(3):249-261.

Gagnaire F, Marignac B, Bonnet P (1998). Relative neurotoxicological properties of five unsaturated aliphatic nitriles in rats. Journal of Applied Toxicology. 18(1):25-31.

Gallagher GT, Maull, EA, Kovacs K, Szabo S (1988). Neoplasms in rats ingesting acrylonitrile for two years. Journal of the American College of Toxicology. 7(5):603-615.

Gargas ML, Anderson ME, Teo SKO, Batra R, Fennell TR, Kedderis GL (1995). A Physiologically-based dosimetry description of acrylonitrile and cyanoethylene oxide in the rat. Toxicology and Applied Pharmacology. 134:185-194.

Gincheva N, Stamova N, Hinkova L, Spasovski M, Hristeva V, Muhtarova M (1977) Study of the health status of workers from acrylonitrile department (Abstract). In: Proceedings of the International Symposium on Occupational Health in the Production of Artificial Fibers, Finland, 6–10 June 1977, p. 41.

Gomes RFM, Shardonofsky F, Eidelman DH, Bates JHT (2001). Respiratory mechanics and lung development in the rat from early age to adulthood. Journal of Applied Physiology. 90:1631-1638.

Grahl R (1970). Toxicologie und Wirkungsweise von acrylnitril. Zentralb. Arbeitsmed. 12: 369-378 (Cited in EU RAR).

Granath F, Ehrenberg L, Tornqvist M (1992) Degree of alkylation of macromolecules in vivo from variable exposure. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 284(2):297-306. Cited in van Nieuwenhuyse et al (2014).

Gut I, Nerudova J, Stiborova A, Kopecký J, Frantik E (1985). Acrylonitrile inhalation in rats: II. Excretion of thioethers and thiocyanate in urine. Journal of Hygiene, Epidemiology, Microbiology, and Immunology. 29(1):9-13.

Haber LT and Patterson J (2005). Report of an independent peer review of an acrylonitrile risk assessment. Human and Experimental Toxicology. 24:487-527.

Hachiya N, Sato M, Takizawa Y (1984). Detection of DNA damage in mutagen treated mammalian tissues by alkaline elution assay. Mutation Research. 130:363.

Hachiya N, Tanaka N, Takizawa Y (1986). DNA damages in mammalian tissues, II. DNA single-strand breaks and alkali-liable sites detected by alkaline elution assay. Mutation Research. 164:266.

Hachiya N (1987). Evaluation of chemical genotoxicity by a series of short-term tests. Akita Journal of Medicine. 14:269-292.

Hagmar L (2001). How confident can we be that acrylonitrile is not a human carciniogen? Scandinavian Journal of Work, Environment and Health. 27(1):1-4.

Hashimoto K and Kobayashi T (1961). A case of acute dermatitis caused by contact with acrylonitrile. Q. J. Labor. Res. 9(1-4):21-24.

Hoenig JM and Heisey DM (2001). The abuse of power. American Statistician. 55(1):19-24.

Hogy LL and Guengerich FP (1986). In vivo interaction of acrylonitrile and 2-cyanoethylene oxide with DNA in rats. Cancer Research. 46:3932-3938.

Hood RD (1996). Handbook of developmental toxicology. CRC Press.

HPA [Health Protection Agency] (2007) Acrylonitrile – General Information. UK Health Protection Agency CHAPD HQ, 2007 (Version 1). https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/337523/HPA_ACRYLONITRILE_general_information_v1.pdf

HSE [Health and Safety Executive] (1993) Methods for the Determination of Hazardous Substances (MDHS 72): Volatile Organic Compounds in Air. Health and Safety Executive, Liverpool, UK, March 1993. http://www.hse.gov.uk/pubns/mdhs/pdfs/mdhs72.pdf

HSE [Health and Safety Executive] (1995) Methods for the Determination of Hazardous Substances (MDHS 80): Volatile Organic Compounds in Air. Health and Safety Executive, Liverpool, UK, August 1995. http://www.hse.gov.uk/pubns/mdhs/pdfs/mdhs80.pdf

HSE [Health and Safety Executive] (1997) Methods for the Determination of Hazardous Substances (MDHS 88): Volatile Organic Compounds in Air. Health and Safety Executive, Liverpool, UK, December 1997. http://www.hse.gov.uk/pubns/mdhs/pdfs/mdhs88.pdf

HSE [Health and Safety Executive] (2010) Investigation of Potential Exposure to Carcinogens and Respiratory Sensitisers during Thermal Processing of Plastics. Research Report RR797. Health and Safety Executive 2010. Health and Safety Laboratory, Buxton, UK.

He Z, Li G, Chen J, Huang Y, An T, Zhang C (2015). Pollution characteristics and health risk assessment of volatile organic compounds emitted from different plastic solid waste recycling workshops. Environment International. 77:85-94.

Humiston CG and Frauson LO (1975). 90-day oral toxicity study incorporating acrylonitrile in the drinking water of rats. Midland, MI, Dow Chemical Toxicology Research laboratory, 99 pp.

IARC [International Agency for Research on Cancer] (1979). Acrylonitrile, Acrylic and Modacrylic Fibres, and Acrylonitrile-Butadiene-Styrene and Styrene-Acrylonitrile Copolymers. In: Some Monomers, Plastics and Synthetic Elastomers, and Acrolein. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol 19. Lyon: International Agency for Research on Cancer. Pp. 73-113.

IARC [International Agency for Research on Cancer] (1999). Acrylonitrile. In: Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol 71. Lyon: International Agency for Research on Cancer. Pp.43-108.

IFA [Institute for Occupational Safety and Health of the German Social Accident Insurance] (2010). Exposure to environmental tobacco smoke in German hospitality venues. (617.0-IFA:638.1). Institute für Arbeitsschutz der Deutsche Gesetzliche Unfallcversicherung. Alte Heerstraße 111. 53757 Sankt Augustin. Germany. Available at: http://www.dguv.de/medien/ifa/en/pub/ada/pdf_en/aifa0297e.pdf

Jacob S and Ahmed AE (2003) Acrylonitrile-induced neurotoxicity in normal human astrocytes: oxidative stress and 8-hydroxy-2'-deoxyguanosine formation. Toxicology Mechanisms and Methods. 13:169-179.

Jain RB (2015a). Levels of selected urinary metabolites of volatile organic compounds among children aged 6-11 years. Environmental Research. 142:461-470.

Jain RB (2015b). Distributions of selected urinary metabolites of volatile organic compounds by age, gender, race/ethnicity, and smoking status in a representative sample of U.S. adults. Environmental Toxicology and Pharmacology. 40:471-479.

Jakubowski M, Linhart I, Pielas G, Kopecky J (1987). 2-cyanoethylmercapturic acid (CEMA) in the urine as a possible indicator of exposure to acrylonitrile. British Journal of Industrial Medicine. 44:834-840.

Jiang J, Xu Y, Klaunig JE (1998) Induction of oxidative stress in rat brain by acrylonitrile (ACN). Toxicological Sciences. 46:333-341.

Johannsen FR and Levinskas GJ (2002a). Chronic toxicity and oncogenic dose-response effects of lifetime oral acrylonitrile exposure to Fischer 344 rats. Toxicology Letters. 132(3):221-247.

Johannsen FR and Levinskas GJ (2002b). Comparative chronic toxicity and carcinogenicity of acrylonitrile by drinking water and oral intubation to Spartan Sprague-Dawley rats. Toxicology Letters. 132(3):197-219.

Kamendulis LM, Jiang J, Klaunig JE (1999) Induction of oxidative stress and oxidative damage in rat glial cells by acrylonitrile. Carcinogenesis. 20:1555-1560.

Kaneko Y and Omae K (1992). Effect of Chronic Exposure to Acrylonitrile on Subjective Symptoms. The Keio Journal of Medicine. 41(1):25-32.

Karami S, Boffetta P, Brennan P, Stewart PA, Zaridze D, Matveev V, Janout V, Kollarova H, Bencko, V, Navratilova, M, Szeszenia-Dabrowska N, Mates D, Gromiec JP, Sobotka R, Chow WH, Rothman N, Moore LE (2011) Renal cancer risk and occupational exposure to polycyclic aromatic hydrocarbons and plastics. Journal of Occupational and Environmental Medicine/American College of Occupational and Environmental Medicine. 53(2):218.

Kedderis GL and Batra R (1991). Species differences in the hydrolysis of 2-cyanoethylene oxide, the epoxide metabolite of acrylonitrile. Proceedings of the American Association for Cancer Research. 32:118.

Kedderis GL and Batra R (1993). Species differences in the hydrolysis of 2-cyanoethylene oxide, the epoxide metabolite of acrylonitrile. Carcinogenesis. 14:685-689.

Kedderis GL, Batra R, Koop DR (1993). Epoxidation of acrylonitrile by rat and human cytochromes P450. Chemical Research in Toxicology. 6:866-871

Kedderis GL and Fennell TR (1996). Development of a physiologically based description of acrylonitrile dosimetry. CIIT Activities. Vol 16, No. 1.

Kida K and Thurlbeck WM (1981). Tracheal banding in weanling rats diminishes lung growth and alters lung architecture. Pediatric Research. 15:269-277.

Kirman (2017). Comments submitted by Kirman CR in the public consultation on the proposal by ECHA in support of occupational exposure limit values for Acrylonitrile in the workplace. Comment reference number 30. All public comments submitted in the consultation are available at https://echa.europa.eu/echas-executive-director-requests-to-the-committees-previous-consultations/-/substance-rev/17808/term

Kirman CR, Gargas ML, Marsh GM, Strother DE, Klaunig JE, Collins JJ, Deskin R (2005). Cancer dose-response assessment for acrylonitrile based upon rodent brain tumor incidence: use of epidemiologic, mechanistic, and pharmacokinetic support for non-linearity. Regulatory Toxicology and Pharmacology. 43:85-103.

Kirman CR, Sweeney LM, Gargas ML, Strother DE, Collins JJ and Deskin R (2008). Derivation of noncancer reference values for acrylonitrile. Risk Analysis: An International Journal. 28(5):1375-94.

Kolenda-Roberts HM, Harris N, Singletary E, Hardisty JF (2013). Immunohistochemical characterization of spontaneous and acrylonitrile-induced brain tumors in the rat. Toxicologic Pathology. 41:98-108.

Koopmans MJE and Daamen PAM (1989). Skin sensitisation to acrylonitrile in albino guinea pig (maximisation test). Study 012972 of RCC NOTOX BV, The Netherlands.

Lambert IB, Singer TM, Boucher SE, Douglas GR (2005). Detailed review of transgenic rodent mutation assays. Mutation Research. 590:1-280.

Leonard DL, Vincent LE, Krohne HE (1981). Mutagenicity of acrylonitrile in mouse. Toxicology Letters. 7:329-334.

Licea Perez H, Segerback D, Osterman-Golkar S (1999). Adducts of acrylonitrile with haemoglobin in nonsmokers and in participants in a smoking cessation program. Chemical Research in Toxicology. 12(10):869–873. Cited in WHO 2002.

Lijinsky W and Andrews AW (1980). Mutagenicity of vinyl compounds in Salmonella typhimurium. Teratogenesis, Carcinogenesis, and Mutagenesis. 1:259-267.

Rongzhu L, Ziqiang C, Fusheng J, Collins JJ (2005). Neurobehavioral effects of occupational exposure to acrylonitrile in Chinese workers. Environmental Toxicology and Pharmacology. 19(3):695-700.

Maltoni C, Ciliberti A, Dimaio V (1977). Carcinogenicity bioassays on rats of acrylonitrile administered by inhalation and ingestion. La Medicina del Lavoro (Italian Journal of Occupational Medicine and Health). 68:401-411.

Maltoni C, Ciliberti A, Cotti G, Perino G (1988). Long-term carcinogenicity bioassays on acrylonitrile administered by inhalation and by ingestion to Sprague-Dawley rats. Annals New York Academy of Sciences. 534:179-202.

Marsh GM, Gula MJ, Youk AO, Schall LC (1999). Mortality among chemical plant workers exposed to acrylonitrile and other substances. American Journal of Industrial Medicine. 36(4):423-436.

Marsh GM, Youk AO, Collins JJ (2001). Reevaluation of lung cancer risk in the acrylonitrile cohort study of the National Cancer Institute and the National Institute for Occupational Safety and Health. Scandinavian Journal of Work, Environment and Health. 27(1):5-13.

Marsh GM and Zimmerman SD (2015). Mortality among chemical plant workers exposed to acrylonitrile: 2011 follow-up. Journal of Occupational and Environmental Medicine. 57(2):134-145.

Mastrangelo G, Serena R and Marzia V (1993). Mortality from tumours in workers in an acrylic fibre factory. Occupational Medicine. 43:155-158.

McOmie WA (1949). Comparative toxcity of methacylonitrile and acrylonitrile. Journal of Industrial Hygiene and Toxicology. 31:113-116.

Mehrotra J, Khanna VK, Husain R, Seth PK (1988). Biochemical and Developmental Effects in Rats Following in Utero Exposure to Acrylonitrile. Industrial Health. 26:251-255.

Minet E, Cheung F, Errington G, Sterz K, Scherer G (2011). Urinary excretion of the acrylonitrile metabolite 2-cyanoethylmercapturic acid is correlated with a variety of biomarkers of tobacco smoke exposure and consumption. Biomarkers. 16(1):89-96.

Morita T, Asano N, Awogi T, Sasaki YF, Sato S, Shimada H, Sutou S, Suzuki T, Wakata A, Sofuni T, Hayashi M (1997). Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B) the summary report of the 6th collaborative study by CSGMT/JEMS MMS. Collaborative Study of the Micronucleus Group Test. Mammalian Mutagenicity Study Group. Mutation Research. 389(1):3-122.

Murray FJ, Schwetz BA, Nitschke KD, John JA, Norris JM, Gehring PJ (1978). Teratogenicity of acrylonitrile given to rats by gavage or by inhalation. Food and Cosmetics Toxicology. 16:547-551.

Muto T, Sakurai H, Omae K, Minaguchi H, Tachi M (1992). Health profiles of workers exposed to acrylonitrile. The Keio Journal of Medicine. 41(3):154-60.

Nakagawa Y, Toyoizumi T, Sui H, Ohta R, Kumagai F, Usumi K, Saito Y, Yamakage K (2015). In vivo comet assay of acrylonitrile, 9-aminoacridine hydrochloride monohydrate and ethanol in rats. Mutation Research. 786–788: 104–113.

Nasralla SN, Ghoneim AI, Khalifa AE, Gad MZ, Abdel-Naim AB (2009). Lactoperoxidase catalyzes in vitro activation of acrylonitrile to cyanide. Toxicology Letters. 191(2-3): 347-52.

National Toxicology Program (2016). Acrylonitrile. 14th Report on Carcinogens (RoC). Department of Health and Human Services.

Nazaroff WW and Singer BC (2004). Inhalation of hazardous air pollutants from environmental tobacco smoke in US residences. Journal of Exposure Analysis and Environmental Epidemiology. 14(1):71-S77.

Neal BH, Collins JJ, Strother DE, Lamb JC (2009). Weight-of-the-evidence review of acrylonitrile reproductive and developmental toxicity studies. Critical Reviews in Toxicology. 39(7):589–612.

Nemec MD, Kirkpatrick DT, Sherman J, Van Miller JP, Pershing ML, Strother DE (2008). Two-Generation Reproductive Toxicity Study of Inhaled Acrylonitrile Vapors in Crl: CD(SD) Rats. International Journal of Toxicology. 27:11-29. Testing laboratory: WIL Research Laboratories, LLC, Ohio.

NICNAS [National Industrial Chemical Notification and Assessments Scheme] (2000) Preliminary Assessment of the Use of Acrylonitrile (PEC 10). Commonwealth of Australia.

NIOSH [National Institute for Occupational Safety and Health] (1994) Acrylonitrile: Test Method 1604, Issue 2, dated 15 August 1994. https://www.cdc.gov/niosh/docs/2003-154/pdfs/1604.pdf

NTP [National Toxicology Program] (2001). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Acrylonitrile (CAS No. 107-13-1) in B6C3F1 Mice (Gavage Studies). NTP, Research Triangle Report, NTP TR 506. NIH Publication No. 02-4440. Testing laboratory: Battelle Columbus Operations. Report no.: NTP TR 506.

O'Berg MT (1980). Epidemiologic study of workers exposed to acrylonitrile. Journal of Occupational Medicine. 22:245-252.

O'Berg MT, Chen JL, Burke, CA, Walrath J, Pell S (1985). Epidemiologic study of workers exposed to acrylonitrile: an update. Journal of Occupational Medicine. 27:835-840.

Obe G, Hille A, Jonas R, Schmidt S, Thenhaus U (1985). Tests for the induction of sister-chromatid exchanges in human peripheral lymphocytes in culture. In: Ashby J, de Serres FJ, Draper M, Ishidate Jr M, Margolin BH, Matter BE, Shelby MD (Eds.): Evaluation of short-term tests for carcinogens. Report of the International Programme on Chemical Safety's collaborative study on in vitro assays. Progress in Mutation Research. 5:439-442.

OSHA [Occupational Safety and Health Administration] Method ORG-37 (1982) Acrylonitrile: A sampling and analytical method which has been subjected to the established evaluation procedures of the Organic Methods Evaluation Branch. Methods Development Team. Industrial Hygiene Chemistry Division. OSHA Salt Lake Technical Center. Sandy UT 84070-6406. Available at: https://www.osha.gov/dts/sltc/methods/organic/org037/org037.html

OSHA [Occupational Safety and Health Administration] (1990) Chemical Sampling Information – Acrylonitrile. OSHA https://www.osha.gov/dts/chemicalsampling/data/CH_217300.html

Pouyatos B, Gearhart CA, Fechter LD (2005). Acrylonitrile potentiates hearing loss and cochlear damage induced by moderate noise exposure in rats. Toxicology and Applied Pharmacology. 204(1):46-56.

Pouyatos B, Gearhart C, Nelson-Miller A, Fulton S, Fechter L (2007). Oxidative stress pathways in the potentiation of noise-induced hearing loss by acrylonitrile. Hearing Research. 224(1-2):61-74.

Pu X, Kamendulis LM, Klaunig JE (2006) Acrylonitrile-induced oxidative damage in rat astrocytes. Environmental and Molecular Mutagenesis. 47:631-638.

Pu X, Kamendulis LM, Klaunig JE (2009). Acrylonitrile-induced oxidative stress and oxidative DNA damage in male Sprague-Dawley rats. Toxicological Sciences. 111:64-71.

Pu X, Wang Z, Zhou S, Klaunig JE (2016) Protective effects of antioxidants on acrylonitrile-induced oxidative stress in female F344 rats. Environmental Toxicology. 31:1808-1818.

Quast JF, Enriquez RM, Wade CE, Humiston CG, Schwetz BA (1975). Toxicity and drinking water containing acrylonitrile in rats: results after 12 months. Midland MI, USA, Dow Toxicology Research Laboratory.

Quast JF, Schwetz BA, Balmer MF, Gushow TS, Park CN, McKenna MJ (1980a). A two-year toxicity and oncogenicity study with acrylonitrile following inhalation exposure of rats. Dow Chemical Co., Toxicology Research Laboratory, Midland, MI.

Quast JF, Wade CE, Humiston CG, Carreon RM, Hermann EA, Park CN & Schwetz BA (1980b). A two-year toxicity and oncogenicity study with acrylonitrile incorporated in the drinking water of rats. Toxicology Research Laboratory, Health and Environmental Services, Dow Chemical USA, Midland Michigan for the Chemical Manufacturers Association, Washington DC (unpublished report). Testing laboratory: Toxicology Research Laboratory, Dow.

Quast JF (2002). Two-year toxicity and oncogenicity study with acrylonitrile incorporated in the drinking water of rats. Toxicology Letters. 132:153-196.

Radimer GF, Davis JH, Ackerman AB (1974). Fumigant-induced toxic epidermal necrolysis. Archives of Dermatology. 110(1):103-4.

Rabello-gay MN and Ahmed AE (1980). Acrylonitrile: in vivo cytogenetic studies in mice and rats. Mutation Research/Genetic Toxicology. 79(3):249-255.

RAC and SCOEL (2017). Joint Task Force ECHA Committee for Risk Assessment (RAC) and Scientific Committee on Occupational Exposure Limits (SCOEL) on Scientific aspects and methodologies related to the exposure of chemicals at the workplace, Task 2, 6 December 2017. Available at: https://echa.europa.eu/documents/10162/13579/jtf_opinion_task_2_en.pdf/db8a9a3a-4aa7-601b-bb53-81a5eef93145

Rothman KJ (1994). Cancer occurrence among workers exposed to acrylonitrile. Scandinavian Journal of Work, Environment and Health. 20(5):313-321.

Roy P and Kulkarni AP (1999). Co-oxidation of acrylonitrile by soybean lipoxygenase and partially purified human lung lipoxygenase. Xenobiotica. 29(5):511-31.

Saillenfait AM, Bonnet P, Guenier JP, de Ceaurriz J (1993). Relative Developmental Toxicities of Inhaled Aliphatic Mononitriles in Rats. Fundamental and Applied Toxicology. 20(3):365-375.

Saillenfait AM and Sabate JP (2000). Comparative developmental toxicities of aliphatic nitriles: in vivo and in vitro observations. Toxicology and Applied Pharmacology. 163(2):149-163.

Sakurai H, Onodera M, Utsunomiya T, Minakuchi H, Iwai H, Mutsumura H (1978). Health effects of acrylonitrile in acrylic fibre factories. British Journal of Industrial Medicine. 35:219-225.

Sartorelli E (1966). Acute poisoning due to acrylonitirle. La Medicina del Lavoro (Italian Journal of Occupational Medicine and Health). 57:184-187.

Scélo G, Constantinescu V, Csiki I, Zaridze D, Szeszenia-Dabrowska N, Rudnai P, Lissowska J, Fabiánová E, Cassidy A, Slamova A, Foretova L, Janout V, Fevotte J, Fletcher T, Mannetje A', Brennan P, Boffetta P (2004). Occupational exposure to vinyl chloride, acrylonitrile and styrene and lung cancer risk (Europe). Cancer Causes and Control. 15(5):445-452.

Schettgen T, Broding HC, Angerer J, Drexler H. (2002). Hemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile and acrylamide-biomarkers in occupational and environmental medicine. Toxicology Letters. 134:65–70.

Schettgen T, Rossbach B, Kutting B, Letzel S, Drexler H, Angerer J (2004). Determination of haemoglobin adducts of acrylamide and glycidamide in smoking and non-smoking persons of the general population. International Journal of Hygiene and Environmental Health. 207(6):531-539.

Schwetz BA, Haberstroh HD, Humiston C G, Quast JF, Smith FA, Nitschke KD (1975). Results of a reproduction study in rats maintained on drinking water containing

acrylonitrile (acrylonitrile). Toxicological Research Laboratory, Dow Chemical company, Midland, MI.

SCOEL [Scientific Committee on Occupational Exposure Limits] (2003) Recommendation from Scientific Committee on Occupational Exposure Limits for Acrylonitrile. SCOEL/SUM/104, December 2003

Serota DG, Giles HD, Coyne JM, Hogan DB (1996). Sub-chronic Toxicity Study in B6C3F1 Mice. Testing laboratory: Southern Research Institute, Birmingham, AL. Report no.: SRI-LIF-95- 593-8618-I.

Sharief Y, Brown AM, Backer LC, Campbell JA, Westbrook-Collins B, Stead AG, Allen JW (1986). Sister chromatid exchange and chromosome aberration analyses in mice after in vivo exposure to acrylonitrile, styrene, or butadiene monoxide. Environmental Mutagenesis. 8:439-448.

Simons K, DeSmedt T, Stoveb C, DePaepe P. (2016). Short-term health effects in the general population following a major train accident with acrylonitrile in Belgium. Environmental Research. 148:256–263.

Sponsiello-Wang Z, Sanders E, Weitkunat R (2006). Occupational acrylonitrile exposure and lung cancer: A meta-analysis. Journal of Environmental Science and Health, Part C. 24(2): 257-284.

Sram RJ, Beskid O, Binkova B, Possner P & Smerhovsky Z (2004) Cytogenetic analysis using fluorescence in situ hybridization (FISH) to evaluate occupational exposure to carcinogens. Toxicology Letters. 149:335-344.

Stamova N, Gincheva N, Spasovski M, Bainova A, Ivanova S, Kurkchiev S, Hinkova L, Khristeva V, Mukhtarova M, Karadzhova N, Tsaneva L, Fikova E (1976). Labour hygiene during the production of synthetic fibres “Bulana”. Hygiena Zdravotnictvi, 2, 134–140 (in Russian), as reported in IARC (1999).

Stewart, PA, Zaebst D, Zey JN, Herrick R, Dosemeci M, Hornung R, Bloom T, Potttern L, Miller BA, Blair A (1998). Exposure assessment for a study of workers exposed to acrylonitrile. Scandinavian Journal of Work, Environment and Health. 42-53.

Strother DE and Kirman CR (2011) Threshold analyses for acrylonitrile and brain tumors in rats. The Toxicologist. 477(abstract).

Swaen G, Bloemen L, Twisk J, Scheffers T, Slangen J, Sturmans F (1992). Mortality of workers exposed to acrylonitrile. Journal of Occupational Medicine. 34:801-809.

Swaen GMH, Bloemn LJN, Twisk J, Scheffers et al. (1998) Mortality Update of Workers Exposed to Acrylonitrile in the Netherlands. Scandinavian Journal of Work, Environment and Health. 24(2):10-16.

Swaen GMH, Bloemen LJN, Twisk J, Scheffers T, Slangen JJM, Collins JJ, ten Berge WFJP (2004). Mortality update of workers exposed to acrylonitrile in the Netherlands. Journal of Occupational and Environmental Medicine. 46(7):691-698.

Sweeney LM, Gargas ML, Strother DE, Kedderis GL (2003). Physiologically Based Pharmacokinetic Model Parameter Estimation and Sensitivity and Variability Analyses for Acrylonitrile Disposition in Humans. Toxicological Sciences. 71:27-40.

Symons JM, Kreckmann, KH, Sakr CJ, Kaplan AM, Leonard RC (2008). Mortality among workers exposed to acrylonitrile in fiber production: An update. Journal of Occupational and Environmental Medicine. 50(5):550-560.

Szabo S, Gallagher GT, Silver EH, Maull EA, Horner HC, Komanicky P, Melby JC, McComb DJ, Kovacs K (1984). Subacute and chronic action of acrylonitrile on adrenals and gastrointestinal tract: biochemical, functional and ultrastructural studies in the rat. Journal of Applied Toxicology. 4(3):131-40.

Tandon R, Saxena DK, Chandra SV, Seth PK, Srivastava SP (1988). Testicular effects of acrylonitrile in mice. Toxicology Letters. 42(1):55-63.

Tavares R, Borba H, Monteiro M, Proenca MJ et al. (1996) Monitoring of exposure to acrylonitrile by determination of N-(2-cyanoethyl)valine at the N-terminal position of haemoglobin. Carcinogenesis. 17:2655–2660. Cited in WHO 2002.

TCEQ [Texas Commission on Environmental Quality] (2013) Development Support Document: Acrylonitrile. Office of the Executive Director, TCEQ.

Teruel MA, Blanco MB, Luque GR (2007). Atmospheric fate of acrylic acid and acrylonitrile: rate constants with Cl atoms and OH radicals in the gasphase. Atmospheric Environment. 41(27):5769-5777.

The Sapphire Group, Inc (2004). TOXICOLOGICAL REVIEW OF ACRYLONITRILE (CAS No. 107-13-1). Testing laboratory: Prepared for The AN group, Washington DC. Prepared by The Sapphire Group, Inc. Dayton and Cleveland, OH.

Thier R, Lealter J, Bolt HM (2000). Species differences in acrylonitrile metabolism and toxicity between experimental animals and humans based on observations in human accidental poisonings. Archives of toxicology. 74(4-5):184-189.

Thiess AM, Frentzel-Beyme R, Link R, Wild H (1980). Mortalitatsstudie bei chemiefacharbeitern verschiedener produktionsbetriebe mit exposition auch gegenuber acrylonitril. Zentralbl. Arbeitsmed. Arbeitssch. Prophyl. Ergonomie., 30: 259-267.

US Department of Labor (1978). Occupational exposure to acrylonitrile (vinyl cianide). Final standard. Federal Register, 43: 45762-45819.

US Environmental Protection Agency (1999). Integrated Risk Information System (IRIS) on Acrylonitrile. National Center for Environmental Assessment, Office of Research and Development, Washington DC.

Van Nieuwenhuyse A, Fierens S, De Smedt T, De Cremer K, Vleminckx C, Mertens B, Van Overmeire I, Bader M, De Paepe P, Göen T, Nemery B, Schettgen T, Stove C, Van Oyen H, Van Loco J (2014). Acrylonitrile exposure assessment in the emergency responders of a major train accident in Belgium: A human biomonitoring study A. Toxicology Letters. 231:352–359.

Venitt S, Bushell CT, Osborne M (1977) Mutagenicity of acrylonitrile (cyanoethylene) in Escherichia coli. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 42:283-288.

Vernon PA, Dulak LH, Deskin R (1969). Acute toxicologic evaluation of acrylonitrile. Reported in the Journal of the American College of Toxicology in 1990. 1:114-115.

Vogel RA and Kirkendall WM (1984). Acrylonitrile (vinyl cyanide) poisoning: a case report. Texas Medicine, 80: 48-51 (Cited in EU RAR).

VROM (1984). Criteria document on acrylonitrile. Lucht 29. Ministeries van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer, 1984, 1-129 (Cited in EU RAR).

Wakata A, Miyamae Y, Sato S, Suzuki T, Morita T, Asano N, Awogi T, Kondo K, Hayashi M (1998). Evaluation of the rat micronucleus test with bone marrow and peripheral blood: summary of the 9th collaborative study by CSGMT/JEMS. MMS. Collaborative Study Group for the Micronucleus Test. Environmental Mutagen Society of Japan. Mammalian Mutagenicity Study Group. Environmental and Molecular Mutagenesis. 32(1):84-100.

Walker V and Ghanayem B (2003). Mutagenicity at the HPRT locus of T-cells following exposure of wild-type and cytochrome P4502E1-null mice to acrylonitrile. Toxicologist. 1108 (Abstract).

Walters SJ (2009). Consultant's forum: Should post hoc sample size calculations be done? Pharmaceutical Statistics. 8(2):163-169.

Wang Z, Li Z, Wei X (1995). Study on acrylonitrile to teratogenesis of rat sperm. Journal of Lanzhou Medical College. 21:3.

Warneke C, Roberts JM, Veres P, Gilman J, Kuster WC, Burling I, Yokelson R, de Gouw JA (2011) VOC identification and inter-comparison from laboratory biomass burning using PTR-MS and PIT-MS. International Journal of Mass Spectrometry. 303:6-14.

Werner JB and Carter JT (1981). Mortality of United Kingdom acrylonitrile polymerization workers. British Journal of Industrial Medicine. 38:247-253.

WHO [World Health Organization] (1983). Acrylonitrile. Environonmental Health Criteria, No. 28, IPCS (International Programme on Chemical Safety). WHO, Geneva, 1983. Available at http://www.inchem.org/documents/ehc/ehc/ehc28.htm

WHO [World Health Organization] (2000) Air Quality Guidelines for Europe, 2nd Edition. WHO Regional Publications, European Series, No. 91. WHO, Geneva, 2000.

WHO [World Health Organization] (2002) Concise International Chemical Assessment Document 39 – Acrylonitrile. WHO, Geneva, 2002.

Williams GM, Kobets T, Duan JD, Iatropoulos MJ (2017). Assessment of DNA binding and oxidative DNA damage by acrylonitrile in two rat target tissues of carcinogenicity: implications for the mechanism of action. Chemical Research in Toxicology. 30:1470-1480.

Wilson RH (1944). Health hazards encountered in the manufacture of synthetic rubber. Journal of the American Medical Association. 124: 701-3.

Wilson RH, Hough GV, McCormick WE (1948). Medical problems encountered in the manufacture of American made rubber. Industrial Medicine and Surgery. 17(6): 199-207.

Whysner J, Steward RE, Chen D, Conaway CC, Verna LK, Richie JP, Ali N, Williams GM (1998). Formation of 8-oxodeoxyguanosine in brain DNA of rats exposed to acrylonitrile. Archives of toxicology. 72: 429-438.

Wood SM, Buffler PA, Burau K, Krivanek N (1998). Mortality and morbidity of workers exposed to acrylonitrile in fiber production. Scandinavian Journal of Work, Environment and Health. 24(2):54-62.

Working PK, Bentley KS, Hurtt ME, Mohr KL (1987). Comparison of the dominant lethal effects of acrylonitrile and acrylamide in male Fischer 344 rats. Mutagenesis. 2:215-220.

Woutersen RA (1998). Toxicologic profile of acrylonitrile. Scandinavian journal of work, environment and health. 24(2):5-9.

Yokelson RJ, Karl T, Artaxo P, Blake DR, Christian TJ, Griffith DWT, Guenther A, Hao WM (2007). The Tropical Forest and Fire Emissions Experiment: overview and airborne fire emission factor measurements. Atmospheric Chemistry and Physics. 7:5175–5196.

Zeiger E and Haworth S (1985) Tests with preincubation modifications of the Salmonella/microsome assay. In Ashby J, de Serres FJ, Draper M, Ishidate M, Margolin BH, Matter BE and Shelby MD (Eds) Evaluation of short-term tests for carcinogens. Report of the International Programme on Chemical Safety's collaborative study on in vitro assays. Progress in Mutation Research. 5:187-199.

Zeller H, Hofmann HT, Thiess AM, Hey W (1969). Toxicity of nitriles. Results of animal experiments and industrial experiences during 15 years. Zentralblatt fur Arbeitsmedizin und Arbeitsschutz. 19:225-237.

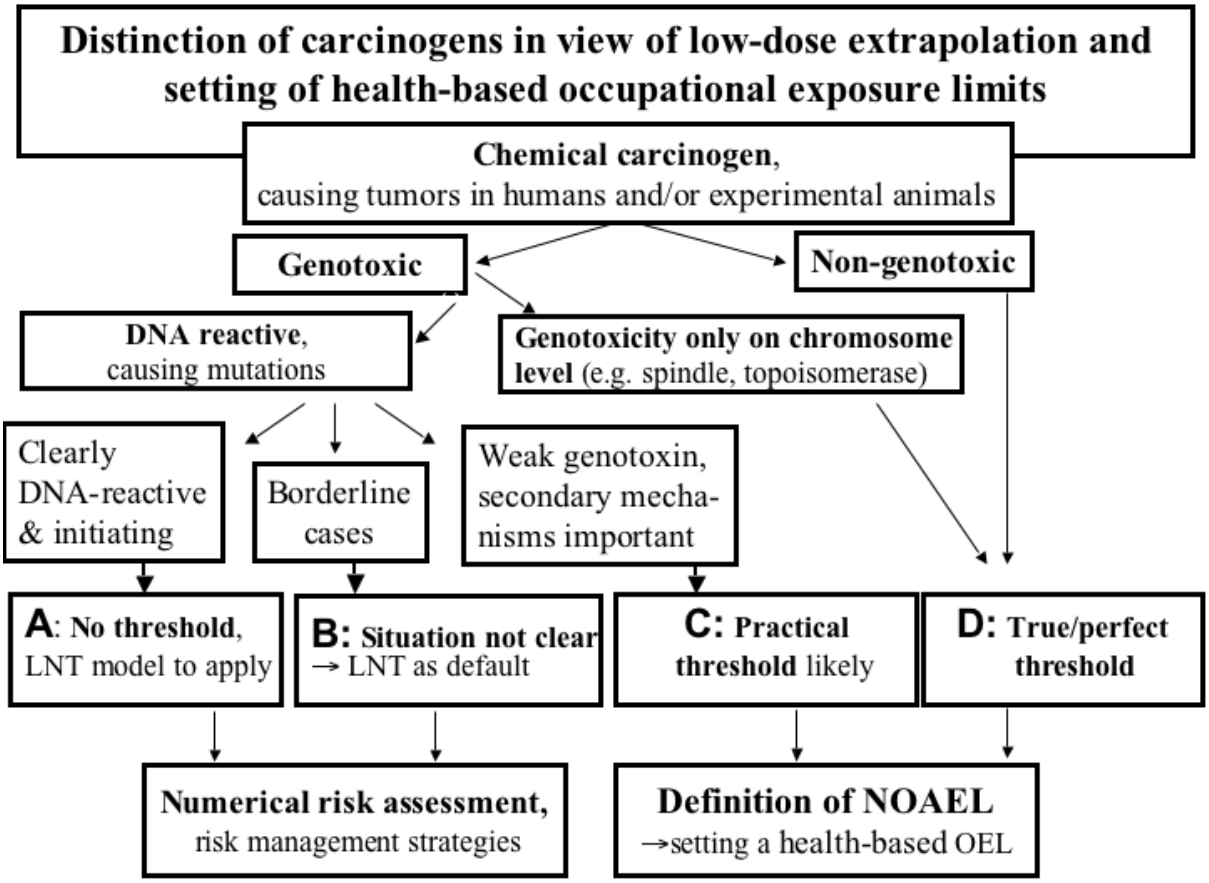
Zhou B and Wang T (1991). Historical cohort investigation of death cause in a chemical fiber factory. J Chin Med Univ. 20:35-37.

Zhurkov VS, Shram RI, Dugan AM (1983). [Analysis of the mutagenic activity of acrylonitrile]. [Article in Russian]. Gigiena i Sanitariia. (1):71-2.

Zimmerman SD, Marsh GM, Youk AO, Talbot E (2015). Evaluation of Potential Confounding by Smoking in the Presence of Misclassified Smoking Data in a Cohort Study of Workers Exposed to Acrylonitrile. Journal of occupational and environmental medicine. 57(2):146-151.

Appendix 1. SCOEL categorisation of carcinogens

Taken from current SCOEL 'Methodology for the Derivation of Occupational Exposure Limits' (SCOEL, 2013; version 7[35]),



Group A: Non-threshold genotoxic carcinogens; for risk low-dose assessment the linear non-threshold (LNT) model appears appropriate.

Group B: Genotoxic carcinogens, for which the existence of a threshold cannot be sufficiently supported at present. In these cases the LNT model may be used as a default assumption, based on the scientific uncertainty.

Group C: Genotoxic carcinogens for which a practical threshold is supported.

Group D: Non-genotoxic carcinogens and non-DNA reactive carcinogens; for these compounds a true ("perfect") threshold is associated with a clearly founded NOAEL.

[35] Available on Commission webpage on SCOEL [http://ec.europa.eu/social/main.jsp?catId=148&intPageId=684&langId=en]

Appendix 2. Key Information from Main Epidemiological Studies

Study	Study period	Number of workers	Person-years	Acrylonitrile use	Exposure assessment	Exposure categories	Analyses	Results	Comments
The NCI Study Blair et al., 1998	1950-1989 Follow-up 96% complete	25460	348642 exposed 196727 unexposed	Fibres Monomer Resins Acrylamide Acrylonitrile	Based on extensive industrial hygiene monitoring when available, process descriptions and changes, and expert judgment when no or little monitoring data available Development of quantitative estimates of exposure by job/department/time period	5 Categories in ppm-years <0.13, >0.13–57, <0.57–1.5, <1.5–8.0, 8.0+	Primary analyses uses cumulative exposure to acrylonitrile with the 5 categories. Other cumulative exposure category cut-points used, metrics (such as number of peaks), and other exposure factors such as respirator use and level of physical activity considered	All causes: Unexposed 0.7 (0.7-0.8) Exposed 0.7 (0.6-0.7) All cancers: Unexposed 0.9 (0.8-1.0) Exposed 0.8 (0.7-0.9) Lung cancer: Unexposed 0.8 (0.6-1.1) Exposed 0.9 (0.8-1.1) <0.13 1.1 (0.7-1.7) >0.13-0.57 1.3 (0.8-2.1) >0.57-1.5 1.2 (0.7-1.9) >1.5-8.0 1.0 (0.6-1.6) >8.0 1.5 (0.9-2.4) Prostate cancer: Unexposed 1.2 (0.7-2.3) Exposed 0.9 (0.6-1.5) <0.13 1.9 (0.7-5.4) >0.13-0.57 0.3 (0.1-2.4) >0.57-1.5 0.7 (0.2-3.3) >1.5-8.0 1.5 (0.5-4.5) >8.0 0.4 (0.1-3.5) Bladder cancer: Unexposed 1.0 (0.4-2.8) Exposed 0.8 (0.4-1.8) <0.13 -- >0.13-0.57 1.0 (0.2-5.6) >0.57-1.5 1.1 (0.2-6.5) >1.5-8.0 0.4 (0.1-4.0) >8.0 0.6 (0.1-5.8) CNS cancers: Unexposed 1.3 (0.7-2.3) Exposed 0.7 (0.4-1.3) <0.13 0.5 (0.1-1.8) >0.13-0.57 0.2 (0.1-1.7) >0.57-1.5 0.5 (0.1-2.3) >1.5-8.0 0.8 (0.1-2.4) >8.0 0.5 (0.1-2.5)	No evidence to indicate exposure to acrylonitrile at levels experienced associated with increased risk for most cancers of interest Excess of lung cancer in highest exposure group, but no dose-response relationship observed (cumulative exposure). No strong dose-response gradient observed by other exposure variables Largest & most statistically powerful study Power to detect moderate excesses for some cancer sites limited Smoking considered, adjustment resulted in slight reduction in risk ratios in highest exposure groups Well-documented procedure to develop qualitative estimates of historical exposure Exposure to other chemicals including benzene, butadiene, formaldehyde styrene, sulphuric acid & vinyl chloride not considered
The UK Study Benn and Osborne, 1998 (Previous reports: Werner & Carter, 1981)	1950-1991 Follow-up 97% complete	2763	63058	Polymerisation & spinning factories producing acrylic fibres & resins	Based on expert judgment and work history	3 Categories based on level and exposure potential: ‘‘high exposure”, ‘‘exposure” and ‘‘possible exposure”	Analyses based on highest exposure levels. There are no cumulative exposure estimates	All causes: 0.84 (0.76-0.93) All cancers: 0.88 (0.74-1.05) Lung cancer: All: 1.03 (0.78-1.34) High exposure: 1.41 (0.95-2.03) Possible exposure: 0.52 (0.23-1.04) Little/no exposure: 0.99 (0.62-1.52) Age group: 15-44 6.25 (0.29-13.85) 45-54: 0.73 (0.19-1.99) 55-64: 1.15 (0.56-2.12) ≥65: 1.56 (0.79-2.79)	No consistent support for hypothesis of causal relationship between acrylonitrile exposure & lung cancer. Increased risk seen in younger age group & workers first exposed after 1968 Limited information on exposure levels, assumed acrylonitrile exposure after 1980 was negligible compared to earlier exposures; questionable quality of source records of work histories (leave dates unknown, therefore estimated)

								Time since 1st exposure (years): <5: 1.01 (0.05-4.93) 5-10: 2.11 (0.67-5.08) 10-15: 1.61 (0.59-3.58) >15 1.30 (0.78-2.04) Length of exposure (years): <5: 1.43 (0.73-2.55) 5-10: 1.32 (0.58-2.61) 10-15: 1.28 (0.52-2.66) >15: 2.00 (0.64-4.82)	Use of national rather than local rates for comparing SMTs compromise inferences that can be drawn Potential for concomitant exposure to styrene & butadiene Power of study to examine effect by exposure categories limited Smoking not considered
The Dutch Study; Swaen et al., 2004 (Previous reports: Swaen et al., 1992; Swaen et al., 1998)	1956-1996 Follow-up 98.8% complete	2842 exposed 3961 unexposed	79205 exposed 134322 unexposed	acrylonitrile production Latex polymer Acrylic fibres acrylonitrile polymers Resins Acrylamide	Based on industrial hygiene monitoring when available, process changes over time, and expert judgment Job-exposure matrix constructed	3 Exposure categories in ppm-years: <1, 1–10, 10+	Primary analyses uses cumulative exposure to acrylonitrile for the 3 categories. Peak exposure (<10 ppm, 10–20 ppm, and >20 ppm) and respiratory use also considered	Lung Cancer: Low exposure (<1ppm-y; latency): <10y: -- 10-20y: 1.03 (0.21-2.97) >20y: 1.00 (0.27-2.53) Total: 0.92 (0.37-1.89) Moderate exposure (1-10ppm-y; latency): <10y: 0.31 (0.40-1.58) 10-20y: 1.29 (0.74-2.09) >20y: 1.04 (0.63-1.63) Total: 1.07 (0.75-1.47) High exposure (10+ppm-y; latency): <10y: 1.20 (0.24-3.44) 10-20y: 1.43 (0.74-2.49) >20y: 0.90 (0.41-1.70) Total: 1.15 (0.75-1.68) Peak exposure (ppm): None: 1.08 (0.65-1.69) <10: 1.11 (0.73-1.63 10-20: 1.02 (0.60-1.61) >20ppm: 1.05 (0.28-2.66) Brain cancer: Low exposure (<1ppm-y; latency): <10y: --- 10-20y: 30.00 (6.03-86.05) >20y: -- Total: 4.29 (0.86-12.29) Moderate exposure (1-10ppm-y; latency): <10y: 2.50 (0.03-12.63) 10-20y: 2.00 (0.23-6.97) >20y: -- Total: 1.11 (0.22-3.19) Peak exposure (ppm): None: 0.77 (0.01-3.89) <10: 2.11 (0.57-5.33) 10-20: 0.77 (0.01-3.89) >20: -- Prostate cancer: Peak exposure (ppm): None: 0.77 (0.09-2.68) <10: 1.25 (0.34-3.16)	Evidence of excess for specific cancers not strong External comparison group (nitrogen fixation plant) exposed over same period; potential chemical exposures not defined, unknown whether profile of this cohort was comparable to acrylonitrile cohort Power to detect dose-response relationships limited Smoking not considered Cause of death of 9 deaths not known Healthy worker effect indicated in both exposed & unexposed cohorts Analysis by peak exposure, respirator use & possible exposure to co-carcinogens indicated no excess risk of site specific cancers Possible misclassification of ACN exposure because of use of current measures to derive past exposures, & use of subjective information about exposure Pooling of data from factories with different sources of ACN production & exposure without adjusting for differences

								10-20: 0.42 (0.01-2.11) >20: 2.00 (0.03-10.11)	
The DuPont Study Symons et al., 2008 (Previous reports: O'Berg et al., 1980; O'Berg et al., 1985; Chen et al., 1987; Wood et al., 1998)	1947-1991 mortality 1956-1991 incidence Follow-up 99.1% complete	2548 11 of original cohort excluded because exposed to ACN <6-months	95657 exposed 1490705 unexposed	Fibres	Based on industrial hygiene monitoring, process descriptions, use of protective equipment, and expert judgment Job-exposure matrix developed	Cumulative exposures in proportional hazards model Latency, duration of exposure, & highest exposure level also considered	Other than use of the proportional hazards model, intensity of exposure (<10 ppm and 10+ ppm) for workers with cumulative exposure greater than 10 ppm-years	All causes: 0.92 (0.86-0.98) All cancers: 0.92 (0.81-1.04) Lung cancer: 0.92 (0.75-1.14) Prostate cancer: 1.02 (0.66-1.51) Urinary organs: 1.29 (0.74-2.09) CNS: 0.74 (0.27-1.62) HR for 100ppm increase in CE: Lung: 0.95 (0.73-1.23) Prostate: 0.78 (0.46-1.32) Urinary: 0.98 (0.53-1.79) CNS: 1.03 (0.38-2.78) HR for lagged CE for 100ppm increase in CE: Lung: 5y: 0.95 (0.70-1.28) 10y: 0.84 (0.59-1.19) 15y: 0.80 (0.53-1.22) Prostate: 5y: 0.86 (0.48-1.53) 10y: 0.98 (0.50-1.92) 15y: 0.95 (0.41-2.22) Urinary: 5y: 1.27 (0.67-2.43) 10y: 1.29 (0.63-2.67) 15y: 1.10 (0.47-2.57) CNS: 5y: 1.38 (0.43-4.47) 10y: 1.55 (0.44-5.47) 15y: 1.96 (0.49-7.84) Mean intensity (≥10ppm cf <10ppm): Lung: 1.09 (0.67-1.77) Prostate: 1.45 (0.56-3.81) Urinary: 0.74 (0.23-2.39)	No association of increased risk observed Good quality information on work practices & industrial hygiene, complete work records, & good follow-up No smoking data Power to detect dose-response relationship limited because of small numbers in sub-categories No monitoring of ACN before 1975, therefore data for early years inferred which could lead to exposure misclassification Concomitant exposure to other chemicals not considered Strong healthy worker effect may be present Cohort limited to male workers & lack of unexposed worker comparison groups to better assess risk of cancers Earlier studies only ones to consider cancer incidence
Lima, Ohio Cohort Marsh & Zimmerman, 2015 (Previous reports: Marsh et al., 1999;Marsh et al., 2001; sub-cohort of Blair et al., 1998)	1960-2011	Total: 2096 Exposed: 789 Unexposed: 1307	Total: 70835 Exposed: 24443 Unexposed: 46392	Monomer Resins Acrylamide	Based on industrial hygiene monitoring; industrial hygienists classified jobs into 20 groups; daily time-weighted exposure estimate assigned to each job group Job-exposure matrix developed	Duration of exposure, DOE (0, >0-4.9; 5.0-13.9; 14.0+; years); Cumulative exposure, CE (0; >0-8.91; 8.92-79.79; 79.80+; ppm-years); Average intensity, AIE (0; >0-3.37; 3.38-9.87; 9.88+; ppm) Time since first exposure, TSFE (0, <20, 20-29, 30+; years); Duration of employment, DOEmp (<10, 10-19, 20+; years)	Relative risk regression modelling; SMR analysis of mortality in relation to exposure categories	Exposed: All causes: 0.68 (0.57-0.80) All cancers: 0.84 (0.63-1.10) Lung cancer: 0.73 (0.41-1.20) Prostate cancer: 1.32 (0.43-3.09) Bladder cancer: 2.27 (0.62-5.80) CNS cancer: - (0.00-2.35) Unexposed: All causes: 0.85 (0.76-0.95) All cancers: 0.97 (0.80-1.17) Lung cancer: 0.84 (0.56-1.20) Prostate cancer: 0.79 (0.21-2.01) Bladder cancer: 1.82 (0.59-4.24) CNS cancer: 0.68 (0.08-2.47) Lung cancer: Unexposed: 1.00 TSFE (RR): <20: 1.43 (0.42-4.82 20-29: 1.45 (0.52-4.03) 30+: 0.75 (0.29-1.93) DOE,TSFE (RR): >5,>20: 1.04 (0.44-2.43) >10,>20: 1.13 (0.46-2.77)	No evidence of increased risk of lung cancer mortality at exposure levels experienced Analysis of sub-cohort of original Blair et al. (1998) cohort Other exposures included asbestos, 1-3-butadiene & depleted uranium, but no information provided on duration, level of or opportunity for exposure to them provided Smoking information collected for 90.3% of original cohort but deemed inaccurate so not included in analysis Possible healthy worker effect Small sample sizes within sub-categories may limit detection of stronger support for an association

								:5,>30: 0.79 (0.27-2.35) >10,>30: 0.73 (0.22-2.47) DOE: >0-4.9: 0.76 (0.27-2.09) 5-14.9: 1.93 (0.77-4.84) 15+: 0.84 (0.29-2.47) CE: >0-8.91: 1.02 (0.38-2.75) 8.92-79.79 1.12 (0.41-3.00) 79.80+: 1.02 (0.38-2.74) AIE: >0-3.37: 0.89 (0.34-2.36) 3.38-9.87: 1.44 (0.53-3.90) 9.88+: 0.96 (0.35-2.62)	

Appendix 3. Post-hoc power calculations for four cohort studies

Combining data from four studies (Blair et al., 1998; Swaen et al., 2004; Symons et al., 2008; Marsh and Zimmerman, 2015) where it was possible to abstract information about an exposed and unexposed population, the methodology described in Dos Santos Silva (1999) was used to estimate power. The calculations indicate that, although individual studies did not have the power to detect an excess cancer risk, adding these four studies together had sufficient power to detect a doubling of risk for cancers of the lung, bladder, prostate and brain. However, there was not enough power in these four studies to detect a 1.5 increase in risk for bladder and brain cancers. Nevertheless, the power would be increased significantly if more studies (i.e. all those in the various meta-analyses) were included in this calculation. For example, Collins and Strother (1999) reported a total of 60 cases of CNS tumours in their meta-analysis (versus 24 cases in the studies used for the power calculation), and there are at least 12 cases of bladder cancers to add from the Benn and Osborne (1998) UK study additional to the 31 cases in the four studies used for the power calculation.

Nevertheless, it should be noted that even if post-hoc sample size or “observed power” calculations are included in statistical software packages they have been criticized, they are a one-to-one function of the p-value attained and therefore adds no new information. In the context of clinical trial research, Walters (2009) concludes that post-hoc power calculations are not recommended, while the confidence intervals of the empirically observed effect should be used in interpreting the data. Hoenig and Heisey (2001) have even considered post-hoc power calculations are fundamentally flawed and again recommend using the confidence intervals for interpretation of empirical data.

Table 19: Power calculations (k*) to assess whether epidemiological studies can detect an increased risk (using methodology described in Dos Santos Silva, 1999)

	Symons et al (2008)		Blair et al (1998)		Marsh & Zimmerman (2015)		Swaen et al (2004)		Total	
	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed	Unexposed
PYAR	95657	1490705	348642	196727	244443.1	46391.5	79205	134322	767947.1	1868146
Lung	88	1329	134	59	15	29	67	160	304	1577
Bladder	16	113	6	4	4	5	5	16	31	138
Brain	6	111	12	11	0	2	6	9	24	133
Prostate	25	382	16	10	5	4	8	24	54	420
Lung	RR=1.5	RR=2.0	RR=1.5	RR=2.0	RR=1.5	RR=2.0	RR=1.5	RR=2.0	RR=1.5	RR=2.0
SE	0.110		0.156		0.318		0.146		0.063	
j+k	3.679	6.296	2.592	4.435	1.273	2.179	2.783	4.762	6.466	11.063
k	1.719	4.336	0.632	2.475	-0.687	0.219	0.823	2.802	4.506	9.103
Bladder	RR=1.5	RR=2.0	RR=1.5	RR=2.0	RR=1.5	RR=2.0	RR=1.5	RR=2.0	RR=1.5	RR=2.0
SE	0.267		0.645		0.671		0.512		0.199	
j+k	1.516	2.594	0.627	1.074	0.604	1.033	0.790	1.353	2.038	3.487
k	-0.444	0.634	-1.333	-0.886	-1.356	-0.927	-1.170	-0.607	0.078	1.527
Brain	RR=1.5	RR=2.0	RR=1.5	RR=2.0	RR=1.5	RR=2.0	RR=1.5	RR=2.0	RR=1.5	RR=2.0
SE	0.419		0.417		n/a		0.527		0.222	
j+k	0.966	1.653	0.970	1.660	n/a	n/a	0.768	1.315	1.826	3.125
k	-0.994	-0.307	-0.990	-0.300	n/a	n/a	-1.192	-0.645	-0.134	1.165
Prostate	RR=1.5	RR=2.0	RR=1.5	RR=2.0	RR=1.5	RR=2.0	RR=1.5	RR=2.0	RR=1.5	RR=2.0
SE	0.206		0.403		0.671		0.408		0.145	
j+k	1.962	3.357	1.005	1.719	0.604	1.033	0.992	1.697	2.801	4.794
k	0.002	1.397	-0.955	-0.241	-1.356	-0.927	-0.968	-0.263	0.841	2.834

* The power of a study is the probability of obtaining a statistically significant result if the true magnitude of the effect is as anticipated (RR of 1.5 or 2.0 in this example). Most often the 5% significance level is used (j=1.96 and CI = +/- 1.96* SE (standard error)). Usually studies are designed aiming at powers above 80% (0.80). Values of k for different power (p): k=1.645, p=0.95; k=1.282, p=0.90; k=0.674, p=0.75; k=0.000, p=0.50; k<0, p<0.50