Texas Commission on Environmental Quality Response to Public Comments Received on the May 2013 Proposed Acrylonitrile Development Support Document

The public comment period for the May 2013 Proposed Development Support Document (DSD) for acrylonitrile (AN) ended in August 2013. The Acrylonitrile (AN) Group, Inc. submitted comments on August 16, 2013. The Texas Commission on Environmental Quality (TCEQ) appreciates the effort put forth by the AN Group to provide technical comments on the proposed DSD for AN. The goal of the TCEQ is to protect human health and welfare based on the most scientifically-defensible approaches possible (as documented in the DSD), and evaluation of these comments furthered that goal. A summary of comments from the AN group provided below, followed by TCEQ responses. The full comments are provided in Appendix 1. Comments on issues that suggest changes in the DSD are addressed whereas comments agreeing with TCEQ's approach are not. TCEQ responses indicate what changes, if any, were made to the DSD in response to the comment.

Upon further review, the DSD has been revised. Information from the DSD that cites or quotes the withdrawn EPA 2011 draft AN IRIS document has been removed. The corresponding references are added (Benn and Osborne 1998, IPCS 2002, Kaneko and Omae 1992, Marsh et al. 2001, NCSAB 2010, Pacifici and Rane 1983, Swaen et al. 2004, Their et al. 2000, WHO 2000). The chronic ReV and ESL for AN have been revised by adopting an updated animal-to-human dosimetric, i.e., a default regional gas dose ratio for the extrathoracic region ($RGDR_{ET}$) = 1 as recommended by USEPA (2012). The $RGDR_{ET}$ of 1 is 3 to 5 times higher than those calculated according to USEPA 1994 RfC Methodology. Accordingly, the chronic ReV and ESL have been revised from 2.2 $\mu g/m^3$ (1 ppb) and 0.7 $\mu g/m^3$ (0.3 ppb) to 7.1 $\mu g/m^3$ (3.3 ppb) and 2.1 $\mu g/m^3$ (1 ppb), respectively.

1. General Comment

Comment No. 1 (Page 1):

The AN Group commented that, overall, TCEQ prepared a quality assessment of AN in the May 2013 proposed DSD.

TCEQ Response:

TCEQ appreciates the AN Group's review and comments.

2. Comments on DSD Chapter 2: Major Sources or Uses and Ambient Air Concentrations

Comment No. 1 (Page 1-2):

The AN Group has recently conducted a review of the available data on the ambient air levels of AN (from EPA databases) and provided this document to TCEQ. Specifically, the AN Group listed several key points from the review and indicated that they may provide some useful supplemental information to Chapter 2 of the DSD (See Appendix 1 for details).

TCEQ Response:

As recommended, the following information has been incorporated into the Chapter 2 of the DSD.

- AN is not exclusively a man-made chemical; it is a combustion by-product from biomass and possibly other sources.
- There are extensive data on AN ambient air monitoring in the U.S. The vast majority of air samples are below the detection limit and those above the detection limit are mostly below 1 ppb (2.2 $\mu g/m^3$) AN in the atmosphere.
- There are some ambient data available for three monitoring stations in Texas in 2007.

3. Comments on DSD Chapter 3: Acute Evaluation

Comment No. 2 (bottom of Page 2 through top of Page 3):

The AN Group has a general comment regarding the referencing of the June 2011 draft USEPA IRIS Toxicology Review of AN. It indicated that, based on the extensive public comments that identified significant concerns with this draft document, USEPA has withdrawn the entire draft assessment for further technical review and revision. The AN Group, therefore, requests that TCEQ remove information from the DSD that cites or quotes the withdrawn EPA 2011 draft AN IRIS document. It believes that the TCEQ DSD is sufficiently complete and robust even with the removal of citations and references to the 2011 draft IRIS document.

TCEQ Response:

As requested, the TCEQ has removed information from the DSD that cites or quotes the withdrawn EPA 2011 draft AN IRIS document. The corresponding references have been added (Benn and Osborne 1998, IPCS 2002, Kaneko and Omae 1992, Marsh et al. 2001, NCSAB 2010, Pacifici and Rane 1983, Swaen et al. 2004, Their et al. 2000, WHO 2000).

Comment No. 3 (Re: Section 3.1 Health-Based Acute ReV and ESL, Page 4):

The AN Group commented that the source of the following statement (Section 3.1 of the DSD) is not clear: *"Exposure to AN above 500 ppm for several minutes (min) is considered lethal to humans"*. The AN Group indicates that it is not aware of reliable data from the references cited in this section or from other references to quantify lethal levels of AN exposure to humans.

TCEQ Response:

The TCEQ agrees with the AN Group's comment. The statement has been removed from Section 3.1 of the DSD.

Comment No. 4 (Re: Section 3.1,2.2, Supporting Animal Studies, bottom of Page 4 through Page 5):

The AN Group further commented that reliable rat data do exist and show lethality following 4-hour exposure to 871 ppm, whereas exposure to 775 ppm for 4 hours did not result in lethality (WIL Research Laboratories, 2005, unpublished). It suggests that TCEQ add this study to Section 3.1.2.2 as the third supporting animal study.

TCEQ Response:

The TCEQ appreciates the AN Groups' comment/suggestion. The WIL Research Laboratories (2005) study has been added to Section 3.1.2.2.3 of the DSD.

Comment No. 5 (Re: Figure 2, Page 5):

The AN Group commented that the header for Figure 2 should be dropped to page 14. The figure would be more readable if it was inverted 180 degrees.

TCEQ Response:

Figure 2 has been revised accordingly.

Comment No.6 (Re: Section 3.1.5 Mode of Action (MOA) Analysis, bottom of Page 5 through top of Page 6):

The AN Group indicated that the statement: *"AN produces a variety of adverse health effects and the MOAs for these different health effects vary"* (Page 17, line 17, proposed DSD) needs to be corrected. It pointed out that while differences have been demonstrated in species sensitivity to the acute effects of AN, variation in effect levels across species is generally attributed to differences in the rates of AN and cyanide metabolism rather than to different MOAs.

TCEQ Response:

The aforementioned statement has been deleted and Section 3.1.5 has been revised. Specifically, the following statements are added: *"AN-induced convulsions, are likely due to the metabolically-released cyanide although the results of metabolism of acute systemic toxicity studies by Benz and Nerland (2005) suggested that only the early convulsion phase may be due to the oxidative metabolism of AN to cyanide and that the terminal convulsions that precede imminent death are due to the toxicity of the parent compound.* Observations in cases of *acute AN intoxication have led to the conclusion that cyanide has been implicated as perhaps playing a larger role in human AN-intoxications than in rodents (Thier et al. 2000), due to differences in oxidative metabolism"*.

Comment No.7 (Re: Section 3.1.5, Page 6):

The AN Group commented that while it is clear that rat nasal tissue has sufficient metabolic activity to generate cyanide from acrylonitrile (Dahl & Waruszewski, 1989), and a potential role for cyanide release in producing the teratogenic effects of AN in hamsters has been proposed (Wilhite *et al*. 1981; Wilhite, 1983), a role for metabolically generated cyanide in producing irritation is a hypothesis that is not well supported by the literature. TCEQ's report cites three sources for this conclusion (Kedderis *et al*. 1996; Saillenfait *et al*. 1993; USEPA, 2007). It further commented that cyanide release from AN requires CYP2E1 (Wang *et al*. 2002; Chanas *et al*. 2003; El Hadri *et al*. 2005). Rat nasal CYP2E1 oxidation rates (measured for styrene) have been reported to be greater in olfactory epithelium than in respiratory epithelium (Green *et al*. 2001), which makes it difficult to reconcile with the location of AN nasal lesions (predominantly in respiratory epithelium). The AN Group recommended that TCEQ reconsider the MOA proposed for irritation, and perhaps borrow from MOA information for portal-of-entry effects following oral exposure (e.g., GSH depletion and gastrointestinal irritation; Ghanayem *et al*. 1985; Ahmed *et al*. 1996).

TCEQ Response:

The TCEQ agrees that a role for metabolically generated cyanide in producing irritation is a hypothesis that is not well supported by the literature. The precise MOA of the acute toxic response, i.e., nasal tissue irritation, is not fully elucidated. As described in the proposed DSD, AN is not expected to be a developmental or reproductive toxicant in the absence of significant maternal toxicity (Section 3.1.3.2.5). The irritation of mucous membranes and headache observed in humans are considered critical effects for acute AN exposure (Section 3.1.7). The MOA of the acute toxic response has been revised to focus on irritation. Two cited sources (Kedderis *et al*. 1996; Saillenfait *et al*. 1993) have been deleted. The MOA information for portal-of-entry effects following oral exposure has been included in Section 3.1.5. Nevertheless, the TCEQ believes that the MOA of cyanide release from metabolism of AN exposure cannot be ruled out especially for nasal tissue irritation following chronic AN exposure. The AN Group commented that rat nasal CYP2E1 oxidation rates (measured for styrene) have been reported to be greater in olfactory epithelium than in respiratory epithelium (Green *et al*. 2001), which makes it difficult to reconcile with the location of AN nasal lesions (predominantly in respiratory epithelium). The comments may be true for transient irritation caused by short-term AN exposure but may not be in cases for chronic AN exposure. As described in Section 4.1.2, overt histopathologic changes in nasal and lung tissues (lesions), not just irritation, were observed in several chronic AN exposure animal studies.

Comment No.8 (Re: Section 3.1.5, bottom of Page 6 through top of Page 7):

The AN Group commented that the statement (last paragraph of Section 3.1 of the DSD) is not clear: *"Adverse effects from acute exposure to AN vary greatly amongst animal species… These effects vary in degrees of severity, target organ, potential carcinogenicity, and critical end-points of effect"*. It indicates that it is not clear what is meant by "*potential carcinogenicity*" in this statement given that it is in regards to adverse effects from acute exposure.

TCEQ Response:

The "*potential carcinogenicity*" has been deleted from the aforementioned statement.

4. Comments on Chapter 4: Chronic Evaluation

Comment No.9 (Re: Section 4.1 Noncarcinogenic Potential, Page 7):

The AN Group commented that the TCEQ proposed DSD states that reproductive effects have been observed in chronic inhalation toxicity studies with AN. It is not aware of any such data and no further details are provided in the TCEQ summary. The AN Group refers TCEQ to Neal et al. (2009) for a comprehensive review of the available reproductive toxicity data for AN.

TCEQ Response:

The "*reproductive effects*" has been deleted from the statement. The WOE review by Neal et al. (2009) has been described in Section 3.1.3.2.4 and the TCEQ has concluded that AN is not expected to be a developmental or reproductive toxicant in the absence of significant maternal toxicity (Section 3.1.3.2.5).

Comment No.10 (Re: Figure 3, bottom of Page 7):

The AN Group commented that Figure 3 would be more readable if it was inverted 180 degrees.

TCEQ Response:

Figure 3 has been revised accordingly.

Comment No.11 (Re: Section 4.1, bottom of Page 7 through Page 8):

The AN Group commented that consistent with TCEQ guidelines, which indicate a preference for using human data (TCEQ 2012), TCEQ should consider including an analysis of available human data for irritation as the primary basis for the ReV/ESL for AN. The AN further indicated that it is recognized that reliance on the human data reflects a trade-off on sources of uncertainty. The human data are uncertain and variable with respect to exposure but the observations are directly relevant, while the rodent data are certain on exposure levels but are uncertain with respect to human relevance.

TCEQ Response:

The TCEQ concurs with the AN Group that the human data are uncertain/variable with respect to exposure but the observations are directly relevant. Three additional epidemiological studies in AN-exposed workers (Sakurai et al. 1978, Muto et al. 1992, and Kaneko and Omae 1992 have been added to Section 4.1.2.2 Supporting Epidemiological Studies. However, as indicated in Section 4.1.2.2, because of potential limitations in the available human epidemiological studies (e.g., lack of reliable exposure data, difficult to assess a dose-response relationship, limitations in study designs, potential confounders), the TCEQ determined to not use these epidemiological studies to develop the chronic ReV for AN.

The TCEQ further concurs with the AN Group that while the rodent data are certain on exposure levels but are uncertain with respect to human relevance. Human and animal studies are consistent in identifying irritation effects following acute and repeated AN exposure. As described in Section 4.1, nasal irritation and neurological effects are the most sensitive endpoints seen in chronic inhalation studies of rats. Human data are difficult to assess in relation to establishment of a dose-response relationship for chronic toxicity. Many of the findings in the animal studies, especially the neurological effects and irritation, reflected the reported findings in workers (EU 2004). Based on the MOA information, Kirman et al. (2008) indicated that the irritation and neurological effects of AN are assumed to be relevant to human. The TCEQ believes that it is appropriate using the Nemec et al. (2008) and Quast et al. (1980) animal studies as key studies. Both studies are well-conducted rat studies on nasal irritation by administered multiple exposure levels and showed dose-effect relations (see Section 4.1.2.1 for details). Therefore, the key studies selected for deriving TCEQ chronic toxicity factors were not changed. Nevertheless, the use of nasal lesions observed in rats as the critical effects for humans is considered conservative.

Comment No.12 (Re: Section 4.1, top of Page 8):

The AN Group further indicated that the NOAELs of 0.5 and 0.53 ppm for mucous membrane irritation from Sakurai et al. (1978) and Muto et al. (1992) have been used by the North Carolina SAB (NCSAB) to support a chronic acceptable ambient level (AAL) of 0.03 mg/m^3 for AN based on irritation as the most sensitive endpoint.

TCEQ Response:

The TCEQ is aware that the NCSAB has re-assessed and established a noncancer chronic exposure AAL for AN at a concentration of 0.03 mg/m^3 based on the NOAELs identified from

Sakurai et al. (1978) and Muto et al. (1992) studies. For the comparison purpose, the NCSAB chronic AAL has been added to Section 4.1.10 Comparison of Various Chronic Toxicity Values.

Comment No.13 (Re: Section 4.1,4, Page 8):

The AN Group commented that TCEQ calculates chronic POD values for 3 key data sets for nasal irritation: (1) hyperplasia of respiratory epithelium in male & female rats (Nemec et al. 2008); (2) hyperplasia of mucous secreting cells in male rats (Quast 1980); and (3) flattening of respiratory epithelial cells in female rats (Quast 1980). TCEQ's use of multiple data sets to support the ReV and ESL values is to be applauded. However, it further commented that because of the relatively small number of animals assessed per test concentration in the key studies (n=10-12), it is difficult to make clear conclusions regarding potential sex differences in sensitivity to nasal lesions in rats. The AN Group suggests that because of the small number of animals examined per test group, and because no consistent pattern has been identified with respect to one sex being more sensitive than the other, TCEQ should (1) consider combining nasal lesion data of Quast across sexes prior to BMD modeling to increase the statistical power of the data set (i.e., similar approach that TCEQ used with the Nemec data); or (2) consider modeling each data set separately and then adopting a geometric mean across sex, nasal lesion type, and/or data set.

TCEQ Response:

The TCEQ appreciates the AN Group's comments. The DSD was not revised based on these comments. Nemec et al. (2000) data showed that F1 males were more sensitive to histological changes than F1 females at 5 ppm exposure level; however, the lesions were not statistically significant in either males or females. In addition, there were no potential sex differences at 15 or 45 ppm. Thus, incidence data from both F1 males and females for hyperplasia in respiratory/transitional epithelium (the most sensitive endpoint) were pooled for BMC modeling (see Section 4.2.4.1). However, as indicated in Section 4.1.2.1.2, the incidence data from the Quast et al. (1980) study showed that males were more sensitive to hyperplasia in the nasal turbinates and mucous secreting cells than females; and females were more sensitive to focal inflammation and flattening of the nasal turbinate tissue at both 20 and 80 ppm exposure level. Because of potential sex differences for these endpoints at 20 and 80 ppm exposure levels (except for hyperplasia of the mucous secreting cells at 80 ppm exposure level), incidence data from both males and females were not pooled for BMC modeling.

As indicated in Section 4.4.1, BMC modeling results showed that the $BMCL_{10}$ values are 2.961, 0.797, 1.247, and 0.564 ppm based on incidence data for four endpoints (Quast et al. 1980), and is 0.919 ppm based on Nemec et al. (2008). The geometric mean for these five $BMCL_{10}$ values is 1.083 ppm. The geometric mean value is not much different from the $BMCL_{10}$ of 0.564 and 0.777 ppm (Quast study) and the $BMCL_{10}$ of 0.919 ppm (Nemec study) that were used as the POD to derive the TCEQ chronic ReV. Therefore, the geometric mean $BMCL_{10}$ would not be used to derive chronic toxicity values for AN.

Comment No. 14 (Re: Section 4.1.6, top of Page 11):

The AN Group commented that TCEQ adopts USEPA (1994) regional gas dose ratio (RGDR) approach for interspecies extrapolation. It indicated that the TCEQ should consider adopting more

recent recommendations from USEPA that a default RGDR for the extrathoracic region ($RGDR_{ET}$) of 1 be used for animal-to-human dosimetric adjustment (USEPA, 2012).

TCEQ Response:

A default $RGDR_{ET} = 1$ has been applied for a rat-to-human adjustment. The $PODs_{HEC}$ were subsequently revised (Section 4.1.6). The corresponding revised chronic ReVs are 3-5 times higher than those previously derived and are consistent with that established by OEHHA (2001) and NCSAB (2010) (Section 4.1.9).

Comment No. 15 (Re: Section 4.1.6, Page 11 through top of Page 12):

The AN Group further commented that if TCEQ opts to continue to apply the RGDR approach, TCEQ should consider alternative values for rat nasal surface area as well as human ventilation rate.

TCEQ Response:

The DSD was not revised based on this comment. As described in the Response to Comment No. 14 above, since a default $RGDR_{ET} = 1$ has been applied for a rat-to-human adjustment, the recommended two alternative values are not needed for RGDR adjustment.

Comment No. 16 (Re: Section 4.1.6.2, top of Page 12):

The AN Group indicated that Section heading of "4.2.6.2" should be numbered "4.1.6.2"

TCEQ Response:

The TCEQ appreciates this clarification. The section heading has been corrected to "4.1.6.2".

Comment No. 17 (Re: Section 4.1.7, top of Page 12):

The AN Group commented that for effects occurring at the point of contact, TCEQ should consider reducing the UF for intraspecies variation (UF_H) from a value of 10 to 3. It indicated that USEPA's standard operating procedures for AEGL development state, *"If evidence is available indicating the mechanism of action, such as direct acting irritation or alkylation is not expected to differ significantly between species an interspecies uncertainty factor of 3 is generally used"* (USEPA, 2000). In deriving their AEGL-2 for AN, USEPA adopted a value of 3 for intraspecies variation, stating, *"the effects associated with acute AN exposure are not likely to vary greatly among individuals; metabolism is not likely to be instrumental in initial minor effects resulting from low-level exposure"* (USEPA, 2007).

TCEQ Response:

The DSD was not revised based on the aforementioned comments. The TCEQ agrees with USEPA that a UF_H be limited to 3 because "the effects associated with acute AN exposure are not likely to vary greatly among individuals; metabolism is not likely to be instrumental in initial minor effects resulting from low-level exposure". Because AEGLs are set at *"threshold exposure limits"* for the general public and are applicable to emergency exposure period from 10 minutes to 8 hours, a UF_H of 3 may be appropriate for the AEGL-2 which was based upon light transient effects in rats following a 2-hour AN inhalation exposure (Dudley and Neal 1942). However, a UF_H of 3 may not be protective for deriving a non-threshold exposure level for AN. Additionally, the critical effects (nasal lesions) observed in the Quast et al. (1980) and Nemec et al. (2008) chronic studies were overt histopathology lesions, not just irritation. Thus, like USEPA (1991)

RfC and OEHHA (2001) REL which were based on the Quast et al. (1980) study, a UF_H of 10 was used for the derivation of TCEQ chronic ReV which was based on the same study. The use of a UF_H of 10 is also consistent with that recommended by a peer review panel of AN risk assessment for a RfC proposed by the AN Group (Haber and Patterson 2005) (see Section 4.1.5).

Comment No. 18 (Re: Section 4.1.7, top of Page 12) - continued:

The AN Group further commented that the need for a full factor of 10 is also reduced by TCEQ's adoption of an upper bound value for human ventilation rate in their application of the RGDR approach (see Comment No. 14 above).

TCEQ Response:

The DSD was not revised based on this comment. Since a default $RGDR_{ET} = 1$ has been applied for a rat-to-human adjustment based on updated animal-to-human dosimetric recommendations in USEPA (2012), the revised chronic ReVs (7.1 and 16 $\mu g/m^3$ from Quast et al. 1980 and Nemec et al. 2000, respectively) are approximately 3 to 5 times higher than the previously proposed (see Section 4.1.5). The TCEQ believes that a full factor of 10 for UF_H is reasonable.

Comment No. 19 (Re: Table 2, line 2, Page 12):

The AN Group indicated that although Quast (1980) evaluated 100 animals per sex per test group for some endpoints; the number of animals evaluated for nasal lesions is considerably smaller (10-12). It requests that entries to this table be revised accordingly.

TCEQ Response:

The TCEQ appreciates this clarification. The number of animals evaluated for nasal lesions has been revised to "10-12" SD female rats per exposure group.

Comment No. 20 (Re: Section 4.2.2.1.2 Maltoni et al. 1988 Study, Page 12):

The AN Group indicated that the Maltoni et al. (1988) study has been the subject of recent pathology working group (PWG) peer review by NTP. The summary report of the NTP (2011) review stated that *"In general, there was good agreement between the Ramazzini Institute (RI) diagnosis and PWG opinions with some minor issues in terminology for neoplasms in the acrylonitrile"*. While USEPA indicated their intention to consider results of the NTP peer review in a 2010 press release (USEPA, 2010), USEPA IRIS chose to not use the Maltoni et al. (1988) tumor results to develop its inhalation unit risk for AN.

TCEQ Response:

The TCEQ appreciates this information. The information has been added to Section 4.2.2.1.2.

Comment No. 21 (Re: Section 4.2.2.3 Carcinogenic MOA, top of Page 13):

The AN Group indicated that USEPA IRIS is re-evaluating the available information on MOA, including comments submitted by the AN Group. A copy of the AN Group comments to EPA regarding the carcinogenic MOA of AN have been previous provided to TCEQ.

TCEQ Response:

Since the TCEQ has removed information from the DSD that cites or quotes the withdrawn EPA 2011 draft AN IRIS document, the DSD was revised based on this comment.

Comment No. 22 (Re: Appendix B, top of Page 13):

BMD results for nasal flattening in female rats (Quast, 1980) are missing. Please provide documentation for all BMD analyses conducted, including those used or considered by TCEQ.

TCEQ Response:

The missing BMD analysis results for nasal flattening in female rats (Quast, 1980) has been added as Table B-4 in Appendix B.

APPENDIX 1

The Acrylonitrile (AN) Group's

Comments Regarding the TCEQ Development Support Document for Acrylonitrile Toxicity Values

THE AN GROUP

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August 16, 2013

Dr. Jong Song Lee
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Texas Commission on Environmental Quality
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RE: Comments to the Texas Commission on Environmental Quality Regarding the May 2013 Proposed Development Support Document for Acrylonitrile

Dear Dr. Lee:

Attached are the Acrylonitrile (AN) Group's comments to the Texas Commission on Environmental Quality (TCEQ) regarding the May 2013 Proposed Development Support Document (DSD) for AN. Please do not hesitate to contact me with any questions or for any of the references mentioned in the comments. I can be reached at 202-419-1504 or at ajaques@regnet.com.

Thank you for your consideration of these comments.

Best Regards,

Andrew Jaques

Enclosure

COMMENTS TO THE TEXAS COMMISSION ON ENVIRONMENTAL QUALITY REGARDING THE MAY 2013 PROPOSED DEVELOPMENT SUPPORT DOCUMENT FOR ACRYLONITRILE

Submitted by the
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August 16, 2013

1. Introduction

The Acrylonitrile (AN) Group is pleased to provide these comments to the Texas Commission on Environmental Quality (TCEQ) in response to the May 2013 Proposed Development Support Document (DSD) for Acrylonitrile (AN). The AN Group is committed to the stewardship of AN, including the development of guidance on the proper handling and safe use and the sponsorship of research directed at understanding the potential health effects associated with AN. The AN Group prides itself on supporting basic and applied research, utilizing state-of-the-art techniques, including sophisticated mode-of-action research to further the understanding of AN toxicity. The AN Group has established a network of academic and other consulting researchers to assist with these endeavors.

Texas is an important state for the AN industry with the majority (~70%) of the U.S. AN monomer production occurring at two manufacturing sites in Texas. In addition, there are several large industrial users of AN in Texas. These facilities employ thousands of people in Texas and products made from reacted AN monomer have enormous benefits to both the U.S. and Texas economies. The AN industry is a net exporter from both Texas and the U.S., contributing billions of dollars annually in U.S. trade surpluses.

Overall, the AN Group believes that TCEQ has prepared a quality assessment of AN in the May 2013 proposed DSD. The AN Group offers the following comments, which we hope will be of assistance to TCEQ in finalizing its DSD for AN. For ease of review, these comments are organized in sections corresponding to the same numbered chapters of the proposed DSD.

2. Comments on DSD Chapter 2: Major Sources or Uses and Ambient Air Concentrations

Chapter 2 of this document provides some useful background on the use and possible exposure levels of AN from the environment. The AN Group has recently conducted a review of the available data on the ambient air levels of AN (from EPA databases) and the potential sources that may contribute to these air concentrations. Information from that review may provide some useful supplemental information to this chapter of the DSD. The AN Group has recently provided this document to TCEQ.

Several key points from the review were:

- Acrylonitrile is not exclusively a man-made chemical; it is a combustion by-product from biomass (Karl *et al*. 2003; Yokelson *et al*. 2008) and possibly other sources.
- AN Toxic Release Inventory (TRI) air emissions have dropped significantly (over 90%) since the initiation of the TRI program, from 1988.
- There are extensive data on AN ambient air monitoring in the U.S. The vast majority of air samples are below the detection limit and those above the

detection limit are mostly below 1 ppb (2.2 µg/m^3) AN in the atmosphere (see summary statistics below).

- There is no apparent correlation between those monitoring stations with the highest AN ambient air levels and being near a TRI air emission source, although the locations of the monitoring stations limits the ability to make close comparisons.
- The AN Group is theorizing that biomass (and perhaps other) combustion sources likely play a significant role in contributing to background levels of AN in the ambient air.

Extracted from Tables 1 and 3 in AN Ambient Air Review (AN Group 2012)

Statistics	Acrylonitrile Detectable Ambient Air Samples (µg/m^3)	
	1997-2007	2008-2009
Sample Size	2599	559
5-percentile	0.074	0.037
25-percentile	0.325	0.088
50-percentile	0.672	0.148
75-percentile	1.150	0.348
95-percentile	2.169	2.522
Mean	0.844	0.519
Max	22.105	8.702

While not specifically covered in the AN Group review, there are some ambient data available for three monitoring stations in Texas. The most recent year in which data were reported from Texas is 2007 (Attachment A summarizes all 2007 AN air monitoring data from EPA). A summary of the Texas results are provided below.

2007 Texas AN Ambient Air Monitoring Data (as reported in EPA AirData)

Monitor	**No. of Observations**	**Mean µg/m^3 (ppb)**	**Max µg/m^3 (ppb)**
Deer Park 1	57	0.119 (0.054)	1.54 (0.7)
Deer Park 2	27	0.213 (0.097)	1.54 (0.7)
Harrison County (Hwy 134)	52	0.035 (0.016)	0.31 (0.14)

3. Comments on DSD Chapter 3: Acute Evaluation

Overall Chapter 3 of the proposed DSD is well organized and the derivations of the acute reference value (ReV) and effect screening level (ESL) are clear and well supported. The AN Group comments on this chapter are summarized below. The AN Group also has a general comment that impacts both Chapters 3 and 4 of the DSD regarding the referencing of the June 2011 draft U.S. Environmental Protection Agency (EPA)

Integrated Risk Information System (IRIS) Toxicology Review of AN. This is discussed further below.

Withdrawn 2011 Draft EPA IRIS Assessment Should Not Be Cited in DSD

In June 2011, EPA released a draft updated AN IRIS assessment for public review. This draft document was clearly marked *"Do not Cite or Quote"* as EPA recognized that the document had not yet been reviewed by the public or external peer-reviewers. Based on the extensive public comments[1] that identified significant concerns with this draft document, EPA withdrew the entire document for further technical review and revision. It is our understanding that a revised draft assessment is now being prepared by EPA, though any subsequent drafts will then be subject to further review by the public and also the newly formed EPA Science Advisory Board Chemical Assessment Advisory Committee (CAAC). As such, it likely will be at least a year or more from now before EPA issues the new final AN IRIS assessment.

The AN Group, therefore, requests that TCEQ remove information from the DSD that cites or quotes the withdrawn EPA 2011 draft AN IRIS document. It would appear that in most cases, the draft EPA IRIS document can be readily replaced as a reference in the DSD by directly citing the primary literature that EPA used. While this change will seemingly necessitate the removal of the proposed IRIS Reference Concentration (RfC) from the toxicity values comparisons in Section 4, it is important to note that there is the likely possibility that the revised IRIS assessment will contain a different RfC. Likewise, the same possibility exists for EPA to change other derivations (e.g., unit risk factors) in future drafts of the IRIS assessment. The AN Group believes that the DSD is sufficiently complete and robust even with the removal of citations and references to the 2011 draft IRIS document.

The following list includes the places in the proposed DSD that cite the withdrawn EPA 2011 draft IRIS document:

Section 3:
3.1.1 Physical/Chemical Properties, line 5
3.1.2 Key and Supporting Studies, line 13
3.1.3.1 Epidemiological Studies, line 8

Section 4:
4.1 Noncarcinogenic Potential, line 17
4.1.2.1 Key Animal Studies, lines 27-30
4.1.2.2. Supporting Epidemiological Studies, lines 4-9
4.1.2.2.1 Lu et al. (2005) Study, pg. 28, line 35 and pg. 29, line 2
4.1.2.3 Supporting Animal Study (Gagnaire *et al*. 1998), line 5
4.1.8 Possible Child/Adult Differences, lines 27-29

[1] The entire AN IRIS file, including the AN Group comments can be accessed at: http://www.regulations.gov/#!docketDetail;D=EPA-HQ-ORD-2009-0204 .

Table 11. Comparison of AN Chronic Noncancer Toxicity Values
4.1.10.3 USEPA (2011)
4.2.1 Mutagenicity and Genotoxicity, lines 21-27
4.2.2.2 Epidemiological Studies, line 25
4.2.2.2.1 DuPont Studies, pg. 40, lines 32 and 35 and pg. 41, lines 13, 18, and 20
4.2.2.2.2 Blair et al. (1998) Study, pg. 42, line 6 - 14
4.2.2.3 Carcinogenic MOA, lines 22-29 and lines 34-35
4.2.2.4 WOE Classifications, lines 10-13
4.2.2.5 Conclusions, line 27
4.2.3 List of Various Estimated URF Values Derived by USEPA
Table 13. List of Various Inhalation URFs Derived by USEPA
4.2.3.2 USEPA (2011) Human Occupational Data
4.2.3.3 USEPA (2011) Rat Inhalation Data

3.1 Health-Based Acute ReV and ^{acute}ESL

Page 11, line 30: The source of the following statement is not clear: *"Exposure to AN above 500 ppm for several minutes (min) is considered lethal to humans"*. We are not aware of reliable data from the references cited in this section or from other references to quantify lethal levels of AN exposure to humans. Reliable rat data do exist and show lethality following 4-hour exposure to 871 ppm, whereas exposure to 775 ppm for 4 hours did not result in lethality (WIL Research Laboratories, 2005). Further details of this rat study are given below in comments on Section 3.1.2.2 – Supporting Animal Studies. We ask that TCEQ reconsider the basis for this statement and/or provide some perspective on its degree of reliability.

Page 12, line 2: Two animal studies are cited as supportive evidence (Dudley & Neal, 1942; Dudley, 1942). A third well-conducted animal study exists (WIL Research Laboratories, 2005) that we believe warrants inclusion. Further details of this rat study are given below in comments on Section 3.1.2.2 below.

3.1.2.2 Supporting Animal Studies

We suggest that TCEQ add the following study to their analysis - "WIL Research Laboratories, 2005, unpublished". A GLP-OECD guideline study sponsored by the Shanghai SECCO Petrochemical Company, Ltd. and SNF SAS examined the acute toxicity of AN in rats (WIL Research Laboratories, 2005). In this study, groups of 5 male and 5 female Crl:CD7(SD) rats (8-12 weeks old; 242-297 g) were exposed (nose-only) for 4 hours to 539, 775, 871, 1006, or 1181 ppm AN (99.9 % purity). The rats were acclimated for 7 days prior to exposure and observed for 14 days after exposure. Exposure was in a two-tiered conventional nose-only exposure system where exposure atmosphere conditions (temperature, oxygen, humidity, etc.) were monitored every 20-30 minutes. The AN test atmosphere was generated by passing compressed nitrogen through the test material to create a vapor which was diluted with compressed air prior to being delivered to the exposure system. Actual AN concentrations were determined by gas chromatography. Mortality data are summarized in the Table below. The study report

provided 4-hour LC50 values of 964 ppm (857-1085 95% CI) for males, 920 ppm (807-1050 95% CI) for females, and 946 ppm (866-1032 95% CI) combined (determined by the method of Litchfield and Wilcoxon, 1949).

Lethality in rats following nose-only inhalation exposure to AN for 4 hours				
Exposure Conc. (ppm)	Mortality During Exposure		Total Mortality	
	M	F	M	F
539	0	0	0	0
775	0	0	0	0
871	0	0	1	3
1006	1	1	3	4
1181	4	3	5	4

Clinical observations immediately following exposure included tremors, ataxia, labored respiration, hypoactivity, decreased defecation, and gasping but there was no apparent exposure concentration-effect relationship. Necropsy findings in dead rats included the presence of a distended, gas-filled jejunum in one female of the 871-ppm group, distended gas-filled stomach in three females in the 871-ppm and 1006-ppm groups, and dark, discoloration of the lungs in one male and one female in the 1181-ppm group. No other findings were noted for rats that died. At scheduled sacrifice, the only finding was dark discoloration of the lungs in one male of the 871-ppm group.

A complete copy of this study report has been provided to TCEQ.

Page 13-14, Figure 2: The header for this figure should be dropped to page 14. The figure would be more readable if it was inverted 180 degrees.

3.1.5 Mode of Action (MOA) Analysis

Page 17, line 27: The TCEQ proposed DSD states that *"AN produces a variety of adverse health effects and the MOAs for these different health effects vary"*. As this statement is made in Chapter 3, it is our assumption that it is intended to characterize the acute effects of AN exposure. Our understanding is that, while differences have been demonstrated in species sensitivity to the acute effects of AN (e.g. Dudley & Neal, 1942; Dudley *et al*. 1942), variation in effect levels across species is generally attributed to differences in the rates of AN and cyanide metabolism rather than to different MOAs. Clinical indications of intoxication are similar across species.

The mechanism of acute systemic toxicity produced by AN exposure has been studied by Benz and Nerland (2005). These investigators administered AN to rats with and without inhibitors of oxidative metabolism to study the role of the parent molecule and metabolically-release cyanide in acute AN intoxication. In rats, it appears that the early convulsive phase is due to the oxidative metabolism of AN to cyanide and that the terminal convulsions that precede imminent death are due to the toxicity of the parent AN molecule. Blocking cyanide release in AN-intoxicated rats increased survival time but

did not substantially alter the eventual lethal outcome. Wang et al. (2002) studied the acute toxicity of AN in CYP2E1 knockout mice, which lack the primary pathway for cyanide formation, and concluded that cyanide plays an essential role in the acute toxicity/mortality of AN. It is important to note that the study by Wang et al. (2002) was of short duration (3 hrs.), and thus did not evaluate the late stage lethal effects reported in rats by Benz and Nerland (2005). Cyanide has been implicated as perhaps playing a larger role in human AN-intoxications than in rodents (Thier *et al*. 2000), due to differences in oxidative metabolism.

Page 17-18, line 36-1: While it is clear that rat nasal tissue has sufficient metabolic activity to generate cyanide from acrylonitrile (Dahl & Waruszewski, 1989), and a potential role for cyanide release in producing the teratogenic effects of AN in hamsters has been proposed (Wilhite *et al*. 1981; Wilhite, 1983), a role for metabolically generated cyanide in producing irritation is a hypothesis that is not well supported by the literature. TCEQ's report cites three sources for this conclusion (Kedderis *et al*. 1996; Saillenfait *et al*. 1993; USEPA, 2007). Kedderis et al. (1996) did not specifically examine cyanide formation or its role in toxicity. The statement in the introduction of Kedderis et al. (1996), "The acute toxic effects of ACN appear to be largely due to metabolically formed cyanide", appears to be related to the discussion of the neurotoxic effects reported by Benz et al. (1990) in the preceding sentence. The study of Saillenfait et al. (1993) is specific to developmental toxicity of various nitriles, and does not support a strong role for cyanide release (and did not confirm the increased malformations reported by Wilhite *et al*. 1981). Specifically, maternal and fetal body weight changes were more pronounced in rats exposed to acrylonitrile than methacrylonitrile (Saillenfait *et al*. 1993), despite likely equal or greater liberation of cyanide from methacrylonitrile via P450 metabolism (El Hadri *et al*. 2005).

Cyanide release from AN requires CYP2E1 (Wang *et al*. 2002; Chanas *et al*. 2003; El Hadri *et al*. 2005). Rat nasal CYP2E1 oxidation rates (measured for styrene) have been reported to be greater in olfactory epithelium than in respiratory epithelium (Green *et al*. 2001), which makes it difficult to reconcile with the location of AN nasal lesions (predominantly in respiratory epithelium). Lastly, USEPA (2007) derived AEGL values for multiple nitrile chemicals (acetonitrile, isobutyronitrile, propionitrile, chloracetonitile, malonitrile). All of these are metabolized to produce cyanide to some extent, however none of the AEGLs derived by EPA are based on nasal irritation/lesions.

We recommend that TCEQ reconsider the MOA proposed for irritation, and perhaps borrow from MOA information for portal-of-entry effects following oral exposure (e.g., GSH depletion and gastrointestinal irritation; Ghanayem *et al*. 1985; Ahmed *et al*. 1996).

Page 18, line 4: The TCEQ proposed DSD states that *"Adverse effects from acute exposure to AN vary greatly amongst animal species… These effects vary in degrees of severity, target organ, potential carcinogenicity, and critical end-points of effect."* As stated earlier, our view is that the pattern of acute effects is generally consistent across species, while the level of exposure producing acute effects differs due largely to differences in the rates of metabolism. Additionally, it is not clear what is meant by

"potential carcinogenicity" in this statement given that it is in regards to adverse effects from acute exposure. We are not aware of information supporting the view that the adverse effects of acute AN exposure include carcinogenicity.

4. Comments on Chapter 4: Chronic Evaluation

As with the previous chapter, the AN Group finds that this chapter is generally well organized and decisions for the quantitative assessments are clearly laid out and supported. Key decisions regarding the selection of the key endpoint (nasal irritation) over other endpoints (neurotoxicity, cancer) are well supported by the available literature. Some important points are provided below for TCEQ to consider as it finalizes the report.

4.1 Noncarcinogenic Potential

Page 22, line 5: The TCEQ proposed DSD states that reproductive effects have been observed in chronic inhalation toxicity studies with AN. We are not aware of any such data and no further details are provided in the TCEQ summary. For a comprehensive review of the available reproductive toxicity data for AN, we refer TCEQ to Neal et al. (2009). The Neal et al. (2009) "Weight-of- the-evidence review of acrylonitrile reproductive and developmental toxicity studies" assessed study strength, characterized toxicity, and identified no-observed-adverse-effect levels. This weight-of-evidence review concludes:

> *"The epidemiological studies do not demonstrate causality and are not sufficiently robust to be used for risk assessment. Rodent developmental studies showed fetotoxicity and malformations at maternally toxic levels; there was no unique developmental susceptibility. NOAELs for oral and inhalation exposures were 10 mg/ kg/day and 12 ppm (6 h/day), respectively. Drinking-water and inhalation reproductive toxicity studies showed no clear effects on reproductive performance or fertility. Maternally toxic concentrations caused decreased pup growth. The drinking-water reproductive NOAEL was 100 ppm (moderate confidence due to study limitations). The inhalation exposure reproductive and neonatal toxicity high confidence NOAEL was 45 ppm (first generation 90 ppm) (6 h/day). The inhalation reproductive toxicity study provides the most robust data for risk assessment. Based on the WoE evaluation, AN is not expected to be a developmental or reproductive toxicant in the absence of significant maternal toxicity."*

Page 27, Figure 3: This figure would be more readable if it is inverted 180 degrees.

Page 28: Consistent with TCEQ guidelines, which indicate a preference for using human data (TCEQ, 2012), TCEQ should consider including an analysis of available human data for irritation as the primary basis for the ReV/ESL for AN. It is recognized that reliance on the human data reflects a trade-off on sources of uncertainty. The human data are uncertain/variable with respect to exposure but the observations are directly relevant, while the rodent data are certain on exposure levels but are uncertain with respect to

human relevance. For example, Sakurai et al. (1978), provided results from 102 workers across 6 acrylic fiber production plants in Japan who were exposed to AN for at least five years. Average exposures were: 0.1 ppm (0.2 mg/m^3) in the three plants with the lowest exposure; 0.5 ppm (1.1 mg/m^3) in two plants characterized as having "intermediate exposure;" and 4.2 ppm (9.1 mg/m^3) in one plant having the highest AN exposures. Physical examination noted a "slightly higher" incidence for signs of irritation (redness of the eyes and throat) and a palpable liver in the highest exposed group (4.2 ppm) relative to that in the control group. No effects were seen in the groups exposed to 0.1 or 0.5 ppm. Muto et al. (1992) found no excessive respiratory symptoms among workers at these same plants with an average exposure of 0.53 ppm. The NOAEL for mucous membrane irritation with chronic acrylonitrile exposure appears to be 0.53 ppm (1.15 mg/m^3). These NOAELs are approximately an order of magnitude higher than BMCL10HEC values calculated by TCEQ from rodent studies, which may reflect in part species differences and/or suggest that refinements are needed in the assumptions used for interspecies extrapolation (discussed further in comments on Pages 31-32). The North Carolina SAB used the NOAELS of 0.5 and 0.53 ppm from Sakurai et al. (1978) and Muto et al. (1992), respectively, to support a chronic safety level of 0.03 mg/m^3 for AN based on irritation as the most sensitive endpoint.

Pages 29-31: In section 4.1.4, TCEQ calculates POD values for 3 key data sets for nasal irritation: (1) hyperplasia of respiratory epithelium in male & female rats (Nemec et al., 2008); (2) hyperplasia of mucous secreting cells in male rats (Quast, 1980); and (3) flattening of respiratory epithelial cells in female rats (Quast, 1980). TCEQ's use of multiple data sets to support the ReV and ESL values is to be applauded. However, because of the relatively small number of animals assessed per test concentration in the key studies (n=10-12), it is difficult to make clear conclusions regarding potential sex differences in sensitivity to nasal lesions in rats. For example, based on the Quast (1980) data, male rats appear to have a higher incidence of some nasal lesions (suppurative rhinitis, respiratory hyperplasia, focal erosion of respiratory mucosa, hyperplasia of mucous secreting cells), while female rats appear to have a higher incidence of other nasal lesions (flattening of respiratory epithelial cells, focal inflammation) (**Table 1**). Similarly, based on the Nemec et al. (2008) data, male rats appear to have a higher incidence of some nasal lesions (hyperplasia of respiratory hyperplasia, squamous metaplasia, sub-acute inflammation), while female rats appear to have a higher incidence of other nasal lesions (olfactory degeneration) (**Table 2**). Each study has its strengths and limitations. Quast (1980) includes lifetime exposures to AN, however, the study was not initially designed to examine nasal lesions (hence only a small subset of animals examined), and evidence of irritation likely occurred well before the time of observation at 2 years. Nemec et al. (2008) includes current histopathology methods, more treatment groups, and coverage across a lower range of exposures. Because of the small number of animals examined per test group, and because no consistent pattern has been identified with respect to one sex being more sensitive than the other, TCEQ should (1) consider combining nasal lesion data of Quast across sexes prior to BMD modeling to increase the statistical power of the data set (i.e., similar approach that TCEQ used with the Nemec data); or (2) consider modeling each data set separately and then adopting a geometric mean across sex, nasal lesion type, and/or data set.

Table 1. Nasal Lesions in Male and Female Rats Exposed to AN (Quast, 1980)*

Male Rats Quast (1980; Table A-29, p 205)								
Conc	n	Suppurative rhinitis	Respiratory hyperplasia	Focal erosion of respiratory mucosa	Squamous metaplasia respiratory epithelium	Flattening of respiratory epithelium	Hyperplasia of mucous secreting cells	Focal inflammation
0	11	0	0	0	0	0	0	0
20	12	1	4	0	1	2	7	1
80	10	5	10	4	7	3	8	1
Female Rats Quast (1980; Table A-33, p 256)								
Conc	n	Suppurative rhinitis	Respiratory hyperplasia	Focal erosion of respiratory mucosa	Squamous metaplasia respiratory epithelium	Flattening of respiratory epithelium	Hyperplasia of mucous secreting cells	Focal inflammation
0	11	1	0	0	0	0	0	2
20	10	0	2	1	2	7	2	6
80	10	2	5	1	5	8	8	7
Combined								
Conc	n	Suppurative rhinitis	Respiratory hyperplasia	Focal erosion of respiratory mucosa	Squamous metaplasia respiratory epithelium	Flattening of respiratory epithelium	Hyperplasia of mucous secreting cells	Focal inflammation
0	22	1	0	0	0	0	0	2
20	22	1	6	1	3	9	9	7
80	20	7	15	5	12	11	16	8

*Highlighted values indicate a potential sex difference in incidence

Table 2. Nasal Lesions in Male and Female Rats Exposed to AN (Nemec *et al*. 2008)*

Male Rats					
Conc	n	Hyperplasia RE	Metaplasia, squamous	Inflammation	Degeneration, OE
0	10	2	0	2	0
5	10	6	2	4	0
15	10	10	8	9	0
45	10	10	8	9	5
Female Rats					
Conc	n	Hyperplasia RE	Metaplasia, squamous	Inflammation	Degeneration, OE
0	10	0	0	0	0
5	10	0	0	0	0
15	10	7	6	6	0
45	10	9	4	3	8
Combined					
Conc	n	Hyperplasia RE	Metaplasia, squamous	Inflammation	Degeneration, OE
0	20	2	0	2	0
5	20	6	2	4	0
15	20	17	14	15	0
45	20	19	12	12	13

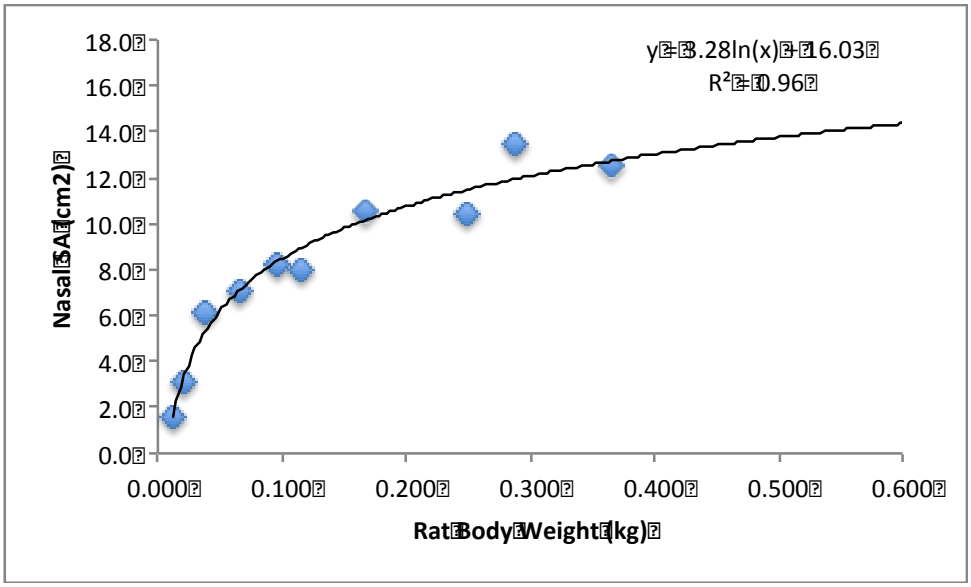
*Highlighted values indicate a potentially higher incidence compared to corresponding data for the other sex

Pages 31-32: In sections 4.1.6 TCEQ adopts USEPA's regional gas dose ratio (RGDR) approach for interspecies extrapolation. However, TCEQ should consider adopting more recent recommendations from USEPA for interspecies extrapolation (USEPA, 2012). Based upon a review of assessments using physiologically based pharmacokinetic (PBPK) and computation fluid dynamic (CFD) models, USEPA concluded that there is "*Strong evidence indicating that in the absence of modeling the default DAF = 1*" for extrathoracic effects. For this reason, we recommend that for calculating human equivalent concentrations from nasal lesion data in rodent studies, TCEQ should adopt an assumption of equal sensitivity across species for AN concentrations in air (i.e., RGDR = 1). This approach is consistent with: (1) observations of ocular/conjunctiva irritation (which is clearly independent of ventilation rates) along with respiratory irritation in exposed workers (Muto *et al*. 1992; Sakurai, 1978); and (2) the approach adopted by USEPA for deriving an AEGL for AN based on irritation (USEPA, 2007).

Alternatively, if TCEQ opts to continue to apply the RGDR approach, TCEQ should consider alternative values:

- *Rat Nasal Surface Area* - Specifically, although TCEQ adopted ventilation rates that varied as a function of body weight using an allometric equation (USEPA, 1988; 1994), for rat nasal surface area TCEQ adopted a constant value (15 cm^2) for all rats, regardless of age/size. Based on the data available in the published literature (Patra, 1987; Schreider, 1983; Gross *et al*. 1982), rat nasal surface area clearly varies as a function of body weight (age) (**Figure 1**). TCEQ should consider replacing the constant nasal surface area value with those that better correspond to the size of the rat evaluated in the toxicity studies.

Figure 1. Rat Nasal Surface Area (cm^2) as a Function of Body Weight (kg) (Patra, 1987; Schreider, 1983; Gross *et al*. 1982).



- *Human Ventilation Rate* - With respect to human values, the ventilation rate of 13.8 L/min (20 m^3/day) corresponds approximately to a 95^{th} percentile value for adults (USEPA, 2011). TCEQ should consider using a central tendency value of 10.5 L/min (15.1 m^3/day) for the RGDR calculation. Alternatively, use of an upper bound ventilation rate here for humans reduces the need to include a full factor of 10 for intraspecies variation (see comment below for page 34, lines 6-8).

Page 32, Line 20: Section heading should be numbered "4.1.6.2".

Page 34, lines 6-8: In deriving an ESL for AN in section 4.1.7, TCEQ included a full factor of 10 to account for intraspecies variation. For effects occurring at the point of contact, TCEQ should consider reducing this uncertainty factor to a value of 3. For example, USEPA's standard operating procedures for AEGL development state, "*If evidence is available indicating the mechanism of action, such as direct acting irritation or alkylation is not expected to differ significantly between species an interspecies uncertainty factor of 3 is generally used"* (USEPA, 2000). In deriving their AEGL-2 for AN, USEPA adopted a value of 3 for intraspecies variation, stating, "*the effects associated with acute AN exposure are not likely to vary greatly among individuals; metabolism is not likely to be instrumental in initial minor effects resulting from low-level exposure*" (USEPA, 2007). The need for a full factor of 10 is also reduced by TCEQ's adoption of an upper bound value for human ventilation rate in their application of the RGDR approach (see comment for page 31-32 above).

Page 36, Table 10, line 2: Although Quast (1980) evaluated 100 animals per sex per test group for some endpoints; the number of animals evaluated for nasal lesions is considerably smaller (10-12). Please revise entries to this table accordingly.

4.2 Carcinogenic Potential

Page 39, line 18: The AN Group would like to make TCEQ aware that this study of AN conducted by the Ramazzini Institute (RI), has been the subject of recent pathology working group (PWG) peer review by National Toxicology Program (NTP). USEPA indicated their intention to consider results of the NTP peer review in a 2010 press release (USEPA, 2010).

A summary report of the NTP review, was subsequently released by NTP (NTP, 2011). In their report, NTP stated:

> *"A pathology review, based on NTP procedures including pathology data review, QA, and PWG, was conducted on selected treatment-related findings in the five chronic rodent cancer studies carried out by RI. In general, there was good agreement between the RI diagnosis and PWG opinions with some minor issues in terminology for neoplasms in the acrylonitrile, VC, and ETBE studies."*

In their 2011 draft document EPA IRIS stated that they chose to not use the Maltoni et al. (1988) tumor results for AN to develop reference values.

Page 42, line 15: It is our understanding that EPA is re-evaluating the available information on MOA, including comments submitted by the AN Group. A copy of the AN Group comments to EPA regarding the carcinogenic MOA of AN have been previous provided to TCEQ.

Appendix B

Page 54: BMD results for nasal flattening in female rats (Quast, 1980) are missing. Please provide documentation for all BMD analyses conducted, including those used or considered by TCEQ.

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Excerpt from REACH 2010 Chemical Safety Report (CSR) on Acrylonitrile

Mutagenicity

Non-human *in vitro*

Method	Results	Remarks	Reference
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) *S. typhimurium* TA 1535 (met. act.: with and without) *S. typhimurium* TA 98 (met. act.: with and without) *S. typhimurium* TA 100 (met. act.: with and without) *S. typhimurium* TA 97 (met. act.: with and without) Doses: Five concentrations of between 100 and 10000 µg/plate and a negative control (0µg/plate). No toxicity was observed at the highest dose of 10000 µg/plate. equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay) NTP protocol	Evaluation of results: positive with metabolic activation (TA100 and TA1535) negative without metabolic activation (TA100 and TA1535) negative (with and without S9 in TA97 and TA98) Test results: positive for *S. typhimurium* TA 100 (all strains/cell types tested); met. act.: with; cytotoxicity: no positive for *S. typhimurium* TA 1535 (all strains/cell types tested); met. act.: with; cytotoxicity: no negative for *S. typhimurium* TA 97 (all strains/cell types tested); met. act.: with and without; cytotoxicity: no negative for *S. typhimurium* TA 98(all strains/cell types tested); met. act.: with and without; cytotoxicity: no	1 (reliable without restriction) key study experimental result **Test material (EC name): acrylonitrile**	NTP (2001b)
mammalian cell gene mutation assay (gene mutation) mouse lymphoma L5178Y cells (met. act.: without) Doses: 3.13, 6.25, 12.5, 25 and 50 nl/ml. The high dose of 50 nl/ml was determined by cytotoxicity (100nl/ml was cytolethal). equivalent or similar to OECD Guideline 476 (*In vitro* Mammalian Cell Gene Mutation Test) NTP protocol	Evaluation of results: positive without metabolic activation Test results: positive for mouse lymphoma L5178Y cells(all strains/cell types tested); met. act.: without; cytotoxicity: no (high dose chosen based on toxicity)	1 (reliable without restriction) key study experimental result **Test material (EC name): acrylonitrile**	NTP (2001c)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation)	Evaluation of results: positive with metabolic	2 (reliable with restrictions)	European Chemicals Bureau

S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 *E. coli* WP2 uvr A *Aspergillus nidulans* Doses: Various concentrations were used in the reviewed studies equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	activation Test results: positive for *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100; met. act.: with positive for *E. coli* WP2 uvr A positive for *Aspergillus nidulans*	weight of evidence review / secondary source **Test material (EC name): acrylonitrile**	(2004b)
mammalian cell gene mutation assay (gene mutation) mammalian cell line, other: various (met. act.: with and without) Doses: Various test concentrations were used The EU RAR summarises the results of a number of studies of various experimental designs.	Evaluation of results: positive with metabolic activation Test results: positive for mouse lymphoma L5178Y cells; met. act.: with positive for human lymphoblastoid cells (TK6); met. act.: with	2 (reliable with restrictions) weight of evidence review / secondary source **Test material (EC name): acrylonitrile**	European Chemicals Bureau (2004b)
bacterial reverse mutation assay (e.g. Ames test) (gene mutation) *S. typhimurium*, other: TA100, TA 98, TA 1535, TA 1537 and TA 1538 (met. act.: with and without) *E. coli*, other: WP2 uvr A and WP2 uvrA/pKM101 (met. act.: with and without) Doses:-S9 mix / +S9 mix ; 0, 100, 250, 500, 1,000, 2,500, 5,000 µg/plate equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: positive (weak positive in TA1535 (+S9)) Test results: positive for *S. typhimurium* TA 1535(strain/cell type: *S. typhimurium* TA 1535); met. act.: with; cytotoxicity: no, but tested up to limit concentrations negative for *S. typhimurium* TA 1535(strain/cell type: *S. typhimurium* TA 1535); met. act.: without; cytotoxicity: no, but tested up to limit concentrations negative for *S. typhimurium*, other: *S. typhimurium* TA 100, TA 98, TA 1537, TA 1538(strain/cell type: *S. typhimurium* TA 100, TA 98, TA 1537, TA 1538); met. act.: with and without; cytotoxicity: no, but tested up to limit concentrations negative for *E. coli*, other: WP2 uvr A and WP2 uvrA/pKM101 (strain/cell type: *E. coli* WP2 uvr A and WP2 uvrA/pKM101); met.	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): acrylonitrile**	Matsushita H & Goto S (1980)

	act.: with and without; cytotoxicity: no, but tested up to limit concentrations		
review of *in vitro* mutagenicity studies performed in bacteria and mammalian cell systems (gene mutation) various systems *in vitro* (met. act.: with and without) Doses: Various This review summarises the available data on the *in vitro* mutagenicity of acrylonitrile in bacterial and mammalian cell systems	Evaluation of results: positive with metabolic activation	2 (reliable with restrictions) supporting study review / secondary source **Test material (EC name): acrylonitrile**	The Sapphire Group (2004a)
sister chromatid exchange assay in mammalian cells (chromosome aberration) Chinese hamster Ovary (CHO) (met. act.: with and without) Doses: Trial 1 without S9: 0.16, 0.5, 1.6, 5, 16 and 50µg/ml. The high dose was limited by toxicity. Trial 2 without S9: 10, 20, 30 and 40 µg/ml. 30µg/ml was lethal in 1/2 tests and 40µg/ml was lethal. Trial 1 with S9: 1.6, 5, 16, 50 and 160 µg/ml. The high dose was limited by toxicity. Trial 2 with S9: 10, 25, 50, 75 and 150 µg/ml. equivalent or similar to OECD Guideline 479 (Genetic Toxicology: *In vitro* Sister Chromatid Exchange Assay in Mammalian Cells) NTP protocol	Evaluation of results: positive (with and without metabolic activation) Test results: positive for Chinese hamster Ovary (CHO)(all strains/cell types tested); met. act.: with and without; cytotoxicity: yes (high concentrations based on toxicity)	1 (reliable without restriction) weight of evidence experimental result **Test material (EC name): acrylonitrile**	NTP (2001d)
in vitro mammalian chromosome aberration test (chromosome aberration) various cell types (met. act.: with and without) Doses: Various The EU RAR summarises and reviews the results of a number of studies of clastogenicity *in vitro*.	Evaluation of results: positive Test results: positive	2 (reliable with restrictions) weight of evidence review / secondary source **Test material (EC name): acrylonitrile**	European Chemicals Bureau (2004b)

in vitro mammalian chromosome aberration test (chromosome aberration) mammalian cell line, other: Chinese hamster lung fibroblasts (CHL cells) (met. act.: with and without) Doses:-S9 mix : 24h continuous treatment : 0, 0.010, 0.015, 0.020, 0.025, 0.030, 0.035 mg/mL 48h continuous treatment : 0, 0.010, 0.015, 0.020, 0.025, 0.030, 0.035 mg/mL -S9 mix (short-term treatment) : 0, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1 mg/mL +S9 mix (short-term treatment) : 0, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1 mg/mL Standard of Chromosomal Aberration Test in Cultured Mammalian Cells (18, March 1987) (Ministry of Labor in Japan)	Evaluation of results: positive Test results: positive for mammalian cell line, other: Chinese hamster lung fibroblasts (CHL cells) (strain/cell type: Chinese hamster lung fibroblasts (CHL cells)); met. act.: with and without; cytotoxicity: yes (24h continuous treatment : 0.035 mg/mL, 48h continuous treatment : above 0.030 mg/mL,-S9 mix (short-term treatment) : 0.1 mg/mL, +S9 mix (short-term treatment) : 0.1 mg/mL)	1 (reliable without restriction) supporting study experimental result **Test material (EC name): acrylonitrile**	Asakura M, Noguchi T, Sugiyama Y, Inoue M & Satake H. (1994)
review of various clastogenicity studies *in vitro* (chromosome aberration) Doses: Various The authors provide an overview of the available *in vitro* data on clastogenicity.	Evaluation of results: positive with metabolic activation Test results: positive (SCE) for rat liver and CHO cell lines (all strains/cell types tested); met. act.: with positive for Chinese hamster lung, liver, and ovary cells (all strains/cell types tested); met. act.: with and without	2 (reliable with restrictions) supporting study review / secondary source **Test material (EC name): acrylonitrile**	The Sapphire Group, Inc (2004a)
various studies of DNA damage and repair *in vitro* (DNA damage and/or repair) various Doses: The reviewed studies used various test concentrations. Summaries and critical reviews of the available DNA damage/repair studies evaluated in the EU RAR are included. The studies are largely non-standard.	Evaluation of results: ambiguous Test results: generally negative results for mammalian cells (HeLA, rat hepatocytes and human mammary epithelial cells); cytotoxicity: yes	2 (reliable with restrictions) weight of evidence review / secondary source **Test material (EC name): acrylonitrile**	European Chemicals Bureau (2004b)

various studies are reported (the results of various genetic effects are investigated in studies in yeast cells) Saccharomyces cerevisiae Doses: Various The results of various genotoxic studies in yeast are reviewed in the RAR	Evaluation of results: positive Test results: positive	2 (reliable with restrictions) weight of evidence review / secondary source **Test material (EC name): acrylonitrile**	European Chemicals Bureau (2004b)
literature review (all types) various Doses: Various The authors review the genotoxicity of acrylonitrile and consider the role of genotoxic or other mechanisms in the carcinogenicity of acrylonitrile.	Evaluation of results: positive Test results: Adducts affecting base pairing were formed in isolated DNA exposed *in vitro* to the metabolite cyanoethylene oxide (CEO). Positive reverse mutagenesis in *Salmonella typhimurium* HisG46 base substitution tester strains by acrylonitrile is attributable to CEO. Other *in vitro* genotoxicity test assays of acrylonitrile have yielded mixed results, without consistent effect of metabolic activation. Some positive genotoxicity data for acrylonitrile appear to result from artefacts or from non-DNA-reactive mechanisms.	2 (reliable with restrictions) weight of evidence Review **Test material (EC name): acrylonitrile**	Whysner J, Ross PM, Conaway CC, Verna LK & Williams GM (1998)
review (review of studies *in vitro*) Doses: Various Review of *in vitro* genotoxicity data, with a focus on the mode of action for the carcinogenicity of this substance.	Evaluation of results: There is abundant evidence in the genotoxicity profile of acrylonitrile to demonstrate that it does induce mutations in several in vitro test systems. It's mutagenicity however, appears to be weak, with most of the positive *in vitro* studies having been conducted at high exposure levels compared to *in vivo* expectations, and in test organisms selected for their susceptibility to mutations.	2 (reliable with restrictions) weight of evidence review / secondary source **Test material (EC name): acrylonitrile**	Acrylonitrile REACH Consortium (2010)

Non-human: *In vivo*

Method	**Results**	**Remarks**	**Reference**
various studies in *Drosophila* (gene mutation) *Drosophila melanogaster*	Evaluation of results: positive Test results:	2 (reliable with restrictions) weight of evidence	European Chemicals Bureau (2004b)

injection, feeding, inhalation The EU RAR summarises the findings of a number of *Drosophila* genotoxicity studies of various designs.	Genotoxicity: positive	review / secondary source **Test material (EC name): acrylonitrile**	
micronucleus assay (chromosome aberration) mouse (NMRI) male intraperitoneal 20 or 30 mg/kg (nominal conc.) Investigation of clastogenicity in mouse bone marrow *in vivo* and in a dominant lethal assay	Evaluation of results: negative Test results: Genotoxicity: negative (male)	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): acrylonitrile**	Leonard A, Garny V, Poncelet F & Mercier M (1981)
micronucleus assay (chromosome aberration) mouse intraperitoneal 20 mg/kg (nominal conc.) Assessment of genetic toxicity in the mouse micronucleus assay and DNA strand break assay	Evaluation of results: negative Test results: Genotoxicity: negative	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): acrylonitrile**	Hachiya N, Sato M & Takizawa Y (1984) Hachiya N, Tanaka N & Takizawa Y (1986) Hachiya N (1987)
micronucleus assay (chromosome aberration) mouse (B6C3F1) male/female oral: gavage 0, 5, 10, 20, 40 or 60 mg/kg bw (nominal in water) NTP protocol	Evaluation of results: negative Test results: Genotoxicity: negative (male/female); toxicity: yes	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): acrylonitrile**	NTP (2001a)
micronucleus assay (chromosome aberration) mouse (NMRI) male intraperitoneal 0, 20, 30 mg/kg bw/d (actual dose) Mouse bone marrow micronucleus assay	Evaluation of results: negative Test results: Genotoxicity: negative (male)	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): acrylonitrile**	Leonard A, Garny V, Poncelet F & Mercier M (1981)
chromosome aberration assay (chromosome aberration) mouse (NMRI, C75B1/6)	Evaluation of results: negative Test results: Genotoxicity: negative (male)	2 (reliable with restrictions) weight of evidence review / secondary	European Chemicals Bureau (2004b)

intraperitoneal 20, 30, 60-100 mg/kg bw (actual dose) Review and summary of published chromosomal aberration studies.		source **Test material (EC name): acrylonitrile**	
dominant lethal assay (chromosome aberration) rat (Fischer 344) male oral: gavage 60 mg/kg bw (actual ingested (gavage)) Dominant lethal assay in rats	Evaluation of results: negative Test results: Genotoxicity: negative (acrylonitrile) (male); toxicity: yes	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): acrylonitrile**	Working PK, Bentley KS, Hurtt ME & Mohr KL (1987)
micronucleus assay (chromosome aberration) mouse (NMRI) male intraperitoneal 0, 20, 30 mg/kg bw/d (actual dose) Mouse bone marrow micronucleus assay	Evaluation of results: negative Test results: Genotoxicity: negative (male)	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): acrylonitrile**	Leonard A, Garny V, Poncelet F & Mercier M (1981)
unscheduled DNA synthesis (DNA damage and/or repair) rat male oral: gavage 60 or 75 mg/kg (nominal conc.) The authors evaluated UDS in rat spermatocytes and hepatocytes *in vivo* by the (preferred) autoradiographic method	Evaluation of results: negative (no evidence of UDS for acrylonitrile; positive for CEO) Test results: Genotoxicity: negative (male)	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): acrylonitrile**	Butterworth BE, Eldridge SR, Sprankle CS, Working PK, Benley KS & Hurtt ME (1992)
unscheduled DNA synthesis (DNA damage and/or repair) rat (Fischer 344) male oral: gavage 50 mg/kg bw (nominal conc.) The authors measured *in vivo* DNA repair in the liver and brain by incorporation of 3H-thymidine during unscheduled DNA synthesis (UDS)	Evaluation of results: positive Test results: Genotoxicity: positive (liver) (male) Genotoxicity: negative (brain) (male)	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): acrylonitrile**	Hogy LL & Guengerich FP (1986)
unscheduled DNA synthesis	Evaluation of results: positive (:	2 (reliable with	Ahmed AE,

(DNA damage and/or repair) rat (Sprague-Dawley) male oral: unspecified 23 or 46 mg/kg bw (actual ingested (gavage)) The authors studied gastric DNA damage and unscheduled DNA synthesis (UDS) in rats	gastric mucosal cells) Test results: Genotoxicity: positive (male)	restrictions) weight of evidence experimental result **Test material (EC name): acrylonitrile**	Nouraldeen AM, Abdel-Rahman SZ & Rajaraman S (1994)
unscheduled DNA synthesis (DNA damage and/or repair) rat and mouse (various) male/female various The EU RAR reviews the findings of a number of studies of UDS *in vivo*.	Evaluation of results: ambiguous (: negative results in studies using autoradiography) Test results: Genotoxicity: ambiguous (male/female)	2 (reliable with restrictions) weight of evidence review / secondary source **Test material (EC name): acrylonitrile**	European Chemicals Bureau (2004b)
sister chromatid exchange assay (DNA damage and/or repair) mouse (C57BL) male intraperitoneal 30, 45 or 60 mg/kg (nominal conc.) The study examined sister chromatid exchange and chromosomal aberrations in bone marrow cells from male mice	Evaluation of results: ambiguous (weakly positive at an intermediate dose level) Test results: Genotoxicity: ambiguous (SCEs at 30 mg/kg) (male); toxicity: yes (at 45 and 60 mg/kg)	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): acrylonitrile**	Sharief Y, Brown AM, Backer LC, Campbell JA, Westbrook-Collins B, Stead AG & Allen JW (1986)
unscheduled DNA synthesis (DNA damage and/or repair) rat (Sprague-Dawley) male oral: gavage 46.5 mg/kg bw (actual ingested) *In vivo* UDS in the lung	Evaluation of results: positive Test results: Genotoxicity: positive (male); toxicity: yes (Hyperplasia of Clara cells and occasional focal perivascular oedema were seen 24 hours after acrylonitrile administration.)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): acrylonitrile**	Ahmed AE, Abdel-Aziz AH, Abdel-Rahman SZ, Haque AK, Nouraldeen AM & Shouman SA (1992)
review (all types of genotoxicity *in vivo*) various species used in the reviewed studies (various) male/female various exposure regimes were used in the reviewed studies Literature review	The most reliable *in vivo* tests of acrylonitrile have been negative, indicating an absence of systemic DNA reactivity *in vivo.* In all of the studies reported, although the exposures used were adequate and the methods were extremely sensitive, no genotoxic effect of acrylonitrile was found to account for the carcinogenicity of acrylonitrile in its target organs. DNA reactivity of acrylonitrile or	2 (reliable with restrictions) weight of evidence Review **Test material (EC name): acrylonitrile**	Whysner J, Ross PM, Conaway CC, Verna LK & Williams GM (1998)

	its metabolites has been detected under certain experimental conditions, but these effects have not been linked to acrylonitrile carcinogenesis in the rat target organs. The existing genotoxicity data for acrylonitrile, therefore, do not provide the mode of action for tumour formation.		
review (all types of genotoxicity *in vivo*) various species used in the reviewed studies (various) male/female various exposure regimes were used in the reviewed studies Literature review	Evaluation of results: The lack of induction of sex-linked recessive lethal mutations in *Drosophila* ranks acrylonitrile with other weak mutagens whose mutagenic effects are inhibited by effective repair. Furthermore, there is a remarkable disparity between the potential of acrylonitrile for inducing chromosome level mutations *in vitro* versus *in vivo*, with none being demonstrated for the latter, even at high doses and non-physiological routes of administration. Acrylonitrile does however induce gene mutations *in vivo* in rodents. Also, molecular epidemiological studies in humans have revealed some reports of acrylonitrile associated gene and chromosome level mutations in exposed worker populations, with the caveat that it has been impossible to exclude confounder effects in some studies. There is also ample evidence in acrylonitrile's genotoxicity profile that it and its CEO metabolite do reach critical cancer target tissues *in vivo*, with administered radio-labelled material being widely distributed among tissues and organs. However, the radioactivity has been bound primarily to proteins. In terms of critical acrylonitrile /CEO specific DNA adducts, only 7OEG has been found, and only in liver by a single laboratory. Therefore, the available evidence indicates that such adducts are either not efficiently produced or are rapidly repaired, at least in brain. This argues against mutations resulting from acrylonitrile's direct, DNA reactivity as a cause of tumours in this target tissue. However, by contrast, several studies have demonstrated that acrylonitrile induces 8oxoG DNA adducts in brain that could result in mutations. Even though	2 (reliable with restrictions) weight of evidence review **Test material (EC name): acrylonitrile**	Acrylonitrile REACH Consortium (2010)

	acrylonitrile, through its CEO metabolite, is a DNA reactive agent and is also mutagenic, it is likely that its mutagenicity is, at least in part, due to its indirect effects in inducing oxidative DNA damage. The implication is that, if mutation is a critical early event in the production of brain tumours, this is most likely secondary to indirect mutagenicity.		
transgenic animal mutagenicity assay (Chromosomal and gene mutation) mouse (CD2F1 (BALB/C - DBA2)) male oral: drinking water Total dose: 505, 1620 and 2350 mg/kg (equivalent to approximate daily doses of 18.04, 57.86 and 83.93 mg/kg, respectively) (nominal in water) The paper reviews all the transgenic mutagenicity studies conducted up to July 2004, with recommendations for development of an OECD test guideline on transgenic rodent mutation assays.	Evaluation of results: negative Test results: Genotoxicity: negative (male) splenic lymphocytes, lung, seminiferous tubules, brain and bone marrow	2 (reliable with restrictions) weight of evidence review of experimental results **Test material (EC name): acrylonitrile**	Lambert IB, Singer TM, Boucher SE & Douglas GR (2005)
literature review (all types of genotoxicity *in vivo* are reviewed) Various (literature review) Various (literature review) Methodological details for individual studies are limited. Review of the genotoxicity data for acrylonitrile, summarising and updating previous reviews	The authors conclude that the most reliable *in vivo* tests performed with acrylonitrile have been negative, indicating an absence of systemic DNA reactivity *in vivo.* In all of the studies reported, although the exposures used were adequate and the methods were extremely sensitive, no genotoxic effect of acrylonitrile was found to account for the carcinogenicity of acrylonitrile in its target organs. DNA reactivity of acrylonitrile or its metabolites has been detected under certain experimental conditions, but these effects have not been linked to acrylonitrile carcinogenesis in the rat target organs. The existing genotoxicity data for acrylonitrile , therefore, do not provide the mode of action for tumour formation.	2 (reliable with restrictions) weight of evidence Review **Test material (EC name): acrylonitrile**	Whysner J, Ross PM, Conaway CC, Verna LK & Williams GM (1998)
literature review (all types of genotoxicity *in vivo* are reviewed)	Test results: Genotoxicity: negative	2 (reliable with restrictions)	Strother (2010)

Various (literature review) Various (literature review) Methodological details for individual studies are limited. Review of the genotoxicity data for acrylonitrile, summarising and updating previous reviews.	Studies that have investigated the occurrence of DNA adducts in rodents exposed to acrylonitrile *in vivo* have only found the 7-OEG adduct in the liver and no adducts in the brain. The brain but not the liver is a target tissue for acrylonitrile-induced carcinogenicity. Other types of DNA adducts have been reported to be formed *in vitro* under conditions of extremely high concentrations and long periods of exposure to acrylonitrile or CEO. Consequently, the only biochemical indication of DNA damage in the brain resulting from *in vivo* acrylonitrile exposure has been an increase in the levels of 8-oxodeoxyguanosine in rats exposed to levels of acrylonitrile in drinking water that produced brain tumours. This is consistent with a role for 8-oxodeoxyguanine in DNA in the formation of brain tumours in rats exposed to acrylonitrile.	weight of evidence literature review **Test material (EC name): acrylonitrile**	

Human information

Method	**Results**	**Remarks**	**Reference**
Study type: biological effect monitoring Type of population: occupational Details on study design: In order to investigate the genotoxic effects of occupational acrylonitrile (acrylonitrile) and dimethylformamide (DMF) exposures, clinical serum and urine parameters and genotoxicological endpoints such as chromosome aberration (CA), sister chromatid exchange (SCE), high frequency SCE (HFC), cell cycle kinetics, and UV-induced unscheduled DNA synthesis (UDS) were followed up three times during a 20-month period in peripheral blood lymphocytes (PBL) of 26 workers (13 maintainers and 13 fibre producers) occupationally exposed to acrylonitrile and/or DMF in a viscose rayon plant, 26 matched control subjects, and six industrial controls (all males). Endpoint addressed: genetic toxicity	Average peak air acrylonitrile and DMF concentrations were over the maximum concentration limits at the time of both investigations. Urine acrylonitrile and monomethyl-formamide (MMF) excretions of the exposed subjects were almost doubled after work shifts. An increase in lymphocyte count (in months 0 and 7), and severe alterations in the liver function were observed in the exposed subjects. In PBLs the proliferative rate index (PRI) was already increased in month 0 compared with the controls. In each study, significant increases in CA and SCE frequencies, as well as increases in UDS were found in PBLs of the exposed subjects. The frequencies of chromatid breaks and acentric fragments further increased in month 7 and remained constantly elevated in month 20. Increased yields of both chromatid and chromosome-	3 (not reliable) supporting study **Test material (EC name): acrylonitrile**	Major J, Hudák A, Kiss G, Jakab MG, Szaniszló J, Náray M, Nagy I & Tompa A (1998)

	type exchange aberrations first appeared in month 20, when HFCs were 2.72 times more frequent in fibre producers than in maintainers. The role of some important biological confounding factors (age, white blood cell count, and haematocrit) and lifestyle confounding factors (smoking and drinking habits) were subjected to an analysis of variance during the second study. Increased CA, SCE, and UDS were found both in control and exposed smokers when current smoking was established on the basis of the serum SCN levels.		
Study type: biological effect monitoring Type of population: occupational Details on study design: In order to investigate the importance of premature centromere division (PCD) in cancer risk assessment, the authors studied the PCD frequency in the PBLs of 400 Hungarian subjects. The various groups comprised 188 control donors and 212 subjects occupationally exposed to different genotoxic chemicals, such as acrylonitrile (acrylonitrile) and/or dimethylformamide (DMF), benzene, cytostatic drugs, ethylene oxide (ETO), mixed exposure in the rubber industry, mixed organic solvents including CCl4, hot oil-mist, bitumen, and polychlorinated biphenyls (PCBs). Data were compared with chromosomal aberration frequencies determined in the same samples. Endpoint addressed: genetic toxicity	The authors report premature centromere division (PCD) yields significantly higher in populations exposed to mixed chemicals, crude oil and cytostatic drugs, compared with controls. PCDs involving more than three chromosomes were also more frequent in ETO-and oil mist-exposed groups than in the other groups. No increase in the incidence of PCDs was seen in workers exposed to acrylonitrile.	2 (reliable with restrictions) supporting study **Test material (EC name): acrylonitrile**	Major J, Jakab MG & Tompa A (1999)

Discussion

Overview

The genetic toxicity of acrylonitrile has been extensively investigated over many years in both standard and non-standard investigative studies *in vitro* and *in vivo*; in isolated DNA, cell cultures, experimental animal and in worker exposure studies. The majority of the studies are non-standard and, individually, many may be considered limited in terms of design. As a consequence of the extensive and historical dataset, it is inevitable that there is some inconsistency between the findings of individual studies. Interpretation of the dataset therefore requires consideration of the weight of evidence from the available studies. The acrylonitrile genotoxicity dataset has previously been reviewed by (among others), Whysner (1998), in the EU RAR (2004) and by The Sapphire Group (2004) and subsequently in an update by Strother (2010).

An overview of the dataset is given below. The significance of the findings of the various studies with regard to the mode of action of carcinogenicity of acrylonitrile seen in chronic rodent bioassays is specifically discussed. For completeness, findings of human studies are also reported.

Studies *in vitro*

Non-mutation studies

The results of studies *in vitro* with acrylonitrile have demonstrated that the substance is capable of binding to proteins and isolated DNA. DNA binding by acrylonitrile was relatively slow and was enhanced by liver microsomes but not by brain microsomes. In contrast, the reactive metabolite 2-cyanoethylene oxide (CEO) was shown to bind rapidly to DNA without the need for metabolic activation. Subsequent investigations have indicated that the apparent low level of binding of acrylonitrile to DNA may be attributable to protein contamination or binding to DNA-associated proteins. Acrylonitrile is capable of producing adducts in isolated DNA, albeit only following very long exposures at biologically irrelevant temperatures, to massive concentrations not representative of those achievable *in vivo*. In contrast, CEO has been shown to produce DNA adducts following much shorter exposure periods and at lower concentrations. The induction of 8oxoG adducts, indicators of oxidative stress, by acrylonitrile exposure has been demonstrated in rat astrocyte DNA but not in rat hepatocyte DNA, and has also been demonstrated in human astrocyte DNA, but only following exposure to much higher concentrations. Many investigators have demonstrated that acrylonitrile and CEO exposure can result in DNA strand breaks, however the methods used in many of these studies had the potential of converting alkali labile sites to DNA stand breaks. Contrasting results in standard and modified (Fapy-G) Comet assays indicate a lack of direct DNA damage by acrylonitrile. A single rec assay in *B. subtilis* shows a positive response for acrylonitrile in the presence of metabolic activation, indicating the induction of DNA strand breaks. The ability of acrylonitrile to induce UDS *in vitro* has been investigated in a number of assays *in vitro*. Studies have used both autoradiography and liquid scintillation techniques, the former being the preferred technique as it enables scheduled (replicative) DNA synthesis to be excluded more reliably. Using liquid scintillation counting, positive responses are reported in cultured human peripheral blood lymphocytes and primary rat hepatocytes; a negative response has been reported in HeLa cells. In contrast, studies using autoradiography report negative responses in rat hepatocytes and human mammary epithelial cells, even at cytotoxic concentrations. A positive response is noted for acrylonitrile and CEO in a study using human mammary epithelial cells and autoradiography; the positive response with acrylonitrile was seen only at very high concentrations. The potential for acrylonitrile to induce sister chromatid exchange (SCE) has been investigated in a number of cell types, although it should be noted that this type of change is without genetic consequence. Positive responses have been reported in CHO cells (with and without metabolic activation) and in cultured human bronchial cells; negative responses are reported in metabolically competent rat liver cells and in human peripheral blood lymphocytes (both with and without metabolic activation).

In summary, studies of non-mutational endpoints *in vitro* have demonstrated that acrylonitrile is capable of binding to DNA and proteins. Protein binding is more extensive and DNA binding is seen only following lengthy exposure to very high concentrations and findings may be attributable to protein contamination. In contrast, the metabolite CEO is shown to bind much more rapidly and avidly to DNA.

Acrylonitrile has, however, been shown to induce the formation of oxidative damage specific DNA adducts, with mutagenic potential. A number of studies have noted the ability of acrylonitrile or CEO to cause DNA strand breakage, however findings may be attributable to the presence of alkali-labile sites. UDS is noted in a number of studies using liquid scintillation counting but generally not in studies using the preferred autoradiography method. The results of SCE studies with acrylonitrile are variable and only positive at unrealistically high concentrations.

Mutation studies

The mutagenicity of acrylonitrile has been investigated in a large number of bacterial mutation assays. The results of studies in *Salmonella* strains sensitive to frameshift mutation (TA97, TA98, TA1537, TA1538) are almost entirely negative, whereas mostly positive results are reported in *Salmonella* strains (TA100, TA1530, TA1535, TA1950) carrying the hisG46 allele and sensitive to GC to AT base pair substitution. It is notable that studies in TA102, which is considered to be sensitive to oxidative damage, have proved to be largely negative. Studies of bacterial mutation in *E. coli* strains have given mixed results, although more recent studies in strains WP2, WP2uvrA, and WP2(PKM101) have more consistently reported positive results in the presence of metabolic activation. WP2 tester strains include an AT base pair as the critical site. Fungal studies in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have given mixed results of gene mutation endpoints but more consistently positive results for chromosomal level mutation, both with and without metabolic activation. A positive result has also been reported for aneuploidy/non-disjunction in *Aspergillus nidulans*.

In mammalian cell studies, a number of positive results are reported for acrylonitrile in L5178Y mouse lymphoma cells (Tk locus) both with and without metabolic activation; negative results are reported for this cell line at the *oua* locus. L5178Y cells are particularly sensitive to mutations, in part because they have a mutation in the P53 tumour suppressor gene, but also because they may be especially sensitive to oxidative damage. The results of studies in other cell lines are variable, with both negative and positive results reported. There is no consistent association with metabolic activation; some studies report positive results with activation only, others both with and without activation. Molecular analyses indicate that point mutations (for CEO involving AT and GC pairs) may predominate over deletion mutations. In mammalian cells, the potential of acrylonitrile to induce clastogenicity has been investigated in human peripheral blood lymphocytes, CHO, CHL and metabolically competent rat liver RL4 cell lines. Many studies have reported positive results for the induction of structural aberrations, with most requiring metabolic activation. There is no evidence for the induction of numerical aberrations.

Studies *in vivo*

Studies in *Drosophila*

Two kinds of mutations have been investigated in *Drosophila s*tudies with acrylonitrile; studies of sex-chromosome aneuploidy and gave a positive result, however further studies with this system show that nitriles can induce aneuploidy due to effects on spindle formation. Two studies of heritable SLRL gene mutation with acrylonitrile have been negative. It is notable that concentrations of acrylonitrile used in the *Drosophila* studies are high compared to those used in mammalian studies.

Non-mutation studies

A number of studies *in vivo* have demonstrated that acrylonitrile and CEO are capable of binding to cellular macromolecules. Early studies may not have sufficiently differentiated DNA binding from binding to protein contaminants. Other studies have shown the induction of specific guanine adducts in liver DNA but not in brain DNA; protein binding in these organs was found to be comparable. DNA adducts specific for oxidative damage (8oxoG) have been identified in the rat, most markedly (out of the organs investigated) in the brain. Other studies show that the induction of adducts is associated with lipid peroxidation, ROS generation and reduced levels of glutathione in the brain but not in the liver. DNA fragmentation has been reported in the brains of rats administered acrylonitrile, with this effect diminished by antioxidant (taurine) co-treatment. Increased levels of lipid peroxidation products have also been reported in the brain and plasma of treated animals. Several studies have reported the induction of UDS responses in rats administered acrylonitrile either by intraperitoneal injection or oral gavage. It is notable that all of the positive studies used liquid scintillation counting to measure UDS. One study showed that the induction of UDS response was associated with glutathione depletion, was increased by depleting glutathione prior to administration and was inhibited by pre-treatment with sulphydryl compounds. A single study using autoradiography (the preferred method) to assess UDS showed a negative response in testis and liver DNA following gavage with acrylonitrile. A single study reports an essentially negative (weak positive) SCE response in mice following the intraperitoneal injection of acrylonitrile.

Mutation studies

Investigation of mutagenicity and clastogenicity in appropriate animal models is of most relevance in terms of carcinogenic potential; the models used generally incorporate relevant toxicokinetic, toxicodynamic and metabolic factors all of which could potentially influence the genetic toxicity potential of the test substance.

Exposure of rats by inhalation to acrylonitrile at concentrations of up to 500 ppm for 90 days did not result in observable effects on cells of the bone marrow (Johnson *et al,* 1978). No effects were observed in the bone marrow cells of mice administered acrylonitrile by gavage at dose levels of up to 21 mg/kg bw/d for up to 30 days, following intraperitoneal injection with dose levels of up to 20 mg/kg bw/d for up to 30 days; similarly no effects were seen in the bone marrow of rats administered acrylonitrile by gavage at a dose level of 40 mg/kg bw/d for 16 days (Rabello-Gay & Ahmed, 1980). Leonard *et al* (1981) showed no induction of bone marrow micronuclei or chromosomal aberrations following the intraperitoneal injection of a single dose of acrylonitrile at a dose level of 20 or 30 mg/kg bw. No increase in the proportion of bone marrow cells was demonstrated in mice following inhalation exposure to dose levels of up to 140 mg/kg bw/d equivalent (Zhurkov *et al,* 1983) or following a single intraperitoneal injection of up to 60 mg/kg bw (Sharief *et al,* 1986). Similar negative effects were seen in mice administered acrylonitrile by single or repeated intraperitoneal injection (10 mg/kg bw) or by single (5, 10 mg/kg bw) or repeated (20 mg/kg bw) gavage dosing (Nesterova *et al,* 1999). The high quality NTP study (NTP, 2001) also showed no evidence of increased micronuclei formation in the peripheral blood NCEs of mice in a 14-week gavage study at dose levels of up to 60 mg/kg bw/d.

A small number of dominant lethal studies performed with acrylonitrile have reported negative results following administration by intraperitoneal injection in mice (Leonard *et al,* 1981), inhalation exposure of mice (Zurkov *et al,* 1983) and in rats following gavage administration (Working *et al,* 1987).

An unpublished abstract of a study of the induction of Hprt mutations in the splenic lymphocytes of mice administered acrylonitrile by gavage for 6 weeks (Walker & Ghanayem, 2003) reports positive results in normal mice at the highest dose level tested of 20 mg/kg bw/d and in CYPE2E1 knock-out mice at the highest dose level tested of 60 mg/kg bw/d (which was lethal to normal mice). Results indicate the requirement for metabolic (or enhancement by) oxidative metabolic activation of mutagenicity and also the involvement of mechanisms other than direct DNA-reactive mutagenicity. An study of Lac Z mutagenicity in the Mutamouse model using administration of acrylonitrile in the drinking water at dose levels of up to 750 ppm for 4 weeks and with a 7-week expression period reports negative findings in all tissues investigated (bone marrow, lung, splenic lymphocytes, male germ cells and brain). This assay detects point mutations, therefore indicating that the positive response in the previous study is attributable to large scale changes.

Cell transformation assays Several cell transformation assays performed in various cell types with acrylonitrile report positive findings. Findings in SHE cells demonstrate a requirement for the oxidative metabolism of acrylonitrile to CEO for a positive response and an association with reactive oxygen species (ROS) generation, reductions in catalase and SOD activity, glutathione depletion and the formation of 8oxoG DNA adducts. Inhibition of P450 activity with ABT prevented cell transformation. Treatment with antioxidants inhibited both the markers of oxidative stress and also cell transformation, strongly suggesting that the cell transformation properties of acrylonitrile are secondary to the induction of oxidative stress. The oxidative metabolism of acrylonitrile generates cyanide, and it is notable that similar findings (oxidative stress and cell transformation) have been reported in SHE cells treated with cyanide. Acrylonitrile has also been shown to inhibit gap junctions in murine lung fibroblasts.

Human studies

A small number of molecular epidemiological studies of acrylonitrile-exposed populations have been performed. The ability of acrylonitrile to bind to specific nucleophilic cites on haemoglobin is utilised in these studies as a biomarker of exposure and a metric of internal dose; CEVal haemoglobin adducts have been used by a number of studies as a sensitive biomarker of exposure. Studies in acrylonitrile-exposed human populations have investigated the induction of chromosome aberration, micronuclei induction, DNA strand breaks, sex chromosome aneuploidy and investigation of sperm parameters as markers of effect. The results of studies investigating effects at the chromosomal level are mixed; one study reports a positive finding but with a response pattern which does not indicate exposure to acrylonitrile as the causative factor. Another positive study reporting chromosomal aberrations is confounded by high levels of illness attributable to other chemical exposures. Other studies report a change in the pattern of chromosomal aberration with no overall increase, or do not provide sufficient detail to facilitate interpretation. A study of Hprt mutations in acrylonitrile-exposed workers used a method known to produce artefacts and is therefore not considered to be reliable. In conclusion, the findings of human genotoxicity studies performed with acrylonitrile are considered to be inconsistent.

Mechanistic considerations

The extensive dataset clearly indicates that acrylonitrile is genotoxic *in vitro*. Acrylonitrile is, however, a weak mutagenic agent. Most positive findings are

reported *in vitro* and involve high exposure levels and test organisms selected for their susceptibility to mutagenesis. *In vivo*, however, there is a dramatic paucity of positive studies. There is only one report of weak gene mutation induction in mice and scattered positive results in humans, many of which can be questioned. The lack of induction of sex-linked recessive lethal mutations in *Drosophila* is comparable with other weak mutagens whose mutagenic effects are inhibited by effective repair systems. Acrylonitrile has the capacity to induce oxidative stress and oxidative DNA damage. This effect (and consequent indirect genotoxicity) may account for the genotoxicity profile of acrylonitrile, indicating a threshold response. The threshold is due to the fact that mutagenicity will only be apparent following exposure to sufficient acrylonitrile to generate oxidative stress and ROS and overwhelm natural cellular defences. Acrylonitrile can also produce other (non-genotoxic) cellular effects associated with carcinogenesis, including cell transformation and the inhibition of gap junctions. Some of the carcinogenicity of acrylonitrile in rodent studies *in vivo* is likely to depend on these non-genotoxic effects, which are also likely to be tissue-dependent. A high incidence of spontaneous tumours in a tissue or organ may reflect a high number of spontaneously initiated cells which require needing only promotion to produce tumours.

Acrylonitrile is a strong irritant. It is a multisite carcinogen in the rat, inducing tumours at the site of contact and in the brain, Zymbal's gland and mammary gland (Whysner *et al*, 1998). *In utero* exposure to acrylonitrile does not shorten the time to tumor development in the rat (Neal *et al*, 2009). In a gavage study in mice, acrylonitrile produced significant increases in forestomach and Harderian gland tumours (NTP, 2001). In contrast, extensive epidemiology investigations of acrylonitrile workers have found no evidence for increased cancer mortality due to acrylonitrile exposure (Symons *et al*, 2008; Cole *et al*, 2008; Bofetta *et al*, 2008). With regard to the genotoxicity data for acrylonitrile, in many instances it is not possible to determine if mutations observed in *in vitro* test systems reflect direct, DNA- reactive effects or indirect effects via oxidative DNA damage as either could have produced these genetic changes. However, some of the *in vitro* mutational changes observed with AN, especially intra-chromosomal recombination and gene conversion in fungi, are highly indicative of oxidative stress mutagenesis. In the case of the aneuploidy reported in *Drosophila*, similar findings have been reported for a wide range of chemicals, including nitriles which are known non-carcinogens, thus these findings are not considered to be relevant to carcinogenicity. The *in vivo* genotoxicity profile of acrylonitrile tends to implicate indirect genotoxicity as a mode of action. Elevated levels of 8oxoG DNA adducts in a variety of tissues, including brain, in several studies, clearly indicates the presence of oxidative DNA damage *in vivo*. This damage resulted from acrylonitrile exposure at concentrations that have produced cancers in bioassays. Furthermore, fragmentation of DNA in brain has also been associated with biomarkers of oxidative stress in acrylonitrile-treated rats, with reduction of both the fragmentation and biomarkers of oxidative stress levels by anti-oxidant treatments. This further implicates oxidative damage in the genesis of brain lesions. The differences between the unpublished *Hprt* and published *lacZ* studies may indicate that many of the mutational events in the *Hprt* gene were due to relatively large scale DNA changes, a finding expected if the mutations resulted from oxidative DNA damage. It is concluded that the genotoxicity profile of acrylonitrile, considered *in toto*, is most compatible with an indirect genotoxic MoA for cancer induction in rodents, rather than a direct, DNA-reactive MoA. There are exposure levels of acrylonitrile below which no oxidative stress, and no oxidative DNA damage, has been detected. These factors, together with the non-linear tumour dose response observed with acrylonitrile in rodent studies and the absence of a causal link

between acrylonitrile exposure and cancer in extensive occupational epidemiology investigations support a view that acrylonitrile is a threshold rodent carcinogen.

EU RAR conclusion on genotoxicity (EU RAR, 2004)

The EU RAR concludes that acrylonitrile has been shown to be weakly mutagenic in *in vitro* systems, indicative of a genotoxic potential. However it was not considered that these findings were reliably reflected in the situation *in vivo*, suggesting that acrylonitrile or its active metabolites do not reach target tissues *in vivo*, possibly due to the detoxification of the epoxide metabolite CEO via a glutathione conjugation pathway which may not exist in *in vitro* test systems. Nevertheless it was concluded that, the body of evidence on the *in vitro* mutagenicity of acrylonitrile, together with the positive results in *Drosophila*, leads to the conclusion that for the purposes of risk assessment, acrylonitrile could be regarded as 'genotoxic or at least mutagenic'.

The following information is taken into account for any hazard / risk assessment:

Acrylonitrile is shown to be weakly DNA-reactive and genotoxic in assays *in vitro* with metabolic activation. Studies in reliable mammalian models *in vivo* do not show any evidence of genotoxicity.

Justification for classification or non classification

Based on the large body of available information, acrylonitrile is not classified with regard to germ cell mutagenicity.

Studies of genotoxic effects in exposed workers have reported mostly negative results; studies that have produced indications of effects have limitations which argue against their use for classification decisions. Adequate *in vivo* experimental data exist to support the classification of acrylonitrile. All published peer reviewed studies of mutational effects in mammalian studies have reported negative results, including studies of germ cell mutagenicity. These include investigations of both chromosome aberrations and micronucleus inductions in rat and mouse somatic cells and studies of heritable chromosome level changes, such as dominant lethal effects, in both species, and chromosome level changes in murine spermatocytes. These studies were conducted independently by several laboratories, spanned a period of over 20 years, and involve exposure by a variety of routes including inhalation, oral and intraperitoneal injection. Acrylonitrile exposure concentrations (although not durations) were at levels comparable to or greater than those that have produced cancer in the various bioassays available. There are unpublished reports of slight increases in Hprt mutant frequencies in lymphocytes of rats and mice treated with acrylonitrile. These results warrant verification given that negative results for LacZ mutations were published from the same mice. Somatic cell mutations and male germ cell aneuploidy have been reported in *Drosophila* at very high exposure levels, while a third mutational endpoint evaluated in *Drosophila*, i.e. that reflecting sex-linked recessive lethal heritable mutations, has been negative as were tests of induction of reciprocal translocations. Nitriles and a wide range of other chemicals have been shown to induce aneuploidy in *Drosophila* sperm. This finding does not appear to correlate with other genotoxicity findings or to be relevant to carcinogenicity.

Carcinogenicity

Oral bioassays

Method	Results	Remarks	Reference
mouse (B6C3F1) male/female oral: gavage 0, 2.5, 10 or 20 mg/kg (nominal conc.) Exposure: Two years. (5 days per week for 104 to 105 weeks.) equivalent or similar to NTP protocol	LOAEL (carcinogenicity): 2.5 mg/kg bw/d (actual dose received) (male) based on: test mat. (Increased tumour incidences (Harderian gland) at 2.5 mg/kg bw/d) NOAEL (carcinogenicity): <2.5 mg/kg bw/d (actual dose received) (male) based on: test mat. (Increased tumour incidences (Harderian gland) at 2.5 mg/kg bw/d) NOAEL (carcinogenicity): 2.5 mg/kg bw/d (actual dose received) (female) based on: test mat. (Increased tumour incidences at 5 mg/kg bw/d and above) Neoplastic effects: yes	2 (reliable with restrictions) weight of evidence experimental result **Test material (EC name): acrylonitrile**	NTP (2001e)
rat (Sprague-Dawley) male/female oral: drinking water 0, 35, 85 or 210 ppm (nominal in water (exposure levels during the first 21 days)) 0, 35, 100 or 300 ppm (nominal in water (exposure levels for the remainder of the study after day 21)) Exposure: 2 years (Daily / continuous-*ad libitum* in drinking water) equivalent or similar to OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	NOAEL (carcinogenicity): <35 ppm (male/female) based on: test mat. (Increased tumour incidences in all treated groups including the lowest dose level of 35 ppm (equivalent to 3.4 and 4.4 mg/kg bw/d in males and females respectively)) LOAEL (carcinogenicity): 35 ppm (male/female) based on: test mat. (Increased tumour incidences in all treated groups including the lowest dose level of 35 ppm (equivalent to 3.4 and 4.4 mg/kg bw/d in males and females respectively)) NOAEL (toxicity): <35 ppm (male/female) based on: test mat. (Reduced bodyweight and food consumption in all treated groups including the lowest dose level of 35 ppm (equivalent to 3.4 and 4.4 mg/kg bw/d in males and females respectively)) LOAEL (toxicity): 35 ppm (male/female) based on: test mat. (Reduced bodyweight and food consumption in all	2 (reliable with restrictions) Weight of Evidence experimental result **Test material (EC name): acrylonitrile**	Quast JF, Wade CE, Humiston CG, Carreon RM, Hermann EA, Park CN & Schwetz BA (1980) Quast JF (2002)

	treated groups including the lowest dose level of 35 ppm (equivalent to 3.4 and 4.4 mg/kg bw/d in males and females respectively)) Neoplastic effects: yes		
rat (Fischer 344) male/female oral: drinking water 0, 100 and 500 ppm (nominal in water) Exposure: 18 months (Daily-*ad libitum* in drinking water) Chronic rat carcinogenicity study specifically investigating brain tumour incidence and histogenesis.	NOAEL (carcinogenicity): <100 ppm (male/female) based on: test mat. (Increased incidences of brain tumours) LOAEL (carcinogenicity): 100 ppm (male/female) based on: test mat. (Increased incidences of brain tumours) NOAEL (toxicity): <100 ppm (male/female) based on: test mat. (Increased mortality, bodyweight effects, signs of toxicity) LOAEL (toxicity): 100 ppm (male/female) based on: test mat. (Increased mortality, bodyweight effects, signs of toxicity) Neoplastic effects: yes (Increased incidences of brain tumours, skin, stomach and Zymbal's gland tumours)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): acrylonitrile**	Bigner DD, Bigner SH, Burger PC, Shelburne JD & Friedman HS (1986)
rat (Sprague-Dawley) male oral: drinking water 0, 20, 100 and 500 ppm (nominal in water) Exposure: 2 years (Daily / *ad libitum* in drinking water) Oral 2 year carcinogenicity study in rats; drinking water administration.	NOAEL (carcinogenicity): 20 ppm (male) based on: test mat. (Zymbal's gland tumours at 100 and 500 ppm) LOAEL (carcinogenicity): 100 ppm (male) based on: test mat. (Zymbal's gland tumours at 100 and 500 ppm) NOAEL (toxicity): 20 ppm (male) based on: test mat. (Reduced weight gain & food consumption at 100 and 500 ppm) LOAEL (toxicity): 100 ppm (male) based on: test mat. (Reduced weight gain & food consumption at 100 and 500 ppm) Neoplastic effects: yes (: increased tumour incidences (Zymbal's gland) and forestomach papillomas)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): acrylonitrile**	Gallagher GT, Maull EA. Kovacs K & Szabo S (1988)
rat (Fischer 344) male/female	NOAEL (carcinogenicity):	2 (reliable with	Biodynamics

oral: drinking water 0, 1, 3, 10, 30 and 100 ppm. (nominal in water) Exposure: The intended duration was 2 years, however the study was terminated at 23 months in females because of low survival rates. The males were exposed for 26 months. (Daily-*ad libitum* in drinking water.) equivalent or similar to OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	3 ppm (male/female) based on: test mat. (Increased tumour incidences) NOAEL (toxicity): 3 ppm (male/female) based on: test mat. (Local gastric irritation) Neoplastic effects: yes (increased incidences of mammary gland carcinoma, astrocytoma, forestomach tumours, Zymbal's gland tumours)	restrictions) Weight of Evidence experimental result **Test material (EC name): acrylonitrile**	(1980a) Johannsen FR & Levinskas GJ (2002a)
rat (Sprague-Dawley) male/female oral: drinking water 0, 1 and 100 ppm (nominal in water) Exposure: The intended duration was 2 years, however the study was terminated at 19 months in females and 22 months in males because of low survival rates. (Daily-*ad libitum* drinking water) equivalent or similar to OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	NOAEL (carcinogenicity): 1 ppm (male/female) Neoplastic effects: yes (increased tumour incidences at 100 ppm)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): acrylonitrile**	Biodynamics (1980b) Johannsen FR & Levinskas GJ (2002b)
rat (Sprague-Dawley) male/female oral: gavage 0, 0.1, 10 mg/kg bw/d (actual ingested) Exposure: The intended duration was 2 years, however the study was terminated at 19 months in females and 22 months in males because of low survival rates. (Daily) equivalent or similar to OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	NOAEL (carcinogenicity): 0.1 mg/kg bw/d (actual dose received) (male/female) based on: test mat. (No effects at this dose level) LOAEL (carcinogenicity): 10 mg/kg bw/d (actual dose received) (male/female) based on: test mat. (Increased tumour incidence) NOAEL (toxicity): 0.1 mg/kg bw/d (actual dose received) (male/female) based on: test mat. (No effects at this dose level) LOAEL (toxicity): 10 mg/kg bw/d (actual dose received) (male/female) based on: test mat. (Mortality, bodyweights, food consumption) Neoplastic effects: yes (increased tumour incidences at 10 mg/kg bw/d)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): acrylonitrile**	Johannsen FR & Levinskas GJ (2002b)
rat (Sprague-Dawley) male/female	LOAEL (carcinogenicity): 5 mg/kg bw/d (actual dose received) (male/female) based	2 (reliable with restrictions)	Maltoni C, Ciliberti A &

oral: gavage 5 mg/kg bw (nominal conc.) Exposure: 52 weeks (3 times weekly for 52 weeks) 1 year oral carcinogenicity study	on: test mat. Neoplastic effects: yes (: increased incidences of mammary gland and forestomach tumours)	supporting study experimental result **Test material (EC name): acrylonitrile**	Dimaio V (1977) Maltoni C, Ciliberti A, Cotti G & Perino G (1988)
rat (Sprague-Dawley) male/female three-generation study oral: drinking water 0, 100 or 500 ppm (nominal in water) Exposure: 100 days prior to mating, throughout the mating period and the subsequent gestation and lactation phases in females. (Daily) Three-generation reproduction study in rats, test substance administered in the drinking water	A low incidence of astrocytomas and Zymbal's gland tumours was found in all generations. Tumour incidence was slightly greater in the F1 animals that had perinatal exposure compared to the F0 animals that did not, but was very similar in the F0 animals that did not have exposure *in utero* or during lactation and the F2 animals that did. It should be noted that the number of animals evaluated was low compared to a standard carcinogenicity evaluation. The findings of this study suggest that *in utero* or perinatal exposure to acrylonitrile does not lead to an increased incidence of astrocytomas compared to that seen after adult exposure alone.	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): acrylonitrile**	Beliles RP, Paulin HJ, Makris NG & Weir RJ (1980) Friedman MA & Beliles RP (2002)

Animal Bioassays; Inhalation

Method	**Results**	**Remarks**	**Reference**
rat (Sprague-Dawley) male/female whole body 0, 20 and 80 ppm (nominal conc.) 0, 44, 176 mg/m^3 (nominal conc.) Exposure: 2 years (6 hours per day, 5 days per week.) equivalent or similar to OECD Guideline 453 (Combined Chronic Toxicity / Carcinogenicity Studies)	LOAEC (carcinogenicity): 20 ppm (male/female) based on: test mat. (Increased brain tumour incidence at 20 ppm) NOAEC (carcinogenicity): <20 ppm (male/female) based on: test mat. (Increased brain tumour incidence at 20 ppm) LOAEC (toxicity): 20 ppm (male/female) based on: test mat. (Local nasal irritant effects) Neoplastic effects: yes	2 (reliable with restrictions) key study experimental result **Test material (EC name): acrylonitrile**	Quast JF, Schwetz, DJ, Balmer MF, Gushow TS, Park CN & McKenna MJ (1980)
rat (Sprague-Dawley) male/female 5, 10, 20 and 40 ppm (nominal conc.) Exposure: 12 months (4 hours/day, 5	LOAEC (carcinogenicity): 5 ppm (nominal) (male/female) based on: test mat. (Borderline increase in tumours at 5 ppm)	2 (reliable with restrictions) supporting study	Maltoni C, Ciliberti A & Dimaio V (1977) Maltoni C, Ciliberti A, Cotti

days/week) One year inhalation carcinogenicity study in rats	NOAEC (carcinogenicity): <5 ppm (nominal) (male/female) based on: test mat. (Borderline increase in tumours at 5 ppm) Neoplastic effects: yes	experimental result **Test material (EC name): acrylonitrile**	G & Perino G (1988)
rat (Sprague-Dawley) male/female inhalation: vapour (whole body) Comprehensive review of the carcinogenicity of acrylonitrile by an expert working group. Acrylonitrile was previously considered by Working Groups in 1979 and 1987. The 1999 review incorporates new data which is taken into consideration for the evaluation of the potential for carcinogenicity.	Regarding the animal carcinogenicity data, The IARC monograph noted that acrylonitrile has been tested for carcinogenicity in one study in rats by inhalation with pre- and postnatal exposure. This study confirmed the findings of increased incidences of glial cell tumours of the central nervous system found in several previous studies that had not been fully reported and also found increases in malignant mammary tumours, Zymbal gland carcinomas, benign and malignant hepatocellular tumours and extrahepatic angiosarcomas. Acrylonitrile forms adducts with proteins and glutathione. It also forms DNA adducts *in vitro*, but only after cytochrome P450 bioactivation, most likely through its epoxide metabolite (cyanoethylene oxide), which is also formed *in vivo*. Acrylonitrile–haemoglobin adducts have been detected in exposed workers. Both acrylonitrile and cyanoethylene oxide can conjugate with glutathione, leading to detoxification of these reactive compounds. At high doses of acrylonitrile, as used in animal studies, glutathione in certain tissues may be depleted. Such glutathione depletion will probably not occur at low-level human exposure. Acrylonitrile is mutagenic in vitro; in *Salmonella* systems, bioactivation (to cyanoethylene oxide) is required, but in *Escherichia coli* and in rodent systems, bioactivation by an added microsomal system is not required. The results of genotoxicity experiments *in*	2 (reliable with restrictions) supporting study review **Test material (EC name): acrylonitrile**	IARC (WHO) (1999)

	vivo have in most cases been negative, although acrylonitrile is mutagenic in *Drosophila*. Regarding the human carcinogenicity data, the IARC monograph notes that the potential carcinogenicity of acrylonitrile in occupationally exposed populations has been investigated in several epidemiological studies. Studies carried out in the 1970s and 1980s suggested a possible increased risk of lung cancer among workers exposed to acrylonitrile. However, these were inconclusive because of one or more of the following actual or potential problems: small sample sizes, insufficient length of follow-up, incompleteness of follow-up, inadequate exposure assessment, potential confounding by other occupational carcinogens, and potential confounding by smoking. Consequently, larger and better studies were undertaken, in most cases building upon the same cohorts that had previously been assembled. Four such studies (two in the United States, one in the United Kingdom and one in the Netherlands) were carried out and these now provide the most relevant, informative data on which to base an evaluation. All of the studies made some attempt to establish exposure levels, although for the British study, this was rather cruder than for the others. The two studies from the United States were carried out in similar industries, but the range of cumulative exposure values was quite different between the two, raising questions about the inter-study comparability of methods of exposure assessment. The four studies employed different strategies for comparing exposed with		

	unexposed. While the British study used a classic SMR comparison with national rates, the Dutch study did the same, but also compared the exposed with a different unexposed cohort. One of the studies from the United States compared the exposed with national rates and with rates of mortality and incidence in other plants of the same large company. The other compared the exposed with workers in the same plants who were unexposed to acrylonitrile. Typically, in each study, a number of analyses were carried out, varying comparison groups and other parameters. The IARC monograph concludes that was no significant excess risk for any type of cancer when all exposed workers were compared with unexposed, or with an external comparison population. Further, when the study subjects were subdivided by levels of exposure (cumulative exposure when feasible), for no site but lung was there any hint that risk increased with exposure. For lung cancer, there was an indication that workers with the highest exposures had relative risk estimates greater than 1.0. This finding was strongest in the largest of the studies, which had one of the most intensive exposure assessment protocols, but the other studies gave either negative or only weakly supportive results. Even in the largest study (where the relative risk in the highest exposure quintile ranged from 1.2 to 1.7 depending on the parameters in the analysis), the finding was not consistently statistically significant; there was no coherent dose–response pattern throughout the range of exposures and the risk in the highest decile of exposure was lower than that in the		

	second highest decile. On balance and given the largely unsupportive findings from the other studies, the evidence from this one study was not considered to be sufficiently strong to conclude that there was a credible association between acrylonitrile and lung cancer. Thus, the earlier indications of an increased risk among workers exposed to acrylonitrile were not confirmed by the recent, more informative studies.		

5.8.1.3. Carcinogenicity: dermal

No data are available and none are required under REACH. The carcinogenicity of acrylonitrile has been investigated extensively in a large number of studies using inhalation and oral routes of exposure. Further investigation of carcinogenicity using dermal exposure is not required and will be limited by the local (irritant and sensitisation) effects on the substance.

5.8.1.4. Carcinogenicity: other routes

No data are available and none are required under REACH. The carcinogenicity of acrylonitrile has been investigated extensively in a large number of studies using relevant (inhalation and oral) routes of exposure. Further investigation of carcinogenicity using other exposure routes is not required and cannot be justified on scientific grounds.

5.8.2. Human information

The exposure-related observations in humans are summarised in the following table:

Method	**Results**	**Remarks**	**Reference**
Study type: cohort study (retrospective) Details on study design: A large mortality study of industrial workers exposed to acrylonitrile was conducted by NCI/NIOSH using a cohort of 25,460 workers (18,079 white men; 4,293 white women; 2,191 non-white men; 897 non-white women) employed at eight acrylonitrile producing or processing plants in the US. Exposures at the eight plants started between 1952 and 1965. Two of the plants included those evaluated previously by Collins *et al* (1989), as well as those evaluated in unpublished studies by Zack (1980) and Gaffey and Strauss (1981). These workers, employed from the 1950s to 1983, were followed up until 1989 to	Analyses by various indicators of exposure including cumulative (ppm-years), average, peak, intensity, duration, and lagged exposure revealed no elevated risk of cancers of the stomach, brain, breast, prostate, or lymphatic and hematopoietic system. Relative risks (and 95% confidence intervals) for lung cancer from lowest to highest quintile for cumulative exposure were 1.1 (0.7 to 1.7), 1.3 (0.8 to 2.1), 1.2 (0.7 to 1.9), 1.0 (0.6 to 1.6), and 1.5 (0.9 to 2.4), respectively. Several analyses were done on culumative exposure. When the data were	2 (reliable with restrictions) weight of evidence **Test material (EC name): acrylonitrile**	Blair A, Stewart PA, Zaebst D, Pottern L, Zey J, Bloom T, Miller B, Ward E & Lublin J (1998)

determine their vital status and cause of death. Endpoint addressed: carcinogenicity	restricted to workers with more than 20 years since their first exposure to acrylonitrile, the relative risks (and 95% confidence intervals) were 1.1 (0.6 to 2.2), 1.0 (0.5 to 0.1), 1.2 (0.6 to 2.2), 1.2 (0.6 to 2.1) and 2.1 (1.2 to 3.8), respectively, achieving statistical significance for the highest quintile, compared to the unexposed group of workers. However, the linear trend for the relative risk in this analysis was not significant, and when 20 year lagging of exposure was employed, no significant relative risk was observed. No increased risk was observed for lung cancer when external comparisons are used. To evaluate RRs at a wider range of cumulative exposure, analyses were also conducted for decile categories. The RR did not continue to increase in a dose-response fashion, and actually decreased from 1.7 at the ninth decile to 1.3 at the tenth decile. When confounding effects from tobacco use were accounted for, the RR for lung cancer was reduced in the upper quintile slightly (from 1.5 to 1.4). Despite finding a significant increase in lung cancer mortality in workers from the highest quintile, analyses of the exposure-response data did not provide a strong or consistent evidence for a causal relationship. Separate analyses for wage and salaried workers, long-term and short-term workers, fibre and non-fibre plants, and by individual plants revealed no clear exposure-response patterns and tests for trend were not statistically significant. The authors conclude that the "excess of lung cancer in the highest quintile of cumulative exposure may indicate carcinogenic activity at the highest exposure levels , but analysis by exposure-response did not provide strong or consistent evidence.."		
Study type: cohort study (retrospective)	The SMR values for all causes of death combined, based on US	2 (reliable with restrictions)	Marsh GM, Youk AO &

Type of population: occupational Details on study design: The authors obtained a copy of the NCI-NIOSH study data (Blair *et al*, 1998). The file included individual demographic, work history and acrylonitrile exposure data for 25460 workers employed in 1 or more of 8 plants that produced or used acrylonitrile from 1952 to 1983 in 1 or more of 8 plants in the US. The study group was followed during 1989 for vital status and cause of death. Priori interest cancer sites were defined as: lung, stomach, brain, breast, prostate and the lymphatic and haematopoietic systems. Endpoint addressed: carcinogenicity	and regional rates, were less than 1.0 for all the categories of exposed workers. For both external comparisons, the largest SMR values were observed for workers who were unexposed or in the lower exposure categories. The SMR values for lung cancer were uniformly much lower when based on regional mortality rates than when based on US rates. The regional rate-based SMR values were all less than 1.0 (range 0.92 to 0.64). The findings based on the internal rate ratios indicated an increased risk of lung cancer mortality in 4 of the 5 acrylonitrile exposure categories, with the largest excess observed in the highest exposure category (RR 1.5, 95% CI 0.9-2.4). No evidence of a linear exposure-response relationship for lung cancer was observed with either the internal or the external comparison groups. For the remaining cancer sites, the pattern of findings was similar between the US and regional rate-based SMR values, and between the SMR and rate ratio analyses. Only a few of the SMR values or rate ratios were greater than 1.0 for the remaining sites, and there was little or no evidence of an exposure-response relationship for any of these cancer sites. In most cases, the SMR values and the internal rate ratios tended to be larger in the lower acrylonitrile exposure categories and smaller among more highly exposed workers. No evidence of a linear exposure-response relationship for lung cancer was observed in any of the examined categories of time since the first exposure. In only 2 plants was an excess observed among the combined acrylonitrile-exposed workers when they were compared with the unexposed plant (plant 3, SMR 1.60 and plant 4, SMR 1.39). The 2 largest SMR values were observed in the highest acrylonitrile exposure category (≥8.0 ppm-years) for plant 3	weight of evidence **Test material (EC name): acrylonitrile**	Collins JJ (2001)

	(SMR 2.70, 95% CI 0.7-6.9) and plant 4 (SMR 2.68, 95% CI 1.3-4.9). The authors conclude that the "study provides little evidence that acrylonitrile increases the mortality risk of cancers of a priori interest, including lung cancer."		
Study type: cohort study (retrospective) Type of population: occupational Details on study design: The authors studied the mortality of UK acrylonitrile workers. This study is an extended and updated study of a previous study (Werner & Carter, 1981). This study included 2763 male workers exposed to acrylonitrile for at least one year between 1950 and 1978 at one of the six UK factories involved in the polymerisation of acrylonitrile and the spinning of acrylic fibres (63,058 person-years, 72% from one plant-Factory 5). The follow-up period lasted until December 31, 1991. Mortality rates were compared to national rates for England and Wales (except one factory in Scotland, where Scottish rates were used). Endpoint addressed: carcinogenicity	The results showed that, overall, there was a deficit in mortality for the combined analysis population, reflecting a significant deficit in circulatory disease deaths, and deficits in most other causes of death. Factory 5 showed deficits from various causes of death while the other plants combined showed non-significant increases. All cancers combined showed a deficit (a total of 121 cancer deaths were observed, compared to 137.12 expected), and for most individual cancer sites (including lung and stomach), the observed numbers were close to the expected numbers. When the workers were stratified by age, lung cancer mortality showed an increased SMR in the 15 to 44 and 45 to 54 age groups (SMR = 284.4, 95% CI = 104.4 to 618.9; SMR =148.7, 95% CI = 83.2 to 245.2) and a deficit for the older age groups. However, further analysis of these lung cancer excesses showed that the excess in the age group 45 to 54 was in Factory 5, while the excess in the 15 to 44 age group was in the other factories. While analysis by length of employment showed a significant tendency for increased all cause and circulatory disease mortality with time, there was no similar trend for cancer. Five lung cancer deaths (0.8 expected) were reported among those with highest exposure and under 45 years of age (SMR = 6.1, 95% CI 2.0-14.6), and among those highly exposed first employed after 1969 (SMR = 2.7, 95% CI 1.1-5.5). With respect to mortality by job category, workers were counted in a category if they worked in it for	2 (reliable with restrictions) weight of evidence **Test material (EC name): acrylonitrile**	Benn T & Osborne K (1998)

	one year or more; workers who changed jobs may thus appear in more than one category. In the two 'high exposure' categories (polymer or control room worker and spinner), cancer SMRs were raised but not significantly so. In the 'end of line' workers (a category used exclusively in Factory 5), the SMR for all cancers was significantly reduced. On grouping the workers according to the level of acrylonitrile exposure categories that they had worked in (i.e. high, possible, and little or no exposure), the SMR for each of the examined causes (other than respiratory disease) was higher in the high exposure group than in the other two groups. The highest cancer SMRs were found in the high exposure group for total cancer (115.8), stomach cancer (166.3), and respiratory cancer (141.1); however, only stomach cancer showed a clear and statistically significant trend across the three groups. However, this finding was based on small numbers. For lung cancer, the SMR was lowest in the middle group. It should be noted, however, that information regarding the smoking habits of the workers was lacking.		
Study type: cohort study (retrospective) Type of population: occupational Details on study design: A cohort of 2842 workers from eight chemical plants situated within the Netherlands was investigated. The control group consisted of 3961 unexposed workers from a nitrogen fixation plant. The chemical workers were exposed to acrylonitrile for at least six months between January 1, 1956 and July 1, 1979, and followed until 1988. The mean observation time was about 17 years, and the observed cancer mortality in the exposed cohort was similar to the expected mortality. Specific analyses were carried out to investigate dose-response relationships and latency for total mortality and lung cancer mortality.	Overall, in the exposed cohort, there were 134 deaths observed, with 172.2 expected according to national mortality rates (SMR = 0.78). Approximately the same ratio of observed to expected deaths was found for the unexposed cohort as for the exposed cohort. There were 42 cancer deaths in the exposed cohort, with an expected number of 50.8 (SMR= 0.83). No significant increases in mortality were reported for any specific cancer type. For lung cancer, there were 16 deaths in the exposed cohort compared with 19.5 expected (SMR= 0.82). Workers were stratified according to exposure level (<1, 1-10, >10 ppm-years) and latency (<10, 10-20, >20 years). When lung cancer risk was	2 (reliable with restrictions) weight of evidence **Test material (EC name): acrylonitrile**	Swaen GMH, Bloemen LJN, Twisk J, Scheffers T, Slangen JJM & Sturmans F (1992)

Endpoint addressed: carcinogenicity	examined according to three categories of dose, there was a marginal increase (eight deaths versus 7.2 expected) in the highest category and deficits for the lower dose categories. The authors attributed the rising SMR by dose category to a waning of the healthy worker effect for longer-term employees. This interpretation was supported by the trend in the ratio of observed deaths for all causes combined to expected deaths by dose category; for low dose (<1 ppm-years), the ratio was 0.67; for moderate dose (1-10 ppm-years) it was 0.78; and for high dose (≥10 ppm-years) it was 0.83. There were two prostate cancer deaths in the exposed cohort, compared with 1.22 expected. These two deaths occurred in the group with the highest exposure and the longest 'latency.' No significant trends with either exposure level or latency were observed.		
Study type: cohort study (retrospective) Type of population: occupational Details on study design: This study is a follow-up to the previous study of Swaen *et al* (1992). The same group of 6803 workers (2842 exposed and 3961 unexposed controls in eight chemical companies) were followed through January 1, 1996 to assess mortality. Workers were exposed to acrylonitrile for at least six months prior to July 1, 1979. The control group consisted of workers at a nitrogen fixation plant employed over the same time interval as the study cohort. All 6803 workers were followed-up for mortality with causes of death obtained from the existing Dutch Central Bureau of Statistics. The follow-up was 99.6% complete and 99.3% of the deaths by cause were ascertained. acrylonitrile exposure at the plants began in 1959 to 1973. Endpoint addressed: carcinogenicity	Of the 6803 study subjects, 6774 could be completely followed, either until the end of the follow-up period, until the date of emigration, or until the date of death. This resulted in a completeness of follow-up of 99.6%. For nine (0.7%) deceased study subjects, it was not possible to trace the actual cause of death, either because the person had died abroad or because it was not possible to link the record with the cause of death file. Adjustments for differences in age distribution, follow-up period, and temporal changes in background mortality rates were made using SMRs for a range of separate causes of death. In the total study population, 1273 deaths were observed (an approximate doubling of the observed number of deaths (n= 706) in the earlier study). The number of deaths in the exposed group increased from 134 to 290. In either group, the observed mortality is still lower than the expected, an indication of the healthy worker effect; however, no direct comparisons were	2 (reliable with restrictions) weight of evidence **Test material (EC name): acrylonitrile**	Swaen GMH, Bloemen LJN, Twisk J, Scheffers T, Slangen JJM, ten Berge WFJP & Sturmans F (1998)

	made between the exposed and unexposed group, which might have yielded larger relative risks. Stratification of the workers according to three levels of exposure and three latency periods (i.e. latency was defined as time since the particular exposure group was entered) did not reveal any significant increases in terms of overall mortality. A total of 97 cancer deaths were reported in the exposed group (110.78 expected). As in the earlier study, stratification of the workers according to three levels of exposure and three latency periods did not reveal any significant increases. As with total mortality, cumulative dose-effect relationships were investigated after classifying the exposed workers into three exposure categories and three latency periods.		
Study type: cohort study (retrospective) Type of population: occupational Details on study design: The study is a more recent follow-up of the previously investigated Dutch cohort, and included investigations up to 1/1/2001. The completeness of follow-up was 98.8%. Endpoint addressed: carcinogenicity	A total of 146 cancer deaths were reported in the exposed group (164.5 expected). The results show that although there are some small fluctuations in cancer mortality, there does not appear to be any cancer excess related to occupational exposure to acrylonitrile. The SMR for trachea and lung cancer overall was 107.2 (95% CI= 83.1 to 136.1). In the low, medium, and high exposure category, the SMRs for lung cancer were 92.1 (95% CI = 36.9 to 189.0), 106.5 (95% CI = 74.6 to 147.4), and 114 (95% CI not reported), respectively. Overall, no excess lung cancer was observed in this cohort based on peak exposures, respirator use, or exposure to other carcinogens.	2 (reliable with restrictions) weight of evidence **Test material (EC name): acrylonitrile**	Swaen GM, Bloemen LJ, Twisk J, Scheffers T, Slangen JJ, Collins JJ & ten Berge WF(2004)
Study type: meta-analysis Details on study design: The stated purpose of the study was to assess the published epidemiological evidence regarding the possible carcinogenicity of acrylonitrile, while focusing on respiratory cancer. A total of 12 published papers that appeared to be epidemiologic studies of workers exposed to acrylonitrile with data on cancer morbidity or mortality were	Approximately the same number of cancer deaths was observed as was expected according to general population mortality rates (standardized mortality ratio 1.03, 90% confidence interval 0.92-1.1 5).	2 (reliable with restrictions) weight of evidence **Test material (EC name): acrylonitrile**	Rothman KJ (1994)

identified. Endpoint addressed: carcinogenicity			
Study type: cohort study (retrospective) Type of population: occupational Details on study design: Two DuPont facilities responsible for developing and manufacturing Orlon acrylic fibre were located in Waynesboro, Virginia (Benger Laboratories and the Waynesboro Plant) and Camden, South Carolina (May Plants 1 and 2). Development work began in 1947 at Benger Laboratories. Production started at May Plant 1 in 1950 and expanded to May Plant 2 in late 1952. Increased demand for Orlon acrylic fibre led to the construction of the Waynesboro facility, which operated from 1958 through 1990. May Plant 1 operated until 1985 while May Plant 2 remained in production until 1991. The manufacturing process was similar at the two sites. The cohort included 2548 male workers with at least 6 months of acrylonitrile exposure at either plant from 1947 through 1991. This update includes mortality events occurring through December 31, 2002. Mortality was determined through the DuPont Epidemiology Registry that has been maintained for all active and pensioned employees since 1957. All employees in the cohort regardless of pensioned status had vital status verified by the National Death Index using social security number as an identifier. Because of incomplete data, cohort members were assumed to be white because 93% of 1083 workers with known race designation in the combined cohort are white. Endpoint addressed: carcinogenicity	Acrylonitrile cohort workers had a slightly higher proportion of deaths due to malignant neoplasms but a lower fraction of cardiovascular disease mortality relative to the DuPont region 7 employees. In comparison with expected deaths based on the US general population, the SMR for all deaths and for all malignant neoplasms are significantly decreased. Expected deaths based on DuPont region 7 mortality yield relatively higher SMR; however, overall mortality is significantly less than expected and all cancer mortality is decreased. For cancer mortality endpoints of a priori concern including respiratory system and prostate cancers, SMR are not significantly increased based on DuPont region 7 comparisons (SMR for respiratory system cancer =91, 95% CI: 74, 113; SMR for prostate cancer =102, 95% CI: 66, 151). In conclusion, there is no evidence for increased cancer mortality among highly exposed acrylic fibre production workers. The analytic findings of this extended mortality update correspond to previous mortality risk estimates for this cohort, as well as to relative risks for cancer mortality from other occupational cohorts exposed to acrylonitrile. No cause-specific mortality outcome of a priori interest, including respiratory system cancer and prostate cancer, was found to be associated with increased acrylonitrile exposure after five decades of follow-up. The authors conclude that no mortality outcome of a priori interest, principally respiratory system cancer, is associated with increased acrylonitrile exposure.	2 (reliable with restrictions) supporting study **Test material (EC name): acrylonitrile**	Symons JM, Kreckmann KH, Sakr CJ, Kaplan AM & Leonard RC (2008)
Study type: meta-analysis Type of population: occupational	Eight estimates with national reference, six with regional reference, one with plant reference, and two rate ratio	2 (reliable with restrictions) weight of	Sponsiello-Wang Z, Sanders E & Weitkunat R

Details on study design: Relevant studies for inclusion in the meta-analysis were identified primarily through literature searches. Inclusion criteria were: the study design was cohort or case-control, the publication contained data on occupational acrylonitrile exposure and lung cancer mortality or morbidity (including respiratory cancer) information, and rate ratios as well as confidence interval estimates were reported, or sufficient data to compute them were provided. Studies lacking description of the examined samples were excluded. When more than one report on a population was available, the most complete or most recent update was considered. Information was collected from the publications using a data abstraction form. Variables recorded included: study design, period, location, maximal follow-up years, cohort/sample size, exposure assessment, type of effect measure, analysis methods, effect size, precision, adjustment variables, and others. Respiratory cancer estimates were used when no data were available on lung cancer estimates. When multiple effect estimates were reported, the one which the authors relied on for the assessment was generally selected. Overall estimates were selected rather than estimates relating to subgroups. Adjusted estimates were selected rather than estimates relating to subgroups. Adjusted estimates were preferred to unadjusted ones. To compare the lung cancer risk of acrylonitrile in exposed workers with that from national and regional populations as well as with non-exposed workers, standard mortality ratios (SMR) based on national, regional and plant-specific expected numbers of cases were aggregated separately in exposed groups. A pooled SMR from all mortality studies with both national and regional references was calculated. Overall estimates were calculated for rate ratios (RRs) stemming from two studies with internal control groups in order to obtain an as direct as possible comparison between exposed and non-exposed workers. Additionally, these two studies were combined with one study reporting an SMR based on the plant-specific expected number. To aggregate SMR-based estimates with studies reporting RR, SMR of exposed	estimates were available. Significant heterogeneity was not detected in any of the analyses, therefore there was a close co-response of overall estimates of fixed and random effects. Publication bias was estimated to be 0.93 (95% CI 0.84-1.02). Three cohort studies presented mortality data for unexposed workers; no heterogeneity was detected among these three studies, and the overall fixed effects estimate was found to be 0.78. This result was thought to indicate the presence of a healthy worker effect in the unexposed samples, which in turn suggested that the overall estimate of SMR in exposed workers might underestimate the risk of acrylonitrile exposure. It was concluded from the overall estimate, that this effect might have a magnitude of ~20% reduction in estimated risk in non-exposed workers as compared to national and regional reference populations. The fixed effect overall rate ratio estimate of all 11 RSMR and RR risk estimates indicated a 25% increase in lung cancer mortality in acrylonitrile exposed workers as compared to unexposed workers. With a probability of 95%, the increase is at least 9%. The plant reference SMR of one of the studies could be expected not to be affected by a healthy worker effect; it could be substituted for the corresponding RSMR in the meta-analysis. An additional overall estimate was therefore calculated on the aggregated RR and RSMR, of which one RSMR was replaced by the plant reference based SMR. The overall estimate was found to be 1.28 (95% CI 1.11-1.47). Since lung cancer survival rate is low, the overall lung cancer rate ratio was also estimated by additionally taking into account one study which reported an OR based on morbidity data. No significant heterogeneity was	evidence **Test material (EC name): acrylonitrile**	(2006)

groups were divided by an overall estimate obtained from 3 SMS of unexposed groups to yield ratios of SMR (RSMR). Endpoint addressed: carcinogenicity	detected. The overall estimate was significantly larger than unity, as indicated by the 95% CI internal (1.11-4.36). In contrast to the former SMR based analyses, these analyses indicated that there is a increased risk of lung cancer associated with occupational acrylonitrile exposure. To further explore the chances of a bias in the overall RSMR estimate, the authors conducted an additional analysis using only those 3 (out of 11) studies which had internal control groups. It was assumed that in these studies, where both the exposed and unexposed control groups were from the same companies and periods, difference age distributions on the cohort pairs would be even less likely than in the total set of exposed and reference cohorts. To further reduce the chances for a biased estimate, the RSMS calculation was based on the cohort pairs (exposed divided by unexposed) of each of the 3 studies. The fixed effects meta-analysis of the resulting 3 RSMR yielded an overall effect estimate of 1.27 with 95% CI of 1.03-1.56 and no heterogeneity (P = 0.4888). This result was close to the result based on all 11 studies and also indicated a significantly elevated risk. Both analyses suggested an increased risk of lung cancer associated with occupational acrylonitrile exposure of more than 20%, being close to the result found in the 2 cohort studies which reported regression based relative rates using internal control groups, therefore not being affected by SMR-specific biases.		
Study type: review Type of population: occupational Details on study design: The authors identified a total of 26 epidemiology studies which examined mortality and/or incidence rates among persons with acrylonitrile exposure. Where cohorts have been updated the most recent data were relied upon but descriptions of the	In the NCI study, the all causes of deaths combined SMR for exposed workers was 70 (60-70) and for unexposed workers was 70 (70-80). For all cancers combined the SMR for exposed workers was 80 (70-90) and for unexposed workers the SMR was 90 (80-100). This study, because of its size and strengths, does not provide support for the	2 (reliable with restrictions) weight of evidence **Test material (EC name): acrylonitrile**	Cole P, Mandel JS & Collins JJ (2008)

earlier publications are provided for background and rationale. Results are provided for all causes of death and all cancers. Detailed results and discussions are provided for the cancers which have received the most attention and for which some positive results have been reported. Endpoint addressed: carcinogenicity	notion that acrylonitrile is carcinogenic to highly exposed workers. The authors conclude that the DuPont study has some limitations, including having race data on only 58% of the cohort, the lack of smoking data, and cancer incidence data only for active workers. However, these limitations are thought unlikely to alter the conclusion of the study that there was no evidence for increased cancer mortality due to acrylonitrile among highly exposed acrylic fibre production workers. Despite its limitations, the Dutch study provides considerable support for the view that acrylonitrile is not carcinogenic for human beings and is not associated with an increase in mortality from any major cause of death. Interpretation of the UK study of acrylonitrile workers is problematic. The results appear questionable but, as presented, do not support the presence of a carcinogen at the plants studied.		
Study type: meta-analysis Type of population: occupational Details on study design: A meta-analysis was conducted using the information available from 25 epidemiology studies (published and unpublished) of workers exposed to acrylonitrile. The data were analyzed using meta-analysis techniques to assess the findings for 10 cancer sites. The predominant focus in the available studies was on worker mortality rates. All but four of the studies assessed were industrial cohort studies, with the remaining four being two nested industrial case-control studies and two general population case-control studies. Endpoint addressed: carcinogenicity	Based on the results from the 14 unique study groups, mortality from all causes was about 20% less than general population rates and the results were heterogeneous ($p < 0.00001$). A total of 783 cancer deaths were observed. Cancer mortality rates were less than expected for total cancers (mRR= 0.9, 95% CI= 0.8 to 0.9). No significant increases were noted for the mortality rates by specific cancer type. All specific forms of cancer mortality were at or below expected levels with the exception of bladder cancer (mRR = 1.4, 95% CI = 0.9 to 2.0). All specific causes of cancer death were homogeneous across studies with the exception of colon cancer ($p = 0.00062$). The cancer incidence studies gave similar results to the mortality studies. Most cancer incidence rates were at expected levels with the	2 (reliable with restrictions) weight of evidence **Test material (EC name): acrylonitrile**	Collins JJ & Aquavella JF(1998)

	possible exception of prostate cancer (mRR = 1.4, 95% CI = 0.8 to 2.6). The incidence rates from the three studies for all cancers were homogeneous. For lung cancer mortality, cumulative relative risk by date of the study before 1992 among acrylonitrile workers was slightly greater than 1.0. This could be a chance finding or reflect an early preference for publication of positive findings. However, after 1992, the cumulative lung cancer rates are at expected levels. The early studies were smaller than the four more recent studies as evidenced by the wide confidence intervals. The 1998 studies of Blair *et al*, Wood *et al*, Swaen *et al*, and Benn and Osborne all have narrow confidence intervals and the SMRs are close to 1.0. The lung cancer mRR for these recent studies is 0.9 (95% CI= 0.9 to 1.1). Several of the acrylonitrile studies used in this meta-analysis examined worker mortality rates by level of exposure. It would be useful to separate workers with low and brief exposures from more highly exposed workers to assess the risk of chronic exposure on mortality and cancer risk. However, only seven studies examined cancer risk by level of exposure and most of these evaluations are for lung cancer. These seven studies, which present data for highly exposed workers, had null rates for lung cancer overall (mRR = 1.0, 95% CI = 0.9 to 1.1) compared to studies that did not specifically examine workers with higher exposure (mRR = 0.7, 95% CI = 0.4 to 1.4). The highest exposed workers in the seven studies produced a mRR of 1.2 (95% CI = 1.0 to 1.5). None of the studies, however, found a lung cancer mortality trend with exposure level. Three of the studies made semi-quantitative estimates of likely exposure that		

	allowed for the examination of workers with comparable high exposures. These three studies, which estimated the likely exposure, had an mRR for lung cancer of 0.9 (95% CI = 0.8 to 1.0) compared to an mRR of 1.1 (95% CI = 0.9 to 1.4) for the studies that did not include an estimation of likely levels of exposure. On combining the greater than 8 ppm years category in the Blair *et al.* study, the 10 to 50, 50 to 100, and 100+ ppm-years categories in the Wood *et al.* study, and the 10+ ppm-years category in the Swaen *et al.* study, the mRR for these studies for lung cancer was 1.1 (95% CI= 0.9 to 1.4). Eight studies considered latency periods of 15 years or longer for lung cancer. Studies that considered latency had an mRR of 1.0 (95% CI= 0.9 to 1.1) compared to an mRR of 0.9 (95% CI = 0.7 to 1.1) for those studies that did not. Only the studies of Blair *et al.* (RR= 1.3, 95% CI = 1.0 to 1.63) and Delzell and Monson (SMR = 1.7, 95% CI = 0.7 to 3.5) give any indication of elevated rates in workers with longer follow-up time. The six other studies report SMRs equal to or less than 1.0 for this category. The mRR for this category is 1.2 (95% CI = 1.0 to 1.4).		
Study type: literature review Type of population: occupational Details on study design: The authors review the available extensive dataset on the carcinogenicity of acrylonitrile Endpoint addressed: carcinogenicity	The weight of the evidence available suggests either that acrylonitrile is not a human carcinogen or that it produces only small increases in cancer risk among humans	2 (reliable with restrictions) weight of evidence **Test material (EC name): acrylonitrile**	Coggon D & Cole C (1998)
Study type: case control study (retrospective) Type of population: occupational Details on study design: The study had a case–control design and was conducted in 15 centres in six Central and Eastern Europe countries and in Liverpool (United Kingdom). The 15 participating centres from Central and Eastern Europe were Bucharest (Romania), Budapest and	Overall, 21729 different jobs were held by study subjects with an average of 3.7 jobs for cases and 3.6 jobs for controls. The proportion of subjects who never held an employment was 0.8% among both cases and controls. A total of 159 jobs were assessed to be exposed to vinyl chloride, 74 to acrylonitrile and 123 to styrene, of which 63 were assessed with	2 (reliable with restrictions) weight of evidence **Test material (EC name): acrylonitrile**	Scelo G, Constantinescu V, Csiki I, Zaridze D, Szeszenia-Dabrowska N, Rudnai P, Lissowska J, Fabianova E, Cassidy E, Slamova A, Foretova A,

four areas of Hungary (Borsod, Heves, Szabolcs and Szolnok), Lodz and Warsaw (Poland), Moscow (Russia), three areas of Slovakia (Banska Bystrica, Bratislava and Nitra), and Brno, Olomouc and Prague (Czech Republic). In each area a series of newly diagnosed cases of primary lung cancer was recruited during 1998–2002 together with a set of controls that were frequency matched to cases on age and gender. The age and sex distributions of cases and controls were similar but not identical because this set of controls served as a referent population for more than one cancer site. Cases were recruited in hospitals that treated essentially all lung cancer in each area, except in Russia, where cases were recruited in a cancer hospital and in a dispensary. Eligible cases were selected among hospitalized subjects, within three months of diagnosis: as a result, only 13 cases died before interview and next-of-kin interviews were not required. Cases had lived in the study area since at least one year. Identification of cases occurred through an active search of clinical and pathological departments. Inclusion in the study depended on histological or cytological confirmation. Most centres recruited hospital controls while in two centres (one in Poland and one in the UK) population controls were selected. Hospital controls were recruited in general public hospitals serving the same areas from which the cases arose; patients admitted for cancer or tobacco-related diseases were excluded. Population controls were selected from population registers in Poland and from registers of General Practitioners in the UK. Three thousand four hundred and three cases and 3670 controls were eligible to participate in the study. Five hundred and forty two (15.9%) eligible cases were not included in the study (27 had been discharged from hospital before the interview, 53 were too ill to be interviewed, 13 had died before the interview and 449 refused to participate). Five hundred and fifty two (15.0%) eligible controls were not included in the study (16 had been discharged from hospital before the interview, 21 were too ill to be interviewed, two had died before interview, 511 refused to participate and two were excluded from the analyses because age was missing). The study	high confidence for vinyl chloride, 46 for acrylonitrile and 58 for styrene. The proportion of ever-exposed cases was 2.3% for vinyl chloride, 1.4% for acrylonitrile and 1.8% for styrene; these figures were respectively 1.8%, 0.6% and 1.5% among controls. Most exposures occurred before 1980, i.e. more than 20 years before diagnosis or interview. The number of jobs assigned to high intensity was low and most assessments were made with medium or high confidence. Exposure to the three agents occurred mainly in the manufacture of rubber and plastic products: 18% of vinyl chloride exposed jobs (3% in the manufacture of rubber and 15% in plastic), 23% of acrylonitrile (19% in rubber and 4% in plastic) and 28% of styrene (15% in rubber and 13% in plastic). Acrylonitrile exposure also often occurred in the manufacture of footwear (22% of exposed jobs). The three exposures were correlated: 22% and 28% of jobs exposed to vinyl chloride and styrene respectively were also exposed to the other agent and 57% of jobs exposed to acrylonitrile were also exposed to styrene; 13 (4.8%) out of 272 jobs exposed to at least one agent were exposed to all three. For cumulative exposure, Pearson correlation coefficients were 0.84 (p<10)4) between vinyl chloride and acrylonitrile, 0.82 (p<10)4) between vinyl chloride and styrene and 0.90 (p<10)4) between acrylonitrile and styrene. A total of 39 cases and 20 controls were classified as exposed to acrylonitrile (OR=2.20; 95% CI: 1.11–4.36). When analyses were restricted to jobs with high-confidence exposure, the OR was 2.39 (95% CI: 0.97–5.84). As more than half of the subjects exposed to acrylonitrile were also exposed to styrene, the analysis on subjects not exposed to styrene (17 cases and 10 controls) was repeated; the		Janout V, Fevotte J, Fletcher T, Mannetje A, Brennan P & Boffetta P (2004)

population included in analyses comprised 2861 cases and 3118 controls. Endpoint addressed: carcinogenicity	results were not different but confidence intervals were wider since the number of exposed cases and controls was smaller (OR=2.08; 95% CI: 0.82–5.27). There were too few subjects exposed to acrylonitrile within each country to conduct country-specific analyses. In order to provide some indication on whether the results are consistent across countries however, analysis excluding the country with the largest number of exposed subjects (Hungary) was performed with 17 exposed cases and eight exposed controls. The resulting OR was 2.91 (95% CI: 1.21–6.96). A linear increasing trend in ORs was observed for weighted duration and cumulative exposure to acrylonitrile (p=0.05 and p=0.06 respectively). Using a 20-year lag did not appreciably change the results. The increased risk was restricted to young individual: ORs for ever exposure were 2.79 (95% CI: 1.01–7.70) for subjects up to 60 years of age at diagnosis or interview and 1.02 (95% CI: 0.35–2.92) for older people (p=0.4 for age-exposure interaction test). The increased risk was present for both squamous cell carcinomas (OR=1.97, 95% CI: 0.79–4.94) and adenocarcinomas (OR=1.85, 95% CI: 0.66–5.20). It was particularly strong for undifferentiated and unspecified tumours (OR=6.25; 95% CI: 1.97–19.80).		
Study type: cohort study (retrospective) Type of population: occupational Details on study design: O'Berg (1980) followed 1345 male workers employed in a DuPont synthetic textile (Orlon) factory from 1950 to 1966. The study had a minimum follow-up period of ten years (1976). The follow-up study (O'Berg *et al*, 1985) had a longer follow-up period of 15 years (1981-1983). Endpoint addressed: carcinogenicity	In the original study, a total of 89 deaths were observed, compared with an expected number of 77.4 based on DuPont mortality rates, and 121.1, based on United States rates. A total of 25 cases of cancer were found, compared with 20.5 expected according to the DuPont rates. There were 8 cases of respiratory cancer, with 4.4 expected, and 3 cases of prostate cancer, with 0.9 expected. All of the cancer cases, except for one non-respiratory cancer, occurred	2 (reliable with restrictions) supporting study **Test material (EC name): acrylonitrile**	O'Berg MT (1980) O'Berg MT, Chen JL, Burke, CA, Walrath J & Pell S (1985)

	among 1,128 workers with 6 or more months exposure (SIR = 126, SMR = 113). A trend of increased cancer incidence was seen with increased duration of exposure and increased length of follow-up time. The excess of respiratory cancer incidence was statistically significant and remained so upon evaluation of the contribution of smoking (5 observed as opposed to 1.6 expected, p-value = 0.02). In the follow-up study, the cancer incidence findings were not very different from the cancer mortality findings. There were a total of 43 incident cases of cancer observed versus 36.7 expected according to company-wide rates. For lung cancer there were 10 cases observed versus 7.2 expected. There were fewer incident lung cancer cases than deaths because of the differing time periods for follow-up (the incidence data refer to the period 1956-1983, whereas the mortality data refer to 1950-1981). The respiratory cancer incidence was no longer significantly increased based on DuPont Company rate. For prostate cancer 6 cases observed and 1.8 expected. All 6 cases of prostate cancer occurred among wage workers, for whom the expected number was 1.5. Prostate cancer incidence was significantly increased, but no significant relationship between incidence and cumulative exposure was found. There were 7 cases of lymphopoietic cancer versus 3.7 expected, and for the wage workers the respective numbers were 6 versus 2.9 expected. There was no significant increase in cancer mortality based on US rates.		
Study type: cohort study (retrospective) Type of population: occupational Details on study design: Cancer morbidity and mortality was investigated in 1083 workers who started work between 1944 and 1970 at a DuPont textile fibre plant, US. The observation period for latency covered the period from 1944 until 1981 for mortality based	There were 92 deaths observed in the cohort during the follow-up period, substantially fewer than the 124.0 expected on the basis of DuPont mortality rates and the 177.2 expected on the basis of rates for all white males in the United States. The deficit in deaths was more striking for salaried employees than for wage employees. Among the	2 (reliable with restrictions) supporting study **Test material (EC name): acrylonitrile**	Chen JL, Walrath J, O'Berg MT, Burke CA & Pell S. (1987)

on US and DuPont rates and from 1944 until 1983 for morbidity based on DuPont rates. The follow-up period for the estimation of expected deaths covered only the period from 1957 until 1981. Endpoint addressed: carcinogenicity	wage workers, there were 18 cancer deaths versus 20.4 expected from the DuPont rates and 24.1 expected from the United States rates. There were 7 deaths from lung cancer among the wage workers, compared with 7.9 expected on the basis of the DuPont rates. No significant excess in cancer mortality was observed, based upon both US and DuPont rates. Respiratory cancer incidence was not increased. There were 37 cases of cancer identified during the period 1956-1983. However there was no excess seen for lung cancer (5 cases observed versus 6.9 expected, p-value = 0.82), but there was an excess for prostate cancer incidence, in that 5 prostate cancer morbidity cases were observed compared to the expected 1.9 (p-value = 0.04), based on DuPont rates.		
Study type: cohort study (retrospective) Type of population: occupational Details on study design: This study combined and updated the O'Berg (O'Berg, 1980; O'Berg *et al,* 1985) and Chen *et al* (1987) studies to study the 2,559 Orlon male workers from both plants over the time period from 1944 to 1991. The study assessed the risk of cancer mortality and incidence in the cohort with a vital status follow-up 99% complete through 1991. Endpoint addressed: carcinogenicity	Overall mortality in the exposed cohort proved to be lower than expected compared to both the US population and the DuPont employee population (observed = 454, SMRs = 69 (95% CI = 62 to 75) and 91 (95% CI = 84 to 99) for the US and DuPont, respectively) All cancer death rates with the exception of prostate cancer were lower than the US and DuPont population referents (observed = 126, SMRs = 78 (95% CI = 65 to 93) and 86 (95% CI = 71 to 102) for the US and DuPont, respectively). The SMRs for prostate were slightly elevated, but were not significantly different from expected (observed = 11, SMRs = 129 (95% CI = 64 to 230) and 106 (95% CI = 53 to 189) for the US and DuPont, respectively). Similarly, the SMRs for brain and CNS cancer were slightly elevated, but not significantly so (observed = 6, SMR = 113 (95%CI=41-247) compared to U.S. referents). Other specific cancers were also not significantly elevated from referent populations and were in some cases significantly lower	2 (reliable with restrictions) supporting study **Test material (EC name): acrylonitrile**	Wood SM, Buffler PA, Burau K & Krivanek N (1998)

	than expected (i.e. respiratory cancer-observed = 47, SMRs = 74 (95% CI = 55 to 99) and 89 (95% CI = 65 to 181) for the US and DuPont, respectively). Other results showing the same trend include digestive cancer (observed = 27, SMRs = 69 [95% CI = 45 to 100] and 72 [95% CI = 48 to 105] for the US and DuPont, respectively), urinary cancer (observed = 7, SMRs = 91 [95% CI = 36 to 187] and 90 [95% CI = 36 to 185] for the US and DuPont, respectively), and lymphatic or hematopoietic cancers (observed = 9, SMRs = 57 [95% CI = 26 to 109] and 55 [95% CI = 25 to 105] for the US and DuPont, respectively). Analyses of all cancers, and prostate, respiratory, and digestive cancer mortality by indices of exposure also did not show any significantly associated increases nor a consistent pattern suggestive of a dose-response relationship. Based on DuPont rates, the SMR for prostate cancer mortality showed an inverse association with latency, duration of exposure, highest exposure, and cumulative exposure. The patterns in cancer incidence were also studied and were dissimilar to that seen in the mortality study in some cases. While total cancer incidence did not show a dose-response relationship with latency, duration, exposure, or cumulative exposure, prostate cancer did show a trend with latency and exposure (although not with duration or cumulative exposure). Lung cancer incidence and deaths were generally less than expected by exposure duration and across cumulative exposure categories.		

Summary and discussion of carcinogenicity

Discussion

The carcinogenicity of acrylonitrile has been investigated in a relatively large number of animal studies using administration in the drinking water, by gavage and by

inhalation. While the studies are of variable quality, the results of the studies are consistently positive. A number of the studies show development of tumours in similar tissue types. The tumours of the CNS and in particular the brain in rats is consistent. However a number of studies show dose levels at which tumour incidence is not discernibly higher than the control. Numerous epidemiology studies investigating cancer incidence in exposed workers are also available and are discussed in detail elsewhere in this dossier.

Mouse gavage study: NTP (2001)

Clear evidence of the carcinogenicity was seen in this study in male and female B6C3F1 mice administered acrylonitrile by gavage at dose levels of 0, 2.5, 10 or 20 mg/kg bw/d for 2 years. Reduced survival was seen at 20 mg/kg bw/d. Evidence of local gastric irritation was seen at 20 mg/kg bw/d. Increased incidences of forestomach tumours were seen in both sexes at 10 and 20 mg/kg bw/d. Harderian gland hyperplasia was increased in males at 10 mg/kg bw/d; increased incidences of Harderian gland tumours were seen in all treated groups of males and in females at 10 and 20 mg/kg bw/d. Ovarian and bronchio-alveolar tumour incidences were increased in females at 10 mg/kg bw/d. It is noteworthy that the mouse did not show any incidence of the brain tumour which were consistently observed in the rat bioassays.

Rat drinking-water study: Quast (1980, 2002)

Male and female Sprague-Dawley rats were administered acrylonitrile in drinking water at concentrations of 0, 35, 100 or 300 ppm. Early mortality was noted in both sexes at 300 ppm. Reduced weight gain and food consumption were seen in all treatment groups, with evidence of local gastric irritation. Increased tumour incidences were seen in one or more dose levels in the brain, Zymbal's gland, forestomach, tongue, small intestine and mammary gland.

Specific investigations: other studies

Carcinogenicity mode of action

A large number of studies have been performed in order to further elucidate the carcinogenic mode of action of acrylonitrile. Studies have focussed on the induction of oxidative stress and cell transformation. The dataset on cell transformation has also been reviewed in the EU RAR (2004), by the Sapphire Group (2004) and more recently by Strother (2010).

The EU RAR (2004) reviewed the extensive dataset on the potential of acrylonitrile to cause cell transformation which is informative in relation to the potential of acrylonitrile to cause carcinogenicity by non-genotoxic mechanisms. It concludes that the various studies summarised in this review document indicate that acrylonitrile has the ability to cause cell transformation; it further states that cell transformation appears to be secondary to oxidative damage and is dependent on metabolic activation. The Sapphire Group review (2004) also notes that the available data indicate that acrylonitrile has the ability to cause cell transformation as a consequence of a reduction in cellular antioxidant status. The extensive investigations of cell transformation show that the induction of oxidative stress by acrylonitrile is dependent on its metabolism to CEO and cyanide. The draft report of the North Carolina Science Advisory Board (NCSAB, 2010) also concludes that the mechanistic work by Pu *et al* suggests that ROS related to toxicity induced by high doses of acrylonitrile appear to play a critical role in its carcinogenicity in the highly sensitive rat brain. The NCSAB also state there is relevant evidence that acrylonitrile possesses

genotoxic and carcinogenic activity by acting indirectly in the production of brain tumours in the rat, and that this may possibly occur via a high-dose mechanism involving oxidative stress and changes in gap junction communication.

In a study in cultured human astrocytes, Jacob & Ahmed (2003) conclude that a redox imbalance may play a major role in acrylonitrile-induced neurotoxicity, which is indicated by compromised antioxidant defence mechanisms (depletion of GSH, increase in GSSG, inhibition of catalase, increased ROS formation and TNF-α secretion), resulting in oxidative DNA damage. In a series of studies in cultured Syrian Hamster Embryo cells, Zhang *et al* (2002) suggest that the induction of oxidative stress by acrylonitrile involves a temporal decrease in antioxidants and increase in xanthine oxidase activity that is mediated by the oxidative metabolism of acrylonitrile.

Pu *et al* (2009) investigated oxidative stress and DNA damage in rats exposed to acrylonitrile in the drinking water. No significant increase in direct DNA strand breaks was observed in brain and WBC from acrylonitrile-treated rats. However, oxidative DNA damage (fpg comet and 8'hydroxyl-2-deoxyguanosine) in brain and WBC was increased in a dose-dependent manner. In addition, plasma levels of reactive oxygen species (ROS) increased in rats administered acrylonitrile. Dietary supplementation with NAC prevented acrylonitrile-induced oxidative DNA damage in brain and WBC. A slight, but significant, decrease in the GSH: GSSG ratio was seen in brain at acrylonitrile doses of >30 ppm. The authors conclude that the results of this study provide support for a mode of action for acrylonitrile-induced astrocytomas involving the induction of oxidative stress and damage. Significant associations were seen between oxidative DNA damage in WBC and brain, ROS formation in plasma, and the reported tumour incidences. Since oxidative DNA damage in brain correlated with oxidative damage in WBC, the results suggest that monitoring WBC DNA damage maybe a useful tool to assess acrylonitrile-induced oxidative stress in humans.

Esmat *et al* (2007) demonstrated that acrylonitrile induces oxidative stress in cultured rat glial cells, depleting reduced GSH and caused lipid peroxidation. The anti-oxidant compound N-acetylcysteine was shown to inhibit the oxidative effects of acrylonitrile. In contrast, the administration of dietary antioxidants did not have any notable effect on the levels of cyanoethylvaline-globin adducts in female rats administered acrylonitrile in the drinking water for 28 days (Snyder, 2010). A similar study reported by Klaunig & Forney (2010) demonstrated increased 8-OHdG formation in rat brain and oxidative DNA damage (but not DNA strand breakage) in peripheral white blood cells.

Klaunig & Forney (2010) investigated the effects of selected antioxidants on acrylonitrile-induced oxidative stress was investigated in female F344 rats. Groups of rats were administered acrylonitrile at 0 or 100 ppm in the drinking water for 28 days; groups received basal diet or diet supplemented with the anti-oxidants Vitamin E (0.05%), Green tea polyphenols (0.4%), N-acetyl cysteine (0.3%), sodium selenite (0.1 mg/kg) or taurine (10 g/kg). Bodyweights were measured weekly; liver weights were measured at termination. Total anti-oxidant capacity, malondialdehyde and 8-OHdG levels were measured in samples of brain tissue. Direct and oxidative DNA damage were assessed in white blood cells by Comet assay. Acrylonitrile induced oxidative stress in the brain of female F344 rats following the administration of 100 ppm in drinking water for 28 days, as shown by increased 8-OHdG formation. Acrylonitrile also induced oxidative DNA damage, but not DNA strand breakage in

white blood cells. Dietary supplementation with Vitamin E, green tea polyphenols, N-acetyl cysteine in diets prevented or reduced acrylonitrile-induced 8-OHdG formation. Dietary supplementation with selenium or taurine gave no significant protection against acrylonitrile-induced oxidative stress. In contrast, supplementation of all five antioxidants in diets prevented oxidative DNA damage induced by acrylonitrile in white blood cells. Acrylonitrile did not cause significant changes in total antioxidant capacity and malondialdehyde level in brain tissues.

Using blood samples taken from the same rats in the study by Klaunig & Forney2010, Snyder *et al (*2010) carried out a study to determine cyanoethylvaline (CEVal) haemoglobin adducts in blood samples following administration of acrylonitrile in drinking water, with or without antioxidant supplements in the diet. Globin CEVal adducts were measured in terminal blood samples by LC/MS-MS. Globin CEVal levels were markedly increased by the administration of acrylonitrile, however the administration of the dietary antioxidants did not have any notable effect on CEVal levels.

The following information is taken into account for any hazard / risk assessment:

The results of a number of cell transformation assays indicate that acrylonitrile has the potential to reduce antioxidant status, increase xanthine oxidase activity and cause cell transformation. The results of recent studies provide evidence for the involvement of oxidative stress in the mode of action of acrylonitrile carcinogenesis in rodent studies.

Rat drinking-water study: Bigner *et al* (1986)

In a study specifically designed to investigate the incidence and origin of brain tumours, male and female F344 rats were administered acrylonitrile in the drinking water at concentrations of 100 and 500 ppm. Treated groups showed effects on mortality and weight gain, with increased incidences of clinical signs consistent with neurotoxicity. Increased incidences of brain tumours were seen in both treated groups, with increased incidences of tumours in other organs (skin, stomach, Zymbal's gland) also noted. Although the brain tumours noted in this study morphologically closely resembled astrocytic tumours commonly seen in this rat strain, specific staining did not reveal the presence of GFAP thus indicating a different cellular origin.

Rat drinking-water study: Gallagher *et al* (1988)

Male CD rats (20/group) were administered acrylonitrile in the drinking water at concentrations of 0, 20, 100 or 500 ppm for two years. Increased mortality was seen at 500 ppm, with bodyweight effects seen at 100 and 500 ppm. Increased incidences of Zymbal's gland tumours were seen at 100 and 500 ppm, with forestomach papillomatous changes also noted at 500 ppm and considered likely to be secondary to local irritation. The incidences of tumours in other organs and tissues were not affected by treatment; however the small group size may have limited the power of the study to detect carcinogenicity.

F344 rat drinking-water study: Johannsen & Levinskas (2002)

In this study, acrylonitrile was administered in the drinking water for approximately 2 years to groups of 100 male and 100 female F344 rats at nominal concentrations of 1, 3, 10, 30 and 100 ppm. Two additional groups, each of 100 males and 100 females, were used as untreated controls. The average daily intake was 0, 0.1, 0.3, 0.8, 2.5 or 8.4 mg/kg bw/d respectively, for males and 0, 0.1, 0.4, 1.3, 3.7 or 10.9 mg/kg bw/d respectively for females. Clinical biochemistry, interim necropsies, organ weights and microscopic evaluation of tissues and organs were performed on 10 rats/sex/group following treatment for 6, 12 and 18 months and at study termination. Females were sacrificed after treatment for 24 months; males after treatment for 26 months. A consistent decrease in survival, lower body weight and reduced water intake and small reductions in haematological parameters were observed in both sexes at 100 ppm. Increased numbers of early deaths were observed in males at 10 ppm and females at 30 ppm. Relative organ weights at various study intervals were consistently elevated in the high dose group; findings are attributable to lower body weights. At the same intervals, mean absolute weights were either comparable to controls or only slightly elevated and few changes in weight ratios were seen when organ weights were compared with brain weights. No biochemical changes suggested a treatment-related effect. An increase in urine specific gravity in 100 ppm male rats reflected the reduced water consumption by this group. The only significant non-neoplastic finding observed histologically was a dose-related increase in hyperplasia/hyperkeratosis in squamous cells of the forestomach in male and female rats at concentrations of 3 ppm and higher. This observation correlated with the induction of treatment-related squamous cell tumours (papillomas and carcinomas) of the forestomach seen primarily in rats in these groups. Mammary gland carcinomas were observed only in female groups. Both sexes given 10 ppm acrylonitrile or more had astrocytomas of the brain/spinal cord and adenomas/carcinomas of the Zymbal's gland. There were no discernable differences in the development of tumours in the low dose group compared to the controls.

Spartan rat drinking-water study: Johannsen & Levinskas (2002)

Spartan Sprague-Dawley rats (100/sex/group) were administered acrylonitrile continually at concentrations of 0 (controls), 1 or 100 ppm in the drinking water. The equivalent mean doses of acrylonitrile were 0, 0.09 and 8.0 mg/kg bw/d in males; 0, 0.15 and 10.7 mg/kg bw/d in females. Groups of ten rats/sex were sacrificed at 6, 12 and 18 months and at study termination. Ophthalmoscopic, haematological, clinical biochemistry, urinalysis and full histopathological exams were performed on control and high dose groups and in lower dose groups, as required, to define dose-responses of observed effects. All animals were necropsied and underwent microscopic examination of target tissues, including brain, ear canal, stomach, spinal cord and any grossly observable tissue masses. High dose male and female rats exhibited statistically decreased bodyweights. Food consumption and water intake were also reduced. Due to increased deaths in groups of high dose rats, surviving males and females were terminated after 22 and 19 months, respectively. Small, sometimes statistically significant, reductions in haemoglobin, haematocrit and erythrocyte count were observed in male and female rats at 100 ppm drinking water. Organ weight findings were not observed and there were no changes in clinical biochemistry. Absolute kidney weights were increased in high dose female rats only. Male and female rats from high dose groups had a higher incidence of palpable masses of the head and the non-glandular stomach and, in females only, the mammary region. In both sexes, treatment-related tumours of the central nervous system (brain, spinal cord), ear canal, and gastrointestinal tract were observed in rats administered 100 ppm. There were no discernable differences in the development of tumours in the low dose group compared to the controls.

Spartan rat gavage study: Johannsen & Levinskas (2002)

Spartan Sprague-Dawley rats (100/sex/group) were administered lifetime oral doses of acrylonitrile by gavage at 0, 0.1 or 10 mg/kg bw/d, 7 days per week. The doses selected were designed to approximate the same daily intake of acrylonitrile in the drinking water study by the same authors. Groups of ten rats/sex were sacrificed at 6, 12 and 18 months and at study term. Ophthalmoscopic, haematological, clinical biochemistry, urinalysis and full histopathological exams were performed on control and high dose groups. Similar tests were done in lower dose groups, as required, to define dose-responses of observed effects. All animals were necropsied and underwent microscopic examination of target tissues, including brain, ear canal, stomach, spinal cord and any observable tissue masses. High dose male and female rats exhibited statistically decreased body weights. Food consumption and water intake were unaffected. Due to increased deaths in groups of high dose rats, all test groups were terminated after 20 months of treatment. Small, sometimes statistically significant, reductions in haemoglobin, haematocrit and erythrocyte count were observed in male and female rats in the high dose group. There were increases in absolute or relative organ weight ratios for liver and adrenal in the high dose groups, but could not be correlated with acrylonitrile toxicity in the absence of adverse clinical biochemistry or microscopic findings. Absolute kidney weights were increased in high dose male and female rats. Male and female rats from the high dose group had a higher incidence of palpable masses of the head and the non-glandular stomach and, in females only, the mammary region. In both sexes, treatment-related tumours of the central nervous system (brain, spinal cord), ear canal and

gastrointestinal tract, and in females only, the mammary gland were observed in rats administered 10 mg/kg bw/d. There were no discernable differences in the development of tumours in the low dose group compared to the controls.

Comparison of results with the drinking water study

Spartan rats from the gavage study had a substantially higher incidence of acrylonitrile-related site-specific tumours than rats of the same strain administered similar dose levels of acrylonitrile via their drinking water study counterparts. While a similar spectrum of tumours was produced by both dosing regimens, there were some notable differences in organ-specific incidence of tumours. Astrocytomas of the brain and spinal cord were found at a higher incidence in those rats exposed continuously to acrylonitrile administered in the drinking water compared to bolus dosing by gavage. Conversely, a higher incidence of squamous cell carcinomas/papillomas of the forestomach and adenocarcinomas of the intestine and, in females only, carcinomas of the mammary gland were observed in high dose rats receiving acrylonitrile by gavage. An increase in the degree of severity of forestomach hyperplasia was observed in all high dose groups of animals, irrespective of mode of administration. These effects were more pronounced, were correlated with a much higher incidence of forestomach tumours, and were identified earlier (12 months) in the gavage study in which there was direct tissue contact with a more concentrated acrylonitrile solution.

Rat gavage study (Maltoni *et al*, 1977; 1988)

Acrylonitrile was administered orally to male and female Sprague-Dawley rats by gavage in olive oil, at a single daily dose of 5 mg/kg bw 3 times weekly for 52 weeks. The rats were then observed until spontaneous death occurred. There were no effects on survival or body weight of the test animals. No treatment-related histological changes were observed in liver, kidneys and lung. Acrylonitrile administration did not affect the percentage of animals bearing benign and malignant tumours, the number of animals bearing malignant tumours only, the number of total malignant tumours per 100 animals or the incidence of Zymbal's gland carcinomas, extra hepatic angiosarcomas, hepatomas and encephalic gliomas. The only increase in incidence of tumours was in the mammary gland and forestomach of female rats.

Rat inhalation study (Quast *et al*, 1980a)

Male and female Spartan Sprague-Dawley rats were exposed to acrylonitrile by inhalation at concentrations of 20 or 80 ppm, 6 hours/day, 5 days/week for 2 years. Controls were exposed to air only. Additional animals were included for interim sacrifice at 6 and 12 months. A statistically significant increase in mortality was observed within the first year in both male and female rats administered 80 ppm and in the females of the 20 ppm group during the last 10 weeks of the study. The apparent increase in the reported mortality for the 20 ppm females was principally due to early sacrifice of rats with large, benign, mammary gland tumours. These tumours are known to occur spontaneously in this strain at a high rate, but in this experiment the tumours were observed earlier and more frequently, and became larger in exposed animals. Primary treatment-related effects were observed in the nasal turbinate mucosa of all rats examined in the 80 ppm group as well as in most of the rats in the 20 ppm group. The changes in both groups were qualitatively similar but much less severe in the 20 ppm group than in the 80 ppm group. The main tumours observed in rats exposed to acrylonitrile were microscopic brain tumours and Zymbal's gland tumours.

Rat inhalation study: Maltoni *et al* (1977, 1988)

The effects of inhalation exposure to 5, 10, 20 and 40 ppm acrylonitrile, 4 hours/day, 5 days/week for 12 months were investigated in groups of male and female Sprague-Dawley rats. One group of untreated rats acted as controls. After the 12 month exposure period, rats were kept under observation until spontaneous death. There were no apparent effects on mortality or body weights throughout the study. A statistically significant increase in the percentage of animals bearing benign and malignant tumours ($p < 0.01$), malignant tumours alone ($p < 0.01$) and in the number of total malignant tumours per 100 animals was found in several treated groups, although a strong dose response relationship was not established. Slight to moderate increases in tumour incidence were observed in the mammary gland, forestomach and CNS, but none of these were statistically significant. No increase in Zymbal's gland tumours, extra-hepatic angiosarcomas and hepatomas was observed, 3/60 and 2/60 cephalic gliomas were observed in animals exposed to the two highest concentrations of acrylonitrile. Whilst this finding did not achieve statistical significance, it is biologically significant given that the brain was clearly shown to be the target organ in rats following oral administration. The authors suggested that the carcinogenicity of acrylonitrile was influenced by the age of the animals at the start of treatment, and was dependent on the concentration administered and duration of treatment. The results were considered by the authors to indicate a borderline carcinogenic effect.

Three-generation study: Friedman & Beliles (2002)

Although not primarily intended to assess carcinogenicity, this three-generation toxicity drinking water study in Sprague-Dawley rats identifies increased tumour incidences. The study evaluated tumour pathology for selected tissues in F_0, F_1, and F_2 adult females exposed for 20 weeks after weaning of the second littering of pups (giving a total exposure period of approximately 1 year). This evaluation was conducted to assess whether there was any increased susceptibility to specific tumour types resulting from perinatal exposure to acrylonitrile. The brain was examined in all adult females surviving to scheduled necropsy; the majority of other tissues were evaluated histopathologically only if they demonstrated gross lesions or masses. A low incidence of astrocytomas and Zymbal's gland tumours was found in all generations. Tumour incidence was slightly greater in the F1 animals that had perinatal exposure compared to the F0 animals that did not, but was very similar in the F0 animals that did not have exposure *in utero* or during lactation and the F2 animals that did. It should be noted that the number of animals evaluated was low compared to a standard carcinogenicity evaluation. The findings of this study suggest that *in utero* or perinatal exposure to acrylonitrile does not lead to an increased incidence of astrocytomas compared to that seen after adult exposure alone.

Tumour incidence in female rats after approximately 1 year drinking water exposure

Generation	Dose (ppm)	Total tumours/masses	Astrocytomas	Zymbal's gland tumours
F0	0	0/19	0	0
	100	3/20	1	0
	500	6/24	2	2
F1	0	1/20[a]	0	0
	100	3/19	1	2
	500	9/17[b]	4*	3*
F2	0	0/20	0	0
	100	2/20[c]	1	0
	500	7/20[d]	1	3

**statistically significant ($p \leq 0.05$); [a]Uterine papilloma; [b]Includes 1 adenocarcinoma of the leg and 1 mammary adenocarcinoma; [c]Includes 1 mammary fibroadenoma; [d]Includes 3 mammary fibroadenomas*

Human epidemiological studies

The numerous epidemiology studies investigating cancer incidence and/or mortality in exposed workers are individually summarised here and elsewhere. Some individual studies have reported associations between acrylonitrile exposure and increased cancer incidence; however the studies have limitations and more recent updates of individual cohorts have not identified any increase in cancer risk consistently across studies. Recent meta-analyses and reviews have concluded that the existing epidemiology data do not support an increased risk of cancer resulting from acrylonitrile exposure.

Reviews

The peer review of acrylonitrile performed by TERA in 2004 (Haber & Paterson, 2005), noted that the acrylonitrile database contains unusually extensive epidemiology data. It was concluded that 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. Overall, the epidemiological data was noted by the TERA review to have three striking features: (1) the size and completeness of the database; (2) the lack of consistently positive findings across studies; and (3) the lack of a clear dose-response relationship for human cancer. Overall, the panel concluded that the epidemiology data do not support an association between acrylonitrile and increased cancer risk in humans, but that such an association could not be ruled out completely. It was concluded that linear extrapolation from the animal data was not supported by the available epidemiology data and that the overall weight of the evidence suggests that acrylonitrile may be carcinogenic to humans at high doses based on extrapolation from rat studies, but the cancer risk associated with the low levels to which humans have been exposed in occupational settings is negligible. In an assessment of the mechanism and dose-response relationship for carcinogenicity, Kirman *et al* (2005) also concluded, based on a weight of evidence approach, that the data support a non-linear extrapolation.

The data on the carcinogenicity of acrylonitrile was also reviewed by the IARC in their re-evaluation of the classification of the substance. Regarding the animal carcinogenicity data, The IARC monograph noted that acrylonitrile has been tested for carcinogenicity in one study in rats by inhalation with pre- and postnatal exposure. This study confirmed the findings of increased incidences of glial cell tumours of the central nervous system found in several previous studies that had not been fully reported and also found increases in malignant mammary tumours, Zymbal gland carcinomas, benign and malignant hepatocellular tumours and extrahepatic angiosarcomas. Acrylonitrile forms adducts with proteins and glutathione. It also forms DNA adducts *in vitro*, but only after cytochrome P450 bioactivation, most likely through its epoxide metabolite (cyanoethylene oxide), which is also formed *in vivo*. Acrylonitrile–haemoglobin adducts have been detected in exposed workers. Both acrylonitrile and cyanoethylene oxide can conjugate with glutathione, leading to detoxification of these reactive compounds. At high doses of acrylonitrile, as used in animal studies, glutathione in certain tissues may be depleted. Such glutathione depletion will probably not occur at low-level human exposure. Acrylonitrile is mutagenic in vitro; in *Salmonella* systems, bioactivation (to cyanoethylene oxide) is required, but in *Escherichia coli* and in rodent systems, bioactivation by an added microsomal system is not required. The results of genotoxicity experiments *in vivo* have in most cases been negative, although acrylonitrile is mutagenic in *Drosophila*. Regarding the human carcinogenicity data, the IARC monograph notes that the potential carcinogenicity of acrylonitrile in occupationally exposed populations has been investigated in several epidemiological studies. Studies carried out in the 1970s and 1980s suggested a possible increased risk of lung cancer among workers exposed to acrylonitrile. However, these were inconclusive because of one or more of the following actual or potential problems: small sample sizes, insufficient length of follow-up, incompleteness of follow-up, inadequate exposure assessment, potential confounding by other occupational carcinogens, and potential confounding by smoking. Consequently, larger and better studies were undertaken, in most cases building upon the same cohorts that had previously been assembled. Four such studies (two in the United States, one in the United Kingdom and one in the Netherlands) were carried out and these now provide the most relevant, informative data on which to base an evaluation. All of the studies made some attempt to establish exposure levels, although for the British study, this was rather cruder than for the others. The two studies from the United States were carried out in similar industries, but the range of cumulative exposure values was quite different between the two, raising questions about the inter-study comparability of methods of exposure assessment. The four studies employed different strategies for comparing exposed with unexposed. While the British study used a classic SMR comparison with national rates, the Dutch study did the same, but also compared the exposed with a different unexposed cohort. One of the studies from the United States compared the exposed with national rates and with rates of mortality and incidence in other plants of the same large company. The other compared the exposed with workers in the same plants who were unexposed to acrylonitrile. Typically, in each study, a number of analyses were carried out, varying comparison groups and other parameters. The IARC monograph concludes that was no significant excess risk for any type of cancer when all exposed workers were compared with unexposed, or with an external comparison population. Further, when the study subjects were subdivided by levels of exposure (cumulative exposure when feasible), for no site but lung was there any hint that risk increased with exposure. For lung cancer, there was an indication that workers with the highest exposures had relative risk estimates greater than 1.0. This finding was strongest in the largest of the studies, which had one of the most intensive exposure assessment protocols, but the

other studies gave either negative or only weakly supportive results. Even in the largest study (where the relative risk in the highest exposure quintile ranged from 1.2 to 1.7 depending on the parameters in the analysis), the finding was not consistently statistically significant; there was no coherent dose–response pattern throughout the range of exposures and the risk in the highest decile of exposure was lower than that in the second highest decile. On balance and given the largely unsupportive findings from the other studies, the evidence from this one study was not considered to be sufficiently strong to conclude that there was a credible association between acrylonitrile and lung cancer. Thus, the earlier indications of an increased risk among workers exposed to acrylonitrile were not confirmed by the recent, more informative studies.

Cole et al (2008) reviewed several retrospective cohort epidemiology studies evaluating a number of health outcomes in workers exposed to acrylonitrile. The authors reviewed epidemiology studies published since 1970 and identified through Ovid and MEDLINE. A total of 26 studies which examined mortality and/or incidence rates among persons with AN exposure were identified. Where cohorts have been updated the most recent data were relied upon but descriptions of the earlier publications are provided for background and rationale. Results are provided for all causes of death and all cancers. Detailed results and discussions are provided for the cancers which have received the most attention and for which some positive results have been reported. These include lung, bladder, prostate and central nervous system cancers. In this review the four most informative cohort studies are evaluated and it is apparent that the results do not support a causal relationship between AN exposure and all cancers or any specific type of cancer. The authors also note that the IARC actually downgraded acrylonitrile from 'probably carcinogenic' to 'possibly carcinogenic to humans', finding that 'the earlier indications of an increased risk among workers exposed to acrylonitrile were not confirmed by the recent, more informative studies'. This review of the epidemiology data is consistent with the conclusions of the earlier IARC review which found no consistent findings of increased cancer risk across studies.

Boffetta *et al* (2008) describe examples from cancer epidemiology of what they consider to be likely false-positive findings. The conditions under which such results may occur are discussed. General guidelines or principles, including the endorsement of editorial policies requiring the prominent listing of study caveats, which may help reduce the reporting of misleading results are proposed. The authors consider that 'increased epistemological humility' regarding findings in epidemiology would go a long way to diminishing the detrimental effects of false-positive results on the allocation of limited research resources, on the advancement of knowledge of the causes and prevention of cancer, and on the scientific reputation of epidemiology and would help to prevent oversimplified interpretations of results by the media and the public. As one of a number of examples presented in this paper, a cumulative meta-analysis of the initial (1978) findings and the 15 subsequent studies of acrylonitrile and lung cancer that were published in 1980-1998 was performed. A steady decrease over time in the reported overall relative risk estimate of lung cancer in acrylonitrile workers was identified; the authors conclude that the initial finding in the 1978 study of a link between acrylonitrile exposure and lung cancer was a false positive result.

Summary of carcinogenicity

It is clear that acrylonitrile is carcinogenic at numerous sites in rodent bioassays: all rodent studies demonstrate a positive response. In contrast, the weight of evidence

from numerous epidemiological studies does not support an association between worker exposure to acrylonitrile and increased cancer risk. The reason for the marked difference between occupationally exposed humans and the results of the animal studies is unclear, however this could be due to either the MoA in rats not being relevant to humans, or that acrylonitrile carcinogenicity is a threshold effect and human exposure does not exceed this threshold.

The following information is taken into account for any hazard / risk assessment:

The carcinogenicity of acrylonitrile has been investigated in a large number of studies in rats and mice, using oral (gavage, drinking water) and inhalation exposure. The results of the studies indicate that acrylonitrile is a multi-site carcinogen in rodent species. In contrast, the weight of evidence from numerous well conducted epidemiological studies does not support an association between worker exposure to acrylonitrile and increased cancer risk.

Value used for CSA (route: inhalation):

Target organs: digestive: stomach; neurologic: brain (multiple sections); glandular: mammary gland; glandular: other

Justification for classification or non classification

Acrylonitrile was classified as a category 2 carcinogen: (R45) 'May cause cancer' under Directive 67/548/EEC. This classification equates to the GHS Cancer Category 1B according to Annex VI of the CLP Regulation. However recent reviews of the extensive epidemiology dataset have concluded that there is a lack of association between acrylonitrile exposure and increased cancer risk in exposed workers.

Given the consistent and clear observations of a lack of effect in occupationally exposed humans, it would seem more appropriate to reinterpret the hazard as a 'suspected human carcinogen' (GHS Category 2), consistent with the IARC classification of acrylonitrile. The reason for the difference between occupationally exposed humans and the results of the animal studies is unclear, however this could be due to either the MoA in rats not being relevant to humans, or that acrylonitrile carcinogenicity is a threshold effect and human exposure does not exceed this threshold.

Although research is presently unable to fully define a mode of action for acrylonitrile carcinogenicity, the existence of a threshold principle is entirely plausible based on the existing data. Kirman *et al* (2005) were able to show the link between occupational human exposure and the results of the rodent cancer assays by modelling the exposure concentrations based on internal CEO levels. This demonstrated that even the highest occupationally relevant exposure concentrations in humans (which are now no longer permitted since changes in legislation), gave rise to an internal concentration at the very lowest animal exposure levels where significant cancer risk was not apparent in the animals. Whilst a cancer risk in humans at high concentrations cannot presently be entirely ruled out, the occupational exposures presently imposed are clearly below a threshold for cancer. At the time of the EU RAR (2004), there was little mechanistic evidence to support the threshold carcinogen hypothesis; therefore acrylonitrile was conservatively classed as a non-threshold (direct DNA acting) carcinogen. As well as recent updates to the epidemiology dataset, numerous recent mechanistic studies (see special investigations section) have highlighted the link between effects in rat tissue and oxidative stress and the cell transformation capacity of acrylonitrile. Mechanistic data therefore strongly indicate that the

carcinogenicity of acrylonitrile occurs through an indirect mechanism secondary to the induction of oxidative stress in the target tissue, through the (non-genotoxic) transformation of initiated cells, or through a combination of these threshold mechanisms.

IARC consideration of acrylonitrile

It is notable that the IARC (Monograph 71; 1999) downgraded their carcinogenicity classification of acrylonitrile to Group 2B (possibly carcinogenic to humans). This assessment was based on a consideration of the genotoxicity data, animal carcinogenicity and human epidemiological data. It was concluded that, while acrylonitrile was mutagenic *in vitro*, the results of studies *in vivo* were largely negative. The clear evidence of carcinogenicity in studies in experimental animals was not considered to be reflected in the epidemiology. The IARC concluded that, on balance, and given the largely unsupportive findings from the other epidemiology studies, the evidence of an increased incidence of lung cancer reported in exposed workers in one early study was not considered to be sufficiently strong to conclude that there was a credible association between acrylonitrile exposure and lung cancer. The earlier indications of an increased cancer risk in workers exposed to acrylonitrile were therefore not confirmed by the more recent studies, which were also considered to be more informative. The IARC Group 2B classification is comparable to the GHS Category 2 classification for carcinogenicity.

Specific investigations: other studies

Carcinogenicity mode of action

A large number of studies have been performed in order to further elucidate the carcinogenic mode of action of acrylonitrile. Studies have focussed on the induction of oxidative stress and cell transformation. The dataset on cell transformation has also been reviewed in the EU RAR (2004), by the Sapphire Group (2004) and more recently by Strother (2010).

The EU RAR (2004) reviewed the extensive dataset on the potential of acrylonitrile to cause cell transformation which is informative in relation to the potential of acrylonitrile to cause carcinogenicity by non-genotoxic mechanisms. It concludes that the various studies summarised in this review document indicate that acrylonitrile has the ability to cause cell transformation; it further states that cell transformation appears to be secondary to oxidative damage and is dependent on metabolic activation. The Sapphire Group review (2004) also notes that the available data indicate that acrylonitrile has the ability to cause cell transformation as a consequence of a reduction in cellular antioxidant status. The extensive investigations of cell transformation show that the induction of oxidative stress by acrylonitrile is dependent on its metabolism to CEO and cyanide. The draft report of the North Carolina Science Advisory Board (NCSAB, 2010) also concludes that the mechanistic work by Pu *et al* suggests that ROS related to toxicity induced by high doses of acrylonitrile appear to play a critical role in its carcinogenicity in the highly sensitive rat brain. The NCSAB also state there is relevant evidence that acrylonitrile possesses genotoxic and carcinogenic activity by acting indirectly in the production of brain tumours in the rat, and that this may possibly occur via a high-dose mechanism involving oxidative stress and changes in gap junction communication.

In a study in cultured human astrocytes, Jacob & Ahmed (2003) conclude that a redox imbalance may play a major role in acrylonitrile-induced neurotoxicity, which is indicated by compromised antioxidant defence mechanisms (depletion of GSH, increase in GSSG, inhibition of catalase, increased ROS formation and TNF-α secretion), resulting in oxidative DNA damage. In a series of studies in cultured Syrian Hamster Embryo cells, Zhang *et al* (2002) suggest that the induction of oxidative stress by acrylonitrile involves a temporal decrease in antioxidants and increase in xanthine oxidase activity that is mediated by the oxidative metabolism of acrylonitrile.

Pu *et al* (2009) investigated oxidative stress and DNA damage in rats exposed to acrylonitrile in the drinking water. No significant increase in direct DNA strand breaks was observed in brain and WBC from acrylonitrile-treated rats. However, oxidative DNA damage (fpg comet and 8'hydroxyl-2-deoxyguanosine) in brain and WBC was increased in a dose-dependent manner. In addition, plasma levels of reactive oxygen species (ROS) increased in rats administered acrylonitrile. Dietary supplementation with NAC prevented acrylonitrile-induced oxidative DNA damage in brain and WBC. A slight, but significant, decrease in the GSH: GSSG ratio was seen in brain at acrylonitrile doses of >30 ppm. The authors conclude that the results of this study provide support for a mode of action for acrylonitrile-induced astrocytomas involving the induction of oxidative stress and damage. Significant associations were seen between oxidative DNA damage in WBC and brain, ROS formation in plasma, and the reported tumour incidences. Since oxidative DNA damage in brain correlated with oxidative damage in WBC, the results suggest that monitoring WBC DNA damage maybe a useful tool to assess acrylonitrile-induced oxidative stress in humans.

Esmat *et al* (2007) demonstrated that acrylonitrile induces oxidative stress in cultured rat glial cells, depleting reduced GSH and caused lipid peroxidation. The anti-oxidant compound N-acetylcysteine was shown to inhibit the oxidative effects of acrylonitrile. In contrast, the administration of dietary antioxidants did not have any notable effect on the levels of cyanoethylvaline-globin adducts in female rats administered acrylonitrile in the drinking water for 28 days (Snyder, 2010). A similar study reported by Klaunig & Forney (2010) demonstrated increased 8-OHdG formation in rat brain and oxidative DNA damage (but not DNA strand breakage) in peripheral white blood cells.

Klaunig & Forney (2010) investigated the effects of selected antioxidants on acrylonitrile-induced oxidative stress was investigated in female F344 rats. Groups of rats were administered acrylonitrile at 0 or 100 ppm in the drinking water for 28 days; groups received basal diet or diet supplemented with the anti-oxidants Vitamin E (0.05%), Green tea polyphenols (0.4%), N-acetyl cysteine (0.3%), sodium selenite (0.1 mg/kg) or taurine (10 g/kg). Bodyweights were measured weekly; liver weights were measured at termination. Total anti-oxidant capacity, malondialdehyde and 8-OHdG levels were measured in samples of brain tissue. Direct and oxidative DNA damage were assessed in white blood cells by Comet assay. Acrylonitrile induced oxidative stress in the brain of female F344 rats following the administration of 100 ppm in drinking water for 28 days, as shown by increased 8-OHdG formation. Acrylonitrile also induced oxidative DNA damage, but not DNA strand breakage in white blood cells. Dietary supplementation with Vitamin E, green tea polyphenols, N-acetyl cysteine in diets prevented or reduced acrylonitrile-induced 8-OHdG formation. Dietary supplementation with selenium or taurine gave no significant protection against acrylonitrile-induced oxidative stress. In contrast, supplementation of all five

antioxidants in diets prevented oxidative DNA damage induced by acrylonitrile in white blood cells. Acrylonitrile did not cause significant changes in total antioxidant capacity and malondialdehyde level in brain tissues.

Using blood samples taken from the same rats in the study by Klaunig & Forney2010, Snyder *et al (*2010) carried out a study to determine cyanoethylvaline (CEVal) haemoglobin adducts in blood samples following administration of acrylonitrile in drinking water, with or without antioxidant supplements in the diet. Globin CEVal adducts were measured in terminal blood samples by LC/MS-MS. Globin CEVal levels were markedly increased by the administration of acrylonitrile, however the administration of the dietary antioxidants did not have any notable effect on CEVal levels.The following information is taken into account for any hazard / risk assessment:

The results of a number of cell transformation assays indicate that acrylonitrile has the potential to reduce antioxidant status, increase xanthine oxidase activity and cause cell transformation. The results of recent studies provide evidence for the involvement of oxidative stress in the mode of action of acrylonitrile carcinogenesis in rodent studies.

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